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STUDIES IN REPRODUCTIVE ENDOCRINOLOGY

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DECLARATION

This is to certify that this thesis entitled "Studies in Reproductive Endocrinology" presented for the degree of Doctor of Science in Medicine to the faculty of Medicine at the University of the Witwatersrand is my own work. It has not been presented at any other university.

Irving M Spitz

DEDICATION

This thesis is dedicated to the memory of my beloved father Harry Spitz.

He was a devoted son, father, grandfather and brother. He dedicated his life to the healing of the sick but was also a Zionist, philosopher, humanitarian, historian and music lover. His shining example of what it is possible to accomplish in life will forever remain imprinted in my mind and govern all my actions.

He was a man, take him for all in all,
I shall not look upon his like again.

His life was gentle; and the elements
So mix'd in him that Nature might stand up
And say to all the world "This was a man!"

W. Shakespeare

ACKNOWLEDGMENTS

First and foremost, I would like to acknowledge the help and devotion of Diane, my wife and partner in life. Without her patience, encouragement and tolerance, none of this work could have been accomplished. I also wish to thank all the patients and volunteers who devoted their time to undergo testing. Finally, I owe a debt of gratitude to my colleagues for their help and co-operation.

PREFACE:

OUTLINE AND CATEGORIES OF SUBMITTED PUBLICATIONS

For this work, I have selected 30 of my publications to portray various aspects of reproductive endocrinology which I have studied over the past 25 years. These publications are divided into two categories which correspond to distinct periods of my career.

1. Studies in patients with isolated pituitary deficiency and testicular failure. These investigations commenced in 1971 during my Fellowship in Endocrinology under the direction of the late Dr. David Rabinowitz at the Department of Endocrinology, Hadassah University Hospital, Jerusalem, Israel. Dr. Rabinowitz left Israel in 1972 and I continued these studies at the same institution until 1976. From 1977 till 1982, all studies were conducted under my direction in the Department of Endocrinology and Metabolism at Shaare Zedek Medical Center in Jerusalem.

2. Studies with synthetic hormone agonists and antagonists. These were conducted from 1983-1996 while I was a Senior Scientist at the Center for Biomedical Research at The Population Council, New York.

Listed below are the peer-review journals in which these articles were published.

Journal of Clinical Endocrinology and Metabolism (11)*

New England Journal of Medicine (6)

American Journal of Obstetrics and Gynecology (2)

Clinical Endocrinology (2)

Contraception (2)

Acta Endocrinologica (1)

* the number of articles appearing in the particular journal is shown in brackets

Annual Review of Pharmacology and Toxicology (1)

Archives of Internal Medicine (1)

Fertility and Sterility (1)

Journal of Neurosurgery (1)

Journal of Steroid Biochemistry (1)

Metabolism (1)

INTRODUCTION:

SCOPE OF SUBMITTED PUBLICATIONS

1. Studies in patients with isolated pituitary hormone deficiency

The hypothalamic-portal blood system was first described by Poppa and Fielding in 1930 (1)*. It was Green and Harris (2) who initially proposed that the hypothalamus controlled anterior pituitary function by secreting regulatory factors into the hypothalamic-portal circulation. The ultimate proof of this hypothesis was supplied in 1970 by the groups of Roger Guillemin (3) and Andrew Schally (4) who, working independently, isolated and synthesized the first hypothalamic releasing hormone known as thyrotropin-releasing hormone (TRH). Subsequently Schally and coworkers (5) isolated and synthesized luteinizing hormone-releasing hormone (LHRH). This was shown to be a decapeptide and it stimulated the secretion of both pituitary gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH). Since LHRH releases LH as well as FSH, it has become customary to refer to this hypothalamic releasing hormone as Gonadotropin hormone releasing hormone (GnRH).

Soon after synthetic GnRH became available, my attention was drawn to the evaluation of patients who presented with isolated anterior pituitary hormone deficiency. This may be defined as selective deficiency of a pituitary hormone with the rest of the gland retaining normal function. One such example is isolated bihormonal gonadotropin deficiency, which is also known as hypogonadotropic hypogonadism. This is characterized by low circulatory levels of both LH and FSH.

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* The number in brackets refers to the chronological list of references cited from the literature. All are listed at the end of this Introduction.

As a consequence of the gonadotropin deficiency, these subjects have low levels of sex steroids and present with hypogonadism. The aim of our evaluation was to determine whether the isolated gonadotropin deficiency originated because of a lesion at the level of the pituitary, the hypothalamus or even higher level. Publications 1 and 2** were among the earliest reported at the time which showed that female and male subjects presenting with this syndrome had a heterogeneous response to the administration of GnRH. In the majority of these subjects, there was elevation of LH and FSH following GnRH, but when compared to controls, the response was impaired. It was thus concluded that in the majority of these subjects, the site of the lesion was at the hypothalamus or above.

Meanwhile, it had been shown that TRH stimulated the secretion not only of thyrotropin (TSH) but also of prolactin (6). It had been known for many years that prolactin secretion was under tonic inhibition by dopamine (7,8) and that dopaminergic antagonists such as metoclopramide and chlorpromazine were potent stimulators of prolactin secretion (9). Further evaluation of subjects with isolated gonadotropin deficiency showed that these subjects had low basal prolactin levels with an impaired prolactin response to stimulation with TRH, chlorpromazine and metoclopramide (publications 3 and 4). Female (but not male) patients with isolated gonadotropin deficiency also demonstrated subtle defects in TSH secretion characterized by an impaired thyrotropin response to TRH in the presence of normal levels of circulating thyroid hormones (publication 5).

The question arose as to whether these defects in prolactin and thyrotropin secretion were an inherent component of the syndrome. An alternate explanation was that they represented a

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** These refer to the personal publications forming the body of this thesis and are listed chronologically in the following section.

consequence of the low circulatory estradiol levels. It was known that both prolactin and thyrotropin secretion were stimulated by estrogens (8.10,11). To test the hypothesis, we treated female patients with exogenous ethinyl estradiol or conjugated estrogens. Male patients were given human chorionic gonadotropin (hCG) which increased both serum testosterone and estradiol levels. In both sexes, there was normalization of basal prolactin levels and restoration of the impaired prolactin responses to TRH and metoclopramide (publications 3 and 4). However, constant treatment was required to retain normal prolactin responsiveness and its cessation in both males and females was associated with a reduction of both basal and stimulated prolactin levels (publications 3 and 4). The impaired thyrotropin response to TRH observed in the female patients with this syndrome was also normalized following administration of estrogens (publication 5). The results of these studies supported the conclusion that the altered prolactin and thyrotropin secretory patterns characteristic of isolated gonadotropin deficiency were not an integral component of the syndrome. Rather they were reversible and represented a consequence of the low levels of serum estradiol. In contrast to these subtle defects in prolactin and thyrotropin secretion, patients with isolated bihormonal gonadotropin deficiency had intact growth hormone and adrenocorticotropin (ACTH) secretion (publication 1).

We have utilized the impaired prolactin response to TRH in isolated gonadotropin deficiency as an ancillary aid in differentiating this condition from delayed puberty. Both conditions are characterized by an impaired gonadotropin response to GnRH (12). In contrast to isolated gonadotropin deficiency, however, the prolactin response to TRH was normal in delayed puberty (publication 6).

Although isolated bihormonal gonadotropin deficiency is the commonest type of selective pituitary hormone deficiency, isolated deficiency of a single gonadotropin is extremely uncommon. During these investigations, the first patient was described who presented with isolated deficiency of FSH. She had low to undetectable levels of FSH in the presence of high levels of circulating LH (publication 7). This was the first subject described in which antibodies to FSH developed following administration of FSH. The preparation used was human menopausal gonadotropins (hMG) also known as menotropins (publications 8 and 9).

There have been several reports describing subjects who presented with selective deficiency of other pituitary hormones such as growth hormone, thyrotropin and ACTH (13). However, isolated prolactin deficiency is distinctly uncommon. Publication 10 documents the first subject to be described who presented with unequivocal evidence of isolated prolactin deficiency. This subject had low basal prolactin levels and an absent prolactin response to classical stimuli of prolactin secretion. The remainder of the pituitary function was intact. The clinical presentation of this patient was one of short stature and delayed puberty. The administration of hCG produced an increase in height and sexual maturation. However, following treatment, there was still an absent prolactin response to stimulation.

To determine possible relationships between prolactin secretion and reproductive function in other clinical situations, we turned our attention to the evaluation of patients with primary testicular disorders.

2. Studies in patients with primary testicular disorders

Patients studied had idiopathic azoospermia or severe oligospermia consequent to primary testicular failure or secondary to exposure to the industrial nematocide, dibromochloropropane. These subjects had high levels of gonadotropins. Testosterone and estradiol levels were normal in

idiopathic but increased in those subjects with azoospermia secondary to dibromochloropropane. In contrast to isolated gonadotropin deficiency, these subjects demonstrated exaggerated prolactin responses to TRH, metoclopramide and chlorpromazine. Basal prolactin levels, however, were normal (publications 3 and 11). This exaggerated prolactin response was only noted with pharmacological stimulation; prolactin levels were normal in response to the physiological stimulus of sleep (publication 12).

The exaggerated prolactin responses could be related to estrogens which are known to increase prolactin levels (8, 10). Indeed estradiol levels were increased in those subjects who had been exposed to dibromochloropropane and the estradiol/testosterone ratio correlated with the increased prolactin response to TRH in the idiopathic group (publications 3 and 11). Moreover following the administration of the antiestrogen clomiphene citrate, there was a reduction in the exaggerated prolactin response confirming that this phenomenon is indeed modulated by estrogens (publication 13) It was thus concluded that the exaggerated prolactin response observed in patients with primary testicular disorders, represented a pharmacological phenomenon and was a consequence of enhanced estrogen activity.

3. Studies with a GnRH agonist

Soon after the discovery of GnRH, scientists turned their attention to the synthesis of agonistic and antagonistic analogs of this decapeptide. Currently many potent long-acting preparations are available (14). Following the administration of a potent agonist, there is an initial burst of both LH and FSH secretion. This is then followed by receptor down-regulation which results in a fall of gonadotropin secretion (14). Thus administration of a potent GnRH agonist has a paradoxical effect and leads to reduction of secretion of sex steroids in both men and women resulting in the development of a hypogonadal state.

One subset of patients with acute intermittent porphyria often have severe exacerbations of their disease often with debilitating attacks in relation to the menstrual cycle. These attacks are presumably hormone induced. We asked ourselves if administration of a long-acting GnRH agonist would alter these cyclical attacks of pain patterns by producing a medical hypogonadism. Parenteral administration of one of these long acting agonists (D-His) blocked these repeated attacks (publication 14). We subsequently developed an effective nasal delivery system for this long acting agonist (publication 15). This form of treatment is now well-established in the treatment of this variant of the disease.

4. Studies with a progesterone antagonist

An unique opportunity to continue our ongoing study of antihormones and hormone antagonists arose in 1981 (15). In that year the first antiprogestin was synthesized by scientists at Roussel UCLAF. This was known as RU 38486. It was later abbreviated to RU 486 and is now known as mifepristone. Of interest is the fact that this synthetic steroid was first recognized as an antiglucocorticoid. Only subsequently was it appreciated that this agent was also a potent antiprogestin (15). Mifepristone binds strongly to both progesterone and glucocorticoid receptors and displays potent antiglucocorticoid and antiprogestin properties. This fascinating compound has enabled us to further our understanding of female reproductive function. The mechanism of action and physiological effects of antiprogestins has been reviewed in publications 16 and 17.

Our initial task was to determine if this agent produced glucocorticoid deficiency since this would limit its use as an antiprogestin. To answer this question, studies were conducted in dogs. Despite blockade of glucocorticoid receptors with high doses of mifepristone, none of the dogs developed any signs of adrenal insufficiency. This was related to the fact that the increase in ACTH and cortisol consequent to mifepristone administration, overcame glucocorticosteroid re-

ceptor blockade (publication 18). An interesting observation was that on repeated challenging some of these animals consistently had higher ACTH and cortisol responses than others. It is not known why these dogs displayed different patterns to mifepristone administration. It could presumably be related to variations in pharmacokinetics, since circulating levels of mifepristone and the mono- and didemethylated derivatives were higher in those animals who demonstrated a greater cortisol response (publication 19). In pharmacokinetic studies carried out in humans, we have shown that mifepristone is rapidly broken down to these metabolites which bind to glucocorticoid and progesterone receptors, and may have antagonistic actions (publication 20).

An attempt was then made to block glucocorticoid receptors by administering high doses of mifepristone to a patient with Cushing's syndrome due to ectopic ACTH syndrome. This ameliorated the clinical and biochemical manifestations of the disease without changing circulating cortisol levels (publication 21). The results of these studies showed that mifepristone was an effective clinical glucocorticoid antagonist.

Mifepristone may also play a role in the treatment of other tumors which have demonstrable steroid receptors (16, 17). One such tumor is the meningioma which has high concentrations of progesterone receptors (17). We showed that there was objective and subjective improvement in five out of 14 patients with inoperable meningioma on treatment with 200 mg mifepristone daily (publication 22). None of these subjects developed any clinical evidence of unequivocal glucocorticoid deficiency.

One of the main actions of progesterone is in the initiation and maintenance of pregnancy. For this reason, an antiprogestin would be expected to produce medical termination of pregnancy. Different dose schedules of mifepristone were used to terminate pregnancy in women with amenorrhea of under 49 days duration (publications 23 and 24). The success ranged from 50 -

86% with doses of 50 mg or 100 mg administered daily for 7 days or with a single 450 mg dose. There was only a 10% success rate with higher doses (200 or 400 mg daily for 4 days). In these studies, there was a dose dependent transient increase in serum cortisol but in none of these subjects was there clinical evidence of hypoadrenalism. There were two major conclusions to this study. Firstly it was apparent that mifepristone is a more potent antiprogestin than it is an antiglucocorticoid and that higher doses of mifepristone were required to produce an antiglucocorticoid as compared to an antiprogestin effect. Secondly, when administered alone, mifepristone was not sufficiently effective as a medical abortifacient to be used routinely in the clinic.

Mifepristone has many other potential applications in female reproductive health. It is well known that together with estradiol, progesterone facilitates the LH surge prior to ovulation (18). The question was posed as to whether mifepristone could block the LH surge and ovulation thus acting like the classical contraceptive pill. Follicular phase mifepristone administration interrupted normal follicular development. This resulted in delay of the LH surge and ovulation (publication 25). Following cessation of mifepristone, there was re-initiation of follicular growth with eventual ovulation. An attempt was made to use intermittent mifepristone administration as a contraceptive agent by blocking ovulation (publication 26). With the doses and regimen used, it was not always possible to block the LH surge. Furthermore, no definite dose response effect was evident. It was concluded that with the regimens evaluated, mifepristone could not consistently inhibit ovulation.

Progesterone is critical for transforming the endometrium from a proliferative to a secretory state. It is the fall of progesterone and estradiol at the end of the menstrual cycle which initiates the process of endometrial sloughing and bleeding. Could mifepristone, acting as an antiprogestin induce endometrial bleeding by withdrawing progesterone support? Mifepristone, ad-

ministered in a dose of 50 mg or higher in the mid or late luteal phase, did indeed produce menstrual bleeding (publications 27 - 30).

Was the menstrual bleeding consequent to mifepristone administration a direct action on the endometrium or secondary to an effect on the hypothalamic-pituitary-ovarian axis? This issue was addressed by administering hCG with or without mifepristone during the luteal phase in normal women. The action of hCG was to simulate early pregnancy by increasing estradiol and progesterone levels and delaying the onset of bleeding (publication 27 and 28). However this treatment regimen did not prevent bleeding induced by an adequate dose of mifepristone. Thus, by maintaining corpus luteum function with exogenous hCG, mifepristone related menstrual bleeding occurred despite high circulating progesterone and estradiol levels. This indicated that antiprogestins act directly at the level of the endometrium (publication 27).

Mid luteal phase administration of mifepristone (50 mg or greater) induced menstrual bleeding within 36 to 72 hours although complete luteolysis only occurred in one third of the subjects. In the remainder, there was a second episode of menstrual bleeding at the time of spontaneous luteolysis (publications 28 and 29). This effect was not dose dependent (publication 29). This variable effect on bleeding prevents the clinical application of antiprogestins in the mid luteal phase.

In contrast, when mifepristone was administered in the late luteal phase, only one bleeding episode was observed. This usually commenced within 24 to 48 hours after mifepristone administration (publication 30). In another study, mifepristone was given for 4 consecutive days prior to the expected menses for three successive cycles. This was preceded and followed by two placebo-treated cycles (publication 30). Bleeding patterns and hormonal profiles were similar between mifepristone and placebo cycles. It was concluded that mifepristone had no major effect on

menstrual cycle events when administered at the time of expected menses. The results of these studies have suggested new therapeutic avenues to be explored in the treatment of female reproductive health. The possibility exists for the use of antiprogestins as monthly-menses-inducers, morning-after-pills or even contraceptives.

5. Summary

We have shown that patients presenting with isolated bihormonal gonadotropin deficiency respond to the administration of GnRH with an impaired elevation of LH and FSH indicating that the site of the lesion is at the hypothalamus or above. These subjects had low basal prolactin levels with an impaired prolactin response to stimulation. Female patients also demonstrated an impaired thyrotropin responses to TRH. These defects in prolactin and thyrotropin secretion were reversible on steroid treatment indicating that they represented a consequence of the low circulatory estradiol levels and were not an inherent component of the syndrome. This impaired prolactin response to TRH in isolated gonadotropin deficiency has been used as an ancillary aid in differentiating this condition from delayed puberty. In contrast to isolated gonadotropin deficiency, the prolactin response to TRH is normal in delayed puberty.

During these investigations, the first patient was described who presented with isolated deficiency of FSH. She developed antibodies to FSH following administration of exogenous FSH. In addition, we had the opportunity to evaluate the first subject who presented with unequivocal evidence of isolated prolactin deficiency.

Following these observations, we turned our attention to the assessment of prolactin secretion in patients with testicular failure who had high circulating levels of gonadotropins. These subjects demonstrated exaggerated prolactin responses to pharmacological but not to physiologi-

cal stimulation. This exaggerated prolactin response was shown to be a consequence of enhanced estrogen activity.

Studies were then conducted with long acting GnRH agonists which induced a medical hypogonadism. One of these agonists, D-His, was administered to patients with acute intermittent porphyria who had severe exacerbations of their disease in relation to the menstrual cycle. This treatment was extremely effective in blocking these repeated debilitating attacks.

The final part of this thesis, was related to the evaluation of the clinical effects of the synthetic steroid mifepristone, which is both an antiprogestin and an antiglucocorticoid. Despite high dose mifepristone administration to dogs, none developed any signs of adrenal insufficiency. This was because the increase in ACTH and cortisol overcame glucocorticosteroid receptor blockade. High doses of mifepristone administered to a patient with Cushing's syndrome due to ectopic ACTH syndrome ameliorated the clinical and biochemical manifestations of the disease. Mifepristone also plays a role in the treatment of other (non-adrenal) tumors which have demonstrable steroid receptors such as meningioma.

Progesterone is critical in the initiation and maintenance of pregnancy. Different dose schedules of mifepristone were used to terminate pregnancy in women with amenorrhea of under 49 days duration and in the majority of these studies, the success ranged from 50 - 86%. In no subject did hypoadrenalism develop which indicates that mifepristone is more potent as an antiprogestin than as an antiglucocorticoid. However when administered by itself, mifepristone is not a clinically effective medical abortifacient agent.

Follicular phase mifepristone administration interrupted normal follicular development, delaying the LH surge and ovulation. With the doses and regimen used, it was not possible to

uniformly inhibit the LH surge. Thus mifepristone could not be used as a contraceptive by blocking ovulation.

Mifepristone administered in the mid or late luteal phase produced menstrual bleeding as a result of a direct action on the endometrium. Mifepristone had no major effect on menstrual cycle events when administered at the time of expected menses. The advent of antiprogestins has indeed opened a new chapter in reproductive biology.

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PUBLICATIONS



ISOLATED GONADOTROPIN DEFICIENCY

A Heterogenous Syndrome

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Abstract Seven female patients with primary amenorrhea were shown to have isolated gonadotropin deficiency. Thyroid and adrenal function and growth hormone secretion were normal. Basal levels of follicle-stimulating hormone, luteinizing hormone and estradiol 17β were below the limit of sensitivity of our assays, and there was no change after intravenous estrogen or oral clomiphene citrate. With luteinizing-hormone-releasing hormone, levels of both follicle-stimulating and luteinizing hormones rose in five subjects, of luteinizing hormone only increased in one subject, and in the remaining patient there was no change in either hormone. In all three patients to whom urinary gonadotropins (menotropins) were administered serum estradiol 17β was elevated. Subsequent therapy with human chorionic gonadotropin led to ovulation, with an increase in serum progesterone, and two patients became pregnant.

The syndrome of isolated gonadotropin deficiency thus appears to be heterogenous although in most patients the pituitary gonadotrope is intact and the defect resides in the hypothalamus. Ovarian responsiveness is retained. (N Engl J Med 290:10-15, 1974)

ISOLATED deficiency of pituitary secretion of the gonadotropins human follicle-stimulating hormone (hFSH) and human luteinizing hormone (hLH) has long been recognized.[1-6] The advent of radioimmunoassay has permitted documentation of subnormal or absent peripheral levels of both hormones in this syndrome ("hypogonadotropic hypogonadism").[7,8] The low circulating hFSH and hLH levels could be consequent upon failure of the gonadotrope or failure of the appropriate signal to reach the gonadotrope via the pituitary portal circulation. Thus, the defect could be in the hypothalamus or at an even higher central level.

The identification of the thyrotropin-releasing hormone (TRH) has helped to pinpoint the nature of the defect in states of thyroid-stimulating hormone (TSH) deficiency. Many patients with idiopathic hypopituitarism, including secondary hypothyroidism, respond with a rise of TSH after TRH, indicating that the thyrotrope is intact.[9,10] Fleischer et al. have reported a positive response to TRH in one patient with isolated TSH deficiency.[10] Kaplan et al.[9] have suggested that most states of idiopathic pituitary deficiency are in fact due to hypothalamic rather than pituitary disease.

Availability of luteinizing-hormone-releasing hormone (LHRH)[11] has allowed similar examination of the site of the lesion in isolated gonadotropin deficiency, and there have already been some reports of its administration in this condition.[12-16] In our study of seven female patients with this syndrome we sought answers to two questions: whether the site of the lesion in the hypothalamic-pituitary unit could be identified; and what was the responsiveness of the ovary, deprived of endogenous gonadotropin, to therapy with human menopausal gonadotropins (hMG) and human chorionic gonadotropin (hCG).

Our results show that the syndrome of isolated gonadotropin deficiency is heterogenous. Whereas none of the patients responded to clomiphene citrate or to conjugated estrogens, responses to LHRH were varied.

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Abbreviations Used

hCG:	human chorionic gonadotropin
hFSH:	human follicle-stimulating hormone
hGH:	human growth hormone
hMG:	human menopausal gonadotropins
LHRH:	luteinizing-hormone-releasing hormone
TRH:	thyrotropin-releasing hormone
TSH:	thyroid-stimulating hormone

In all patients tested, the ovary responded to exogenous gonadotropin therapy.

Material and Methods

Serum hFSH and hLH were measured by radioimmunoassay with use of double-antibody systems.[17,18] Pituitary hLH (IRC2), supplied by Dr. A. S. Hartree, and pituitary hFSH (LER-1366), provided by the National Pituitary Agency, were labeled with radioactive iodine (^{125}I) to specific activities of 50 to 100 μCi per microgram.[19] Rabbit anti-hCG antibody, a gift of Dr. Saul Rosen, was employed in a final dilution of 1:1,500,000, and rabbit anti-hFSH antibody, a gift of Dr. W. D. Odell, in a final dilution of 1:20,000. Goat anti-rabbit gamma globulin was used as the second antibody. The Second International Reference Preparation for Human Menopausal Gonadotropins (2d IRP-hMG), provided by Dr. D. R. Bangham, was used as reference standard for both hFSH and hLH and expressed as mIU 2d IRP-hMG per milliliter of serum. In our radioimmunoassay system 1 ng of Hartree hLH is equivalent to 8 mIU of 2d IRP-hMG, and 1 ng of hFSH (LER-1366) is equivalent to 2.9 mIU of 2d IRP-hMG. The lower limit of sensitivity of the immunoassay for hFSH and hLH varied in different assays between 0.6 and 1.0 mIU per tube. Since we use a maximum of 0.2 ml of serum per tube, this corresponds to a sensitivity of 3.0 to 5.0 mIU per milliliter for hLH and for hFSH. All serum samples from a sequential study were run in duplicate in the same assay. Serum estradiol 17β was measured by a radioimmunoassay[20] using estradiol-17β-6,7-^3H of specific activity 45 Ci per millimole and a sheep antiserum raised against estradiol-17β-bovine serum albumin conjugate, a gift of Dr. F. Dray. The steroids were extracted from serum by ether, and subsequently subjected to chromatography on Sephadex LH 20, and the appropriate fractions were collected for assay of estradiol. "Bound" estradiol was separated from "free" by use of dextran-coated charcoal.[21] All samples were corrected for the procedural loss, which was monitored by internal recovery. The limit of sensitivity of the assay is 15 pg per milliliter.

Serum progesterone was measured by radioimmunoassay[22] with use of progesterone-1α,2β-^3H with a specific activity of 53 Ci per millimole. The antibody raised against a rabbit was kindly supplied by Dr. H. Lindner, Rehoboth, Israel. Immonoassay was performed after ether extraction, and separation was effected with dextran-coated charcoal.[21]

Growth hormone (hGH) was measured by an immunoassay[23] employing a single antibody system with separation of "bound" from "free" hormone by dextran charcoal.[24]

Serum 11-hydroxy-corticosteroids ("cortisol") were assayed by the fluorometric method of Mattingly.[24]

All test procedures were begun between 7:30 and 8:00 a.m. after an overnight fast. A needle was inserted into an antecubital vein and was kept patent by the slow administration of physiologic saline. After a basal control period of at least 60 minutes, during which serial blood samples were taken, the test substance was given intravenously via a three-way stopcock, and periodic blood sampling continued.

LHRH Test

Unless otherwise stated, 100 μg of LHRH was rapidly injected, and blood samples taken at intervals of approximately 15 minutes for a period of up to two hours. On occasion, further doses were given 60 or 120 minutes after the initial injection, and periodic sampling continued. Several of the subjects were challenged with LHRH on more than one occasion. In normal women, tested during the early follicular phase of the cycle, 100 μg of LHRH effects an increase of two to four times in hLH, and a 50 to 130 per cent increase in hFSH.

Conjugated Estrogen Test

Twenty or 25 mg of conjugated equine estrogen (Premarin) was administered intravenously over two to 10 minutes. Blood samples were then taken at about hourly intervals for one to two days. On the fourth day after conjugated estrogen the blood samples were withdrawn daily or twice daily for up to a week.

Insulin Test

Insulin (0.1 U per kilogram of body weight) was administered intravenously, and blood samples were taken at 15-minute intervals for 90 minutes.

Clomiphene Test

Clomiphene citrate was administered in a dosage of 50 to 150 mg per day for five days. In some subjects the procedure was repeated with larger doses of clomiphene. Blood samples were taken each morning during the clomiphene administration and for a further period up to one month.

Therapy with hMG

In patients treated with hMG (Menotropins, Pergonal 500, Human Post-Menopausal Gonadotrophins, Istituto Farmacologico Serono, Roma and Ikapharm, Limited, Ramat Gan, Israel), the beginning dose was 2 ampoules daily. With use of clinical indexes of estrogenization such as an increase in cervical mucus arborization, the dosage was gradually increased until maximum arborization had been attained for at least three days. Thereafter hCG was administered on two or more successive days. Blood samples were taken at frequent intervals throughout the period of hMG and hCG administration. If undue ovarian enlargement became apparent during the hMG administration, therapy was immediately withheld.

Results

General Clinical and Laboratory Features

The chief complaint among our seven patients was primary amenorrhea. Clinical data are shown in Table 1. With the exception of Case 4 all patients noted development of axillary and pubic hair before receiving therapy. Breast development was observed before estrogen therapy in five patients (Cases 2, 3, 5, 6 and 7); one subject (Case 1) related breast development to the time of estrogen administration, whereas in Case 4, the breasts failed to develop despite intensive systemic and local therapy. This patient also had no axillary hair, although pubic hair did develop spontaneously. All seven patients responded to estrogen therapy with withdrawal bleeding. Of interest was the response of

Table 1. Clinical and Laboratory Details in Isolated Gonadotropin Deficiency.

Case No.	Age	Height		Span	17-Ketosteroids	17-Hydroxycorticosteroids	Thyroxine	Cortisol		hGH after Insulin
		Upper segment	Lower segment					Basal	After insulin	
	yr	cm			mg/24 hr		μg/100 ml			ng/ml
1	22	86	88.5	175.5	4.6	4.8	6.5	10.3	17.2	19
2	29	82.5	88	176	5.7	5.7	6.0	14.8		—
3	27	84	90	176	7.1	7.1	8.9	13.2	20.7	>30
4	24	77	85	168	4.5	3.6	6.6	21.8		>50
5	25	73	94	180	4.0	5.6	7.0	13.5		—
6	22	79	91	180	5.1	2.6	8.2	12.9	19.8	>50
7	26	88	81	167	4.5	5.9	4.0	7.5	22.4	32
Normal value					4-9	3-7.5	3.5-8.0		18-35	7-50

Cases 2, 3 and 7, who had menstrual bleeding after either intramuscular progesterone or oral progestins. No pertinent family history was obtained in any of the subjects. One patient (Case 4) had anosmia. With the exception of Case 7 proportions in all subjects were eunuchoid (Table 1).

Visual fields and x-ray films of skull and pituitary fossa were normal. Buccal smear was positive in all, and chromosomal studies on three subjects showed a 46XX pattern. On laparoscopy, a small, hypoplastic uterus and small, white ovaries were noted. Ovarian biopsy, performed in two patients, showed the presence of primitive primordial follicles. Urinary 17-ketosteroids, 17-hydroxycorticoids and studies of thyroid function (serum thyroxine, "tri-iodothyronine Sephadex," Ames, and radioiodine uptake studies) were all within normal limits.

Secretion of ACTH (assessed indirectly by cortisol levels) was normal in all subjects, and hGH was normal in six of the seven subjects who were tested. With induction of hypoglycemia by insulin, hGH levels rose to peak values of 19 ng per milliliter or higher, and cortisol levels rose from basal values of 7.5 to 13.2 to maximal levels of 17.2 to 22.4 μg per 100 ml. Cases 2, 4 and 5 showed random cortisol levels of 13.5 μg per 100 ml or greater, and Case 5 showed hGH levels of 9 ng per milliliter and hence further tests were not made.

Specific Hormonal Studies

Basal values of gonadotropins and of estradiol-17β. Basal gonadotropin secretion was assessed in two ways (Table 2): by daily blood samples for several days; and by frequent sampling in particular patients over a number of hours. Basal levels of hFSH and of hLH were undetectable in all subjects except two, in whom one sample out of 26 and 35 respectively showed hLH values of 5 mIU per milliliter. Basal values of serum estradiol-17β were all below 20 pg per milliliter. Among normal women, mean values of estradiol 17β of 30 pg per milliliter were found during the early proliferative phase, which increased to peak values of 180 pg per milliliter one day before the hLH surge, with a plateau of 105 pg per milliliter, during the secretory phase of the cycle.

Response to clomiphene citrate. All patients were challenged with clomiphene citrate. Two patients (Cases 2 and 7) reported no rise in basal body temperature and no menstrual bleeding after the tablets. Of the other subjects detailed measurements of hFSH and hLH were made in five, and of estradiol-17β in three over a four-week period after clomiphene. There was little or no change in serum gonadotropins or estradiol (Table 2). Case 4 had nine to 12 samples analyzed for hLH and hFSH on a day before and a day after clomiphene. Levels were all undetectable on both days. Figure 1 shows gonadotropin and estradiol-17β levels in that patient after clomiphene citrate.

Responses to conjugated estrogens. Intravenous conjugated estrogens failed to effect a noteworthy rise in gonadotropin levels in any of the five patients tested (Table 2). The response in Case 1, which was characteristic of our group of patients, is shown in Figure 2.

Table 2. Serum Levels of hFSH and hLH in Isolated Gonadotropin Deficiency.

Case No.	hFSH		hLH	
	No. of determinations*	Concentration†	No. of determinations*	Concentration†
		mIU/ml		mIU/ml
1:				
Basal	26 (0)	—	26 (1)	5
After clomiphene citrate‡	21 (1)	7	20 (1)	7
	20 (1)	7	21 (1)	6
After conjugated estrogens	21 (1)	4	21 (3)	6,6,6
2:				
Basal	1 (0)	—	1 (0)	—
After conjugated estrogens	8 (0)	—	8 (0)	—
3:				
Basal	10 (0)	—	3 (0)	—
After clomiphene citrate	19 (0)	—	20 (4)	4,4.5,4.5,5
After conjugated estrogens	10 (0)	—	12 (2)	4.5,12.5
4:				
Basal	41 (0)	—	35 (1)	5
After clomphene citrate	29 (0)	—	26 (3)	4,5,7
5:				
Basal	6 (0)	—	6 (0)	—
After clomiphene citrate	15 (0)	—	12 (1)	5
After conjugated estrogens	16 (0)	—	19 (1)	4.5
6:				
Basal	12 (0)	—	14 (0)	—
After clomiphene citrate	6 (0)	—	18 (0)	—
7:				
Basal	9 (0)	—	1 (0)	—
After conjugated estrogens	8 (0)	—	8 (1)	6

*Figures in parentheses represent no. of determinations above assay sensitivity.
†Absolute values of FSH & LH above assay sensitivity.
‡2 courses (250 mg & 500 mg total dose over 5 days).

Response to LHRH (Fig. 3 and 4). All patients received

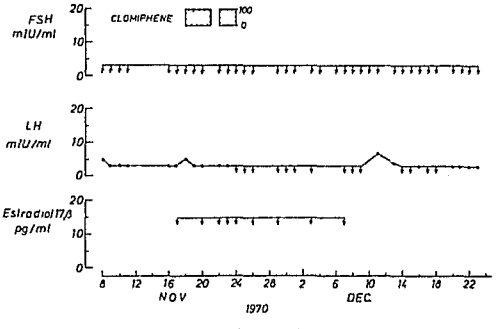


Figure 1. Serum FSH, LH and Estradiol-17β Levels in Case 4 after Clomiphene Citrate.

In this and subsequent figures a horizontal line with an arrow pointing down indicates that values are below the sensitivity of the method.

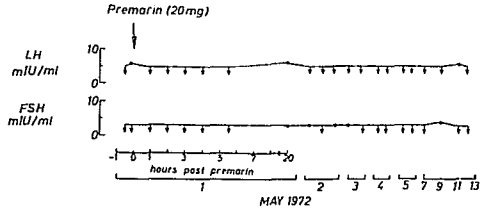


Figure 2. Serum FSH and LH Levels in Case 1 after Conjugated Estrogens (Premarin) Given Intravenously.

LHRH on at least one occasion. Administration of LHRH was not accompanied by any side effects. The following responses were observed. Group 1 (five

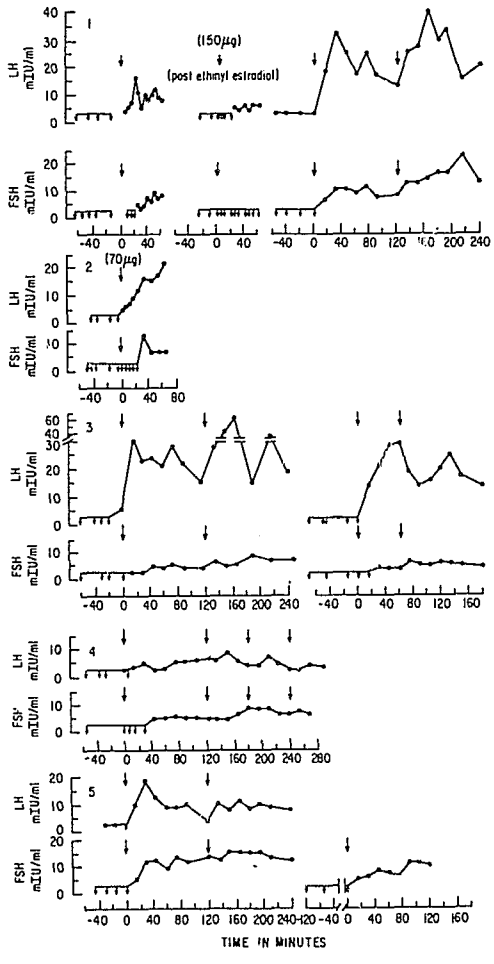


Figure 3. Serum FSH and LH Levels in Cases 1 to 5 after LHRH Administration.

The dose was 100 μg unless otherwise indicated. Arrows show time of administration of LHRH.

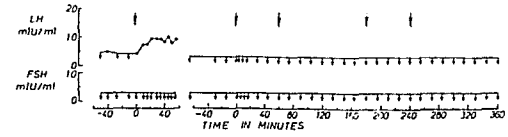


Figure 4. Serum FSH and LH Levels in Case 6 after LHRH Administration.

Large arrows show time of administration of LHRH.

subjects), elevation of hFSH and of hLH (Fig. 3); Group 2 (one subject), inconsistent elevation of hLH only (Fig. 4); and Group 3 (one subject), no response of hFSH or hLH.

Among Group 1 subjects, definite elevation of hLH was apparent by 15 minutes, and peak responses occurred between 15 and 60 minutes (Fig. 3). A second pulse of LHRH, when the hormone was given 120 minutes after the first, was equally effective in eliciting an LH response. In Case 3, when the second pulse was given 60 minutes after the first, the LH response appeared somewhat delayed.

Elevation of hFSH occurred 20 to 40 minutes after LHRH. In no patient had hFSH levels returned to base line by 120 minutes. A second pulse given at 120 minutes elicited an increase in hFSH levels similar to the first test. Case 1 was given ethinyl estradiol (0.1 mg) orally for one week, and the LHRH test was repeated. There was no rise in hFSH, and the hLH response appeared attenuated.

Case 6 showed an interesting pattern (Fig. 4). On the first occasion there was elevation of hLH only. When she was tested subsequently, despite four pulses of LHRH, there was no rise in either hLH or hFSH.

Response to menotropins. We have data on the three married subjects to whom menotropins were administered on at least two occasions. Cases 2 and 7 have become pregnant. In all three subjects, menotropins and hCG resulted in an increase in serum estradiol-17β to levels of between 150 and 750 pg per milliliter and in progesterone of 3.3 and >20 ng per milliliter.

Figure 5 indicates the changes in hormonal levels in Case 7 during therapy with menotropins. Serum estradiol-17β levels were 89 pg per milliliter on the day of hCG administration. Further elevation was noted on the following day when a second injection of hCG was given. Twenty-four hours later, levels decreased precipitously and then rose to 151 pg per milliliter.

On the same day, serum progesterone was 3.3 ng per milliliter. Three days later the patient had menstrual bleeding. Treatment with menotropins during the following month was successful in allowing her to become pregnant.

Discussion

The syndrome of isolated gonadotropin deficiency is characterized in female subjects by primary amenorrhea, low or absent peripheral levels of hFSH, hLH and estradiol-17β, and normal hGH, ACTH and TSH secretion. Breast development is variable, al-

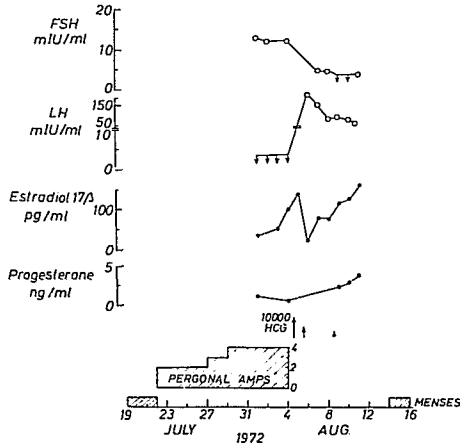


Figure 5. Serum FSH, LH, Estradiol-17β and Progesterone Levels in Case 7 after hMG Pergonal Amps and hCG Therapy.

though only one patient attributed breast growth to previous estrogen therapy. One subject failed to show breast enlargement despite intensive estrogen therapy. She was also the only patient in our series with anosmia. A family history of deficiency was not obtained in any patient, although other family members have often been affected in isolated gonadotropin deficiency.[2,3,5,6]

We have attempted to define the site of the lesion within the hypothalamic-pituitary unit in our patients. The following possibilities were considered: a primary lesion of the gonadotrope (such as failure of hormone synthesis); and LHRH deficiency (e.g., failure of LHRH synthesis or release or both).

On the basis of the responses to LHRH, we could distinguish three groups: Group 1, hFSH and hLH release after LHRH: Group 2, inconsistent release of hLH only after LHRH; and Group 3, failure of release of hFSH or hLH after LHRH.

Thus, in all but one subject, the gonadotrope was qualitatively responsive to LHRH. The lesion in Groups 1 and 2 does not appear to reside primarily in the pituitary but at some higher level. In these patients clomiphene citrate and conjugated estrogens (which may effect release of LHRH) did not influence gonadotropin levels. LHRH effected release of only one of the gonadotropins (hLH) in Group 2. This action may be a consequence of prolonged releasing hormone "deprivation" on the pituitary cell, which could also account for her inconsistent response after LHRH with respect to LH. Alternatively, there may be failure of release of a second FSH releasing hormone from the hypothalamus. The one patient (Group 3) who failed completely to respond to LHRH may have a defect at the pituitary level, or failure to respond to LHRH may also be due to secondary pituitary changes consequent upon chronic LHRH deprivation. We have recently described a patient with isolated FSH deficiency of probable pituitary origin. The hLH levels were extremely high,[25] and free α-chain (the common subunit of the glycoprotein hormones hFSH, hLH, TSH and hCG) was present in serum in the basal state and after LHRH stimulation.[26] It may be fruitful to search for the presence of free α-chain in the serum in states of isolated gonadotropin deficiency.

We have assessed the responsiveness of the dormant ovaries of three of our patients to exogenous gonadotropins. In all, a rise in estradiol-17β and in progesterone levels was observed after menotropins. Two patients are pregnant. Bardin et al. have described a "double defect" in isolated gonadotropin deficiency,[27] and we have also seen one such male patient. Thus, although we have been alerted to a similar situation among female patients, we have as yet not observed such a defect.

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HETEROGENEITY OF GONADOTROPIN RESPONSE TO LHRH IN HYPOGONADOTROPIC HYPOGONADISM

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ABSTRACT. A group of six hypogonadal males with low serum levels of FSH and LH, and otherwise normal pituitary function, underwent tests in which 100-300 ug of LHRH [Luteinizing Hormone Releasing Hormone] was injected rapidly intravenously. All four possible combinations of serum LH and FSH responses were observed. That is, failure of FSH and LH to rise; an increase in both FSH and LH; and an increase in FSH only, or in LH only. The heterogeneity of the LH and FSH responses to LHRH indicates the complexity of the etiology of this group of disorders, whose clinical expression is hypogonadotropic hypogonadism.

INTRODUCTION

LHRH has been shown to cause release of LH and of FSH in both adults and in prepubertal children [1]. The rapid intravenous injection of LHRH is a potentially useful test for evaluation of the site of the defect in hypogonadal patients with low serum levels of LH and of FSH. Absence of a gonadotropin response to LHRH suggests a pituitary lesion, whereas a rise in gonadotropins after LHRH indicates that the gonadotrope is intact and points to the presence of an hypothalamic defect. We have found, however, that both in male and female hypogonadal patients with low serum gonadotropins, the LH and FSH responses to LHRH are heterogeneous.

We report results of LHRH tests among six male subjects with hypogonadotropic hypogonadism in whom all four possible combinations of LH and FSH responses occurred.

MATERIAL AND METHODS

LHRH provocative test. After an overnight fast, an indwelling needle was inserted into a brachial vein and 0.9% saline was infused slowly. Blood samples were taken at approximately 15 minute intervals during the hour prior to the injection of LHRH, and over the two hours following the injection. LHRH [Ayerst] was injected rapidly intravenously in a dosage of 100-300 ug. Serum FSH and LH were measured by a modification of the double antibody radioimmunoassay method of Odell et al [2,3], using ^{125}I labelled FSH and LH, and rabbit anti hFSH and rabbit anti hCG antisera. Goat anti rabbit IgG was used as the second antibody. Results were expressed in mIU/ml of 2nd IRP-HMG. The FSH [LER 1366] and LH [LER 960] used for labelling, and the rabbit anti hFSH antibody were generously supplied by the National Pituitary Agency, NIH, USA. Rabbit anti hCG was generously supplied by Dr. S. Rosen. The lower limit of sensitivity for both LH & FSH assays was 3 mIU/ml.

RESULTS

Six males with low or absent levels of serum gonadotropins underwent a total of ten LHRH provocative tests. All patients had been shown to have normal thyroid and adrenal function, and normal growth hormone responses to insulin hypoglycemia.

Table 1 gives particulars of the patients and a summary of their FSH and LH responses to LHRH. All possible combinations of LH and FSH responses occurred after LHRH. Fig. 1 shows examples of each type of response to LHRH. That is, a definite rise in both FSH and LH [AP]; a rise in FSH only [GL]; a rise in LH only [DS] and no response in either FSH and LH [EB].

Patients EB [tested twice], AP [tested twice] and MS [tested 3 times] showed essentially identical qualitative responses with each test [Table 1].

DISCUSSION

Schally et al [4] believe that the single hormone LHRH controls the release of both FSH and of LH from the pituitary. It remains possible, however, that there are other hormonal factors acting on the pituitary, perhaps in the regulation of FSH release. Regardless of this controversy, it is well established that the synthetic decapeptide LHRH effects release of both FSH and LH in normal adults, males and females. This has provided an important tool in the evaluation of clinical syndromes of hypogonadism associated with low circulating levels of FSH and LH. In these syndromes, the defect may reside at the pituitary level, or may be consequent upon a defect in the hypothalamus or at some higher level. Costom et al[5] have shown that in most patients with TSH deficiency, the thyrotrope is intact since the exhibition of Thyrotropin Releasing Hormone [TRH] leads to a rise in serum TSH levels.

We have similarly evaluated the response to LHRH of a group of patients with hypogonadotropic hypogonadism. The striking feature of the results displayed in Table 1 and Fig. 1, is their heterogeneity. There was no significant rise in serum FSH and LH in patients EB and MS; both FSH and LH were increased in AP; LH only rose in CS and DS; and FSH only rose in GL. EB had anosmia and undescended testes and failed to respond to exogenous hCG [Human Chorionic Gonadotropin]. He probably represents an example of the "double defect" described by Bardin et al[6]. DS had a clinical and testicular histologic picture compatible with "fertile eunuch syndrome." AP, CS, MS and GL were typical examples of hypogonadotropic hypogonadism. Even among these patients the responses were disparate, and it seems probable that we are dealing with disorders of complex etiology. Similar conclusions have been reached by Lunenfeld et al [7]. There are several reservations necessary, however, in interpreting the results of one pulse of LHRH. Schally et al [8] have shown that LHRH also increases net synthesis of gonadotropin by pituitary gland, incubated in vitro. Theoretically, therefore, the gonadotrope unstimulated by LHRH may require considerable "priming" with LHRH before it is capable of effecting release. Hence a negative response to one pulse of LHRH may not indicate with certainty that the gonadotrope is defective.

A further note of caution arises because of uncertainty whether LHRH is the sole gonadotropin releasing hormone. If there were, for example, an FSH Releasing Hormone, there may be a rise in serum FSH after exhibition of this material and not after LHRH. Theoretically then, we could err in calling

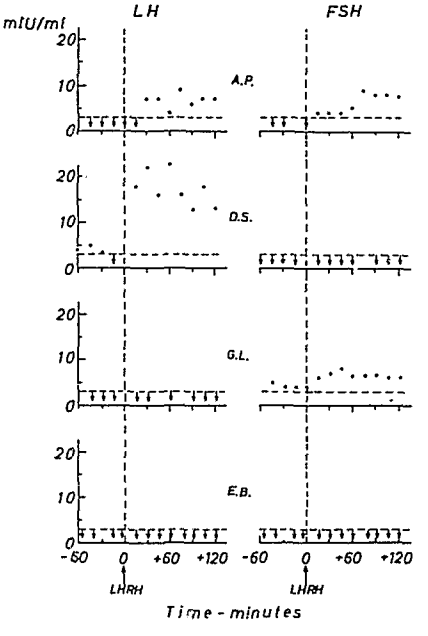


Fig. 1. The LH and FSH responses to the rapid I.V. injection of LHRH in 4 hypogonadal males. 300 ug of LHRH wc. given at time 0 in all cases except DS who received 200 ug. LH and FSH values are in mIU of 2nd IRP-HMG. All values below 3mIU are designated by an arrow pointing downwards.

TABLE 1. Clinical details of six patients with hypogonadism associated with undetectable or subnormal serum FSH and LH levels.

Name	Age	Ht. cm.	Wt. kg.	Special Clinical Features and Testicular Biopsy	Dose	LHRH TEST Maximal Increase of FSH [mIU/ml]	LHRH TEST Maximal Increase of LH [mIU/ml]
EB	31	180	74.5	Anosmia: Bilateral undescended testes. Biopsy: Infantile testis; no signs of Leydig cell differentiation. No response to exogenous hCG.	100μg 300μg	0 0	2 0
MS	21	177	68.5	Infantile Testis; no signs of differentiations of interstitial cells.	100 μg 200 μg 300 μg	0 0 0	0 0 0
AP	19	170	58	Infantile testis;no signs of differentiation of interstitial cells.	100 μg 300 μg	5 6	12 6
CS	23	164	58	No biopsy. Left testis undescended surgically brought into scrotum.	300 μg	2	28
GL	16.5	189	73	Infantile testis; No signs of differentiation of interstitial cells.	300 μg	7	0
DS	18	184	103.5	Normal sized tubules with good evidence of advancing spermatogenesis. Leydig cells not present in the interstitium.	200 μg	0	19

the gonadotrope defective because of a negative response to LHRH. Despite such reservations, the quatitative agreement between tests was impressive in the 3 patients on whom we made repeat studies.

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Impaired Prolactin Response to Thyrotropin-Releasing Hormone in Isolated Gonadotropin Deficiency and Exaggerated Response in Primary Testicular Failure*

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ABSTRACT. Six males with isolated gonadotropin deficiency (group I) and 6 with primary testicular failure (group II) received LRH (100 μg) and TRH (200 μg) 30 min later. Their responses were compared to 15 male controls. In group I, basal LH, FSH, testosterone, and estradiol values were all less than the controls ($P < 0.001$). In group II, basal gonadotropins were elevated (LH, $P < 0.05$; FSH, $P < 0.001$), although testosterone and estradiol values were similar to the controls. The peak gonadotropin responses to LRH were impaired in group I ($P < 0.001$) and exaggerated in group II ($P < 0.001$). Mean (±SEM) basal PRL levels in group I were 7.. ± 0.5 vs. 11.0 ± 1.0 ng/ml in the controls ($P < 0.01$). Levels in group II were 14.3 ± 1.6 ng/ml and were not significantly different from controls. The mean peak PRL response to TRH in controls was 35.8 ± 3.9 ng/ml. In group I, the peak was 13.4 ± 1.5 ng/ml ($P < 0.001$), and it was 77.4 ± 9.0 ng/ml in group II ($P < 0.001$).

Long term treatment with hCG in three subjects of group I and testosterone enanthate in one additional subject was associated with a rise in basal testosterone, estradiol, and PRL levels and restoration of PRL response to TRH. Cessation of hCG treatment in two of these subjects was accompanied by a return to the pretreatment state. These results suggest that the impaired PRL response in isolated gonadotropin deficiency may not be an integral component of the syndrome but, rather, a consequence of the altered steroid milieu.

A direct correlation existed between the estradiol to testosterone ratio and the peak PRL response to TRH in group II and the controls. In the same two groups, an inverse correlation also existed between basal testosterone and peak PRL response to TRH. This suggests that the exaggerated PRL response in primary testicular failure may be related to a relative increase in estradiol or reduction in testosterone. (*J Clin Endocrinol Metab* 48: 941, 1979)

THE RELATIONSHIP of hyperprolactinemia to hypogonadism is well known (1). There is, however, relatively little literature on the PRL response to dynamic stimuli in subjects with hypogonadism and normal basal PRL levels. Moreover, the reported data are conflicting. Thus, in the syndrome of isolated bihormonal deficiency of LH and FSH, *i.e.* isolated gonadotropin deficiency (IGD), both intact and attenuated PRL responses to TRH have been described (2-4). A controversy also exists about Klinefelter's syndrome, as both normal (5) and exaggerated PRL responses to TRH have been reported (6).

In this paper, we have assessed PRL dynamics in two groups of patients. One had IGD and the other primary testicular failure. Our results show that in IGD patients, basal PRL levels are low and there is an attenuated PRL response to TRH. These abnormalities can be corrected with hCG or testosterone (T) treatment. In contrast, patients with primary testicular failure have exaggerated PRL responses to TRH.

Materials and Methods

Group I

This group comprised six men, aged 18-36 yr, who presented with the syndrome of IGD. The chief complaint in these subjects was failure to mature sexually, absence of beard growth, and minimal penile development. Subjects complained of lack of erections and nocturnal emissions. All subjects had small testes and eunuchoid features. Anosmia was present in one subject. Three of the subjects had received previous treatment with T enanthate for various periods. Two of these treated subjects had subsequently developed mild gynecomastia. One of them had also received a course of human menopausal gonadotropins (hMG; Pergonal) and hCG (7). None of the subjects had received any treatment for at least 6 months before testing. The remaining three subjects had not been given any previous hormonal therapy.

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Group II

This group comprised six subjects with primary testicular failure. Four of the subjects, aged 29–38 yr, presented with severe oligospermia of unknown etiology, with sperm counts ranging from 2–10 million/cc. Testicular biopsy in two of these subjects showed that most of the tubules were sclerosed and hyalinized, but Leydig cells were intact. All four subjects had small atrophic testes but normal secondary sex characteristics. They claimed to have frequent erections and ejaculations. Intercourse was reported to be normal. None of the subjects had received any previous hormonal treatment. The fifth subject, aged 18 yr, presented with bilateral cryptorchidism. On examination, the scrotum was developed but empty. The penis was normal, as were the secondary sex characteristics. Subsequent to the evaluation, he underwent orchipexy and two abdominal testes were brought down into the scrotum. The sixth patient, aged 20 yr, had mumps orchitis at the age of 14 yr. Because of failure of development of secondary sex characteristics, he received T enanthate in oil for 12 months at the age of 18 yr. This was stopped 7 months before the present evaluation. On examination at the time of testing he had an absent right testis and a small left testis. His penis was normal in size and the secondary sex characteristics were normal. Semen analysis was not obtained in the last two subjects. In all of these six subjects, chromosomal analysis showed the normal male pattern.

Group III

This group comprised 15 normal male controls, aged 18–45 yr. Physical examination was normal in all of these subjects.

Experimental design

The precise nature of the study was explained to each subject, who then gave written consent. None of the subjects was taking drugs or medication at the time of the study. The test was commenced between 0800–0830 h, after an overnight fast. A needle inserted into an antecubital vein was kept patent by the slow administration of physiological saline. Two or more blood samples were drawn during a basal control period of 15–30 min and then the test substances were given by rapid iv injection. All of the subjects received LRH (100 μg) at zero time and TRH (200 μg) at 30 min. Blood samples were taken at frequent intervals after each pulse.

Three subjects from group I were treated with long term hCG (5000 IU im twice weekly), and repeated TRH testing was performed after commencing treatment. Subject 1 was treated with hCG for 12 months. Therapy was interrupted for 4 weeks after 10 months while the patient was in the army reserves. It was subsequently reinstituted and finally stopped after a further 5 weeks. TRH was administered before treatment and 1 month before stopping hCG.

Subject 2 received hCG for 12 months with the exception of two short periods. TRH was given on three occasions: before treatment, after 10 months of hCG administration, and 2 months after stopping treatment. In these two IGD subjects, serial bloods were taken during the course of hCG therapy for determination of PRL, T, and 17β-estradiol (E_2).

Subject 3 received hCG for 2 yr and the response to TRH was determined before the treatment, at the end of the treatment schedule, and then again 1 month after cessation of treatment. A fourth IGD subject was treated with T enanthate (200 mg administered im every 2 weeks for 2 yr) and rechallenged with TRH at the end of this period. TRH was given 48 h after T injection.

Methods

Serum LH, FSH, PRL, T, and E_2 were determined by previously described methods (8, 9). Pituitary LH, FSH, and PRL as well as their respective antisera were kindly supplied by the National Pituitary Agency, NIAMDD (Bethesda, MD). The Second International Reference Preparation for hMG provided by the Medical Research Council was used as the reference standard for both LH and FSH. Standard for PRL was also supplied by the Council.

To help avoid interassay variations, samples from subjects in all three groups were included in each assay. When a single subject was given more than one dynamic test, all samples were measured in the same assay. In those two IGD subjects who sere followed serially over the course of 12 months, all PRL determinations were performed in one assay. E_2 and T were assayed in batches of 15 consecutive samples.

Results

Basal levels (Fig. 1)

Mean (±SEM) basal FSH and LH levels in group III control subjects were 6.4 ± 0.6 and 9.8 ± 0.8, mIU/ml, respectively (Fig. 1). Mean values were: T, 6.25 ± 0.54 ng/ml; E_2, 24.2 ± 1.8 pg/ml; and the E_2:T ratio, 0.0045 ± 0.0006. In group I (IGD), mean LH was 3.1 ± 0.2 mIU/ml, FSH was 3.2 ± 0.2 mIU/ml, T was 1.13 ± 0.3 ng/ml, and E_2 was 9.5 ± 0.7 pg/ml. These values were all significantly less ($P < 0.001$) than in the controls. (Because of the very low steroid levels, no E_2:T ratio was calculated in group I.) In group II (primary testicular failure), mean basal LH was 33.3 ± 9.4 mIU/ml ($P < 0.05$

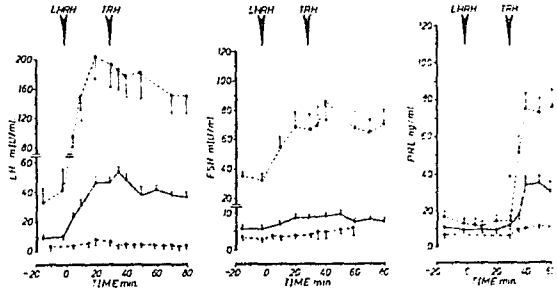


FIG. 1. Mean (±SEM) response of LH (*left panel*), FSH (*middle panel*), and PRL (*right panel*) in the three groups of subjects. LRH (LHRH), was given at zero time and TRH at 30 min. Group I (●- - -●), IGD; group II (●—●), primary testicular failure; group III (●—●), control subjects. See text for details.

vs. controls) and mean FSH was 35.3 ± 3.0 mIU/ml ($P < 0.001$). Serum T was 5.48 ± 1.19 ng/ml, E_2 was 28.2 ± 5.6 pg/ml, and the E_2:T ratio was 0.0057 ± 0.001. These values were not different from the controls. Mean basal PRL levels (which represent a mean of two to four determinations before TRH administration) in control subjects were 11.0 ± 1.0 ng/ml. In group I subjects, mean basal PRL levels were 7.2 ± 0.5 ng/ml ($P < 0.01$ *vs.* controls). Basal values were not significantly different in group II, where mean levels were 14.3 ± 1.6 ng/ml.

Response to releasing hormones (Fig. 1)

In the controls, the mean peak LH response to LRH was 49.4 ± 3.4 mIU/ml and this occurred at 35 min. The peak FSH response of 9.7 ± 0.9 mIU/ml was evident at 50 min (Fig. 1). The maximum LH and FSH responses to LRH in group I were 8.6 ± 5.4 and 4.3 ± 1.3 mIU/ml, respectively ($P < 0.001$ *vs.* controls). In group II, the peak LH response was 202.9 ± 28.4 mIU/ml and the FSH response was 86.4 ± 13.4 mIU/ml ($P < 0.001$ *vs.* controls). The peak PRL response to TRH in the controls was 35.8 ± 3.9 ng/ml, and it occurred 20 min after TRH administration. In group I, there was a markedly impaired PRL response to TRH and the maximum response (13.4 ± 1.5 ng/ml) occurred 20 min after TRH ($P < 0.001$). Group II subjects had significantly increased PRL responses to TRH ($P < 0.001$ *vs.* controls), and the maximum was 77.4 ± 9.0 ng/ml, which occurred 30 min after TRH.

Correlations (Fig. 2)

There was a negative correlation between the individual peak PRL responses to TRH and the basal T levels when the controls and group II (primary testicular failure) subjects were considered together ($r = -0.50$; $P < 0.05$) or when the control subjects were considered alone ($r = -0.62$; $P < 0.05$). When the same two groups were considered together, the peak PRL response to TRH also correlated with the E_2:T ratio ($r = 0.50$; $P < 0.05$) as well as the peak gonadotropin response to LRH (FSH, $r = 0.66$ and $P < 0.01$; LH, $r = 0.69$ and $P < 0.01$).

hCG or T therapy to IGD subjects (Figs. 3 and 4)

Figures 3 and 4 show the T, E_2, and PRL profiles in two IGD subjects who received long term hCG treatment. In the first IGD patient (Fig. 3), within 1 month of commencing hCG, T had increased from 1.4 to 4.3 ng/ml, E_2 from 13 to 20 pg/ml, and PRL from 7.5 to 12 ng/ml. During the course of hCG treatment, T levels (with one exception) ranged from 3–9.5 ng/ml, E_2 from 17–42 pg/ml, and PRL from 11–39 ng/ml. Both E_2 and PRL levels were higher than in control subjects. Basal PRL levels, which had been over 20 ng/ml after 10 months of treatment, gradually decreased to below 10 ng/ml over the succeeding 4 months. During this latter period, hCG had been stopped for 1 month and was then reinstituted. There was a parallel progressive decline in E_2 and T levels. The PRL response to TRH was assessed before treatment (Fig. 3A) and then just before stopping hCG (Fig. 3B). In the first test, basal PRL levels were 8.7 ng/ml and increased to 10.1 ng/ml after TRH. Correspond-

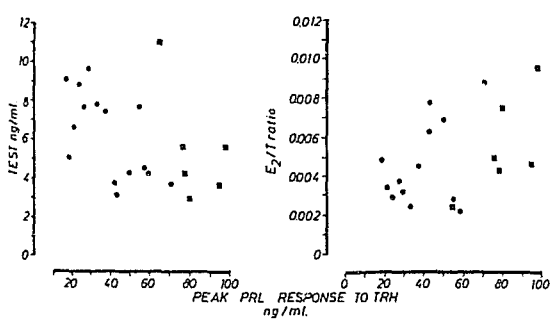


FIG. 2. *Left panel,* Correlation between individual basal T (TEST) and peak PRL response to TRH in primary testicular failure (group II; ▩) and control subjects (group III; ●). *Right panel,* Correlation between the E:T ratio and peak PRL response to TRH in the same two groups. See text for details.

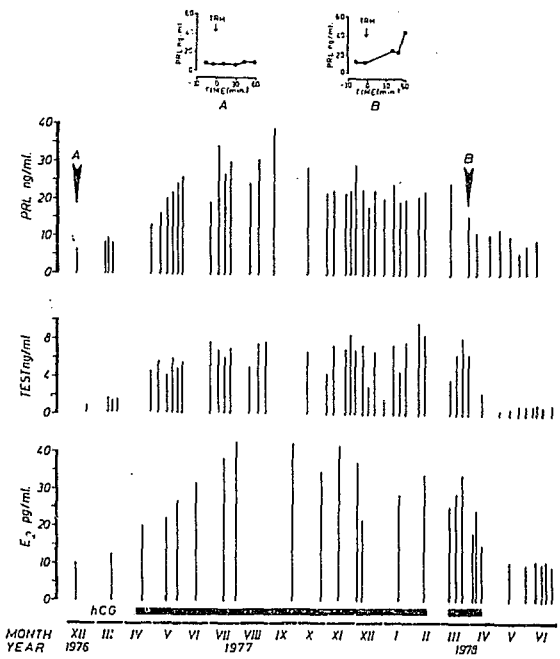


FIG. 3. Histogram representing basal PRL, T (TEST), and E_2 levels during hCG therapy in a single IGD subject. ▩, hCG treatment. For E_2, pools of consecutive serum samples were frequently made. *Inset,* PRL response to TRH before (A) and during (B) hCG administration.

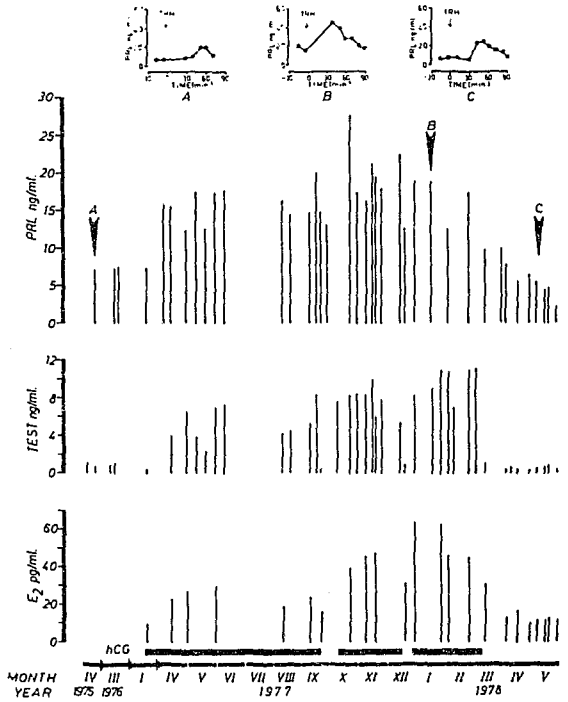


FIG. 4. Histogram representing basal PRL, T (TEST), and E_2 levels during hCG therapy in a second IGD subject. ■, hCG treatment. For E_2, pools of consecutive serum samples were frequently made. *Inset*, PRL response to TRH before (A), during (B), and after (C) hCG administration.

ing levels in the second test were 13 ng/ml (basal) and 44.7 ng/ml (post-TRH).

In the second IGD subject (Fig. 4), basal PRL in the untreated state was 7 ng/ml, E_2 was 8 pg/ml, and T was 0.5 ng/ml. He was assessed only after 3 months of hCG therapy. Basal PRL levels were then 16 ng/ml, E_2 levels were 23 pg/ml, and T levels were 4.0 ng/ml. During the course of hCG treatment, PRL levels fluctuated between 12–27 ng/ml, T levels from 3–11 ng/ml, and E_2 levels from 18–62 pg/ml. As in the previous subject, PRL and E_2 levels were again higher than controls. Within 2 weeks after stopping therapy, there was a rapid decrease in PRL from 17.5 to 8 ng/ml and associated decreases in T and E_2. PRL steadily fell to 2.5 ng/ml. The PRL response to TRH was assessed on three occasions (marked in Fig. 4). Before treatment (Fig. 4A), the peak response to TRH was 19.5 ng/ml. During hCG (Fig. 4B) it was 44.6 ng/ml, and after cessation (Fig. 4C), it was 23.8 ng/ml.

In the third IGD subject, the pre-hCG basal PRL level was 10 ng/ml and the peak response to TRH was 16 ng/ml. After 2 yr of hCG therapy, basal PRL had increased to 20 ng/ml and the response to TRH was 44 ng/ml. One month after cessation of hCG, basal PRL had decreased to 6.6 ng/ml and the peak PRL response to TRH was 22 ng/ml.

In the fourth IGD patient, TRH was readministered 2 yr after continuous T enanthate therapy. Basal PRL levels increased from 8.2 to 15.0 ng/ml and the peak PRL response to TRH increased from 8.0 ng/ml before treatment to 69.8 ng/ml after T enanthate.

Discussion

Our results have shown that untreated male IGD subjects (group I) have significantly decreased basal PRL levels and an attenuated PRL response to TRH. Long term hCG or T treatment was associated with a rise in levels of E_2 and T as well as in basal PRL levels and a restoration of PRL responsiveness to TRH. Conversely, cessation of hCG treatment was accompanied by a rapid decrease of basal serum T, E_2, and PRL levels and a reduction in PRL response to TRH. It is well known that administration of exogenous estrogens increases both basal PRL levels and the PRL response to provocative stimuli (10–12). Hence, it is likely that the elevation in serum E_2 produced by hCG or T treatment alone normalized the PRL response in our subjects. It is not certain in humans if T can directly increase PRL levels. However, T can be aromatized to E_2 (13). This steroid effect appears to be short-lived, and continuous therapy is required to maintain PRL responsiveness.

An impaired PRL rise after TRH has been reported previously in male IGD subjects by Yamaji *et al.* (4). Although they also reported an improvement in PRL response to TRH in some of their IGD subjects who were on T enanthate treatment, this was not always evident. In contrast, W[illers] *et al.* (3) have noted normal basal PRL levels and intact responses to TRH and chlorpromazine in IGD patients on long term hCG or T. We have previously reported that the impaired PRL response to chlorpromazine in untreated female IGD patients can be improved after ethinyl E_2 administration (8, 14). Thus, the restoration of PRL responsiveness in both female and male IGD subjects suggests that the alteration in PRL response in IGD may not be an inherent component of the syndrome but, rather, a consequence of the altered steroid milieu.

A different picture emerged in the six patients with primary testicular failure. Their defect predominantly involved the seminiferous tubules; this was confirmed on testicular biopsy in two patients. All had normal secondary sex characteristics with intact levels of T. Severe oligospermia was presented in four subjects. All had elevated basal gonadotropin levels and exaggerated FSH as well as LH responses to LRH. Presumably, the high LH secretion rate maintained their serum T. These subjects had exaggerated PRL responses to TRH. Similar results have been reported by some authors in patients

with Klinefelter's syndrome (6, 15), although this has not been uniformly confirmed (5, 16, 17).

What is the mechanism underlying these findings? It could be related to an absolute or relative increase in E_2. This has been postulated to occur in Klinefelter's syndrome (6, 18, 19). In the present series, there were no significant differences in basal levels of E_2 or the E_2:T ratio in the controls and subjects with primary testicular failure. There was also no correlation between E_2 levels and the PRL response to TRH. There was, however, a correlation between the E_2:T ratio and the PRL response to TRH in the individual control and primary testicular failure patients. This suggests that the exaggerated PRL response may be related to a relative increase in E_2.

Subtle alterations in T dynamics may also play a role in the exaggerated PRL response. Although there were no differences in basal levels of T in the controls and primary testicular failure patients, a negative correlation did exist between basal T and peak PRL response to TRH in the controls, considered alone or together with the testicular failure subjects. Hence, the exaggerated PRL response may be related to a decrease in T. Although the precise role of PRL in testicular physiology is unknown, recent studies have demonstrated a correlation between serum PRL and T at night and after haloperidol and methyl-TRH administration (20-22). Ambrosi *et al.* (23) noted that the T response to hCG was greater during sulpiride-induced hyperprolactinemia. All of these data suggest that PRL has a stimulatory effect on T secretion. The negative correlation between PRL and T, described above, and the reduction of the exaggerated PRL response to TRH in Klinefelter's syndrome by exogenous T (6) raise the possibility of the existence of a negative feedback loop between T and PRL. The correlation between the peak PRL response to TRH and peak gonadotropin responses to LRH in the testicular failure and control subjects is also compatible with the existence of a feedback loop between the testis and PRL.

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THE DECREASED BASAL AND STIMULATED PROLACTIN LEVELS IN ISOLATED GONADOTROPHIN DEFICIENCY: A CONSEQUENCE OF THE LOW OESTROGEN STATE

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SUMMARY

Prolactin secretion has been evaluated in seven male and six female patients with isolated gonadotrophin deficiency (IGD). The subjects were challenged with the dopaminergic antagonist, metoclopramide (10 mg) and TRH (200 μg) before, during and after cessation of hormonal treatment. Five females received three consecutive 21-day courses of ethinyl oestradiol (0·1 mg daily) at monthly intervals and the remaining subject conjugated oestrogens (Premarin 0·625 mg daily) according to a similar protocol. Treatment of the males with hCG (pregnyl) 5000 iu twice weekly led to a rise in oestradiol and testosterone levels. Two males were receiving pergonal (human menopausal gonadotrophin) in addition. In the untreated state in both males and females, basal oestradiol and PRL levels were decreased as were the PRL responses to metoclopramide and TRH as compared with normal controls. During treatment in both groups, there was an increase in basal PRL levels as well as PRL response to the two stimuli, which became indistinguishable from the controls. Cessation of treatment was associated with a rapid decrease in basal PRL levels and PRL elevation following metoclopramide and TRH. In contrast to the effect of hCG, the administration of two non-aromatizable androgens (mesterolone and fluoxymesterone) had no effect on basal and TRH-induced PRL secretion. The administration of clomiphene citrate during hCG treatment in one male IGD patient produced a decrease in the basal and stimulated PRL response.

It is concluded that the low basal PRL levels and impaired PRL responses to stimulation are not an inherent component of the syndrome of IGD, but a consequence of the abnormal steroid milieu.

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The syndrome of hypogonadotrophic hypogonadism or isolated gonadotrophin deficiency (IGD) is characterized by decreased secretion of the pituitary gonadotrophins, LH and FSH. As a consequence, the gonads are unstimulated and circulatory levels of sex steroids are low (Rabinowitz & Spitz, 1975). There have been controversial reports of prolactin (PRL) secretion in this disorder. Some have observed normal or even elevated basal PRL levels with intact or impaired PRL responses to thyrotrophin releasing hormone (TRH), as well as to the dopaminergic antagonists chlorpromazine and metoclopramide (Antaki *et al.*, 1974; Pertzelan *et al.*, 1977; Yamaji *et al.*, 1977; Winters *et al.*, 1978; Fernandez-Lazala *et al.*, 1979). Our group has reported impaired PRL responses to TRH, chlorpromazine (CPZ) and metoclopramide (MET) in male IGD subjects and an impaired response to CPZ in females (Spitz *et al.*, 1978; 1979; 1981b).

Since oestrogens have the capacity to enhance PRL secretion (Buckman & Peake, 1973; Carlson *et al.*, 1973; Yen *et al.*, 1974), it is conceivable that the impaired PRL secretion might not be an inherent component of the disease process, but rather a consequence of the low circulating sex steroids. Indeed, we have already shown that treatment of two male IGD subjects with human chorionic gonadotrophin restored PRL responsiveness to TRH (Spitz *et al.*, 1979).

The aim of the present study was to evaluate PRL secretion before, during and after cessation of treatment in IGD subjects. Males received human chorionic gonadotrophin (hCG) and females received ethinyl oestradiol. Our results have shown that the low basal PRL levels and impaired PRL response to pharmacological stimulation observed in the untreated state are reversed following elevation of sex steroids after hCG or ethinyl oestradiol administration. With cessation of replacement therapy, the PRL profile again reverts to the pretreatment state.

MATERIALS AND METHODS

Patients and treatment schedules

Seven males aged 18–37 years and six females aged 18–28 years with IGD were selected for the study. Clinical details and results of hypothalamic–pituitary function in some of these subjects in the basal state have been reported (Rabinowitz & Spitz, 1975; Spitz *et al.*, 1978; 1979; 1981b). All the females had received previous cyclic replacement therapy with oestrogens. However, therapy had been stopped 6 months prior to the present investigation. For the study, five IGD females were treated with ethinyl oestradiol 0·05 mg twice daily for 21 days and after stopping for 7 days, the treatment schedule was repeated for another two cycles. The remaining female was given a similar regime with conjugated oestrogens (premarin) 0·625 mg daily instead of ethinyl oestradiol.

Of the male subjects, four had never received treatment and two were evaluated whilst on treatment with hCG (5000 iu twice weekly) and pergonal (human menopausal gonadotrophins) one ampoule twice-weekly. According to the manufacturer's literature (Istituto Farmacologico, Serono, Roma and Ikapharm Ltd, Ramat Gan, Israel), each ampoule of pergonal contains 75 iu of hFSH and 75 iu of hLH. One had received hCG, pergonal and testosterone enanthate previously. However, therapy had been stopped for 6 months.

In the present study, all the males were given hCG (pregnyl) 5000 iu twice weekly. Two males were also treated with the non-aromatizable androgens, mesterolone (150 mg daily)

and one with fluoxymesterone (10 mg daily). Another was also given clomiphene citrate (100 mg daily). The duration of treatment is shown in Fig. 3.

Test procedures

All tests were performed between 0800 and 0830 h after an overnight fast. After explaining the full nature of the proposed study to all subjects, they gave written consent. A needle inserted into an antecubital vein was kept patent by the slow administration of 0·9% saline. Two to three blood samples were taken during a basal control period of 15–20 min and then 10 mg metoclopramide (MET) or 200 μg TRH were given by rapid i.v. injection. Blood samples were then taken at frequent time intervals. The test substances were administered on separate occasions at intervals of at least 3 days in a random fashion. Both male and female patients were challenged with MET or TRH in the untreated state, after 2–3 months of replacement therapy and then one month after completing the course of treatment.

Methods

Serum PRL, testosterone and oestradiol were determined by previously described methods (Spitz *et al.*, 1978; 1979; 1980; 1981b). The PRL antiserum was kindly supplied by the National Pituitary Agency, National Institute of Arthritis, Metabolism and Digestive Diseases (NIAMDD). PRL standard (75/504) was provided by the Division of Biological Standards and Control, Holly Hill, Hampstead, London. To avoid interassay variation, all samples of a specific treatment schedule in each particular patient were measured in the same assay. The results in the female patients were compared with the response in fifteen female controls aged 18–26 years, who were tested in the early follicular phase of the menstrual cycle. Similarly, the results of our male patients were compared to that of twenty-four normal males aged 20–40 years of age. The integrated secretion after TRH administration in the male patients was calculated by linear interpolation.

Table 1. Mean ± SD oestradiol, testosterone and basal PRL levels in male and female IGD patients before, during and following treatment (values in control subjects are also shown)

	17β oestradiol (pg/ml)	Testosterone (ng/ml)	Prolactin (ng/ml)
Females			
IGD (pretreatment)	25·4 ± 8·6 (14)‡ [a]		6·4 ± 2·8 (52)‡ [a,b]
IGD (during treatment)	26·3 ± 7·4 (14)‡ [a]		9·7 ± 4·8 (45)
IGD (post-treatment)	—		4·8 ± 2·2 (19)‡ [a,b]
Controls	105·8 ± 30·9 (12)		9·8 ± 3·2 (44)
Males			
IGD (pretreatment)	13·0 ± 7·3 (6)*[a]‡[b]	0·89 ± 0·25 (16)‡[a,b]	7·1 ± 1·7 (32)‡[a,b]
IGD (during treatment)	61·4 ± 42·7 (30)‡[a]	7·96 ± 4·27 (17)*[a]	13·8 ± 5·1 (88)†[a]
IGD (post-treatment)	11·8 ± 7·6 (26)*[a]‡[b]	0·90 ± 0·45 (19)‡[a,b]	7·4 ± 2·4 (58)†[a]‡[b]
Controls	22·1 ± 6·9 (27)	5·86 ± 1·99 (28)	9·6 ± 4·1 (27)

Number in brackets refers to the number of samples measured.
*$P < 0.05$; †$P < 0.005$; ‡$P < 0.001$.
a compared with controls.
b compared with level during treatment.

Statistical evaluation was performed using the student's paired or unpaired *t* test as appropriate. In the male patients, results were also analysed using the Mann-Whitney test (Siegel, 1956).

RESULTS

Steroids (Table 1)

When compared to controls, basal oestradiol levels were decreased in untreated male and female patients and testosterone levels were decreased in the males. Since ethinyl oestradiol did not compete in our RIA, no changes in oestradiol could be detected during treatment in the female patients. In the males, following hCG administration, there was a marked rise in both testosterone and oestradiol and values exceeded that in the male controls. With the cessation of hCG, both oestradiol and testosterone levels again decreased and were indistinguishable from the pretreatment values.

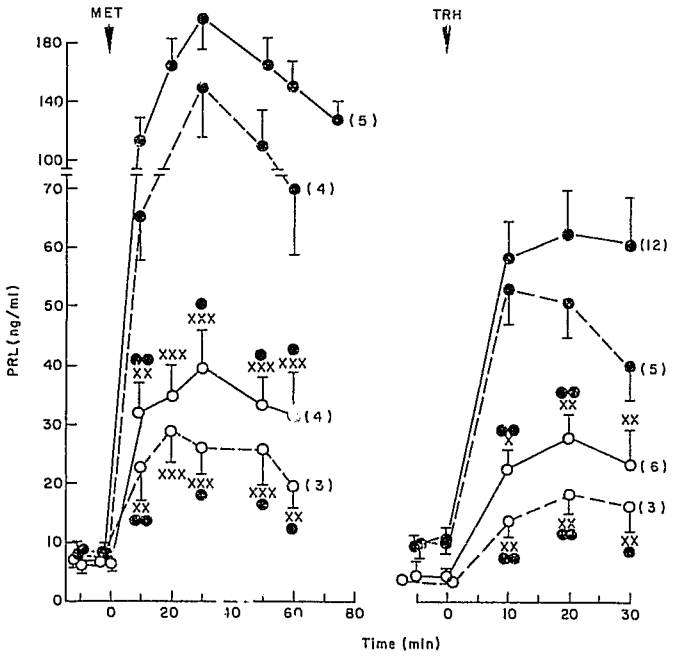


Fig. 1. Mean ± SEM PRL responses to metoclopramide and TRH in female IGD subjects before (O———O), during (●– – –●) and 1 month after cessation of treatment (O– – –O). Values for controls (●———●) are also shown. The number in brackets refers to the number of subjects tested. XXX ●●● $P<0{\cdot}001$; XX ●● $P<0{\cdot}01$; X ● $P<0{\cdot}05$ compared with control subjects (×) or IGD patients during treatment (●).

Basal P[illegible]els (Table 1)

Basal PRL levels in untreated male and female IGD subjects were significantly less than the corresponding controls. During oestrogen treatment, mean basal PRL levels increased in the females to values similar to the controls and significantly greater than pretreatment values. One month after cessation of treatment, basal PRL levels again decreased.

In the male IGD subjects, treatment with hCG increased mean ± SD PRL levels to 13·8 ± 5·1 ng/ml. This was greater than both pretreatment and control values and represents a mild degree of basal hyperprolactinemia. Levels decreased rapidly following cessation of treatment.

Response to stimulation (Figs 1, 2 and 3)

In both female and male controls, the peak PRL response to MET was greater than that to TRH ($P < 0{\cdot}001$). Untreated IGD subjects had significantly impaired PRL responses to both MET and TRH at all time intervals after the administration of the test substances (Figs 1 and 2).

During replacement therapy, there was an increase in PRL response to both MET and TRH as compared to the pretreatment test and the peak responses were not significantly different from the controls. One month after cessation of treatment, there was a reduction in PRL responses to MET and TRH which were not significantly different to the

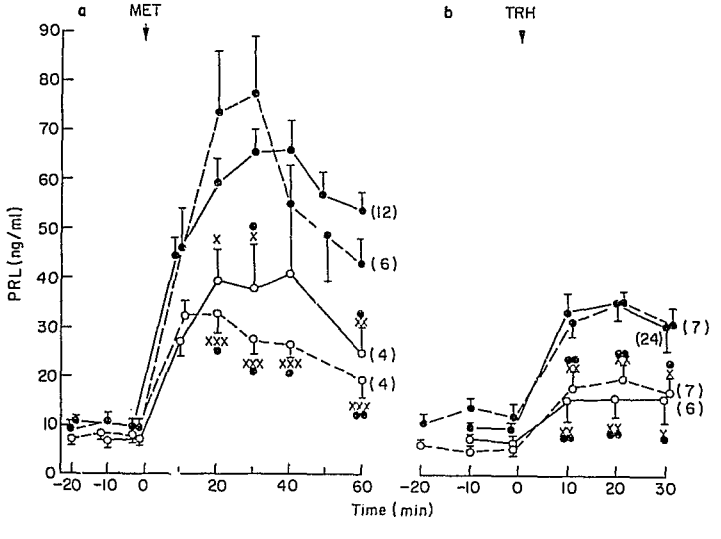


Fig. 2. Mean ± SEM PRL responses to (a) metoclopramide and (b) TRH in male IGD subjects before (o- ——o), during (●- - -●) and 1 month after cessation of hCG treatment (o- - -o). Values for male controls (●———●) are also shown. The number in brackets refers to the number of subjects tested. ×××/●●● $P < 0{\cdot}001$; ××/●● $P < 0{\cdot}01$; ×/● $P < 0{\cdot}05$ compared with control subjects (×) or IGD patients during treatment (●).

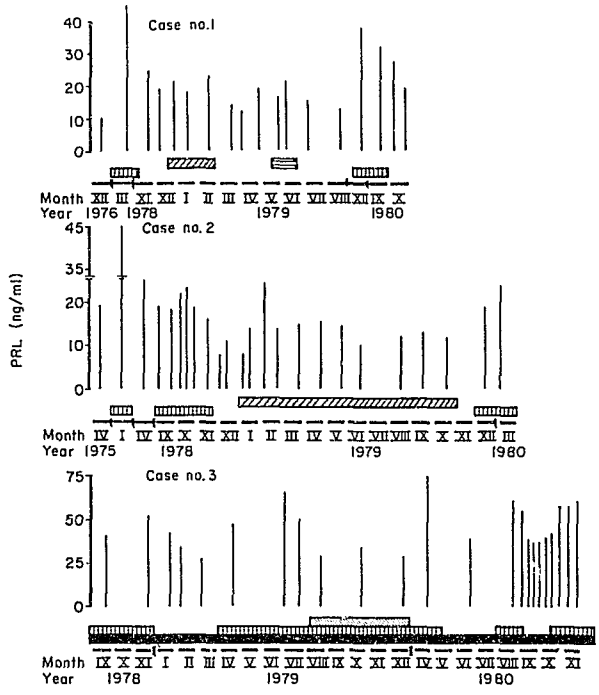


Fig. 3. The peak PRL response to TRH in male IGD case nos 1, 2 and 3. Patients were tested at various intervals in relation to hCG (⊟) therapy. Case no. 1 also received mesterolone (⊡) and fluoxymesterone (▣) and patient no. 2 mesterolone. Patient no. 3 was on pergonal (hMG ■) for the whole period and also received a course of clomiphene citrate (CC ⊡). See text for details.

pretreatment values, but less than the controls or during treatment. This occurred in both male and female patients (Figs 1 and 2).

In three male IGD subjects, the PRL response to TRH decreased progressively following cessation of hCG until it reached pretreatment levels (Fig. 3). The reinstitution of hCG again led to an increase in PRL response in all three subjects (Fig. 3). In one subject who received pergonal in addition to hCG, the peak and integrated PRL responses decreased with cessation of hCG despite continuation of pergonal (Fig. 3). In the same subject, the administration of clomiphene citrate during hCG and pergonal treatment led to a decrease in both basal ($P<0{\cdot}05$) as well as peak ($P<0{\cdot}005$) and integrated ($P<0{\cdot}005$) PRL secretion following TRH as compared with hCG alone (Fig. 3). Following clomiphene withdrawal and with continuation of hCG, basal and stimulated PRL responses again increased (Fig. 3). The administration of mesterolone and fluoxymesterone produced no increase in basal and stimulated PRL levels following TRH as compared with the non-treated periods. In addition, in both cases, basal PRL

levels, as well as peak and integrated responses after TRH, were less during mesterolone ($P < 0{\cdot}05$) than during hCG treatment (Fig. 3).

DISCUSSION

These results have extended our previous observations that untreated male and female IGD subjects have low basal PRL levels with impaired responses to TRH as well as to the dopaminergic antagonist, MET (Spitz *et al.*, 1979; 1981b). In general, our findings are in agreement with our reports although some have observed basal hyperprolactinemia and normal PRL responses in IGD (Antaki *et al.*, 1974; Pertzelan *et al.*, 1977; Yamaji *et al.*, 1977; Winters *et al.*, 1978; Fernandez-Lazala *et al.*, 1979). The reason for these differences is unknown, but may be related to previous therapy of more complex hypothalamic–pituitary disorders. Our group previously reported a dissociation of PRL responsiveness in female IGD subjects characterized by normal PRL elevation following TRH and an impaired response to chlorpromazine (Spitz *et al.*, 1978). The explanation for the discrepancy with the present data is related to the fact that the PRL responses to TRH in our present female control subjects were twice as high as in our previous female controls. In both studies, untreated IGD subjects had similar PRL responses.

The normalization of both basal PRL levels and responses to stimulation during oestrogen therapy in female IGD subjects is explained by the ability of oestrogens to stimulate PRL secretion (Buckman & Peake, 1973; Carlson *et al.*, 1973; Yen *et al.*, 1974). There have been few reports on the effect of oestrogens on PRL responsiveness in female IGD subjects (Pertzelan *et al.*, 1977; Fernandez-Lazala *et al.*, 1979). One study reported increased basal PRL levels, but no increased response to MET (Fernandez-Lazala *et al.*, 1979). In a second study, basal PRL levels were unchanged following treatment although the response to TRH was increased (Pertzelan *et al.*, 1977).

The administration of hCG to our male IGD subjects increased circulating levels of both testosterone and oestradiol. It is well known that hCG induces oestradiol as well as testosterone secretion from the testes (Kelch *et al.*, 1972; Weinstein *et al.*, 1974). Although the stimulatory effect of oestrogens on PRL secretion in the human and rat is well known, the effect of testosterone is not as dramatic and it has been reported to be a weaker stimulant of PRL secretion (Kalra *et al.*, 1973; Shin *et al.*, 1974; Zylber-Haran *et al.*, 1981). There is also controversy about the mode of action of testosterone which may act directly or alternatively via aromatization to oestradiol or reduction to dihydrotestosterone (Nolin *et al.*, 1977). The administration of the non-aromatizable androgens, mesterolone and fluoxymesterone, in two male IGD subjects, did not have any effect on the PRL response to TRH and PRL levels were less than during hCG treatment. It would thus appear that the elevation of oestradiol is the main factor in restoring PRL responsiveness. Further evidence that this is an oestrogen-related phenomenon, is our observation that this increased PRL response was reduced on administration of the oestrogen antagonist, clomiphene citrate during hCG therapy in one IGD subject. We have previously described a similar phenomenon in primary testicular failure with reduction of the exaggerated PRL response to TRH and MET following administration of clomiphene citrate (Spitz *et al.*, 1981a). In contrast to hCG, pergonal had no effect on the impaired PRL response.

It should be emphasized that the doses of ethinyl oestradiol used in the females were supraphysiological; furthermore, the oestradiol and testosterone levels achieved in the

males following hCG were greater than in the controls. The improved PRL responses thus occurred with pharmacological dose schedules. This may account for the occurrence of basal hyperprolactinemia in the male patients during hCG administration. These patients are currently being investigated on more physiological replacement regimens.

Although the precise time required to restore PRL responsiveness to TRH has not been characterized, it was noted within 1 month of commencing hCG therapy. In contradistinction to our observations, Yamaji *et al.* (1977) did not note an improvement in PRL response to TRH in all their IGD subjects following testosterone enanthate treatment. Winters *et al.* (1978), in contrast, reported normal PRL dynamics in IGD patients on long-term hCG or testosterone. However, in a recent study it was reported that untreated IGD subjects had a reduced PRL response to CPZ compared with a treated group (Winters *et al.*, 1980).

In both the male and female IGD subjects, cessation of treatment was associated with a rapid decline in basal PRL levels and decreased PRL responses to MET and TRH. Serial studies showed that basal PRL levels and responsiveness to TRH were already decreased 4 weeks after cessation of therapy. This sequence of events indicates that the lactrotophe requires constant steroid priming to maintain normal responsiveness. In contrast, elderly males with prostatic carcinoma who have undergone bilateral orchidectomy and have low steroid levels, have normal PRL responses to TRH (Le Roith *et al.*, 1981). It should be noted that TRH is a potent stimulus for PRL release in the normal prepubertal child (Foley *et al.*, 1972) and in constitutional delayed puberty, the PRL response to chlorpromazine is normal (Winters *et al.*, 1980). Thus normal PRL responsiveness characterizes delayed puberty in contrast to untreated IGD. This may prove a useful method to distinguish between the two conditions. Even basal gonadotrophins and responses to LHRH do not offer a clear cut distinction between the two groups (Kelch *et al.*, 1980).

In conclusion, it is evident that IGD is characterized by low basal PRL levels and impaired responses to stimulation with TRH, as well as to the dopaminergic antagonist, MET. This is a direct consequence of the oestrogen deficiency and responsiveness can be restored by increasing circulatory oestrogens. However, treatment must be continued to maintain a normal PRL profile and its cessation is associated with a rapid reduction of both basal PRL levels and PRL responses to stimulation.

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Comments

The Thyrotropin (TSH) Profile in Isolated Gonadotropin Deficiency: A Model to Evaluate the Effect of Sex Steroids on TSH Secretion*

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ABSTRACT. This study evaluated the effect of estrogens and androgens on TSH secretion in hypogonadal male and female patients with isolated gonadotropin deficiency (IGD). The IGD subjects were clinically euthyroid and had normal circulating levels of thyroid hormones and T_4-binding globulin (TBG). The patients were challenged with TRH (200 μg) in the untreated state, during treatment, and 1 month after cessation of hormonal replacement therapy. For the study, five females were treated with ethinyl estradiol (0.05 mg twice daily) for 21 days; after stopping for 7 days, the treatment schedule was repeated for another two cycles. The remaining female was given a similar regimen with conjugated estrogens (0.625 mg daily). Five males were treated with hCG (5000 IU twice weekly) for 3 months; two were treated with hCG and Pergonal. The female patients had significantly decreased basal TSH levels as well as impaired TSH responses to TRH. After 3 months of ethinyl estradiol treatment, there was a rise in TBG, total serum T_4 and T_3 levels and a decrease in T_3 resin uptake; the free T_4 index was unchanged. During estrogen administration, there was no change in basal TSH, but there was an increase in the peak TSH response to TRH, which became identical to that of the controls. Cessation of estrogen was associated with a reduction in releasable TSH, and the profile reverted to the pretreatment state. In addition, serum TBG levels, with the associated changes in thyroid hormones, also returned to normal.

The male patients had TSH responses to TRH identical to those of the male controls. After 3 months of hCG treatment, there was a marked rise in serum estradiol as well as testosterone. Serum T_4 was reduced without a change in T_3, T_3 resin uptake, or TBG. Furthermore, there was no alteration in the TSH response to TRH. On the other hand, the administration of ethinyl estradiol (0.1 mg daily for 2 weeks) to two male IGD subjects produced an increase in TBG. This was associated with elevation of serum T_4 and T_3 levels and reduction of T_3 resin uptake. During estradiol administration, there was an increase in the TSH response to TRH.

These data are compatible with the hypothesis that estrogens are required to maintain a normal TSH response to TRH in the female. However, testosterone may counteract the effect of estradiol, which may explain why normal males tend to have a lower TSH response to TRH than females. (*J Clin Endocrinol Metab* **57:** 415, 1983)

BOTH androgens and estrogens have been reported to influence the TSH response to TRH, although there is no uniform concensus on their effects (1–7). Some have reported that estrogens enhance TSH responsiveness (1), whereas others have failed to document any effect (2–4). Androgen administration has also been associated with increased (5), decreased (6), or unaltered (7) TSH responses to TRH. In view of the conflicting data, we have assessed TSH secretion in hypogonadal male and female subjects and monitored their TSH responses to TRH during replacement therapy. As a model, we have selected patients with hypogonadotropic hypogonadism or isolated gonadotropin deficiency (IGD) who were deficient in LH and FSH and had low circulating levels of sex steroids (8–10). Thyroid hormone levels have been reported to be normal in patients with IGD (8–10), and GH and ACTH reserves are intact (8, 10). Basal PRL levels are low, and there is an impaired PRL response after stimulation with TRH and the dopaminergic antagonist metoclopramide (11, 12).

Our results show that female patients have an impaired TSH response to TRH. This was corrected by ethinyl estradiol administration. Male patients, in contrast, had normal TSH responses which were not influenced by hCG but were increased during ethinyl estradiol administration.

Materials and Methods

Test procedures

The TRH tests were performed between 0800–0830 h after an overnight fast. After explaining the full nature of the proposed study to all subjects, they gave written consent. A needle inserted into a antecubital vein was kept patent by the slow

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administration of physiological saline. Two or three blood samples were taken during a basal control period of 15–20 min, and then 200 μg TRH were given by rapid iv injection. Blood samples were taken 10, 20, 30, 40, and, on occasion, 60 mins after TRH administration. With the exception of two males, the patients were challenged with TRH in the untreated state, after 3 months of replacement therapy with estrogens (females) or hCG (males), and then 1 month after completing the course of treatment. Two males were also tested while receiving ethinyl estradiol.

Patients and treatment schedules

Six female patients, aged 18–28 yr, and seven males, aged 18–36 yr, were selected for the study. All subjects were clinically and biochemically euthyroid. None had thyromegaly, and all had a normal thyroid ^{131}I uptake and thyroid scan.

The female patients had primary amenorrhea, and all had responded to estrogen therapy with withdrawal bleeding. All had noted development of axillary and pubic hair before initiation of treatment, but breast development was poor. With one exception, all had eunuchoid body proportions. Their mean ± SD basal LH level was 4.1 ± 1.3 mIU/ml, and the mean ± SD FSH level was 3.5 ± 0.8 mIU/ml. The female patients had received previous cyclic replacement therapy with estrogens. However, therapy had been stopped 6 months before the present investigation. For the study, five IGD females were treated with ethinyl estradiol (0.05 mg twice daily) for 21 days; after stopping for 7 days, the treatment schedule was repeated for another two cycles. The remaining female took conjugated estrogens (Premarin; 0.625 mg daily) instead of ethinyl estradiol. The females were challenged with TRH before, after 3 months of estrogen therapy, and then again 1 month after cessation of treatment.

The chief complaint in the male IGD subjects was failure to mature sexually, absence of beard growth, and minimal penile development with small testes. Five had eunuchoid body proportions. Anosmia was present in one subject. Their mean ± SD basal LH level was 2.8 ± 0.8 mIU/ml, and their mean FSH level was 3.2 ± 0.4 mIU/ml. Four of the male patients had never been treated. One patient had received hCG, Pergonal, and testosterone enanthate previously. However, therapy had been stopped for 6 months.

For the present study, all males were given hCG (Pregnyl; 5000 IU twice weekly). Five of the males were challenged with TRH in the basal state, after 3 months of hCG therapy, and then 1 month after completion of the course. The remaining two males were tested initially after 6 and 12 months of hCG and Pergonal therapy and then 1 month after its cessation. They received hCG (5000 IU twice weekly) and Pergonal (human menopausal gonadotropins; one ampoule twice weekly). According to the manufacturer's literature (Istituto Farmacologico, Serono, Rome, and Ikapharm Ltd., Ramat Gan, Israel), each ampoule of Pergonal contains 75 IU human FSH and 75 IU human LH. Two males were also given a 2-week course of ethinyl estradiol (0.1 mg daily). Patient 1 was evaluated in the basal state, on the last day of ethinyl estradiol treatment, and 1 month after its cessation. Patient 2 was tested while on hCG and then after 2 weeks of combined hCG and ethinyl estradiol treatment (Fig. 1).

The results in the female patients were compared to the responses in 14 female controls, aged 18–26 yr, tested in the early follicular phase of the menstrual cycle. Similarly, the results of our male patients were compared to those of 26 normal males, aged 20–40 yr.

Methods

Serum TSH, testosterone, and estradiol were determined by previously described methods (11–13). Total serum T_4, T_3, and T_3 resin uptake (T_3RU) were measured using kits kindly supplied by Diagnostic Products Corp. (Los Angeles, CA). T_4-binding globulin (TBG) was measured using a RIA kit supplied by CEA Sorin. Steroid, thyroid hormones, and TBG were measured in equal aliquots of the pooled basal samples. The TSH antiserum was kindly supplied by the National Hormone and Pituitary Agency, NIAMDD. TSH standard (68/38) was provided by the Division of Biological Standards and Control (Holly Hill, Hampstead, London, England). The intraassay and interassay coefficients of variation were 3.4% and 10.6%, respectively, and the lower limit of sensitivity (mean ± SD) was 1.1 ± 0.6 μU/ml. To avoid interassay variation, all samples from an individual patient were measured in the same assay.

The integrated TSH secretion after TRH stimulation was measured by calculating the net area under the TSH response curves by the trapezoidal rule (14). Statistical evaluation was performed using Student's paired or unpaired *t* test whenever appropiate.

Results

Steroids (Table 1)

Compared to controls, basal serum estradiol levels were decreased in untreated male and female patients, and serum testosterone levels were decreased in the males. Since ethinyl estradiol did not compete in our RIA, no changes in serum estradiol were detected during treatment in the female patients. In the males, after hCG administration, there was a marked rise in both serum

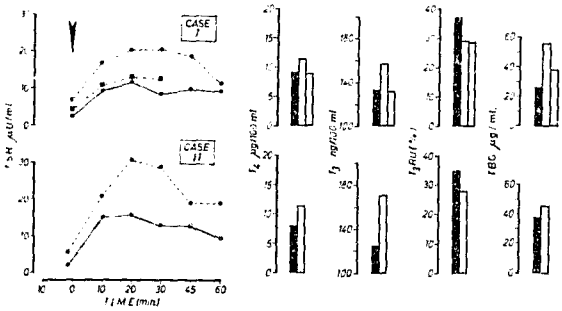


FIG. 1. *Left panel*, TSH response to TRH; *right panel*, serum T_4, T_3, T_3RU, and TBG responses in two male IDG patients before (●—●; ■), during (●- - ●; □), and after (■- · -■; □) ethinyl estradiol treatment. Patient 2 received hCG throughout the period.

TABLE 1. Mean ± SE serum estradiol, testosterone, T_4, T_3, T_3RU, TBG, and integrated TSH secretion in male and female IGD patients before, during, and after treatment; normal ranges of control subjects are also shown

	17β-Estradiol (pg/ml)	Testosterone (ng/ml)	T_4 (μg/100 ml)	T_3 (ng/100 ml)	T_3RU (%)	TBG (μg/ml)	TSH integrated secretion (μU/ml·min)
Females							
IGD							
Pretreatment	25 ± 7		9.9 ± 2.0[a]	130 ± 22	30.6 ± 2.8[b]	25.6 ± 2.9[a]	7.6 ± 3.4[a]
During treatment	26 ± 6		14.3 ± 2.6	197 ± 55	20.3 ± 4.5	41.6 ± 10.5	12.4 ± 2.4
Post treatment			9.9 ± 1.8[a]	116 ± 1[a]	31.9 ± 1.3[b]	22.0 ± 4.3[a]	7.4 ± 4.5
Controls (normal range in follicular phase)	44–125		4.5–12.5	90–210	23–37	15–31	6.1–28.1
Males							
IGD							
Pre-hCG	<15[c]	0.89 ± 0.23[c]	10.2 ± 1.8[a]	117 ± 21	27.5 ± 2.9	23.5 ± 6.2	7.8 ± 3.8
During hCG	56 ± 39	6.9 ± 3.2	7.2 ± 1.8	118 ± 23	28.3 ± 2.9	21.1 ± 3.9	9.2 ± 4.8
Post-hCG	<15[c]	1.2 ± 0.8[c]	10.2 ± 1.8[a]	128 ± 25	27.8 ± 2.5	24.3 ± 2.5	9.7 ± 3.5
Controls (normal range)	15–38	3.0–9.7	4.5–12.5	90–210	27–41	15–31	2.6–14.4

[a] $P < 0.05$ *vs.* levels during treatment.
[b] $P < 0.01$ *vs.* levels during treatment.
[c] $P < 0.001$ *vs.* levels during treatment.

testosterone and estradiol. Mean serum estradiol values exceeded those in the normal men. With the cessation of hCG, both serum estradiol and testosterone levels again decreased and were indistinguishable from the pretreatment values.

Thyroid hormones (Table 1)

Before treatment, both female and male patients had values of serum T_4, T_3, and T_3RU within normal limits. During treatment of the female patients, there was a rise in serum T_4 and T_3 and a decrease in T_3RU. Mean serum T_4 levels were above normal, and T_3RU values were below the normal range. After cessation of treatment, serum thyroid hormone levels returned to pretreatment values.

In the males during treatment, there was a transient decrease in serum T_4, although it remained within the normal range. After cessation of hCG, serum T_4 levels again increased. There were no changes in serum T_3 or T_3RU during hCG therapy. In the two males studied while taking ethinyl estradiol, there was a transient elevation of serum T_4 and T_3 and a decrease in T_3RU (Fig. 1). The free T_4 index, which is the product of the serum T_4 and T_3RU values, did not change during hormonal treatment in either males or females.

TBG levels (Table 1)

There were no differences in basal serum TBG levels in the males and females, and values were within the normal range. During treatment in the females, there was a significant rise in serum TBG. Values decreased after cessation of treatment. There were no changes in serum TBG in the male patients during hCG, although a transient rise was noted in the two males given ethinyl estradiol (Fig. 1).

TSH

Females (Table 1 and Fig. 2). The mean ± SD basal TSH level in the untreated state was 1.8 ± 0.2 μU/ml (this represents the mean of all samples before TRH administration). This value was significantly less ($P < 0.001$) than that in the normal women in whom the mean level was 3.7 ± 1.3 μU/ml. The peak TSH response in the untreated state occurred at 30 min and was 10.8 ± 4.6 μU/ml. In the controls, the peak occurred at 40 min and was 24.3 ± 10.2 μU/ml. Integrated TSH secretion was significantly less in the patients than in the controls ($P < 0.05$). During ethinyl estradiol treatment, there was no change in basal TSH levels; however, there was a marked increase in the TSH response to TRH, and the peak was 19.3 ± 10.5 μU/ml (Fig. 2). The response was similar to that in the controls and greater than the pretreatment response at 30 min ($P < 0.05$). The integrated area was now similar to that in the controls and greater than that before treatment (Table 1). After cessation of treatment, there was no change in basal TSH levels, but the response to TRH decreased. The peak was 10.5 ± 4.9 μU/ml. The profile after cessation of treatment was similar to that in the untreated state, and the integrated secretion was significantly less than that in controls ($P < 0.05$).

Males. The basal TSH level was 2.3 ± 0.6 μU/ml before treatment and similar to the control value (2.0 ± 0.8 μU/ml). No changes were noted in basal TSH after hCG

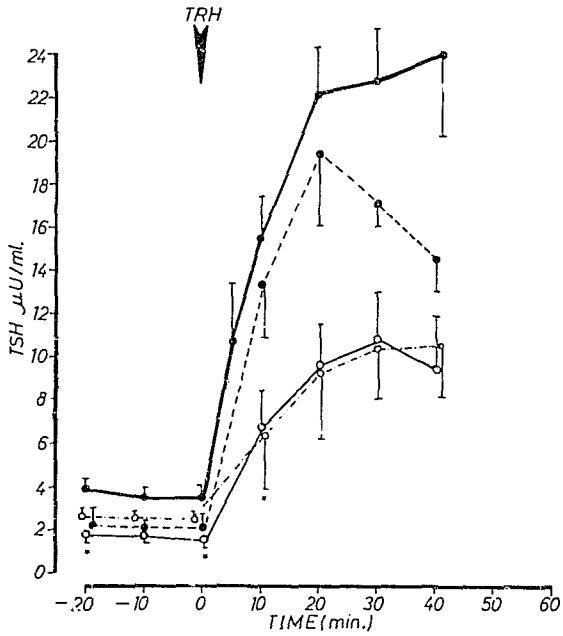


FIG. 2. Mean ± SEM TSH response to TRH in six female IGD subjects before (O—O), during (●- -●), and 1 month after cessation of (O- · -O) estrogen treatment. Values for female controls are also shown (●—●).

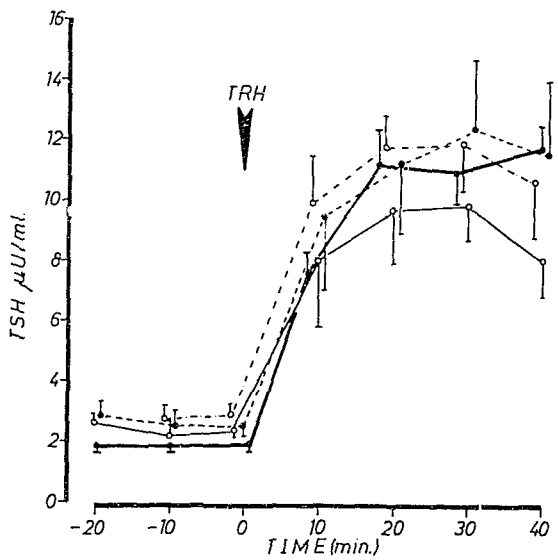


FIG. 3. Mean ± SEM TSH responses to TRH in seven male IGD subjects before (O—O), during (●- -●), and 1 month after cessation of (O- · -O) hCG treatment. Values for male controls (●—●) are also shown.

treatment. The peak TSH response to TRH before treatment was 9.7 ± 2.6 μU/ml compared to 11 ± 5.2 μU/ml in the controls. Corresponding peak levels during and after the cessation of hCG treatment were 12.1 ± 5.1 and 11.8 ± 4.2 μU/ml, respectively. None of these values was significantly different from one another (Fig. 3), nor was the integrated TSH secretion different (Table 1). In the two males challenged with TRH while taking ethinyl estradiol, there was a rise in basal and TRH-induced TSH secretion in both subjects, and the response was similar to that of the normal female. In patient 1, cessation of ethinyl estradiol resulted in reduction of TRH-induced TSH secretion (Fig. 1).

Discussion

The IGD subjects were clinically euthyroid, with no thyromegaly. All had normal circulating levels of thyroid hormones and normal thyroid ^{131}I uptake. Although within the normal range, the female patients had significantly decreased basal TSH levels as well as an impaired TSH response to TRH. This phenomenon has been described previously (9). During ethinyl estradiol treatment, there was a rise in serum T_3 and T_4, with a corresponding reduction of the T_3RU. The free T_4 index was unaltered, and thus, thyroid function did not change. The variations in thyroid hormones were, therefore, consequent to the increase in TBG, a well described phenomenon associated with estrogen treatment (15). After cessation of therapy, TBG decreased, and thyroid hormone values returned to normal.

During treatment of the female patients, there was no change in basal TSH, but there was an increase in the peak TSH response to TRH, which became identical to that in the normal women. This effect is presumably related to the estrogen therapy. It has been previously reported that exogenous estrogens may increase the TSH response to TRH in the male, although this is not a uniform observation and does not occur in normal females (1–4, 16). There is also no firm relationship between TSH secretion and endogenous estrogens. Patients with secondary amenorrhea and low estrogen levels as well as pregnant subjects with high estrogen levels have similar TSH responses to TRH (17, 18). In IGD, constant estrogen priming is required to maintain a normal TSH response to TRH. Its cessation is associated with an immediate reduction in releasable TSH. The TSH and PRL profiles in female IGD patients are thus very similar since the low basal and stimulated PRL levels in IGD are also restored to normal after treatment with ethinyl estradiol (12). Hence, estrogens are required to maintain normal TSH and PRL responsiveness in female IGD patients.

As regards the male patients, they showed a transient

reduction in serum T_4 during hCG therapy. This may be related to the rise in testosterone, which can reduce serum TBG (15, 19). However, no change in serum TBG was noted during hCG therapy. Moreover, serum T_3 and T_3RU values did not change. The effect of testosterone may have been neutralized by the concomitant increase in estradiol during hCG treatment. When the male patients received exogenous ethinyl estradiol, there was an increase in TBG and appropriate changes in thyroid hormone levels.

In accordance with previous observations (20), our male patients had TSH responses to TRH identical to those in normal men. In both of these groups, the TSH response mimicked that of the untreated female patients, but was less than that in the normal women. Although a sex difference in TSH responsiveness has been observed by some workers (16, 21, 22), others have described equivalent responses to TRH in males and females (23, 24). During hCG treatment, despite an increase in estradiol to pharmacological levels, there was no alteration in the TSH response. On the other hand, when ethinyl estradiol was administrated to two male IGD subjects, either alone or together with hCG, there was a marked increase in the TSH response to TRH, which became equivalent to that of the normal females. The TSH results in the male IGD patients should be contrasted to those of PRL. During hCG treatment, there was an increase in both basal and stimulated PRL levels (12).

These results shed some light on the steroid modulation of TSH secretion. Female IGD subjects who have not had any previous exposure to estrogens require this hormone for a normal TSH response to TRH. Once the female pattern has been fully established over a prolonged period, marked decreases in endogenous estrogens, such as in ovarian failure, or increases, such as in pregnancy, do not alter the TSH response to TRH (17, 18). Even exogenous estrogens have no effect (2, 3, 16). Untreated male IGD patients have a TSH profile similar to those of untreated female IGD patients and the normal adult male; their responses, however, are less than those of normal females. hCG administration to these subjects increased both testosterone and estradiol, but did not alter their TSH responses. It is possible that the rise in testosterone counteracted the effect of estradiol in enhancing the TSH response. This is supported by the fact that the administration of ethinyl estradiol to these subjects converted their TSH responses to the normal female pattern. Exogenous estrogen administered to normal males and to those with prostatic carcinoma may increase their TSH responses to TRH (1, 16), although this has not been a uniform observation (4).

Additional evidence for the restraining effect of testosterone on the TSH response to TRH comes from data of patients with testicular failure who have high gonadotropin levels and an increase in their estradiol to testosterone ratios. Their TSH profile consequent to TRH adminstration mimics that of the normal female (13). Nonaromatizable androgens, such as fluoxymesterone, reduce the TSH response in testicular failure (6). It is thus likely that the lower TSH response to TRH in male subjects compared to females is due to the inhibitory effect of testosterone on TSH secretion.

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THE PROLACTIN RESPONSE TO THYROTROPIN-RELEASING HORMONE DIFFERENTIATES ISOLATED GONADOTROPIN DEFICIENCY FROM DELAYED PUBERTY

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IT is currently believed that hypogonadotropic hypogonadism (isolated gonadotropin deficiency) is secondary to lack of delivery of endogenous luteinizing hormone-releasing hormone to the pituitary gonadotrope, whereas delayed puberty is related to retarded activation of the hypothalamic–pituitary axis.[1,2] The distinction between the two conditions poses one of the most difficult diagnostic problems in endocrinology.[2,3] When isolated gonadotropin deficiency is associated

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with anosmia or other congenital anomalies, it is easily differentiated from physiologic delayed puberty.[4] However, many patients present without any somatic abnormalities. In such cases, the usual course is to procrastinate. However, prolonged delay in treatment causes severe social embarrassment for the patient and considerable concern for the parents. Attempts have therefore been made to differentiate early between the two conditions so that isolated gonadotropin deficiency can be appropriately treated.

Serum and urinary levels of gonadotropins are low in both conditions and are thus of limited help in the assessment.[2,3,5] Furthermore, neither the response of gonadotropin to luteinizing hormone-releasing hormone[2,4,6,7] or clomiphene citrate[5] nor the testosterone response to human chorionic gonadotropin[5] can reliably differentiate the two conditions. Changes in sleep-associated episodic secretion of luteinizing hormone, follicle-stimulating hormone, and testosterone are also not consistently helpful in the differential diagnosis.[2,8]

We have previously made the unexpected observation that the prolactin response to thyrotropin-releasing hormone is impaired in isolated gonadotropin deficiency.[9] We therefore decided to assess the response in boys with delayed puberty. In this report we show that in contrast to the response in isolated gonadotropin deficiency, the prolactin response to thyrotropin-releasing hormone is normal in delayed puberty, and that this response may be helpful in differentiating between the two.

Methods

Subjects

Fifteen male subjects with constitutional delayed puberty and 10 with isolated gonadotropin deficiency had initially sought medical attention because their sexual maturation was delayed and they had small external genitalia. The boys with delayed puberty were 13 to 18 years of age and at the time of the study had delayed sexual development for their age (Stage I or II), according to the classification of Tanner.[10] All but two were within 10 per cent of their ideal body weight. Three had eunuchoid body proportions. Their bone ages ranged from 12 to 14 years, and in each case there was a delay of one to two years in skeletal maturation as compared with the chronologic age. After the evaluation, the subjects were observed at six-month intervals, and all underwent spontaneous sexual development.

The other 10 subjects all presented with well-documented isolated gonadotropin deficiency. Four ranged in age from 16 to 20 years, another four were 24 to 25, and the remaining two were 30. Eight of the subjects were eunuchoid, and none was obese. Anosmia was present in one, but there were no other somatic abnormalities, such as cleft palate, cleft lip, unilateral renal agenesis, color blindness, or nerve deafness.[1] Four of the subjects had never been treated. The remainder had had previous therapy with human chorionic gonadotropin or long-acting testosterone. This treatment had been stopped six months before the present investigation. The clinical and laboratory data for some of these subjects have been described previously.[9] All had normal thyroid function and intact adrenal and growth-hormone reserve.

Test Procedure

The tests were performed at 8 a.m., after an overnight fast. After the nature of the study had been explained to them, the subjects or their parents signed written consent forms. A needle inserted into an antecubital vein was kept patent by a slow saline infusion. The boys with delayed puberty were then given a bolus of luteinizing hormone-releasing hormone (100 μg) and thyrotropin-releasing hormone (5 μg per kilogram of body weight, to a maximum of 200 μg). The subjects with isolated gonadotropin deficiency received 100 μg of luteinizing hormone-releasing hormone and 200 μg of thyrotropin-releasing hormone, either as a bolus or, alternatively, on separate days. The response in both patient groups was compared with that in a group of 26 normal men who had also received luteinizing hormone-releasing hormone (100 μg) and thyrotropin-releasing hormone (200 μg). It has been shown previously that the gonadotropin, thyrotropin, and prolactin responses are not altered if the releasing hormones are given together.[11] In all subjects blood samples were taken at 10-minute intervals for 60 minutes. The peak hormonal response (independent of time) was used to compare the responses in the three groups.

Measurements

Serum levels of luteinizing hormone, follicle-stimulating hormone, prolactin, testosterone, and estradiol 17β were determined by previously described methods.[9] Gonadotropin levels were expressed in relation to the second international reference preparation of human menopausal gonadotropins. The actual standard used in the assay was the first international reference preparation of pituitary follicle-stimulating hormone and luteinizing hormone (69/104). This, as well as the prolactin standard (75/504), was kindly provided by the Division of Biological Standards and Control, Hampstead, London, England. Samples of antiserum to luteinizing hormone (final dilution, 1:200,000), follicle-stimulating hormone (1:400,000), and prolactin (1:400,000) were kindly supplied by the National Pituitary Agency of the National Institute of Arthritis, Metabolism, and Digestive Diseases. ^{125}I-labeled luteinizing hormone, follicle-stimulating hormone, and prolactin were purchased from CIS-France. Intraassay and interassay coefficients of variation were as follows: 5.8 per cent and 19.1 per cent (luteinizing hormone); 4.3 per cent and 6.9 per cent (follicle-stimulating hormone); 6.6 per cent and 12.2 per cent (prolactin). Testosterone and estradiol were measured in pooled aliquots of the basal blood samples. For the polypeptide hormones, the mean basal level refers to the mean value of all samples before administration of releasing hormones.

Student's t-test was used to compare responses in patients and controls.

Results

Basal Levels

Basal serum levels of testosterone (mean ±S.D.) were similar in patients with isolated gonadotropin deficiency (1.5±0.8 ng per milliliter) and delayed puberty (1.3±1.2 ng per milliliter) and were significantly lower ($P<0.001$) in both groups than in the normal men (5.9±2.0 ng per milliliter). The majority of the patients in both groups had undetectable levels of estradiol — below 15 pg per milliliter, which is the lower limit of sensitivity of the assay. Mean basal levels of luteinizing hormone were not different in isolated gonadotropin deficiency and delayed puberty, but levels in both groups were lower ($P<0.01$) than in the normal men. Basal levels of follicle-stimulating hormone and prolactin were lower in patients with gonadotropin deficiency ($P<0.001$) than in boys with delayed puberty or in normal men. However, because of the large overlap, low basal levels cannot be used to diagnose isolated gonadotropin deficiency in an individual patient (Fig. 1).

Response to Releasing Hormones

The responses to the releasing hormones in the three groups are shown in Figure 1. The elevation of serum

luteinizing hormone after the injection of luteinizing hormone-releasing hormone was similar in the boys with delayed puberty and the normal men. The increase in luteinizing hormone in the former group was higher than has been reported by others,[6,12,13] although four of our subjects did have a markedly impaired response. It has been shown that the luteinizing hormone response increases sharply with increasing testicular size[13] and after a bone age of 10 to 12 years has been attained.[12] The fact that all our subjects had bone ages of more than 12 years may explain the marked response to luteinizing hormone-releasing hormone. Mean luteinizing hormone responses were significantly decreased in the patients with isolated gonadotropin deficiency ($P<0.001$), although three of these subjects had peak luteinizing hormone responses within the range of response of the normal men and another four had maximum responses within the lower limit of those of the boys with delayed puberty. Thus, only 3 of the 10 subjects with isolated gonadotropin deficiency had peak responses that were lower than those of the boys with delayed puberty and of the normal men.

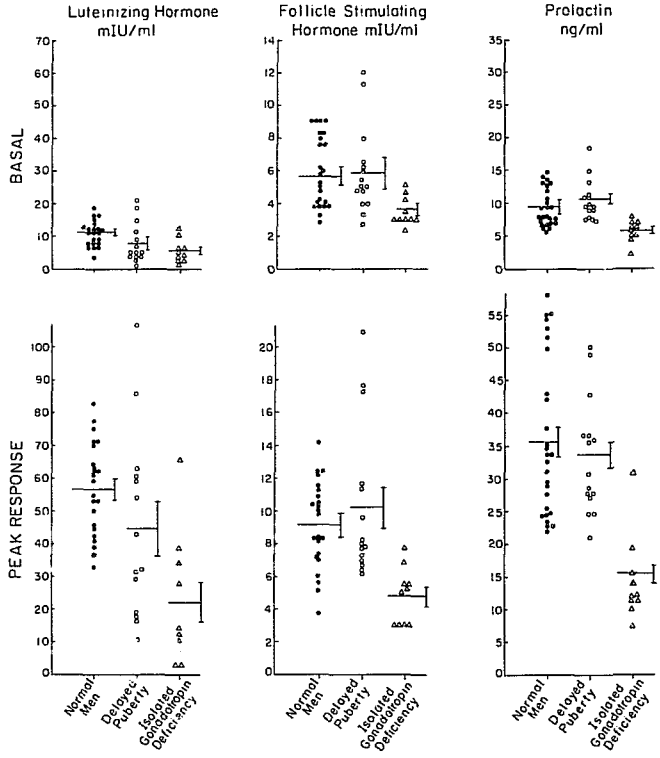


Figure 1. Response to Releasing Hormones in Normal Adult Men, Boys with Delayed Puberty, and Patients with Isolated Gonadotropin Deficiency.

The upper panels show basal hormone levels. The lower panels show the peak gonadotropin responses to luteinizing hormone-releasing hormone and the prolactin responses to thyrotropin-releasing hormone in the three groups. The means (±S.E.M.) are indicated by the horizontal lines and the symbols indicate individual responses.

Elevation of follicle-stimulating hormone was also similar in the boys with delayed puberty and the men, although three of the former did have peak follicle-stimulating hormone responses that exceeded the range of the controls' responses. Such a finding has been reported previously.[12] It should be mentioned that a lack of response of follicle-stimulating hormone to the releasing hormone has also been reported in normal men.[11] The response in our patients with isolated gonadotropin deficiency was markedly impaired ($P<0.001$). Four patients had no increase in follicle-stimulating hormone after stimulation, and the remaining six had peak responses within the range for subjects with delayed puberty and isolated gonadotropin deficiency.

Clearly, these results do not differentiate between isolated gonadotropin deficiency and delayed puberty. Similar observations have been reported by other authors using this releasing hormone.[2,4,6,13,15] The reason for the heterogenous responses to luteinizing hormone-releasing hormone is that some subjects probably have only a partial deficiency of gonadotropins.[3]

The peak elevation of prolactin after injection of thyrotropin-releasing hormone ranged from 22 to 50 ng per milliliter in the boys with delayed puberty and from 22 to 58 ng per milliliter in the normal men. Nine of the patients with isolated gonadotropin deficiency had a prolactin response ranging from 7 to 19 ng per milliliter. Assuming that a prolactin level of at least 22 ng per milliliter after injection with thyrotropin-releasing hormone constitutes the minimal normal response, 9 of the 10 subjects with isolated gonadotropin deficiency had a subnormal increase in prolactin after thyrotropin-releasing hormone. Neither age nor the gonadotropin response to luteinizing hormone-releasing hormone influenced the prolactin response. For example, the four youngest patients with gonadotropin deficiency had peak responses of 12, 13, 18, and 31 ng per milliliter. The four boys with delayed puberty who had low peak responses of luteinizing hormone (<20 mIU per milliliter) were clearly differentiated from the group with isolated gonadotropin deficiency by their normal prolactin response. The four patients with gonadotropin deficiency who had the highest luteinizing hormone peak (>28 mIU per milliliter) were readily distinguished from the delayed-puberty group by vir-

tue of their low prolactin response. These four patients would have been misdiagnosed if the only diagnostic criterion had been impaired luteinizing hormone response. Only one patient with isolated gonadotropin deficiency, a 17-year-old boy, had a normal peak prolactin response of 31 ng per milliliter, despite a low luteinizing hormone response (12 mIU per milliliter).

Discussion

These results indicate that in the individual patient, basal levels of testosterone, gonadotropins, and prolactin, as well as the responses of luteinizing hormone and follicle-stimulating hormone to luteinizing hormone-releasing hormone, were of limited value in differentiating isolated gonadotropin deficiency from delayed puberty and the normal adult male state. The prolactin response was the best discriminant; it reliably identified 9 of 10 subjects with isolated gonadotropin deficiency.

It is worthy of note that the boys with delayed puberty received a higher dose of thyrotropin-releasing hormone per kilogram of body weight than the patients with isolated gonadotropin deficiency. This cannot explain the observed difference, since the studies of Jacobs et al.[16] have shown that the prolactin response to thyrotropin-releasing hormone is maximal at 100 μg and that additional increases in dosage up to 800 μg do not produce a further increment. We have confirmed these results by showing that patients with isolated gonadotropin deficiency do not have higher prolactin responses with doses of thyrotropin-releasing hormone up to 800 μg (unpublished data). In contrast, the thyrotropin response to thyrotropin-releasing hormone is maximal only at 400 μg.[17] For this reason, we were unable to compare the thyrotropin response of prepubertal boys with those of subjects with isolated gonadotropin deficiency and normal men.

The impaired prolactin response in isolated gonadotropin deficiency is not an inherent component of the syndrome but, rather, a manifestation of the low levels of steroids present.[18] The administration of human chorionic gonadotropin raises basal and stimulated prolactin levels to normal. It should be mentioned that constant steroid "priming" is required to maintain normal prolactin responsiveness, and that the cessation of such therapy is associated with an immediate reduction in basal and stimulated prolactin levels.[18] Further studies have shown that an elevation of estradiol, rather than testosterone, is the main factor in restoring prolactin responsiveness in subjects with isolated gonadotropin deficiency. This conclusion is based on the finding that the concomitant administration of the antiestrogen clomiphene citrate during treatment with human chorionic gonadotropin reduces the prolactin response. Furthermore, androgens that cannot be aromatized do not influence the impaired prolactin response.[18]

The precise mechanism underlying the difference in prolactin response to thyrotropin-releasing hormone in isolated gonadotropin deficiency and delayed puberty is not known. It is impossible to ascertain whether levels of circulating endogenous estrogens were lower in the group with gonadotropin deficiency because of the limitations of the existing assays at these low concentrations. Indeed, most of the patients of both groups had undetectable levels of estradiol.

A subtle difference between the estradiol levels in the two groups is a possible explanation for the abnormal results. If a certain estrogen level is required to achieve a prolactin response to thyrotropin-releasing hormone, there must be a sufficient amount in young boys. In unpublished studies we have shown that three children 3 to 8 years of age had normal prolactin responses to thyrotropin-releasing hormone. Extraglandular aromatization of androgens to estrogens is high in the newborn and gradually decreases during childhood.[19] Thus, although testosterone levels were similar in the two groups of patients in the present study, the potential for aromatization may have been much greater in the boys with delayed puberty, since they were younger than the subjects with gonadotropin deficiency. An alternative explanation is that a certain degree of estrogen insensitivity characterizes isolated gonadotropin deficiency.

Previous investigators have shown that there is also a decreased prolactin response to the dopaminergic antagonist chlorpromazine in isolated gonadotropin deficiency and a normal response in delayed puberty.[20] However, chlorpromazine administration may be associated with transient hypotension, and the response is delayed.[21] Preliminary studies indicate that the prolactin response to another dopaminergic antagonist, metoclopramide, can also be used to differentiate delayed puberty from isolated gonadotropin deficiency. A distinct advantage of the test using thyrotropin-releasing hormone is that it is free of serious side effects; it also may be completed within 30 minutes.

Although the precise mechanism underlying the phenomenon is not clear, we suggest that the impaired prolactin response to challenge with thyrotropin-releasing hormone may facilitate the diagnosis of isolated gonadotropin deficiency and differentiate this condition from delayed puberty, which is characterized by a normal prolactin response.

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ISOLATED DEFICIENCY OF FOLLICLE-STIMULATING HORMONE

Clinical and Laboratory Features

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Abstract Isolated deficiency of follicle-stimulating hormone (FSH) was found in a 22-year-old woman with primary amenorrhea. Pituitary growth hormone, ACTH and thyrotropin release, assessed either directly or indirectly, was normal. Serum luteinizing hormone levels were high, generally 50 to 90 mIU per milliliter, whereas serum follicle-stimulating hormone levels were undetectable (less than 3 mIU per milliliter). Serum estradiol-17β concentration was less than 10 pg per milliliter. Ovarian biopsy revealed primordial follicles but without maturation to the stage of antral formation. Administration of menotropins (human menopausal gonadotrophins), which contains urinary follicle-stimulating and luteinizing hormone, led to the appearance of readily detectable levels of follicle-stimulating hormone in the serum (between 10 and 20 mIU per milliliter) that fell to less than 3 mIU per milliliter 48 hours after the last injection of menotropins. Luteinizing hormone release was inhibited by intravenous injection of conjugated estrogen, with rebound to basal high values within three days. Serum luteinizing hormone levels were uninfluenced by clomiphene administration; follicle-stimulating hormone was not measurable because of development of antibodies to it. The defect in our patient may be at the pituitary rather than the hypothalamic level.

DEVELOPMENT of specific radioimmunoassays for measurement in serum of the gonadotrophins follicle-stimulating hormone and luteinizing hormone[1,2] has permitted a better understanding of events during the normal female menstrual cycle and also of clinical disturbances in gonadotrophin release.

Among the causes of primary amenorrhea a deficiency state of both gonadotrophins (that is, of follicle-stimulating hormone and of luteinizing hormone) has been recognized as occurring with or without hypofunction of other pituitary hormones, such as ACTH growth hormone and thyrotropin.[3,4] In the following case isolated deficiency of follicle-stimulating hormone presented as primary amenorrhea.

CLINICAL DESCRIPTION

A 22-year-old woman was first seen at the Hadassah University Hospital in July, 1971, for evaluation of primary amenorrhea. She has a younger sister with regular menstruation. There was no family history of menstrual disturbance, and the past history was noncontributory. Breasts and sexual hair started to develop at the age of 13 years, but breast growth was quite modest. At the age of 17, the patient first sought medical attention because of amenorrhea. For a number of years she was treated intermittently with a sequential estrogen-progesterone preparation (Nogest S), which produced cyclical withdrawal bleeding. Menstrual bleeding did not occur in response to the administration of progestins, of pregnant mare's serum (Gestyl) or of clomiphene. In 1969, at the age of 19, she was given a course of menotropins, or human menopausal gonadotrophins (Pergonal-500). Two ampoules (150 IU of follicle-stimulating hormone and 150 IU of luteinizing hormone, according to the manufacturer's literature) were injected each day for 5 days. She did not menstruate after these injections, and she was told that therapy had not been successful in inducing ovulation.

Physical examination on admission in July, 1971, showed a eunuchoid patient, her height being 163 cm (upper segment, 79 cm) and her span 172 cm. She had poor breast development, but normal axillary and pubic hair. The rest of the examination, including routine blood evaluation, was unremarkable. Olfactory acuity was normal, and there was no galactorrhea. Tests of thyroid function, including protein-bound iodine, serum tri-iodothyronine – Sephadex (Ames) and uptake of radioactive iodine, were within normal limits, as was urinary excretion of 17-ketosteroids and 17-hydroxycorticoids. X-ray films of the skull, including the sella turcica, and chest were normal. The buccal smear was of a female pattern, and the chromosome karyotype in the peripheral white cells was 46-XX. At laparoscopy (July 1971) the uterus was found to be hypoplastic, and the ovaries were small, white and anovulatory. A detailed investigation of the patient was carried out over the next 9 months. In March, 1972, at biopsy of the right ovary, tiny fragments of ovarian tissue were obtained. Histologic examination showed numerous primordial follicles (Fig. 1). There were occasional graafian follicles in a later stage of maturation with 2 or 3 cell layers. Mature follicles showing antral formation were not seen. Step sections of the biopsy material were made, and a single structure resembling a corpus albicans was found. Hilar cells were not present in any of the sections. The overall histologic picture was that of unstimulated follicles.

SPECIAL HORMONAL INVESTIGATIONS

Growth hormone was measured by immunoassay, a single antibody system,[5] with separation of "bound" from "free" hormone by dextran-charcoal,[6] being used. Serum 11-oxycorticosteroids ("cortisol") were assayed by the fluorometric method of Mattingly.[7] Follicle-stimulating and luteinizing hormones were determined by radioimmunoassay with use of a double-antibody system.[1,2] Pituitary luteinizing hormone, generously provided by Dr. A. S. Hartree, was used for iodination purposes. Rabbit anti-HCG antibody, a gift from Dr. Saul Rosen, was employed in a final dilution of 1:500,000. The Second International Reference Preparation of menotropins was used as standard. In our immunoassay system, 1 ng of Hartree luteinizing hormone is equivalent to 8 mIU of the Reference Preparation. Pituitary follicle-stimulating hormone (LER 1366), provided by the National Pituitary Agency, was used for iodination purposes in our assay. Rabbit anti-human follicle-stimulating hormone antibody, a gift of Dr. W. D. Odell, was employed in a final dilution of 1:20,000; 1 ng of (LER 1366) is equivalent to 2.9 mIU of the Reference Preparation in our system. The lower limit of sensitivity in our laboratory is 0.6 mIU per

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Figure 1. Ovarian Biopsy Taken in March, 1972 (Hematoxylin and Eosin Stain × 125), Showing Numerous Primordial Follicles.

tube. Since we use a maximum of 0.2 ml of serum per tube, this corresponds to a sensitivity of 3 mIU of follicle-stimulating hormone per milliliter of serum. Serum estradiol-17β was measured by a radioimmunoassay method.[8] Serum was extracted with ether, and the extract was filtered on a Sephadex-LH 20 column. The estradiol fraction was collected and assayed in an assay system employing tracer ^{3}H-estradiol, and antiestradiol antiserum, a gift of Dr. F. Dray, in a final dilution of 1:90,000. Separation of "bound" from "free" estradiol was achieved by dextran-charcoal precipitation.[6] Procedural losses were monitored by addition of labeled steroid to the plasma.

Results

Results of the special hormonal investigations are shown in Figures 2 and 3.

Serum Growth Hormone and "Cortisol"

Integrity of growth hormone release was evaluated after the administration of insulin (0.1 U per kilogram of body weight). Serum glucose levels fell after insulin from 70 to 30 mg per 100 ml. Growth hormone levels, undetectable before hypoglycemia, rose to 50 ng per milliliter — that is, release was intact (normal range after insulin hypoglycemia, 7 to 50 ng per milliliter). This is probably also true for ACTH release, since cortisol levels in serum rose to 26 μg per 100 ml after insulin (normal range after insulin hypoglycemia, 18 to 35 μg per 100 ml).

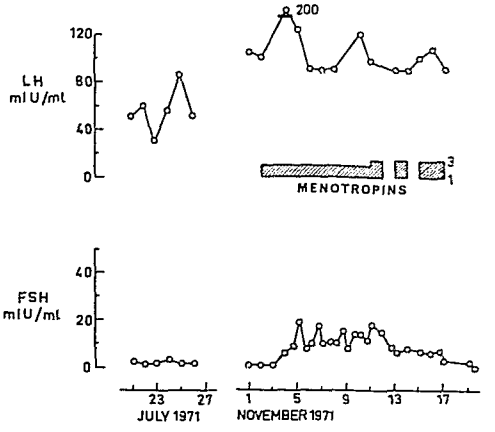


Figure 2. Serum Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) Levels Measured in July and Again in November, 1971, before (November 1 and 2), during (November 3-16) and after (November 17 and 18) Administration of Menotropins.

The dosage is given in ampoules injected; 1 ampoule contains the equivalent of 75 IU of follicle-stimulating hormone and 75 IU of luteinizing hormone.

Serum Luteinizing Hormone, Follicle-Stimulating Hormone and Estradiol-17β

Basal values. Basal values of serum immunoreactive luteinizing hormone were high. The normal ranges for menstruating women in our laboratory are 8 to 25 mIU per milliliter (proliferative phase), 50 to 200 mIU per milliliter (at time of luteinizing hormone surge) and 5 to 15 mIU per milliliter (secretory phase). In our patient, the range of values was very wide, between 30 and 200 mIU per milliliter (Fig. 2 and 3). Of 69 serum samples assayed, 47 were between 50 and 90 mIU per milliliter.

Serum follicle-stimulating hormone, on the other hand, was undetectable (less than 3 mIU per milliliter) when measured repeatedly on six occasions in July, 1971, and again on November 1 and 2, 1971 (Fig. 2). Levels of serum estradiol-17β were undetectable (less than 10 pg per milliliter) in July, 1971.

Response to menotropins. Our patient received a course

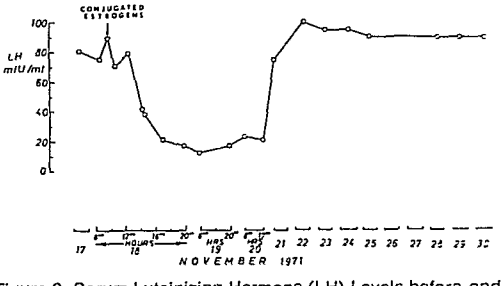


Figure 3. Serum Luteinizing Hormone (LH) Levels before and after Intravenous Administration of Conjugated Equine Estrogens.

of menotropins (Pergonal 500, human post-menopausal gonadotrophins, Istituto Farmacologica Serono, Roma, and Ikapharm Limited, Ramat Gan, Israel). She initially received 2 ampoules daily by intramuscular injection for nine days, and then on November 11, the dose was increased to 3 ampoules. [illegible] dizziness, weakness and nausea, this therapy [illegible] then on November 12 and 14, 1971, and was s[illegible] November 16 (Fig. 2). During the period o[illegible]pins administration, luteinizing hormone leve[illegible] one exception fluctuated between 80 and 120 mIU per milliliter. Levels of follicle-stimulating hormone became detectable in serum on the third morning of therapy (i.e., after two injections of 2 ampoules); levels were recorded during the entire period of administration (Fig. 2), with values fluctuating between 10 and 20 mIU per milliliter. These values are similar to those observed in unpublished observations on other patients who have received comparable dosage schedules of menotropins. On the morning after the last injection (November 17, 1971) levels of follicle-stimulating hormone were at the limit of sensitivity of our method (3 mIU per milliliter), and on the following morning (November 18, 1971) six individual serum samples showed undetectable levels (Fig. 2).

Serum estradiol levels measured on November 16, 1971, were still undetectable (less than 10 pg per milliliter). The only clinical suggestion of enhanced estrogen activity after menotropins administration was obtained from examination of the urinary sediment. Morning sediments were stained by the Papanicolaou method.[9] The base-line maturation index was 100/0/0 — that is, 100 per cent of the cells were parabasal, and there were no intermediate or superficial squamous cells. On November 7, 1971, after 12 ampoules of menotropins, the index had not changed. Four days later, after 21 ampoules, a shift to intermediate and superficial squamous cells, compatible with mild estrogen effects, took place in the maturation index (51/47/3). This change was not maintained, however, since on November 12 the index was 80/20/0 and five days later, it was 90/10/0.

Response to conjugated estrogens. On November 18, 1971, 20 mg of conjugated equine estrogens (Premarin, Ayerst) was administered intravenously (Fig. 3). Some 4½ hours later, luteinizing hormone levels began to fall, reaching a nadir of 13 mIU per milliliter on the following day. Levels remained suppressed for 48 hours and by November 21, had returned to basal high values. Serum follicle-stimulating hormone levels, as mentioned previously, were undetectable on the day of estrogen administration (less than 3 mIU per milliliter). It was not possible to assess endogenous levels further in this patient, since by the following morning (November 19), there was evidence in the patient's serum of an antibody to human follicle-stimulating hormone. Antibody was not detected in serum samples obtained before menotropins administration in November, 1971. The antibody was shown to be specific for human follicle-stimulating hormone. Luteinizing hormone was unable to compete with ^{125}I-labeled follicle-stimulating hormone for binding to the antibody and the patient's serum was not capable of binding tracer quantities of luteinizing hormone.

Response to clomiphene. In January, 1972, clomiphene, 75 mg daily, was given by mouth for five days. There was no change in basal body temperature, and the luteinizing hormone levels remained high, averaging 60 mIU per milliliter. It was not possible to assess endogenous levels of follicle-stimulating hormone at this stage, because of the presence of anti-follicle-stimulating hormone antibody in the patient's serum. No change in the circulating levels of anti-human follicle-stimulating hormone antibody was noted at that time.

Discussion

There is strong evidence in our patient to support the diagnosis of isolated deficiency of follicle-stimulating hormone. Regarding secretion of other pituitary hormones, we did not evaluate prolactin release, but release of growth hormone, measured directly, was intact, and there was good, although indirect, evidence for normal secretion of ACTH and thyrotropin. Serum luteinizing hormone levels were in the postmenopausal range, whereas serum levels of follicle-stimulating hormone were repeatedly undetectable. Circulating levels were readily measured in our patient after she had received two injections each containing 150 IU of follicle-stimulating hormone equivalent.

There are a number of clinical situations in which there is dissociation of gonadotrophin levels in serum with high circulating luteinizing hormone and low or normal follicle-stimulating hormone levels. Yen, Vela and Ryan have reported such findings in the polycystic-ovary syndrome.[10] Judd et al.[11] and our group (in studies not yet published) have observed a similar profile in the feminizing testis syndrome. Our patient clearly does not fall into either of these categories. Adamopoulos and his co-workers[12] have reported low urinary follicle-stimulating hormone levels and high urinary luteinizing hormone levels (as measured by bioassay) in a case of premature ovarian failure. Our own experience with a group of patients with this syndrome is discordant with this report. Serum follicle-stimulating hormone and luteinizing hormone were elevated to levels generally observed in post-menopausal women. Furthermore, whereas ovarian biopsy revealed absence of primordial follicles in the patient with premature ovarian failure, described by Adamopoulos and his colleagues, a quite different picture was seen in our patient.

Biopsy of the patient's right ovary revealed a picture compatible with deficiency of follicle-stimulating hormone. Large numbers of primordial follicles were observed, but there was little maturation of the follicle, without antral formation. The overall picture was that of an ovary unstimulated by the hormone. Such evidence as there was for follicular development (follicles containing up to three cell layers and possible evidence

of corpus albicans) may have been consequent upon the therapy that she had received in 1969.

Primary amenorrhea, absence of circulating follicle-stimulating hormone, and a histologic picture of an ovary unstimulated by the hormone were associated with undetectable levels of circulating estradiol 17β (less than 10 pg per milliliter) and high luteinizing hormone levels. Pituitary growth hormone, ACTH and thyrotropin secretion appeared intact. Prolactin was not evaluated in our patient. The constellation seems to fulfill criteria for a diagnosis of isolated deficiency of follicle-stimulating hormone.

There are several pathologic sites that may account for the absence of circulating follicle-stimulating hormone in our patient. In the first place, the pituitary gland may be incapable of synthesizing the hormone. Secondly, release may be impaired because of failure of release of follicle-stimulating hormone releasing factor from the hypothalamus into the portal circulation supplying the pituitary gland.[13-15] Schally and his colleagues[13,14] have shown that injection of luteinizing hormone releasing factor raises serum levels of luteinizing and follicle-stimulating hormones, and believe that one peptide regulates secretion of both hormones, but there may be other factors of hypothalamic origin regulating release of follicle-stimulating hormone.

It has been shown in children who were previously thought to have "pituitary hypothyroidism," and in whom serum thyrotropin levels were low, that the administration of thyrotropin releasing hormone resulted in a notable rise of thyrotropin in seven out of the eight subjects studied.[16] Martin et al.[17] have reported on two patients in whom a hypothalamic origin for defective pituitary function appeared likely. By analogy with these reports, the defect in our patient may similarly reside at the hypothalamic level, with selective failure of synthesis or release of follicle-stimulating hormone releasing factor. We do not favor this hypothesis, however, irrespective of whether we consider the concept that luteinizing hormone releasing factor is identical with follicle-stimulating hormone releasing factor or that both hormones are controlled by different releasing hormones.

Serum levels of luteinizing hormone were persistently high in our patient. If luteinizing hormone release is being driven by that hormone's releasing factor, and if that factor is identical to follicle-stimulating-hormone releasing factor a hypothalamic cause for absence of circulating follicle-stimulating hormone in our patient is not very likely. This is similarly so if we accept the alternate view that releasing factors for follicle-stimulating and luteinizing hormones are separate hormones. This follows from evidence that luteinizing hormone releasing factor does effect a rise in serum follicle-stimulating hormone levels, even if it is not the "specific" releasing hormone of follicle-stimulating hormone.[14] Since luteinizing hormone releasing factor was presumably functioning in our patient, but levels of follicle-stimulating hormone were undetectable, we lean toward a nonhypothalamic site of her defect.

Accordingly, we suspect but cannot at present prove that absence of circulating follicle-stimulating hormone is consequent upon a pituitary lesion, possibly failure of synthesis of the glycoprotein. This may account for failure of development of immunologic tolerance to the hormone, and subsequent appearance of human anti-human antibody in our patient after menotropins administration. The patient's serum failed to bind luteinizing hormone nor did that hormone compete with labeled follicle-stimulating hormone for binding to serum. In circumstances of absolute follicle-stimulating hormone deficiency, the administration of urinary follicle-stimulating hormone could be viewed as the introduction of a foreign antigen, and, according to classic immunologic concepts, our patient would not have immunologic tolerance to the glycoprotein.

We are indebted to Drs. Jesse Roth, Saul Rosen, G. Seeger Jones and W. D. Odell for helpful criticism and suggestions, to Dr. A. S. Hartree, who generously provided us with purified human luteinizing hormone, to Dr. D. R. Bangham, who provided the 2d IRP-HMG standard, to Mrs. V. Pfeiffer and Miss S. Täpuchi for technical assistance and to Miss Esther Ben David, who performed the estradiol-17β estimations in this patient.

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Development of Anti-human FSH Antibody in a Patient with Isolated FSH Deficiency

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ABSTRACT. Antibodies to human FSH were documented in the serum of a patient previously shown to have isolated FSH deficiency. These antibodies developed subsequent to the administration of a second course of Human Menopausal Gonadotrophins (Pergonal 500). Using the ammonium sulfate precipitation method, antibodies were detected in the first week after cessation of Pergonal 500 therapy. Characterization of the antibody showed that (a) it was associated with the IgG fraction of the patient's serum; (b) addition of excess Pergonal 500 lead to rapid dissociation of ^{125}I-FSH binding to serum; (c) pituitary FSH (LER-1366) and 2nd IRP-hMG, but not Hartree human LH, displaced ^{125}I-FSH tracer bound to serum and (d) there was no binding of tracer insulin, hGH and LH by serum.

Antibody has remained detectable over a 5-month period of observation. (*J Clin Endocrinol Metab* 36: 684, 1973)

HUMAN menopausal gonadotrophins (hMG)[1] have been widely used therapeutically over the last decade in the treatment of infertility. In the United States alone, 3000 courses of therapy have been administered to over 1200 women (1). This paper describes the appearance of specific antibodies to human FSH in the serum of a patient who had received two courses of treatment with hMG (Pergonal 500). To our knowledge the development of antibodies to this agent has hitherto not been described.

The subject CR is a 22-yr-old female with isolated deficiency of FSH (2). She presented with primary amenorrhoea, and on investigation showed persistently elevated levels of serum LH (generally between 50–90 mIU/ml) while serum FSH levels were undetectable; that is, less than 3 mIU/ml or 0.6 mIU per tube, which is the lower limit of sensitivity of our FSH immunoassay in which we employ 0.2 ml serum per tube. hGH release was intact and no abnormalities were detected in thyroid or adrenal function. Pergonal 500 was first administered to the patient in 1969 (2 ampoules per day for 5 days) each ampoule containing, according to the manufacturer, 75 IU of FSH and 75 IU of LH. The second course was administered in November, 1971, and consisted of 30 ampoules given over a 15-day period. The potency of this batch of Pergonal 500 (Human Post-Menopausal Gonadotrophin, Istituto Farmacologico Serono, Roma and Ikapharm Ltd., Ramat Gan, Israel) was checked in our immunoassay system and was found to be close to the manufacturer's stated content of 75 IU FSH per ampoule.

Three days after the completion of the second course of Pergonal 500, her serum showed the apparent appearance of endogenous FSH; that is, there was a fall in the percent of ^{125}I-labeled FSH bound to rabbit anti-human FSH antibody and precipitated by goat anti-rabbit IgG. We suspected that the fall in B/F was in fact due to the development by our patient of an antibody to Pergonal 500. This isoantibody (human anti-human FSH) would bind labeled FSH, but would not be precipitated by our goat anti-rabbit IgG, accounting for the apparent endogenous FSH.

This report describes the characteristics of the human anti-human FSH antibody in our patient.

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[1] ABBREVIATIONS: FSH Follicle Stimulating Hormone; LH Luteinizing Hormone; hMG Human Menopausal Gonadotrophins; hGH Human Growth Hormone; 2nd IRPhMG Second International Reference Preparation–Human Menopausal Gonadotrophins.

Materials and Methods

The immunoassay system for FSH of Odell and his colleagues was employed (3). Human pituitary FSH (LER 1366) was labeled with ^{125}I to a specific activity of 10–50 μCi/μg. Rabbit anti-human FSH antiserum, kindly provided by Dr. W. D. Odell was used in a final dilution of 1:20,000. The 2nd IRPhMG was used as standard. In our system 1 ng LER 1366 is equivalent to 2.9 mIU of 2nd IRPhMG.

A modification of the ammonium sulfate precipitation method of Farr (4) was used to detect the presence of antibodies to FSH in the patient's serum. The buffer employed was phosphate saline buffer (0.01M, pH 7.6) containing 1% bovine serum albumin (PBS-BSA 1%). Normal pooled serum used in some studies had an FSH concentration of 24 mIU/ml. Preliminary experiments established that neither use of serum with high endogenous FSH concentration (100 mIU/ml) nor addition of cold FSH (125 mIU/ml) to normal serum influenced the nonspecific precipitation of ^{125}I-FSH incubated in the absence of antibody.

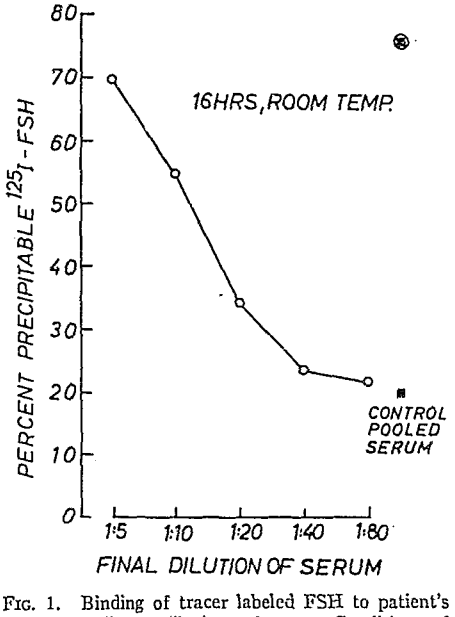


FIG. 1. Binding of tracer labeled FSH to patient's serum at different dilutions of serum. Conditions of incubation are indicated. Also shown is % binding of the tracer FSH employing a rabbit anti-human FSH antibody (Odell) to a final dilution of 1:2000 (■), and "non-specific" binding of tracer to normal serum (▩).

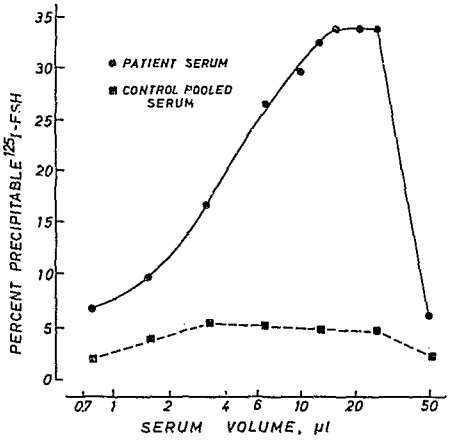


FIG. 2. Precipitation of tracer labeled FSH incubated with increasing volumes of patient's serum by goat anti-human IgG. Included are results with similar volumes of control pooled serum.

Results

Results are shown in Figs. 1–6 and in Table 1.

Quantitation of ammonium sulfate concentration required to effect selective precipitation of ^{125}I-FSH bound to antibody

A series of tubes was set up containing normal pooled serum (0.2 ml), rabbit anti-human FSH (0.1 ml, giving a final dilution of 1:20,000) and labeled FSH (0.1 ml containing 4000 cpm and approximately 0.2 ng FSH). Tubes containing all reagents but without antibody served as controls, and, in both series, the volume was made up to 1 ml with buffer. After incubation at room temperature for 16 hr, a constant volume of ammonium sulfate (1 ml) was added in final concentrations ranging from 5–50%. The tubes were shaken, centifuged at 2000 rpm for 30 min, separated and counted in a Packard autogamma counter. Optimum separation of FSH bound to antibody from free FSH was achieved with 40% ammonium

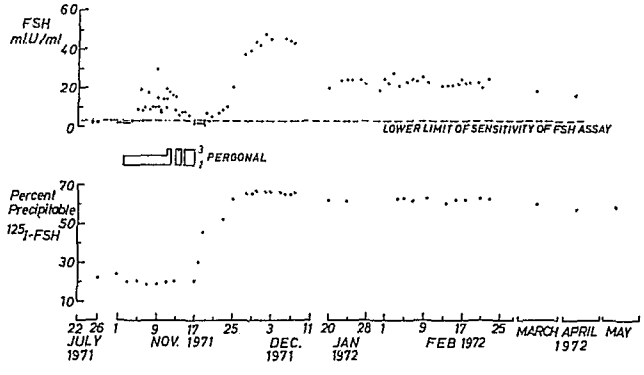


FIG. 3. Sequential study of patient's serum showing appearance of binding of ^{125}I-FSH to her serum (measured by % of tracer precipitable by ammonium sulfate). Parallel appearance of "FSH" in patient's serum is also shown (as measured under routine conditions of the double antibody FSH method).

sulfate, and this was subsequently used for all further experiments. "Non-specific" precipitation of ^{125}I-FSH never exceeded 20%. Passage of labeled FSH over a Sephadex G-75 column prior to incubation decreased this precipitation at all concentrations of ammonium sulfate. The inference is that damaged components present in the labeled product bind non-specifically to serum proteins and are consequently precipitated with ammonium sulfate.

Examination of patient's serum

An aliquot of the patient's serum taken 5 months after the second course of Pergonal 500 (April, 1972) gave an "apparent FSH level" of 17 mIU/ml. Tubes containing 0.2 ml of serum and 0.1 ml of tracer FSH were made up to 1 ml with buffer (*i.e.*, final dilution of serum = 1:5). Progressively decreasing aliquots of the patient's serum were made up to 0.2 ml with normal pooled serum, tracer FSH and buffer was added to give final dilution of the patient's serum up to 1:80. Incubations were carried out at room temperature for 16 hr as detailed above. Under these conditions, rabbit anti-human FSH (in a final dilution of 1:2000) incubated with normal pooled serum showed

TABLE 1. Results of screening of 26 women who had received Pergonal for ability to bind ^{125}I-FSH*

Courses of treatment	Serum examined: During course	1–3 Weeks after course	4–12 Weeks after course
One	4 (12–22%)	3 (11–14%)	3 (11–16%)
Two	7 (10–15%)	4 (16–23%)	1 (16%)
Three	3 (12–20%)		
Four	1 (10%)		

* courses of treatment refers to number of courses of treatment with Pergonal 500 which patient had received at the time that her serum was examined. This is subdivided further into 3 periods either during, 1–3 weeks after, or 4–12 weeks after treatment. The first figure refers to the number of patients in each group. The figures in parentheses are the range of the % ^{125}I-FSH precipitable by ammonium sulfate, after standard incubation as described in the text Normal pooled serum showed 20% non-specific precipitation.

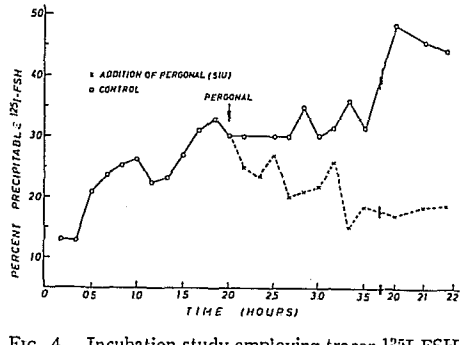


FIG. 4. Incubation study employing tracer ^{125}I-FSH and patient's serum demonstrating (a) time course of binding of tracer to patient's serum, as shown by progressive increase in percent of tracer precipitated by ammonium sulfate, (b) ability of cold FSH (Pergonal 500) to displace tracer FSH from patient's serum, with resultant fall in percent of tracer precipitated by ammonium sulfate.

precipitation of 75% of the tracer FSH while normal pooled serum alone showed 19% precipitation. At 1:5 dilution, the patient's serum precipitated 70% of the tracer and this fell progressively to 21.5% at a dilution of 1:80 (Fig. 1). In contrast, incubation of the patient's serum taken prior to Pergonal 500 administration gave values indistinguishable from control pooled serum. These results demonstrated that the patient's serum had developed the capacity to bind ^{125}I-FSH.

The same serum was then incubated with ^{125}I-FSH, employing between 1–50 µl of

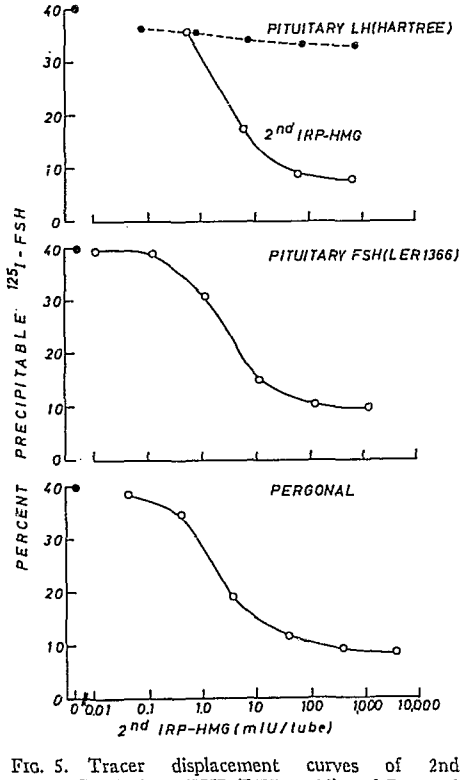


FIG. 5. Tracer displacement curves of 2nd IRPhMG, Pituitary FSH (LER 1366) and Pergonal 500 using tracer ^{125}I FSH and 1:4 dilution (50 µl) of patient's serum as sources of anti-FSH antibody. In top panel results of an experiment using Hartree LH are shown. The LH failed to displace tracer in concentrations up to 1000 mIU.

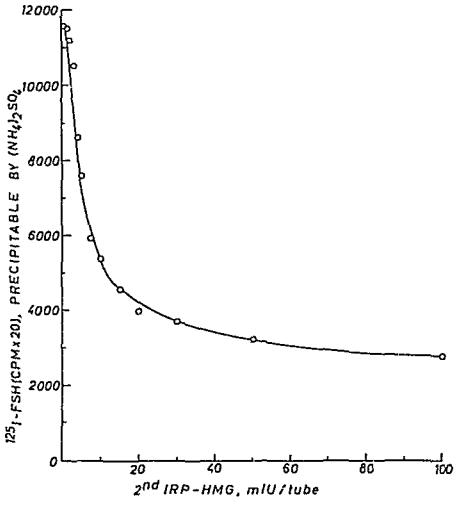


FIG. 6. A tracer displacement curve employing patient's serum, tracer ^{125}I-FSH and 2nd IRPhMG (Fig. 6).

serum and made up with PBS-BSA 1% to a final volume of 200 µl. After remaining at room temperature for 16 hr, 100 µl of goat anti-human IgG (kindly supplied by Dr. E. Shapiro) was added and the tubes were incubated for 1 hr at 37 C and then for 24 hr at room temperature. Following centrifugation, the supernatant was aspirated and both precipitate and supernatant were counted. Normal pooled serum was incubated under identical conditions. Fig. 2 displays the results of this experiment. Maximum precipitation with a constant volume of goat anti-human IgG was achieved using between 15 and 25 µl of patient's serum. Thirty-five per cent of tracer FSH was precipitated. Employing normal pooled serum, 5% or less of tracer FSH was precipitated with all volumes of serum.

We have examined sera from 26 other subjects who had received one or multiple courses of Pergonal 500 for ability to bind ^{125}I-FSH (Table 1). Using the ammonium sulfate precipitation method, values were indistinguishable from that of normal pooled serum.

Sequential profile of patient's anti-FSH antibody

Fig. 3 displays studies of the patient's serum before, during and after Pergonal 500 administration. In these studies the patient's serum was measured in our regular FSH immunoassay. In addition, 25 µl of patient's serum, and tracer FSH to a final volume of 100 µl was incubated at 4 C for four days prior to ammonium sulfate precipitation. Precipitable ^{125}I-FSH in all serum samples both before and during Pergonal 500 treatment was indistinguishable from that of control pooled serum (20%). However, three days after the last injection of Pergonal 500, precipitable radioactivity had risen significantly and ultimately reached a value of 66% 14 days after the last Pergonal 500 injection. Five months later, the percent of precipitable radioactivity had decreased only slightly to 58% (Fig. 3). Serum FSH levels were undetectable before Pergonal 500 therapy, reached values between 10–20 mIU/ml during Pergonal 500 therapy and fell to undetectable levels by the 2nd day after cessation of Pergonal 500. Paralleling the appearance of FSH binding activity in serum, "endogenous FSH" levels were measurable in the patient's serum (Fig. 3), the observation which had first alerted us to the possibility of antibody.

Dissociation of ^{125}I-FSH from antibody by addition of excess cold Pergonal 500

An aliquot of the patient's serum (2.5 ml) was incubated with an equal volume of normal pooled serum and labeled FSH. The volume was made up to 25 ml with buffer. At 10-min intervals, 0.5-ml aliquots were removed from the pool and the radioactivity precipitated by 40% ammonium sulfate was measured. The percent of precipitable counts increased progressively, reaching 31% at 100 min. (Fig. 4). After 120 min of incubation, the pool was divided into two; Pergonal 500 (5IU/ml) was added to one portion, with the second remaining as the continuing unaltered incubation. Aliquots (0.5 ml) from both tubes were centrifuged at 10-min intervals following the addition of ammonium sulfate. Progressive displacement of the tracer in the pool containing Pergonal 500 was evident (Fig. 4). Ninety minutes after the addition of Pergonal 500, binding in the latter had fallen to 18.5%, whereas the second unaltered pool showed 31% precipitable radioactivity. After 20 hr the figures were 17 and 48% for Pergonal 500 enriched and control tubes respectively. Since "non-specific tracer precipitation" (i.e. precipitation of ^{125}I-FSH in the absence of antibody) usually varied between 18 and 20%, addition of excess cold Pergonal 500 caused close to maximal displacement of label from the patient's serum antibody.

Tracer displacement curves utilizing patient's serum as antibody

In these experiments, 50 µl of serum was incubated with 25 µl of tracer FSH, 75 µl of buffer and 50 µl of cold hormone in the desired concentration for 4 days at 4 C. Separation of bound and free hormone was performed employing 40% ammonium sulfate precipitation. In this series of experiments, "non specific" precipitation was 8% and maximal precipitation in the absence of cold hormone was 40%. Fig. 5 displays standard curves obtained with 2nd IRPhMG, pituitary FSH (LER 1366) and Pergonal 500. These three preparations give identical tracer displacement curves, when data were expressed in terms of 2nd IRP-hMG equivalents; 50% inhibition of binding was achieved in the presence of 3 to 5 mIU FSH equivalent with each of the three preparations.

Fig. 6 displays a more detailed standard curve constructed with 2nd IRPhMG employing identical incubation conditions to those described above.

Specificity of the anti-FSH antibody

Aliquots of the patient's serum were incubated with tracer quantities of labeled LH, insulin and hGH. Preliminary studies had shown that with these three tracers, optimal separation of antibody-bound from free hor-

mone occurred with a 35% concentration of ammonium sulfate. The patient's serum failed to bind tracer quantities of LH, insulin or hGH. An experiment was also made to determine whether LH would displace labeled FSH bound by the patient's serum. Addition of up to 125 ng of Hartree LH failed to effect displacement (Fig. 5 top panel). Under our assay conditions for LH 1 ng of Hartree LH is equivalent to 8 mIU 2nd IRPhMG.

The patient had also received a course of Pregnant Mare's Serum in the past [Gestyl]. Gestyl in concentrations up to 5 IU failed to displace labeled FSH from the patient's serum.

Discussion

We have shown the appearance of anti-human FSH antibody in the serum of a patient with isolated deficiency of FSH, shortly after she had completed a second course of Pergonal 500 therapy. The evidence is based on the following experiments. First, the patient's serum bound labeled FSH. This was shown by the ammonium sulfate precipitation method of Farr (4). We established that the antibody was associated with the IgG fraction of her serum since ^{125}I-FSH bound to the patient's serum was precipitated by goat anti-human IgG (Fig. 2). Since we are uncertain that the antibody employed was entirely specific for IgG, we do not exclude the possibility that the patient's antibody may have included other immunoglobulin classes. The patient's serum did not bind tracer LH, hGH or insulin.

Second, addition of excess cold Pergonal 500 inhibited binding, and also reversed binding of labeled FSH to the antibody. Third, using the patient's serum as antibody, 2nd IRPhMG competed with tracer FSH for binding to antibody. Similar curves were obtained with pituitary FSH (LER-1366) and commercial Pergonal 500.

On the other hand, additions of up to 1000 mIU of Hartree LH was unable to compete with tracer FSH for binding to antibody.

No antibody was detected in serum prior to administration of Pergonal 500 in November, 1971, but detectable levels of antibody became apparent after completion of her second course. It is likely that the course of Pergonal 500 in November, 1971, acted as a booster, following priming with initial administration of the agent two years previously.

Formation of antibody to FSH could be related to structural differences between the native urinary hMG and endogenous FSH. Alternately the preparation of the urinary hMG could have altered the molecule and rendered it antigenic. We were, however, unable to detect antibodies to FSH in a further series of 26 women who had received single or multiple courses of Pergonal 500 (Table 1). Furthermore, despite the thousands of courses of Pergonal 500 administered in many different centers, we are unaware of other instances of development of antibodies to Pergonal 500. The explanation for the patient's propensity to develop anti FSH antibody may reside in the fact that she was shown to have an isolated deficiency of circulating FSH. Consequently, to this subject FSH is a foreign antigen and, according to classical immunological concepts, she would have no immunologic tolerance to the glycoprotein. Pergonal 500, of course, also contains urinary LH, but no binding of tracer LH was present in the patient's serum. Neither did large quantities of cold LH compete with tracer FSH for binding to the patient's antibody. It may be fruitful to examine subjects with total gonadotropin deficit (i.e., FSH and LH) treated with Pergonal 500 for gonadotropin binding antibodies.

The ubiquitous presence of insulin antibodies in insulin-treated diabetics has for the most part not been associated with a substantial therapeutic problem (6). This is also true in some cases of hGH deficiency in whom hGH antibodies have developed but with little discernible effect on response to exogenous hGH (5). The possible consequences of FSH antibody on the therapeutic effects of Pergonal 500 in our patient remain speculative.

Acknowledgments

We are grateful to Dr. J. Roth, Dr. E. Shapiro, and Dr. L. Frohman for extremely helpful advice and criticism. We thank Dr. B. Bercovici for referring the patient for study. Mrs. V. Pfeiffer and Miss S. Tapuchi rendered expert technical assistance.

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Isolated Deficiency of Follicle-Stimulating Hormone: Further Studies

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ABSTRACT. We record further studies over the past 2 yr on a unique female subject with isolated follicle-stimulating hormone (hFSH) deficiency, who developed human anti-hFSH antibodies after treatment with exogenous urinary gonadotropins. Administration of LRH resulted in a significant rise in serum hLH, but hFSH levels remained undetectable. "α Subunit" (the common α chain of the glycoprotein hormones) was detectable in basal samples obtained from our patient, and rose sharply after LRH. This is concordant with the hypothesis that the defect in our subject may be in the synthesis of the β chain of hFSH, but it does not exclude other possibilities. The concentration of hFSH antibodies in the patient's serum has been monitored and her response to a further course of exogenous gonadotropins is recorded. The antiserum exhibits specificity for the hFSH molecule; the α and the β chains of hFSH are virtually inert in competing with tracer ^{125}I-hFSH for binding to the antibody. (*J Clin Endocrinol Metab* 40: 790, 1975)

WE have previously reported isolated deficiency of hFSH[1] in a young woman with primary amenorrhoea (1), and we have described the appearance of antibodies against hFSH in her serum after she had received treatment with menotropins (human menopausal gonadotropins, Pergonal 500, Ikapharm, Israel) (2). In this communication we describe (a) the patient's response to LRH (b) studies of common α subunit of glycoprotein hormones in her serum (c) the time course of anti-hFSH antibodies in her serum over 2.5 yr and (d) further characterization of this unique antiserum.

Materials and Methods

A full clinical description of the patient has been given elsewhere (1). Briefly, she is a 23-yr-old eunuchoid young woman who has never menstruated spontaneously. She received an initial course of Pergonal 500 in 1969. We saw her first in 1971, after establishing the diagnosis of hFSH deficiency, we treated her with 30 ampoules (75 IU each) of Pergonal 500. Anti-hFSH antibodies were detected shortly thereafter. In January 1973, she received a 6-day course of 9 ampoules of Pergonal 500, and bloods were drawn intermittently through 1974. The LRH test was performed by injecting 100 μg of LRH (synthetic Ayerst preparation 29031) rapidly intravenously. Bloods were taken at frequent intervals before and after the injection.

Serum hFSH and hLH were measured by radioimmunoassay (3,4). The common chain of α glycoprotein hormone ("α subunit") was quantified on serum samples which had been chromatographed over Sephadex G-100. The "mixed antibody" system of Benveniste *et al.* (5–7) was employed, and Canfield hCGα (CR-117) served as the unlabeled standard. Details of this assay with a comparison to the Vaitukaitis homologous α subunit assay are given elsewhere (7). Anti-hFSH antibody levels were measured by the ammonium sulfate precipitation method of Farr (8,2). hFSH (LER1575-C) was a gift of the National Pituitary Agency and hFSHα and hFSHβ were generously supplied by Dr. L. E. Reichert, Jr.

Results

A. *Response to LRH* (Fig. 1). LRH caused a rapid increase in hLH levels, whereas hFSH was undetectable (<3 mIU/ml) throughout.

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[1] Abbreviations: hFSH = human follicle stimulating hormone; hLH = human luteinizing hormone; hTSH = human thyrotropin; LRH = luteinizing hormone-releasing hormone; menotropins = human menopausal gonadotropins (hMG), Pergonal 500; α subunit = common α chain of glycoprotein hormones (hFSH, hLH, and hTSH).

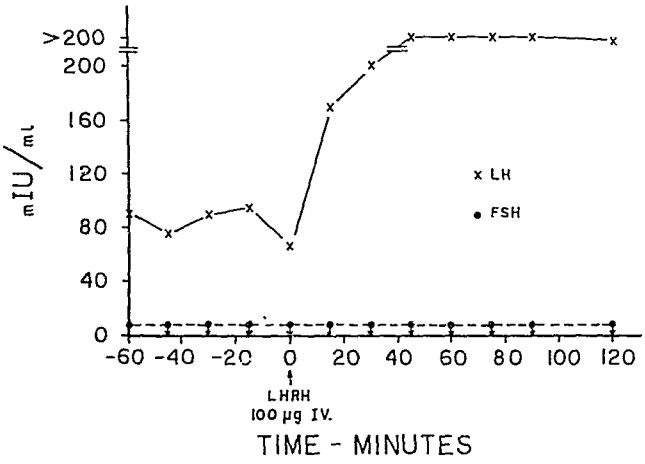


FIG. 1. Serum hLH and hFSH levels after LRH (100 μg) given by intravenous injection. hLH levels rose, while hFSH levels remained undetectable.

B. *Presence of α subunit* (Fig. 2). Four ml of serum, taken before LRH injection, was run on a long 2.2 × 85 cm Sephadex G-100 column, and the concentrations of hLH and α subunit were measured in the fractions eluted. Immunoassayable hLH eluted with a $K_{av} = 0.20$, and a small peak of α subunit was present ($K_{av} = 0.34$). Figure 2 also shows results of a serum sample obtained from our patient 15 min after LRH, and identically processed. A larger hLH peak was observed at a $K_{av} = 0.20$ (data not shown). Also the concentration of α subunit ($K_{av} = 0.34$) was greatly elevated.

C. *Time course of anti-hFSH antibody levels*. Antibody levels were measured over a 2.5-yr period in our patient, during which time she received two courses of menotropins, separated by a 14-month interval (Fig. 3). Prior to November 1971 antibodies were undetectable in serum, but two days after the end of the November 1971 course of menotropins antibody was de-

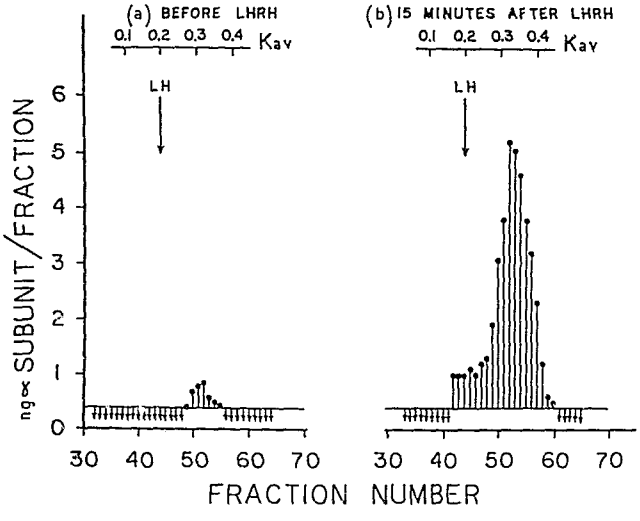


FIG. 2. Serum "α subunit" levels measured in fractions of the patient's serum after chromatography on Sephadex G-100 before and 15 min after LRH injection. A distinct rise in "α subunit" was observed after LRH.

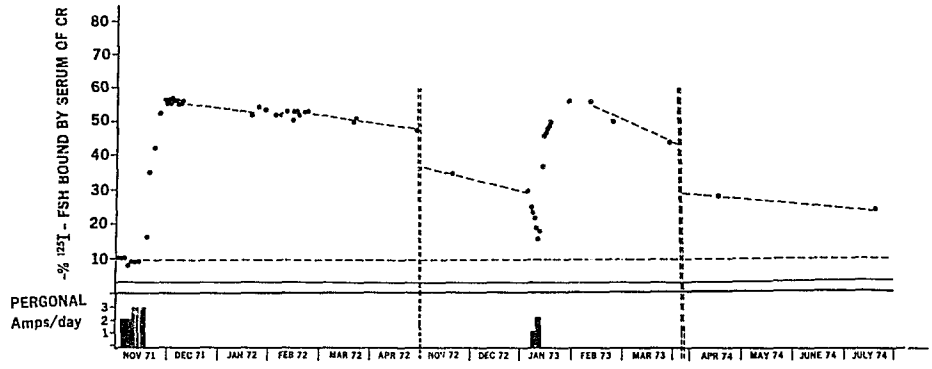


FIG. 3. Measurement of anti-hFSH antibody (measured by binding of tracer hFSH by patient's serum) in our patient between November 1971 and July 1974. The patient received exogenous menotropins (Pergonal) from November 3–17, 1971, and from January 5–12, 1973. The broken horizontal line represents the fraction of tracer ^{125}I-hFSH added to control serum which is precipitated by ammonium sulfate; that is, this is the "blank" value above which binding of tracer to the patient's serum is significant.

tected and rose rapidly over the next 10 days. Over the following year, serum antibody levels slowly declined but remained detectable. A second course of menotropins was given in January 1973. Bloods were collected over the next 18 months, the sera were held frozen, and all samples were analyzed on the same lot of labeled hFSH. A rapid rise in antibody was observed after cessation of therapy (Fig. 3), and antibody remained detectable through July 1974. It was observed that FSH antibody

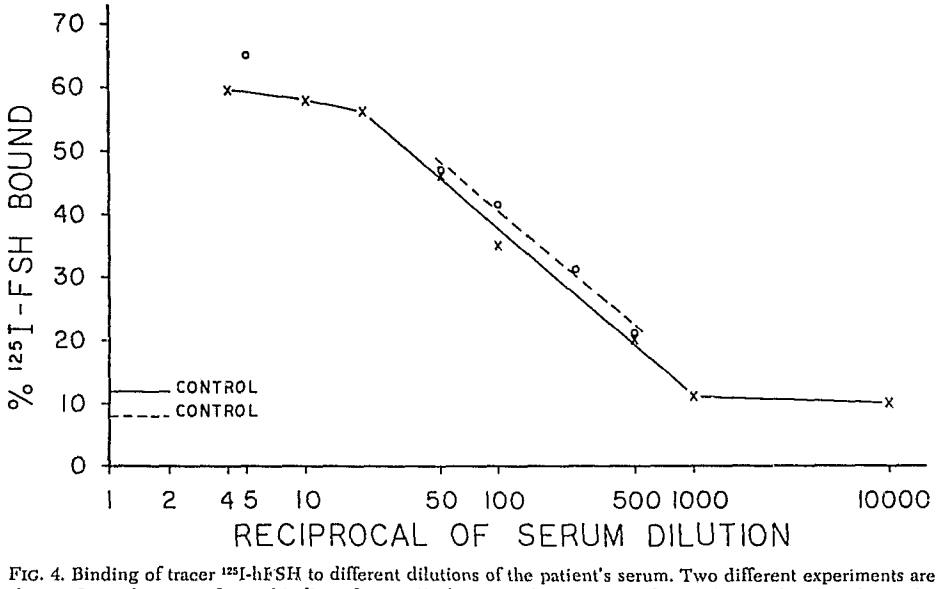


FIG. 4. Binding of tracer ^{125}I-hFSH to different dilutions of the patient's serum. Two different experiments are shown. Control — — — refers to binding of tracer ^{125}I-hFSH in the presence of normal control serum, that is the fraction of tracer ^{125}I-hFSH precipitated by ammonium sulfate in the presence of normal serum.

levels fell during Pergonal therapy. It is possible that hFSH, entering the circulation after intramuscular Pergonal, bound to endogenous antibody and saturated the latter such that it was ineffectual in binding added tracer ^{125}I-hFSH.

D. *Antibody dilution experiments*. Figure 4 displays results of two experiments in which the binding of ^{125}I-hFSH was checked against differing dilutions of the patients' serum. A 50% fall in specific binding occurred at a dilution of serum of 1:120 to 1:150. That is, the antibody titer was not very high.

E. *Binding of FSHα and FSHβ by hFSH antibody* (Fig. 5). We have tested the ability of two batches of α and β subunits of hFSH to compete with tracer hFSH for binding to the human anti-hFSH antiserum. On the first occasion (8/15/72), hFSHβ competed with a potency of about 5% compared to hFSH, and competition by hFSHα was negligible (Fig. 5). On the second test (7/23/73) both hFSHα and hFSHβ were virtually inert (Fig. 5). This may reflect differences in the purity of the FSH subunits: the later Reichert preparation may have had less contamination with native hFSH. In any event, it is clear that the human antiserum is directed against the hFSH molecule, and recognizes the subunits extremely poorly.

Discussion

We have made further observations on a unique female patient with isolated hFSH

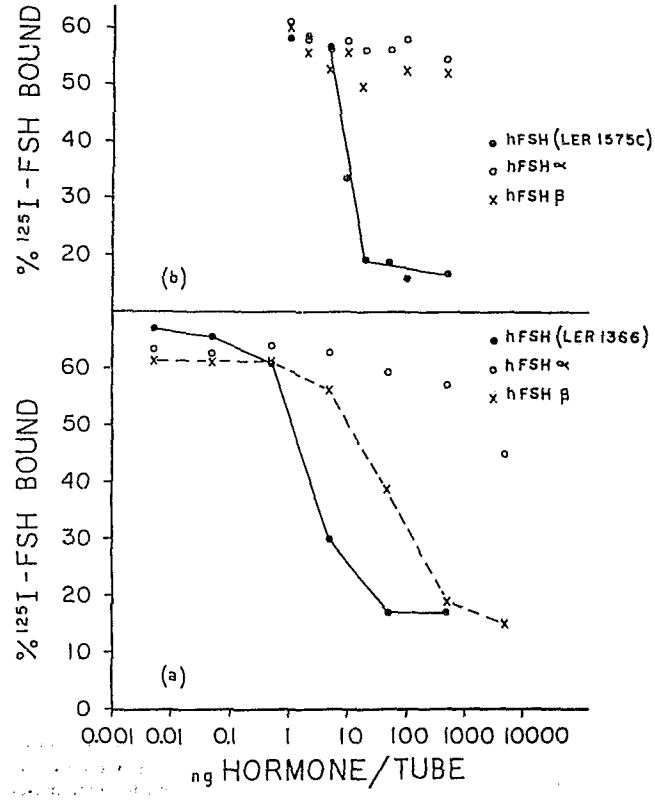


FIG. 5. A comparison of the potency of hFSH, hFSHα and hFSHβ to displace tracer ^{125}I-hFSH from human anti-hFSH antibody. Two experiments are shown with different batches of Reichert hFSHα and hFSHβ.

deficiency. Administration of LRH was not followed by the appearance of hFSH in peripheral serum (Fig. 1). This is concordant with the view that her defect resides at the pituitary, and not at an hypothalamic or even higher level (1). We suspect that her defect is in synthesis of the FSH molecule, specifically in the synthesis of the β subunit. There is adequacy of hLH release, based on direct measurements of the hormone in serum, and probably also of hTSH release, based on the normal indices of thyroid function. It is reasonable therefore to conclude that she can synthesize the α subunit of hLH and hTSH, and presumably then also of hFSH. This hypothesis is strengthened by our finding of α subunit in basal samples of her serum, with marked elevation of its concentration within minutes of LRH administration (Fig. 2). The concentration of α subunit 15 min after LRH is higher than that observed in normal cycling women, but similar values are found in some subjects with gonadal failure (6). Our immunoassay does not distinguish among the α subunits, and we could thus be measuring the α subunit uniquely related to, say, hLH synthesis. We cannot then with certainty exclude the possibility that our patient has a global defect in FSH synthesis (α and β subunits) or even a deficiency of a specific FSH releasing hormone.

It may well be, however, that we are dealing with a congenital inability to synthesize FSHβ (or the synthesis of a molecule not recognized by us as FSH). It is known that the fetal pituitary secretes hFSH (9), and thus an opportunity is present for potentially immunologically competent cells to recognize hFSH as "self." Since maternal hFSH secretion is inhibited during pregnancy, absence of endogenous fetal hFSH secretion could result in exogenous FSH being viewed as "foreign" and, on classic immunologic grounds, this would account for the development of anti-hFSH antibody in this patient. We have examined for the presence of anti-hFSH antibodies in sera from a large number of women who have received one or multiple courses of Pergonal. These were uniformly negative (2). To our knowledge this patient uniquely demonstrates human anti-hFSH antibody.

We have tested sequentially for the presence of hFSH antiserum in our patient. A short course of exogenous menotropins in January 1973 was followed by a prompt rise in her antibody concentration (as measured by percent binding of an hFSH tracer to her serum). Her titer was never high (Fig. 4), but the antiserum showed great specificity; neither hFSHα nor hFSHβ competed for binding to any considerable extent (Fig. 5). This contrasts with many antibodies employed in radioimmunoassays for the glycoprotein hormones, including the rabbit anti-hFSH antiserum distributed by the National Pituitary Agency, which do not discriminate well between the native molecule and the isolated β subunit (10).

Acknowledgments

We thank the Ayerst Company for generous gifts of LRH, and Dr. L. E. Reichert Jr for invaluable supplies of hFSHα and hFSHβ.

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Diminished Prolactin Reserve: A Case Report*

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ABSTRACT. A 17 year old male patient presented with short stature and delayed puberty. Investigations showed normal thyroid function and intact TSH response to TSH-releasing hormone (TRH). Although basal levels of LH were low, both LH and FSH rose following the administration of LH-releasing hormone (LHRH). ACTH secretion assessed indirectly by the cortisol response to insulin hypoglycemia was normal. Growth hormone levels increased following the onset of sleep, as well as after the administration of insulin, L-dopa and L-arginine. Basal levels of prolactin were low (2–5 ng/ml) compared with 5–12 ng/ml in controls. There was a markedly impaired prolactin response to TRH (maximum rise above basal values of 3 ng/ml compared to a rise of 12–29 ng/ml in controls). Prolactin levels did not rise after the administration of chlorpromazine or L-arginine. There was some suppression of prolactin levels after L-dopa. Similar patterns of prolactin are seen in panhypopituitarism, where they are usually associated with other hypophyseal hormonal deficiencies. The diminished prolactin reserve demonstrated in this subject in the presence of intact function of the remainder of the anterior pituitary is compatible with the diagnosis of diminished prolactin reserve. (*J Clin Endocrinol Metab* 45: 412, 1977)

ISOLATED hormonal deficiency of hypothalamic-pituitary origin is defined as impaired secretion of a single hormone with retention of the remaining hypophyseal functions. Syndromes have been described which selectively involve the secretion of growth hormone (GH), adrenocorticotropin (ACTH) and thyrotropin (TSH) (1,2). The commonest variety of selective hormonal deficiency, however, is isolated bihormonal gonadotropin deficiency (IGD) (1). This involves both pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (3) although recently, we described a patient who presented with selective FSH deficiency (4).

Isolated prolactin deficiency is an uncommon condition, having previously been described by Turkington in two cases (5). In these subjects prolactin was measured by an *in vitro* bioassay technique and minimal dynamic stimuli of prolactin secretion were used. Carlson *et al.* have also recently reported a large kindred who presented with a combination of prolactin deficiency and pseudohypoparathyroidism (6).

In this report, we describe a young male patient who presented with short stature, low basal levels of prolactin and minimal prolactin response to dynamic stimuli. The remaining anterior pituitary functions were intact. These features fulfil criteria for the diagnosis of diminished prolactin reserve.

Case Report

The patient, a seventeen year old male of Sephardic Jewish origin, was referred to the Endocrine Clinic for evaluation of short stature and delayed puberty. He was the third of six siblings. There was no consanguinity, the father having been born in Turkey and the mother in Persia. Both parents and all other siblings were healthy. The mother and remaining siblings were of average height. The heights of the father and paternal grandmother were 155 and 154 cm, respectively. The father, who was the smallest of five siblings, commenced puberty at the age of fifteen years.

Past history of the propositus showed that the mother's pregnancy and delivery were uneventful. At the age of eighteen months, esotropia of the left eye was noted. Choroiditis was dianosed and at the age of two years investiga-

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tions were performed, but the cause of the choroiditis could not be determined. Toxoplasmosis was excluded. In 1968 and 1971, the patient underwent two operations for correction of the estropia. The final diagnosis was blindness of the left eye due to healed choroiditis of undetermined etiology.

The patient otherwise enjoyed good health. His psychomotor development was normal. The family became aware of his short stature only at the age of thirteen years, until then his growth was comparable to that of his peers. The rest of the systematic inquiry was non-contributory.

On examination, the patient's height was 150 cm, corresponding to a height age of twelve years and ten months. His weight was 44 kg equivalent to a weight age of fourteen years. He had mild obesity. There was a functional grade 1/6 ejection systolic murmur over the left parasternal area. Vision was normal in the right eye, but there was blindness in the left eye. Fundoscopy of the right eye showed mild peripapillary atrophy. In the left eye there was severe chorioretinal atrophy, peripapillary edema of the optic disc area and localized retinal detachment. Findings were consistent with a diagnosis of healed choroiditis. Penis and testes were normal, prepuberal. There were no other signs of pubertal development, his puberty corresponding to Stage 1 according to the classification of Tanner. Urinalysis including microscopy, culture, pH, electrolytes, osmolarity and chromatography for amino acids was normal. The 24 h secretion of 17 ketosteroids and 17 hydroxicorticoids was normal. Blood count and electrolytes, as well as levels of serum albumin, globulin, cholesterol, bilirubin, alkaline phosphatase, transaminases, calcium and phosphate, were within normal limits. Serum thyroxine and T_3 Sephadex retention (Ames) were normal. EKG and x-rays of chest and skull, including special views of the pituitary fossa were intact. His bone age corresponded to ten years. The complement fixation test for Toxoplasmosis (Sabin-Feldman) was negative on repeated occasions. There were no clinical or laboratory features of pseudohypoparathyroidism.

Specific test procedures

These were commenced between 0800–0830 h after an overnight fast. A needle inserted into an anticubital vein was kept patent by the slow administration of normal saline. Two or more blood samples were withdrawn during a control period of fifteen minutes and then the test substance was injected iv *via* a three stopcock and periodic blood sampling continued.

1. TRH tests. The patient was given TRH on three different occasions. Initially, 200 μg TRH was administered by rapid iv injection. The patient was subsequently challenged with another single pulse of 400 μg TRH. Finally he was given a further dose of 200 μg TRH. This last test was preceeded by three days of diethylstilbestrol treatment (5.0 mg daily) and insulin (0.1 U/kg) was given together with the TRH. It has been shown previously that 200 μg TRH is adequate to induce maximum prolactin release (7).

2. LHRH test. The patient was given repeated pulses of 100 μg LHRH at zero time, 60 and 90 min.

3. CPZ test. Following a basal control period, 25 mg CPZ was injected im and blood samples collected.

The response to the initial test with 200 μg TRH, as well as to the LHRH test and to CPZ was compared to identical protocols which were administered to six normal male controls whose ages ranged from 18–24 years.

4. Additional tests. Several blood samples were also taken after the oral administration of L-dopa (500 mg), the infusion of L-arginine (0.5 g/kg) and after the subject fell asleep.

The latter two tests were performed after three days of estrogen pretreatment.

Materials and Methods

Serum FSH, LH, TSH, GH and plasma II hydroxicorticoids (cortisol) were determined by previously described methods (3). Pituitary LH (IRC 2) was supplied by Dr. A. S. Hartree. Pituitary FSH (LER 1366), TSH and GH, as well as, antisera to LH, FSH, TSH and GH were kindly supplied by the National Pituitary Agency, National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD). The Second International Reference preparation for human menopausal gonadotropins (2nd IRPHMG), provided by Dr. D. R. Bangham, was used as reference standard for both LH and FSH. Standard for TSH was supplied by the Medical Research Council and GH by the National Pituitary Agency.

Prolactin (PRL) was measured by a homologous radioimmunoassay utilizing human prolactin (HPR -V-L-S No. 2) and anti hPRL antibody (rabbit), kindly supplied by the National Pituitary Agency (NIAMDD). The antibody was used at a final dilution of 1:200.000. Labelled prolactin I^{125} was purchased from CEA, France. Displacement curves parallel to the NIH standard were obtained with a series of 5 plasma samples of known prolactin concentration, kindly supplied by Dr. H. Friesen as well as with multiple dilutions of plasma from a patient with galactorrhea and the Research Standard A provided by the Medical Research Council. The NIH standard was equilibrated in terms of plasma samples of known prolactin concentration. In three separate assays, the latter gave values exactly one-half of those of the NIH. Results have been expressed from the corrected NIH standard. Using 0.1 ml serum in a total assay volume of 1.0 ml, the lower limit of sensitivity of the assay is 2 ng/ml. In this assay, the mean ±SD in normal females is 14.9 ± 5.3 ng/ml, in normal males 8.8 ± 2.1 ng/ml and in children aged ten to seventeen years, basal prolactin levels range from 4–14 ng/ml.

Prolactin was measured on all blood samples; GH on all samples with the exception of the LHRH and TRH tests. LH and FSH were estimated after the administration of LHRH and TSH after TRH. Cortisol was measured under basal conditions, 60 min following the administration of 250 μg aqueous synacthen (Ciba) and after insulin hypoglycemia.

Results

The results of the special investigations are shown in Table 1 and Fig. 1–3.

Growth hormone (Table 1, Fig. 1)

In response to L-dopa, the infusion of L-arginine, insulin hypoglycemia and the onset of nocturnal sleep, GH levels rose to maximum levels of 4.4, 7.1, 12.0 and 23 ng/ml respectively. The infusion of arginine also produced the normal expected rise in serum insulin levels.

TSH (Fig. 2)

Basal TSH levels ranged from 2–2.5 μU/ml which is the limit of sensitivity of the TSH assay. On each occasion when TRH was administered, there was a normal TSH response, the peak ranging from 11–28 μU/ml.

LH and FSH

Basal levels of LH were low (3 mIU/ml) whereas FSH levels ranged from 6–7 mIU/ml. When compared to controls, there was a normal gonadotropin response to LHRH, the maximum levels attained were 40 mIU/ml for LH and 12.0 mIU/ml for FSH.

TABLE 1. Clinical data

Test procedure	Parameter	Time (min) −15	0	15	30	45	60	90	120
Insulin hypoglycemia	Glucose (mg/100 ml)	77	76	27	30	46	57	74	82
	GH (ng/ml)	1.6	2.4	1.5	4.1	5.5	5.2	12.0	8.5
	Cortisol (μg/100 ml)		29				61		
	Prolactin (ng/ml)*	2.3	2.8	4.0	5.2	4.2	2.6	2.7	2.8
Arginine infusion	GH (ng/ml)		1.6	7.1	5.0	4.2	2.0	2.2	
	Insulin (μU/ml)		3	17	18	8	4	4	
	Prolactin (ng/ml)		5	5	5	5	5	5	
L-dopa	GH (ng/ml)		1.1			1.9	1.5	4.4	
	Prolactin (ng/ml)		5			6	3	3	
Chlorpromazine	Prolactin (ng/ml)	3.0		2.9	2.9	3.0	2.7	2.7	
Synacthen	Cortisol (μg/100 ml)		13				39		

* 200 μg TRH administered together with insulin.

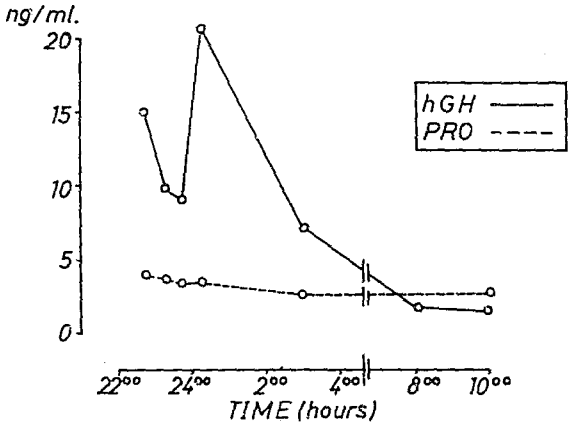


FIG. 1. Prolactin and GH responses during sleep.

Cortisol (Table 1)

Basal cortisol values ranged from 18–30 μg/100 ml. There was a normal response to synacthen and to insulin induced hypoglycemia.

Prolactin (Table 1, Figs. 1, 2, 3)

Basal prolactin levels ranged from 2–5 ng/ml. These levels are significantly decreased when compared to values in our normal male controls which ranged from 5–12 ng/ml ($P < 0.001$).

The peak prolactin response to TRH in male subjects ranged from 21–37 ng/ml with an absolute rise of 12–29 ng/ml. The patient was challenged with TRH on three occasions. When given 200 μg TRH, the maximum levels of prolactin obtained were 6 ng/ml (absolute rise of 2 ng/ml). Neither a larger dose of TRH (400 μg) nor pretreatment for three days with diethylstilbestrol produced a marked increase response to TRH (Fig. 2). On this occasion, insulin was administered together with TRH and this produced the greatest prolactin response, the peak rise being 3 ng/ml. In contrast to its effect on prolactin, the administration of TRH consistently produced an elevation of TSH (Fig. 2).

Whereas GH levels rose following the onset of nocturnal sleep, as well as after insulin induced hypoglycemia and arginine infusion, there was no increase in prolactin levels, (Table 1, Fig. 1). Moreover, unlike the response in the controls, prolactin levels did not increase after the administration of CPZ (Fig. 3). There was, however, minimal suppression after L-dopa (Table 1).

It should be mentioned that blood samples of the patient following both TRH and CPZ were run in several different assays together with stimulation tests from normal individuals.

Studies in the father

Because he presented with short stature, the father was also evaluated. He was given a combined infusion of 100 μg LRF and 200 μg TRH. This was followed 60 min later by insulin (0.1 U/kg). There was normal secretion of FSH, LH, TSH, GH and prolactin.

Discussion

We have documented diminished prolactin reserve in this patient. When compared to our normal male controls, basal prolactin levels were low, but measurable. There was a markedly attenuated response following 200 μg and 400 μg of TRH. This tripeptide elevates prolactin by acting directly at the level of the lactotrope

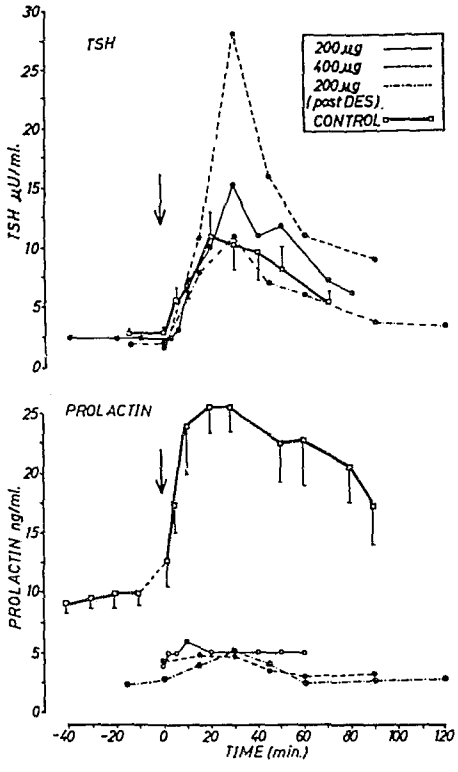


FIG. 2. TSH and prolactin responses to the administration of TRH in the patient and controls. See text for details. In the controls mean ± SE are shown.

(7–9). When TRH was given following estrogen pretreatment, however, there was a minimal response. The slightly augmented response following estrogens is in accordance with previous observations on the effects of this steroid on prolactin secretion (10–12). Prolactin also failed to rise following chlorpromazine, which induces dopaminergic blockade acting at either the hypothalamic or pituitary level (10,13, 14–17). There was also no prolactin response to arginine infusion, insulin hypoglycemia or to the onset of sleep; stimuli which also provoke prolactin secretion in normal subjects (18–20). The precise mechanism as to how these agents elevate prolactin is not known with certainty. Prolactin levels were also slightly depressed following the administration of L-dopa (13).

The results indicate that the prolactin deficiency is not absolute. A comparable situation exists in isolated gonadotropin deficiency (IGD) where low basal gonadotropin levels often occur in association with a blunted LH and FSH response to LHRH (21).

Although the patient did have a delay in puberal development, LH and FSH levels rose following the administration of LHRH and were similar to those of normal male controls. GH secretion was also normal following both pharmacological and physiological stimuli. Although ACTH was not

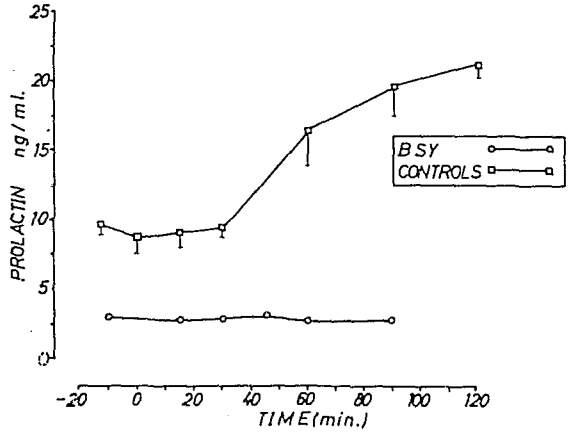


FIG. 3. Prolactin response to chlorpromazine in the patient and control subjects. In the controls mean ± SE are shown.

measured directly, the cortisol rise following insulin hypoglycemia is strong evidence of normal ACTH secretory potential. All thyroid function tests were normal, including the TSH response to TRH. A dissociation of prolactin and TSH secretion after TRH stimulation, has been documented previously, but in these other reports prolactin levels rose after TRH, while TSH remained unchanged (22,23).

Low basal prolactin levels with an impaired response to TRH, CPZ and L-dopa have been described in other patients (24, 25). However, these subjects have invariably presented with panhypopituitarism. In contrast, prolactin deficiency existed in our patient as an isolated phenomenon.

As far as we can ascertain, with the exception of one report by Carlson *et al.* (6), this constellation of findings of decreased prolactin reserve, together with normal LH, FSH, GH, TSH and ACTH secretion is unique. Our patient, however, differed from those reported by Carlson *et al.*, in that there was no evidence for pseudohypoparathyroidism. Although Turkington did describe low basal prolactin levels and absence of a rise following CPZ in two other patients, the prolactin response to TRH was not evaluated (5). Jacobs and Daughaday have reported that they have yet to encounter a patient deficient in prolactin, without being deficient in all other pituitary hormones as well (26). With the exception of the present case, our experience is similar.

Could the prolactin deficiency explain the clinical findings? Studies in mammals have shown that prolactin does have an effect on somatic growth (27). Ovine prolactin has metabolic actions similar to growth hormone (28). Moreover, prolactin can also influence sexual development (27). It has been suggested that prolactin acts by "conditioning" the responsiveness of various target organs to the tropic action of other hormones (29). Nevertheless, it is unlikely that the clinical symptomology in this patient is related to prolactin deficiency. Studies in the father, who also had short stature, demonstrated a normal prolactin response to TRH. It has also been shown that children with constitutional short stature have normal prolactin dynamics (30).

The localization of the lesion in the patient is unknown. It could reside at or above the level of the hypothalamus, possibly resulting from PIF secretion or persistent dopaminergic stimulation. Alternatively, there could be a deficiency of prolactin releasing factor (PRF). The lesion could also be localized to the lactotrope of the pituitary. There could be an absolute reduction in the number of lactotropes. Alternatively, if the lactotropes are present, there could be a lack of synthesis, inhibition of release or release of an immunologically altered prolactin molecule.

Addendum

Since the initial evaluation, the patient has received a short course of hCG (500 IU twice weekly for three weeks). There was a dramatic response and over six months there was a steady advancement in his puberty. At the last examination, this corresponded to stage IV according to Tanner. His height had increased to 1.61 cm. The prolactin response to TRH, however, was unchanged. Basal levels were 4.6 ng/ml and following 200 μg TRH, only increased to 5.2 ng/ml.

Acknowledgments

We thank Dr. H. Friesen who kindly provided us with lyophylized serum samples of known prolactin concentration and Professors S. Glick, C. R. Kleeman and D. Rabinowitz for their helpful comments and criticism. The LHRH used in the study was provided by Dr. M. Gahwyler, Ayerst Laboratories. We also wish to thank Mrs. Y. Selektar, Mrs. Z. Shemesh and Mrs. M. Shananas for their excellent technical assistance.

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The following three publications complement those in the first section since they document the dynamics of prolactin secretion in a group of patients presenting with hypogonadism which is peripheral rather than central in origin.

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The Exaggerated Prolactin Response to Thyrotropin-Releasing Hormone and Metoclopramide in 1,2-Dibromo-3-Chloropropane-Induced Azoospermia*

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ABSTRACT. Twelve males with azoospermia secondary to exposure to the nematocide 1,2-dibromo-3-chloropropane were challenged with iv LRH (100 μg), TRH (200 μg), and metoclopramide (MET; 10 mg) administered 30 min apart. When compared to 24 male controls, both basal FSH and LH levels as well as peak gonadotropin responses to LRH were increased in the azoospermic group. The patients also had increased total estradiol (E_2) and testosterone (T) as well as testosterone-binding globulin levels. Free T levels, however, were not significantly different from the controls.

Basal PRL levels were similar in the two groups. However, the peak PRL responses to both TRH and MET were significantly increased in the azoospermic subjects ($P < 0.001$). In both groups, the PRL response to MET was greater than to TRH. In the individual control and azoospermic subjects, there was no correlation between the PRL response and E_2, T, or the E_2 to T ratio. However, a positive correlation did exist between testosterone-binding globulin levels and the PRL response to TRH and MET. Although the precise mechanism underlying the PRL hyperresponsiveness is unknown, it may be an estrogen-induced phenomenon. (*J Clin Endocriol Metab* 52: 38, 1981)

AN EXAGGERATED PRL response to TRH has been documented in patients with primary testicular failure (1, 2). Although the precise mechanism is unknown, we have shown that this enhanced PRL response in primary testicular failure may be related in part to subtle alteration in estradiol (E_2)-testosterone (T) relationships (1).

To extend our observations into the mechanism underlying the PRL hyperresponsiveness in primary testicular failure, we have evaluated 12 male subjects who were exposed to the industrial chemical 1,2-dibromo-3-chloropropane (DBCP), an agent which supposedly produces selective atrophy of the germinal epithelium (3). In addition to TRH, these subjects were also challenged with the dopaminergic antagonist metoclopramide (MET). Our results have shown that an exaggerated PRL response to both TRH and MET characterizes DBCP-induced azoospermia.

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Materials and Methods

Patients

Twelve males, aged 22–50 yr, presented to the infertility clinic with azoospermia after exposure to DBCP. Eight of the subjects had fathered children before exposure; the remainder had normal male karyotype chromosomal patterns. All subjects had intact secondary sexual characteristics with normal testicular volume and claimed to have frequent erections and ejaculations. Intercourse was reported to be normal.

Bilateral open testicular biopsy was performed under general anesthesia in each case. This demonstrated complete atrophy of the seminiferous epithelium, which was lined exclusively by Sertoli cells. Some tubules were completely hyalinized. Leydig cells were noted in abundance (3).

Experimental protocol

The study was performed from 2–5 yr after the last exposure. Duration of exposure in each subject varied from 100–6700 h (3), and none had received any form of therapy before the study.

After an overnight fast, a needle was inserted into an antecubital vein. This was kept patent by the slow administration of normal saline. Three blood samples were drawn during a 30-min equilibrium period. All subjects then received 100 μg LRH, 200 μg TRH, and 10 mg MET at 30-min intervals. All agents

were administered by rapid iv injection. Blood samples were drawn at 10-min intervals after each agent and continued for 60 min after the MET injection. The responses of the patients were compared to those of 24 healthy controls, aged 20–40 yr, who received the same protocol. Informed consent for the test procedure was obtained from patients and controls.

Methods

Serum LH, FSH, PRL, 17β-estradiol (E_2), testosterone (T), T-binding globulin (TeBG), and free T levels were determined by previously described methods (4, 5). Pituitary LH, FSH, and PRL, as well as their respective antisera, were kindly supplied by the National Pituitary Agency, NIAMDD. Standards for PRL and the Second International Reference Preparation for human menopausal gonadotropin were provided by the Division of Biological Standards and Control (Holly Hill, Hampstead, London, England). Total E_2, T, and TeBG were measured after pooling equal volumes of the three basal samples. Results were analyzed using Student's *t* test.

Results

Gonadotropins (Fig. 1)

The mean (±SD) basal FSH level in the control group was 6.1 ± 1.8 mIU/ml and the mean LH level was 11.1 ± 2.5 mIU/ml. In the patients, basal FSH values were considerably increased (31.3 ± 13.3 mIU/ml; $P < 0.001$ compared to the controls). Although the LH level was also elevated (22.4 ± 7.9 mIU/ml; $P < 0.005$ compared to controls), the values were not as high as those for FSH.

In the controls, the peak LH and FSH responses to LRH occurred at 40 min and were 49.7 ± 14.5 mIU/ml for LH and 8.3 ± 2.9 mIU/ml for FSH. In the patients, the maximum response occurred at 30 min. The mean peak LH was 124.0 ± 39.8 mIU/ml ($P < 0.001$ compared to the controls). The FSH peak was 59.5 ± 25.9 mIU/ml ($P < 0.001$ compared to the controls). At all time intervals after LRH, both gonadotropin responses were greater in patients than controls ($P < 0.001$). Mean total E_2, total T, and TeBG levels were significantly elevated in DBCP-exposed subjects compared to the control levels. Calculated free T levels, however, were similar in both groups (Table 1).

PRL

Basal PRL levels in the controls were 10.6 ± 3.1 ng/ml. The mean peak response to TRH was 32.1 ± 10.7 ng/ml and occurred 20 min after TRH administration. Thereafter, PRL levels declined slightly. After MET administration, PRL levels again rose promptly. The mean peak was 75.5 ± 22.3 ng/ml and occurred 30 min after MET. In the controls, the peak response to MET was significantly greater than to TRH ($P < 0.005$), and PRL levels were still elevated 60 min after MET administration. In the azoospermic group, the basal PRL level was 14.5 ± 11.7 ng/ml. This was not significantly different from the control value. The peak response to TRH was evident at 20 min (70.8 ± 17.9 ng/ml). The peak response to MET was also seen at 20 min (117.7 ± 26.6 ng/ml). As in the controls, the peak response to MET was greater than to TRH ($P < 0.001$). Moreover, at all times, the responses to both TRH and MET were greater in patients than in controls ($P < 0.001$).

Correlations

When individual controls and DBCP-exposed patients were considered together, the peak PRL response to TRH correlated with both basal FSH ($r = 0.62$; $P <$

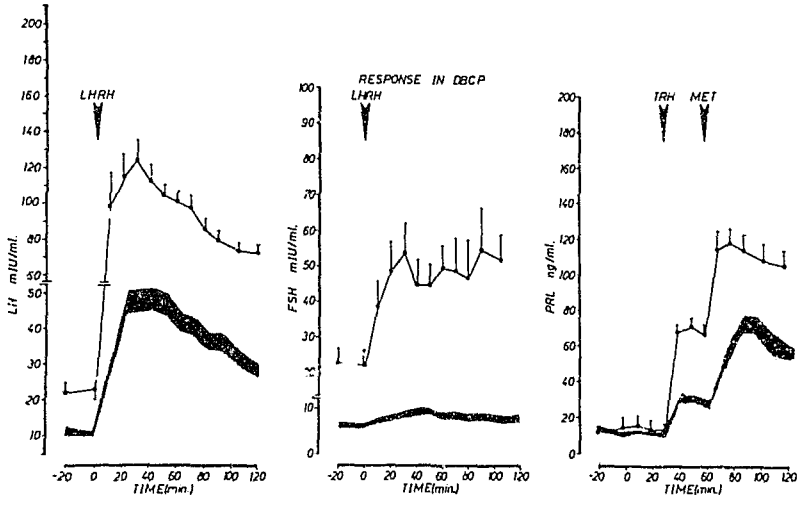


FIG. 1. Mean (±SEM) response of LH (*left panel*), FSH (*middle panel*), and PRL (*right panel*) in the two groups. LRH was given at time 0, TRH was given at 30 min, and MET was given at 60 min. ●—●, DBCP azoospermic group. The control group is depicted by the *shaded area* (see text for details).

TABLE 1. E_2, total T, TeBG, and calculated free T levels in DBCP-exposed subjects and controls

	E_2 (pg/ml)	Total T (ng/ml)	TeBG (nmol/liter)	Free T (pmol/liter)
DBCP				
Mean	30.4[a]	8.9[b]	26.4[c]	930
±SD	4.7	1.0	7.2	430
Control				
Mean	22.1	5.9	16.0	590
±SD	6.9	2.0	8.0	240

[a] $P < 0.05$ *vs.* controls.
[b] $P < 0.005$ *vs.* controls.
[c] $P < 0.01$ *vs.* controls.

0.001) and LH (r = 0.49; $P < 0.005$) as well as peak LH (r = 0.63; $P < 0.001$) and FSH (r = 0.63; $P < 0.01$) responses to LRH. There were similar correlations between the peak PRL responses to MET and basal and peak gonadotropin responses to LRH. When the two groups were considered together, there was a correlation between TeBG levels and the peak PRL response to both TRH and MET (r = 0.49; $P < 0.05$). However, there was no correlation between peak PRL responses and basal E_2, T, and E_2 to T ratios.

Discussion

The industrial chemical DBCP, which is used as a nematocide, has been described as selectively impairing spermatogenesis (3, 6). In all cases, testicular biopsy showed complete absence of all stages of spermatogenesis, with Sertoli cells alone being noted on light microscopy (3). Leydig cells appeared normal in regard to both morphology and number. The normal testicular volume in the presence of such severe atrophy of seminiferous tubules is surprising and is difficult to explain. The seminiferous tubule damage may explain the high basal FSH levels and exaggerated response to LRH, a phenomenon which has been documented previously (7). Basal LH levels were also increased, although not to the same degree as FSH. There was also an increased LH response to LRH. This LH pattern occurred in the presence of intact T levels and a normal light microscopic appearance of Leydig cells. There are a number of reports of germinal cell aplasia accompanied by increased basal LH levels or exaggerated responses to LRH (7–9). This suggests that Leydig cells are also involved in the pathological process. Further evidence of probable Leydig cell involvement in azoospermia was demonstrated by Baker *et al.* (10), who showed impaired testosterone responses to hCG. In our patients, total T levels were increased. This was probably related to the elevation in TeBG levels, as free T levels were normal.

Despite normal basal PRL levels in our subjects, there was an exaggerated PRL response to both TRH and MET. In controls and patients, the PRL response to MET was greater than to TRH, a phenomenon which has been described (11, 12). Previous reports have also documented PRL hyperresponsiveness to TRH in testicular failure (1) and in Klinefelter's syndrome (2), but this has not been a uniform observation (13). The positive correlation noted between gonadotropins and peak PRL responses to TRH and MET suggests that the PRL profile may be related to the elevated gonadotropin levels. However, the absence of an exaggerated PRL response to TRH in male castrates (14) and the reduction in pituitary PRL content and PRL response to MET in castrated male rats (15) implies that high gonadotropins alone are not a dominant factor in the exaggerated PRL response and suggests that a testicular product is modulating PRL secretion.

We have previously noted that, in subjects with primary testicular failure, the exaggerated PRL response to TRH is directly related to the E_2 to T ratio (1). In the present series, however, there was no correlation between peak PRL responses and total E_2 or the E_2 to T ratio. Despite this, the exaggerated PRL response in DBCP-induced azoospermia could be related to estrogens, since mean E_2 levels were higher than in controls and it is well known that estrogens can produce hyperprolactinemia (16) as well as an increase in TeBG (17). The increase in TeBG levels may in part explain the observed elevation in total T. In addition, the elevated TeBG levels in the DBCP group suggests a state of relative estrogen excess compared to the control group. Moreover, the observed correlation between the TeBG concentration and the peak PRL response is consistent with a role for estrogen in mediating this response. The concept that the exaggerated PRL response is related to estrogens is further strengthened by our recent observations that the antiestrogen clomiphene citrate attenuates the exaggerated PRL response to TRH in azoospermia (14).

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Dissociation Between Sleep-Related and TRH-Induced Prolactin Secretion in Seminiferous Tubule Failure

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Prolactin (PRL) secretion has been measured during sleep and following TRH administration in 8 patients aged 24–39 yr with seminiferous tubule failure and 36 controls. Basal LH levels were 25.7 ± 14.7 mIU/ml in the patients compared to 11.5 ± 4.2 mIU/ml in the controls ($p < 0.01$). Corresponding FSH levels were 26.2 ± 10.7 mIU/ml and 5.9 ± 2.1 mIU/ml ($p < 0.001$). Mean estradiol 17B and testosterone levels were similar in the 2 groups. The mean PRL secretion during sleep was 16.5 ± 11.7 ng/ml in the patients and not different in 11 of the controls (12.4 ± 3.2 ng/ml). One patient had a mean nocturnal PRL concentration of 44.1 ng/ml. In both groups, the mean sleep related PRL concentration was greater than that during waking hours. The average number of peaks in the 2 groups was similar. In the same patients, the peak PRL response to TRH (200 ug IV) was 81.9 ± 18.8 ng/ml as compared to 32.1 ± 10.7 ng/ml in the controls ($p < 0.001$). It is concluded that PRL concentrations following pharmacological stimulation are increased in seminiferous tubule failure, whereas levels are normal in relation to the physiological stimulus of sleep.

WE HAVE previously shown that patients with primary testicular failure and elevated gonadotropin levels have exaggerated prolactin (PRL) responses following administration of thyrotropin releasing hormone (TRH) and the dopaminergic antagonists metoclopramide and chlorpromazine. Basal PRL levels, however, were within the normal range.[1-4] Since these stimuli for PRL secretion are pharmacological, the precise physiological relevance of this enhanced PRL response is questionable.

It is well known that there is a circadian rhythm of PRL secretion related to the sleep-wake cycle.[5-8] Augmented PRL secretion characteristically begins 60 minutes after sleep onset and continues with consistent further increases of concentrations through the night to reach peak values during the latter part of the sleep period. Levels then decrease abruptly within one hour of waking. The aim of the present study was to evaluate PRL secretion during EEG monitored sleep in patients with primary testicular failure and seminiferous tubule failure. Our results have shown that the magnitude of the PRL elevation in sleep in these subjects is similar to that of normal controls.

MATERIALS AND METHODS

The patient group comprised 8 males aged 24–39 yr of age who had been referred to our infertility clinic. Azoospermia was evident in 6 patients and the remaining two had severe oligospermia with sperm counts below 6 million/ml. In none was there clinical involvement of Leydig cell function and all had intact secondary sex characteristics with normal libido and potency. Testicular volume was reduced in all 8 subjects. None had any clinically detectable endocrinopathy and all had chromatin negative buccal smears. The control group comprised 36 healthy males aged 19–35 yr.

Study Protocol

Informed consent was obtained in each case. All patients and 11 of the controls slept for 2 nights in the sleep laboratory at their habitual sleep time, while simultaneous electroencephalogram (EEG), electrooculogram and electromyogram recordings were obtained. Subjects arrived at the center at 6 p.m. During the first night, the subjects received a continuous saline infusion. On the second night, blood samples were collected at 15 min intervals in controls and at 20 min intervals in the patients. After sleep stages were scored,[9] the minutes of each sleep stage per sample interval were computed according to standard procedures.[10-11] The mean sleep PRL concentration represents the mean of the samples falling within this period. Similar analyses were done for the mean waking PRL concentrations.

On a subsequent occasion, all 8 patients and 28 of the controls were also challenged with TRH (200 ug) administered IV at 8 a.m. with blood samples being taken at 10, 20, and 30 min after the TRH.

The Student's t test was used to calculate the differences between mean sleep and waking periods as well as between patients and controls.

Methods

Serum LH, FSH, PRL, testosterone (T), 17B-estradiol (E_2) and cortisol were determined by previously described methods.[1-4] LH and FSH levels were expressed by reference to the Second International Reference Preparation of human menopausal gonadotropins. The actual standard used in the assay was the International Reference Preparation of pituitary FSH and LH (69/104). This standard and the PRL standard (75/504) were kindly provided by the Division of Biological Standards and Control, Hampstead, London, England. Antisera to LH, FSH, and PRL were kindly supplied by the National Pituitary Agency, National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, Md.

RESULTS

In the control subjects, the mean ± SD basal LH and FSH levels were 11.5 ± 4.2 mIU/ml and 5.9 ± 2.1 mIU/ml, respectively. In the patients, mean LH

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values were 25.7 ± 14.7 mIU/ml ($p < 0.01$ compared to the controls) and FSH levels were 26.2 ± 10.7 mIU/ml ($p < 0.001$ compared to the controls). The peak LH responses to 100 ug LHRH were 57.1 ± 13.9 mIU/ml in the controls and 196.6 ± 77.6 mIU/ml in the patients ($p < 0.001$). Corresponding peak FSH levels were 9.4 ± 2.7 mIU/ml and 58.3 ± 20.0 mIU/ml ($p < 0.001$). Mean ± SD serum testosterone levels were similar in the patients (6.1 ± 2.9 ng/ml) and controls (5.9 ± 2.0 ng/ml). Estradiol 17B (E_2) levels were also not different in the patients (26.3 ± 6.3 pg/ml) and controls (27.1 ± 6.9 pg/ml).

The individual mean sleep and waking PRL concentrations of all the patients and controls are shown in Table 1. Mean levels during the whole study in the 2 groups are shown in Fig. 1. It is evident that PRL concentrations rose in sleep in both groups. Three of the 8 patients had mean sleep concentrations exceeding 16 ng/ml. One subject, in fact, had a sleep value of 44.1 ng/ml. His results are shown in Fig. 2, together with GH, LH, FSH, cortisol, sleep stages and REM. Only one control had a PRL concentration greater than 16 ng/ml. The mean ± SD sleep PRL concentration was 16.5 ± 11.7 ng/ml in all the patients and 12.4 ± 3.2 ng/ml in the controls. These levels were not different from one another. Mean waking PRL concentrations were 9.0 ± 2.7 ng/ml in the patients and 8.9 ± 1.8 ng/ml in the controls. Neither of these values were different from one another. However, mean sleep levels exceeded mean waking levels in both patients ($p < 0.05$) and controls ($p < 0.01$). In each subject, peaks of PRL secretion were seen during sleep. These have been defined as maximum levels exceeding 1.75 SD of the mean individual PRL concentrations. The average number of peaks was 1.3 in the patients and 1.6 in the controls.

Table 1. Mean Sleep and Waking PRL Concentrations in Patients With Seminiferous Tubule Failure and Controls

Mean PRL Concentrations (ng/ml)					
Patients			Controls		
Case no.	Sleep	Waking	Case no.	Sleep	Waking
1	44.1	11.7	1	9.8	8.2
2	16.2	11.4	2	15.7	11.0
3	18.8	8.5	3	10.8	7.5
4	11.8	—	4	14.6	—
5	11.9	12.2	5	9.2	8.1
6	9.1	7.1	6	13.6	7.2
7	10.7	5.8	7	13.2	10.4
8	9.2	6.5	8	19.4	12.2
			9	10.7	8.8
			10	10.7	9.4
			11	9.2	6.6
Total Mean	16.5	9.0		12.4	8.9
± SD	11.7	2.7		3.2	1.8

Spectral analysis revealed that in both groups, most of the variance was concentrated at the slow frequencies of less than 3 cycles a day. Cross correlation analysis between PRL levels and the sleep stage time series did not reveal any consistent temporal relationship between any of the sleep stages including REM and PRL. The average Pearson product correlation

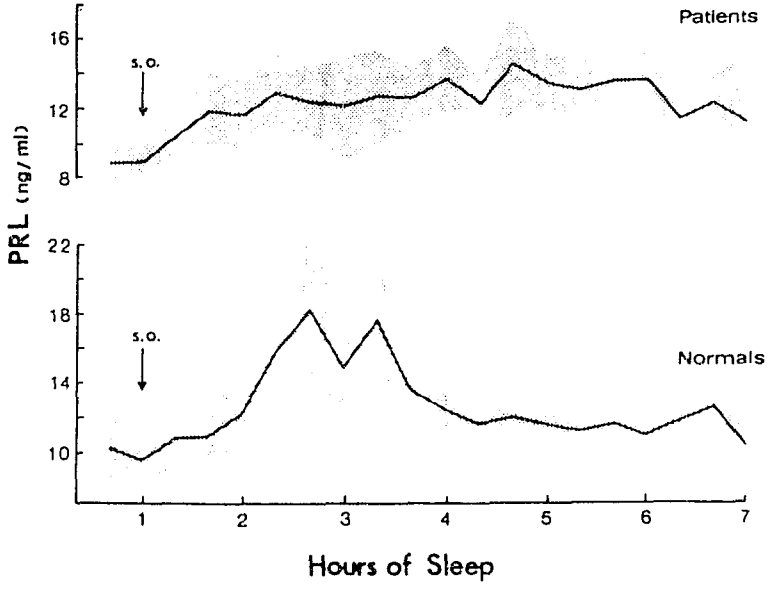


Fig. 1. Mean ± SEM PRL levels during the nocturnal study in the patients with seminiferous tubule failure (upper panel) and the control subjects (lower panel). Patient No. 1 has been omitted. S.O. = Sleep onset.

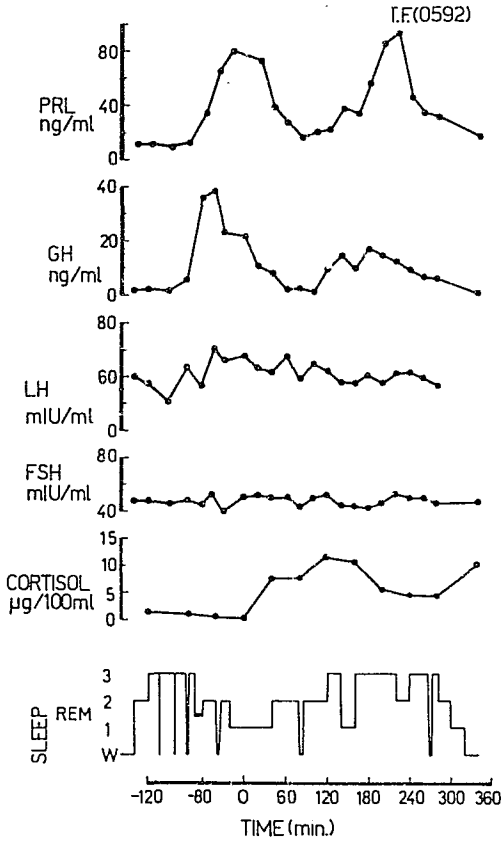


Fig. 2. PRL, GH, LH, FSH, cortisol, sleep stages and REM in patient No. 1. Time 0 = 12 midnight. GH and cortisol show the normal nocturnal profile. There is no specific pattern for LH or FSH.

coefficients between PRL levels and the sleep stages did not approach statistical significance.

In the TRH study, there were no differences in basal PRL levels in the patients and the controls. Following TRH, the peak PRL response occurred at the 20 min sample in both groups. This was 81.9 ± 18.8 ng/ml in the patients and 32.1 ± 10.7 ng/ml in the controls ($p < 0.001$).

DISCUSSION

Our patients demonstrated seminiferous tubule failure in association with elevated LH and FSH, but normal testosterone and estradiol levels. Elevation of LH together with normal testosterone is an indication of a state of compensated Leydig cell failure.[14] This state can be more clearly documented by the demonstration of a subnormal response of testosterone to hCG stimulation.[15] We have documented this hormonal profile previously in our other patients with primary testicular failure[2] as well as in those with azoospermia secondary to exposure to dibromochloropropane.[4] Burman et al. have also observed this in their patients with Klinefelter's syndrome.[16]

The increased PRL secretion occurring at night confirms observations made by previous workers.[5-8] Although it has been suggested that nocturnal rises of PRL secretion are related to sleep stages within a minima in REM and an ascent to maxima in non-REM segments,[7] this was not evident in either patients or controls and other workers have also failed to observe this.[17] Increased nocturnal PRL secretion was noted in both patients and controls and there were no differences in mean sleep levels or the number of PRL peaks in the two groups. Thus, patients with seminiferous tubule failure have basal PRL concentrations during both the day and at night, which are similar to the controls. In contrast, these patients had an exaggerated PRL response following TRH administration. This latter observation has been documented previously by our group as well as by other workers and also occurs with other pharmacological stimuli.[1-4,16] Total E_2 levels were not elevated in these patients and have not been observed to be uniformly increased in our other patients with primary testicular failure and hypergonadotropic hypogonadism.[1-3] Despite this, we have suggested that the exaggerated PRL response following pharmacological stimulation may be estrogen mediated.[1-4] The main evidence for this is that the increased PRL response is reduced following administration of the estrogen antagonist clomiphene citrate.[18] It is possible that free E_2 levels are increased in these subjects.

Our findings in these male patients are very comparable to observations in normal women. The latter have increased PRL responses following administration of TRH as well as other stimuli,[19,20] although basal PRL levels during the day and night are not greater than in men.[21,22] Markedly elevated estrogen levels such as occur, for example, in pregnancy however, are associated with increased basal PRL concentrations.[21-23] This data suggests that changes in estrogen in the physiological range mainly modulates the PRL response to pharmacological stimuli, i.e., provoked PRL secretion and has less effect on tonic basal PRL levels. The reason for the exaggerated PRL rise following pharmacological stimulation and the normal response to sleep, is probably related to the strength of the stimulus. Pharmacological stimuli are employed at their maximally effective doses and are very potent. On the other hand, PRL levels achieved in sleep are lower. Thus, in a situation of mild to moderate PRL cell hyperplasia, the ambient diurnal and nocturnal

levels are not elevated, but with pharmacological provocation, hyperresponsivity is detected. Assuming that the increase in PRL response in seminiferous tubule failure is related to subtle changes in estrogens, this could explain why nocturnal PRL levels are similar to controls. On the other hand, there is an increased provoked response following TRH as well as other stimuli.

It is well known that hyperprolactinemia has an inimical effect on gonadal function in both the male and the female.[23] PRL concentrations are normal under physiological conditions in seminiferous tubule failure. This suggests that the exaggerated PRL responses noted on pharmacological stimulation are a result of the testicular disorder and in themselves, have little effect on testicular function.

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Clomiphene Attenuates the Exaggerated Prolactin Response to Thyrotropin-Releasing Hormone and Metoclopramide Occurring in Primary Testicular Failure*

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ABSTRACT. This study has evaluated the effect of the estrogen antagonist clomiphene citrate on the exaggerated PRL response to TRH and the dopaminergic antagonist metoclopramide (MET) in 16 patients with primary testicular failure and elevated basal and peak gonadotropin responses to LRH. Subjects were challenged with LRH (100 μg), TRH (200 μg), and MET (10 mg), given iv at 30-min intervals in the basal state and after clomiphene citrate, administered as a daily dose of 100 mg for 4 weeks (10 subjects), 200 mg for 4 weeks (3 subjects), and 100 mg for 8 weeks (3 subjects). The patients were subdivided into 2 groups on the basis of the PRL response to TRH and MET, which was exaggerated in 12 subjects (group I) and similar to the controls in 4 subjects (group II). Mean basal LH, FSH, and LH (but not FSH) responses to LRH were increased post clomiphene treatment in both groups. Testosterone levels were normal in both groups, but 17β-estradiol (E_2) levels were increased in group I. After clomiphene administration, both testosterone and E_2 increased in group I subjects. Basal PRL levels were normal in both groups, and there was no change after clomiphene treatment. However, the exaggerated PRL responses to both TRH and MET in group I subjects were decreased after clomiphene administration. Nevertheless, levels were still greater than those in the controls. In contrast, in group II subjects, the PRL response to TRH or MET was not altered by clomiphene. The reduction in the exaggerated PRL response to TRH and MET consequent to clomiphene administration in group I subjects implies that this phenomenon is likely to be estrogen induced. This is supported by the high E_2 levels in this group of patients. (*J Clin Endocrinol Metab* 52: 289, 1981)

AN EXAGGERATED PRL response to TRH has been described in patients with primary testicular failure as well as in subjects with dibromo chloropropane-induced azoospermia (1–3). Since estrogens have the capacity to increase PRL (4, 5), it was suggested that this enhanced PRL response might be an estrogen-induced phenomenon (1–3). The present study was therefore designed to assess the PRL responses to stimulation after blockade of the estrogen receptor with the nonsteroidal estrogen antagonist clomiphene citrate (6–10). Accordingly, patients with primary testicular failure were challenged with TRH and an additional PRL-producing stimulus, the dopaminergic antagonist metoclopramide (MET), both before and after the administration of clomiphene.

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Materials and Methods

The patients comprised 16 males, aged 24–41 yr, referred to the infertility clinic. On the basis of their PRL responses to TRH and MET, the subjects were subdivided into two groups. Group I comprised 12 patients with exaggerated PRL responses to both stimuli. Group II was made up of 4 subjects with PRL responses to TRH and MET similar to the controls.

Azoospermia was evident in seven patients of group I and one subject of group II. The remaining subjects had severe oligospermia, with sperm counts below 6 million/ml. In none was there clinical involvement of Leydig cell function, and all had intact secondary sex characteristics with normal libido and potency. Testicular volume was reduced in eight subjects of group I and three of group II. None of the subjects had any clinically detectable endocrinopathy, and all had chromatin-negative buccal smears. In those subjects in whom testicular histology was available, it showed germinal cell arrest with absence of spermatogenesis beyond the primary spermatocyte stage in one subject of group I. In three subjects of group I and

1 subject of group II, there was marked seminiferous tubule hyalinization. In two subjects of group I, the appearance was characteristic of germinal cell aplasia (Sertoli cell-only syndrome). In one subject of each of the groups, there was a mixed picture, with the majority of tubules being lined by Sertoli cells but some tubules showing evidence of hypospermatogenesis. Testicular failure was secondary to mumps orchitis in one subject of group II, and bilateral cryptorchidism had been surgically corrected in three subjects of group I. The remainder had idiopathic primary testicular failure. None of the subjects had received any form of hormonal therapy for at least 6 months before testing.

Study protocol

Informed consent was obtained in each case. After an overnight fast, an indwelling needle was inserted into an antecubital vein and kept patent with a slow infusion of physiological saline. Two or 3 baseline samples were withdrawn via a 3-way stopcock from the cannulated vein during a 30-min equilibration period. Thereafter, 100 μg LRH, 200 μg TRH, and 10 mg MET were injected by rapid iv administration at 30-min intervals; blood samples were taken every 10 min. All subjects received both LRH, and TRH, and only 11 received the MET injection.

After completion of the test, subjects were given clomiphene citrate according to the following protocols. 1) Eight subjects of group I and 2 of group II received 50 mg clomiphene twice daily for 1 month. 2) Two subjects from group I and 1 from group II received 100 mg clomiphene twice daily for 1 month. 3) The remaining 3 subjects received 50 mg clomiphene twice daily for 2 months. On the last day of the clomiphene course, the LRH-TRH-MET protocol was repeated. Both tests were subsequently determined in the same RIA and the results were compared to those from a group of 28 normal male controls, aged 20–40 yr, who received the same protocol, but without clomiphene.

Methods

LH, FSH, PRL, 17β-estradiol (E_2), and testosterone (T) were measured as previously described (1). Antisera to LH, FSH, and PRL were supplied by the NIAMDD. Standards for LH, FSH, and PRL were supplied by the Division of Biological Standards and Control (Hampstead, London, England). Integrated LH, FSH, and PRL secretion after stimulation was calculated by linear interpolation (120 min after LRH for LH and FSH; 30 min after TRH and 60 min after MET for PRL). Incremental secretion was expressed as the difference between the area under the curve after stimulation and the basal area. All results were analyzed using paired and unpaired Student's *t* tests as appropriate.

Results

Since there were no differences in responses with the three dose schedules of clomiphene, all of the results of each specific group have been pooled.

Gonadotropins, T, and E_2 levels (Table 1)

Mean basal LH and FSH levels and peak gonadotropin responses to LRH were higher ($P < 0.001$) in patients of both groups I and II than in controls. Although also elevated, basal LH levels in group II were only slightly increased compared to controls ($P < 0.05$). In subjects of the two groups, both basal LH and FSH levels as well as peak and integrated gonadotropin responses to LRH were increased in the test performed after clomiphene administration (Table 1). However, the Δ peak (i.e. the difference between the peak response and the basal level) as well as the incremental responses after LRH were increased with LH but not with FSH (Table 1). This indicates that only the LH, not the FSH, response to LRH was augmented after clomiphene administration.

Mean (±SD) T levels were 6.7 ± 2.5 ng/ml in group I, 4.4 ± 1.5 ng/ml in group II, and 5.9 ± 2.0 ng/ml in the controls. There were no differences in mean T levels between the two patient groups or between the patients and controls. Mean E_2 levels in group I subjects (45.6 ± 6.7 pg/ml) were significantly higher ($P < 0.001$) compared to levels in the controls (22.1 ± 6.9 pg/ml). Values were 28.0 ± 10.2 pg/ml in group II subjects, not significantly different from those in the controls.

In group I after clomiphene administration T levels increased to 11.8 ± 4.9 ng/ml, which was significantly different ($P < 0.01$) from the preclomiphene level. E_2 values increased to 105.6 ± 19.3 pg/ml ($P < 0.001$, compared to the initial level). Adequate data postclomiphene treatment were not available for group II subjects.

PRL (Table 1 and Fig. 1)

Mean basal PRL levels were 10.8 ± 2.2 ng/ml in group I before clomiphene and 9.2 ± 2.9 ng/ml after clomiphene. Corresponding levels in group II were 8.4 ± 2.2 and 7.6 ± 2.1 ng/ml, respectively. PRL levels were 9.6 ± 4.1 ng/ml in the controls. It is evident that the basal PRL levels were similar to the control levels in both patient groups and that there were no changes after clomiphene treatment.

The administration of TRH, followed 30 min later by MET, was characterized by two distinct PRL peaks in both normal subjects and those with primary testicular failure. In all instances, the peak after MET exceeded that after TRH (Fig. 1).

In group I, the mean (±SD) peak PRL response to TRH was 66.8 ± 18.2 ng/ml before clomiphene. This decreased to 49.1 ± 15.7 ng/ml after clomiphene ($P < 0.001$). Peak PRL responses to MET decreased from 174.2 ± 56.4 ng/ml before clomiphene to 127.9 ± 26.8 ng/ml after clomiphene ($P < 0.05$). Integrated PRL secretion after TRH as well as that after MET were also decreased after clomiphene administration (Table 1). The de-

TABLE 1. Integrated and incremental secretion of LH, FSH, and PRL subjects of groups I and II before and after clomiphene

	LH			FSH			PRL		
	Basal (mIU/ml)	Integrated secretion (mIU/ml·min)	Incremental secretion (mIU/ml·min)	Basal (mIU/ml·min)	Integrated secretion (mIU/ml·min)	Incremental secretion (mIU/ml·min)	Basal (ng/ml)	Integrated secretion (ng/ml·min)	
								TRH	MET
Group I									
Pre									
Mean	24.7	1169.0	930.0	21.2	427.4	215.5	10.8	500.3	1645.2
±SD	13.2	553.1	485.2	9.7	165.4	97.0	2.2	139.6	447.4
Post									
Mean	68.6	2262.1	1553.0	48.4	717.1	233.0	9.2	382.8	1236.2
±SD	48.7	782.4	644.3	33.3	301.6	255.9	2.9	120.2	283.2
Pre *vs.* Post[a]	<0.01	<0.001	<0.01	<0.02	<0.01	NS	NS	<0.05	<0.05
Group II									
Pre									
Mean	18.8	822.5	634.5	26.3	460.2	196.9	8.4	227.3	533.0
±SD	12.4	262.9	210.2	10.1	135.0	92.7	2.2	35.5	180.6
Post									
Mean	59.3	1681.8	1088.8	48.6	762.2	240.1	7.6	203.6	472.0
±SD	23.4	254.2	259.7	18.5	205.8	158.7	2.1	81.0	120.3
Pre *vs.* Post[a]	<0.05	<0.01	<0.05	<0.02	<0.05	NS	NS	NS	NS
Controls									
Mean	11.5	410.5	297.4	5.9	76.2	17.8	9.1	267.6	630.3
±SD	4.2	106.4	91.4	2.1	23.0	16.2	4.1	35.3	192.8

Pre, Before clomiphene treatment; Post, after clomiphene treatment.
[a] *P* value for Pre *vs.* Post groups.

creased responses were evident 10, 20, and 30 min after TRH and 30 and 45 min after MET (Fig. 1). However, even after clomiphene, the peak PRL responses to both TRH and MET in group I subjects were still greater than those in the controls (Fig. 1).

In group II subjects who were defined as having PRL profiles after TRH and MET which were not different from the controls, there were no changes in PRL responses in the tests performed before and after clomiphene administration. The maximum increase after TRH was 29.6 ± 7.5 ng/ml before and 26.4 ± 8.3 ng/ml after clomiphene. Corresponding levels after MET were 60.7 ± 14.4 and 61.1 ± 17.8 ng/ml, respectively. The integrated responses to TRH and MET were not different in the two tests (Table 1 and Fig. 1).

Discussion

In the human, clomiphene behaves as an antiestrogen in normally estrogenized states and as a weak estrogen agonist in the estrogen-depleted state (7). It has been shown to occupy specific cytoplasmic estrogen receptor sites (11). Its antiestrogenic activity has been explained by its capacity to compete for these estrogen receptor sites in the hypothalamic-pituitary system (6–10). Our results have shown that clomiphene administration for 4 weeks or longer to patients with primary testicular failure and elevated LH and FSH levels is associated with a rise in basal gonadotropins. There was also an increased LH, but not FSH, response to LRH. Increased T and E_2 levels were also noted after clomiphene treatment. It has been well documented that clomiphene as well as the antiestrogen tamoxifen elevate LH, FSH, T, and E_2 in male subjects (12–24). However, its effect on the gonadotropin responses to LRH is more controversial, and decreased, increased, and unaltered gonadotropin release have been described (16–22). These discordant findings can best be explained by the variable time interval of clomiphene pretreatment. While an impaired gonadotropin response to LRH is common early on (17, 18, 20), this has invariably returned to normal or become increased by 7–10 days (16, 19, 21, 22); by 7 weeks, an exaggerated response has been described (24).

Although increased gonadotropin levels after clomiphene treatment were evident in all of our patients, the two groups behaved differently in regard to PRL. Group I subjects, who had exaggerated PRL responses to TRH and MET, showed decreased responses in the test performed after clomiphene pretreatment. Responses to TRH and MET were, nevertheless, still greater than those in the controls. This group also had increased mean E_2 levels. Group II subjects, who had normal E_2 levels, in contrast, demonstrated PRL responses to TRH and MET in their initial test which were not different from

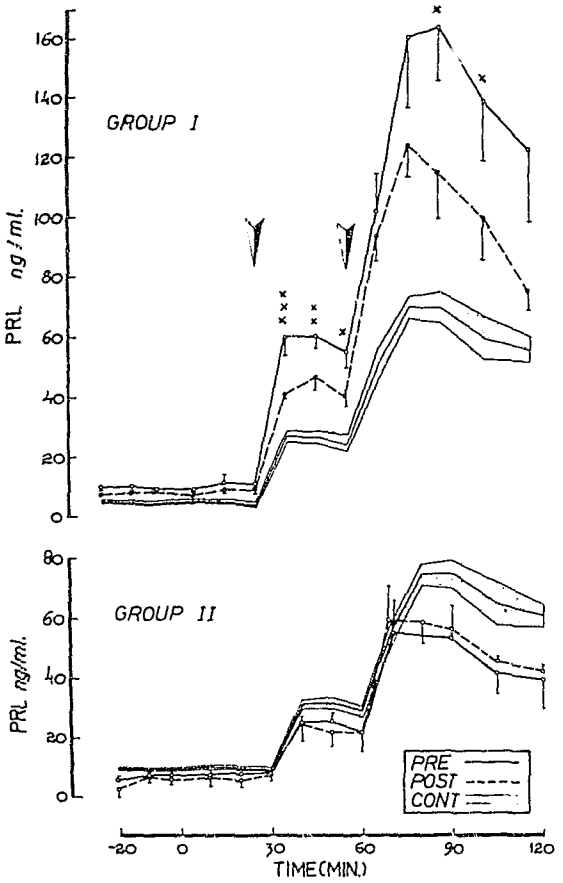


FIG. 1. PRL response to TRH and MET given at 30 and 60 min to patients with testicular failure before clomiphene (——) and after clomiphene (– – –). Group I subjects are shown in the *upper panel* and Group II subjects are shown in the *lower panel*. Control levels are shown by the *shaded areas*. Values given are the mean ± SEM. *P* values comparing PRL responses before clomiphene and after clomiphene: ×××, $P < 0.001$; ××, $P < 0.01$; ×, $P < 0.05$.

the control responses. There was no change in PRL response after clomiphene treatment. These subjects' normal PRL responses are probably related to a lesser degree of testicular failure, as shown by lower LH levels (3). Clomiphene did not produce changes in basal PRL levels in either group.

In common with our group II subjects, clomiphene does not alter the PRL response to TRH, MET, or sulpiride in normal men (21, 22). However, after clomiphene administration,the PRL response to TRH has been reported to be decreased in postmenopausal women (25), and basal levels are decreased in females with hyperprolactinemia (26) or in anovulation (27) and, on occasion, in puerperal women (28, 29). Administration of the antiestrogens tamoxifen, nafoxidene, or MER 25 to rats decreased the estrogen-stimulated PRL response in the gonadectomized rat and may also decrease the proestrous surge in intact females (30–32).

The implication of these results is that blockade of the estrogen receptors by clomiphene is associated with a reduction of the exaggerated PRL response to TRH and MET in those patients with elevated mean E_2 levels. This indicates that the mechanism of their deranged PRL profile may well be estrogen induced. This phenomenon presumably does not operate in those subjects whose mean E_2 levels are intact or in the normal male. In this connection, it was previously shown that in primary testicular failure, the estradiol to testosterone ratio correlated with the exaggerated PRL response to TRH (1).

The increased PRL responses in group I did not revert fully to normal after clomiphene. This could be related to inappropriate dosage or duration of treatment with clomiphene. However, it must also be stressed that there is still some controversy as to whether the effects of clomiphene are specific (24). Although a relationship between clomiphene and androgens has been described (12, 13), *in vitro* studies with the rat ventral prostrate have shown that clomiphene does not compete for androgen-binding sites (33). Although androgen and estrogen receptor sites appear to be distinct (34), some reports indicate that androgen may compete specifically with E_2 for the estrogen receptor sites in different tissues of rats as well as in humans (35, 36). Thus, the clomiphene-induced rise in T may have altered the PRL response. However, it is more likely that the exaggerated PRL response noted in patients with primary testicular failure is an estrogen-induced phenomenon.

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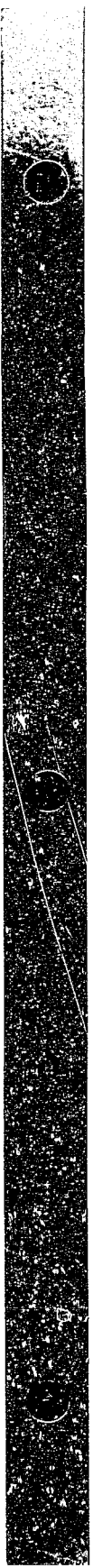
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In contrast to the clinical studies described in all the papers cited above, the following two publications represent the response to a situation of medically-induced hypogonadism. This was produced by administration of a long acting GnRH agonist known as D-His. The first publication describes the response to a parenteral and the second to an intranasal preparation of this GnRH agonist.

The following two publications complement those in the first section since they document the dynamics of prolactin secretion in a group of patients presenting with hypogonadism which is peripheral rather than central in origin.



PREVENTION OF CYCLICAL ATTACKS OF ACUTE INTERMITTENT PORPHYRIA WITH A LONG-ACTING AGONIST OF LUTEINIZING HORMONE–RELEASING HORMONE

KARL E. ANDERSON, M.D., IRVING M. SPITZ, M.D., SHIGERU SASSA, M.D., C. WAYNE BARDIN, M.D. AND ATTALLAH KAPPAS, M.D.

THE genetic disease acute intermittent porphyria may be exacerbated by a number of factors that are distinct from the inherited deficiency of the heme pathway enzyme, porphobilinogen deaminase (also known as uroporphyrinogen I synthase). These factors include endogenous steroid hormones and their metabolites, drugs, and nutrition.[1-5] In some women with acute intermittent porphyria, exacerbations of symptoms occur in relation to the menstrual cycle, and in a minority, disabling premenstrual attacks occur regularly with every cycle.[1,2,5-9]

We report on a patient in whom premenstrual exacerbations of acute intermittent porphyria, which had previously occurred every month for at least two years, were prevented by daily administration of a long-acting agonistic analogue of luteinizing hormone–releasing hormone (LHRH). Exogenous steroid preparations and other therapies either had been unsuccessful or had exacerbated the disease. The beneficial response in this patient raises the possibility that women with frequent attacks of porphyria related to cyclical ovarian hormone secretion may be effectively treated with LHRH agonists to suppress endogenous production of hormones that are provocative in this disease.

CASE REPORT

The patient was 36 years old when first seen at Rockefeller University Hospital. Menarche had occurred at age 14, and thereafter, she had had regular 28-day menstrual cycles, with an uneventful pregnancy at age 18. At age 21 she began having intermittent attacks of abdominal pain occurring regularly in relation to her menstrual cycle; for at least two years these attacks occurred with every cycle. Pain began at or soon after the time of ovulation, increased in intensity until the onset of bleeding, and then gradually subsided. The diagnosis of acute intermittent porphyria was made elsewhere at age 31, when the patient had severe abdominal pain and muscle weakness after treatment of seizure-like episodes with phenytoin, a drug known to be harmful in this disease.[1,2] At that time she had severe neuropathy and required mechanical respiratory assistance. Although she recovered considerably over the next 3½ years, and was subsequently in reasonably good health, she continued to have monthly attacks of abdominal pain even in the absence of harmful drugs and while maintaining an adequate intake of calories and carbohydrates. These attacks were usually treated at home but were sometimes severe enough to require hospitalization. Treatment with repeated intravenous hematin infusions had been attempted but discontinued because of frequent phlebitis at the infusion sites. Danazol, medroxyprogesterone acetate (Provera), and various estrogen–progestin combinations had also been given during the past 10 years, but none had relieved her symptoms, and some of these agents had precipitated attacks of porphyria.

Physical examination at Rockefeller University Hospital revealed no marked abnormalities. Electromyographic and nerve-conduction studies revealed no evidence of peripheral neuropathy. The diagnosis of acute intermittent porphyria was confirmed by the findings of deficient porphobilinogen deaminase activity in erythrocytes (17 nmol of uroporphyrinogen formed per hour per milliliter of erythrocytes; normal value [mean ±S.D.], 30.8±5.6) and increased urinary δ-aminolevulinic acid (14 mg per 24 hours; normal range, 0 to 7) and porphobilinogen (38 mg per 24 hours; normal range, 0 to 4). Decreased levels of erythrocyte porphobilinogen deaminase were also found in the patient's daughter, mother, and two sisters.

The LHRH agonist [(ImBzl)-D-His^6,Pro^9-NET]LHRH (D-His) was synthesized in the Peptide Biology Laboratory of the Salk Institute, La Jolla, California. A dose of 5 μg per day was administered subcutaneously for eight months. Plasma levels of progesterone, estradiol, luteinizing hormone, and follicle-stimulating hormone,[10,11] as well as erythrocyte porphobilinogen deaminase[12] and urinary δ-aminolevulinic acid and porphobilinogen, were measured as described previously.[13] D-His was shown not to interfere with the hormone assays.

We considered the possibility that D-His itself might induce hepatic heme biosynthesis; like a variety of steroids and drugs that have such an inducing effect, it could thus be potentially dangerous in acute intermittent porphyria. Therefore, before D-His was administered to the patient, the effects of the drug were studied in chick embryo liver in ovo and in culture. Chick-embryo hepatic systems have been widely used to screen drugs and hormonal steroids that may pose a risk in patients with porphyria.[1,14-17] For example, the inducing effect of danazol, a harmful drug in porphyria,[8] is demonstrable in chick-embryo liver[15] but has not been [illegible] adult rat liver.[8] The avian-embryo hepatic-cell-culture system is also highly sensitive to the permissive effects of such hormones as insulin, corticosteroids, and triiodothyronine.[1,17] We found that D-His did not stimulate porphyrin accumulation in avian-embryo liver either directly or with a small priming dose of 1,4-dihydro-3,5-dicarbethoxycollidine (which accentuates porphyrin accumulation in liver by inhibiting the enzyme ferrochelatase),[14] nor did the compound induce porphyrins or have a permissive effect[1,17] on the induction of porphyrins by allylisopropylacetamide (a known potent inducer) in the chick-embryo hepatic-cell-culture system (data not shown). These findings provided some assurance that D-His may be administered without exacerbating porphyria.

Figure 1 shows the times of menses and the appearance of porphyric symptoms in the patient for a period of 8 weeks before and 32 weeks after the start of treatment with D-His, as well as plasma hormone levels beginning with the time of treatment. Treatment was begun on Day 11 of a menstrual cycle — a time when plasma estradiol levels were already increased. The increased estrogen levels triggered a luteinizing hormone surge, which occurred on Day 13 and was followed by a rise in plasma progesterone levels (Fig. 1);

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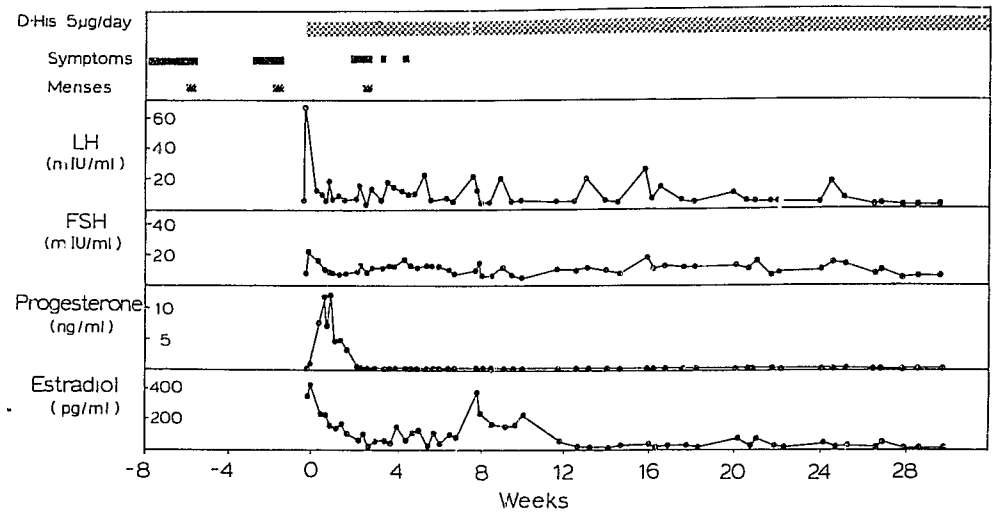


Figure 1. Course of Symptoms, Menses, and Plasma Hormone Levels in a Patient with Acute Intermittent Porphyria, during Eight Months of Treatment with a Luteinizing Hormone–Releasing Hormone (LHRH) Agonist (D-His).

Cyclical premenstrual attacks had occurred monthly for at least two years before treatment. D-His was started in midcycle and did not initially prevent ovulation, menses, or symptoms of porphyria. However, for seven months thereafter, porphyric symptoms and menses did not occur, and hormone levels were low, indicating pituitary–ovarian suppression. LH denotes luteinizing hormone, and FSH follicle-stimulating hormone.

these hormonal changes are consistent with ovulation. Menstrual bleeding occurred on Day 28 of this cycle. Severe symptoms of porphyria developed during the late portion of this cycle, as they had regularly with every cycle for the previous two years. D-His treatment thus failed to prevent ovulation, presumably because the treatment was started when the cycle was too advanced.

During the following seven months of continued administration of D-His in a dose of 5 μg daily by subcutaneous injection, menses did not occur, and progesterone levels remained below 0.2 ng per milliliter, which was consistent with the absence of ovulation and corpus luteum formation. Estradiol values showed a single transient increase and then progressively decreased to hypogonadal levels (Fig. 1). Gonadotropin levels showed several intermittent increases, but the values, especially those of luteinizing hormone, decreased steadily. During this time, there were no symptoms of porphyria, and urinary output of porphobilinogen and δ-aminolevulinic acid remained relatively constant (data not shown). The only side effects that the patient noted during treatment were a slight decrease in breast size and moderately severe hot flushes.

After completion of this study, D-His injections were discontinued, and a trial of intranasal D-His was initiated. The initial dose chosen was not optimal, and menses and premenstrual attacks of porphyria have recurred, with increases in estradiol and progesterone levels. Further studies are being performed to determine the optimal intranasal dose of D-His needed to prevent ovulation in the patient.

Discussion

Long-acting agonistic analogues of LHRH, a decapeptide released by the hypothalamus, have inhibitory effects on reproductive function.[18,19] When administered repeatedly, these agonists apparently desensitize the pituitary to the subsequent effects of both native LHRH and the analogue, resulting in reduced secretion of luteinizing and follicle-stimulating hormones.[20] These observations have suggested the use of LHRH agonists as contraceptive agents to inhibit spermatogenesis[21] and to block ovulation.[22] Administration by intranasal spray[22] is feasible for long-term use.

Prevention of premenstrual porphyric attacks by endocrine manipulation has been difficult, although it has been achieved in some patients by treatment with oral contraceptives, progestins, or androgens.[6,7] In others, attacks have been precipitated by these agents.[8,23-25] Because our patient was having disabling attacks associated with the menstrual cycle, it seemed desirable to suppress ovarian hormone secretion, preferably without administering exogenous steroid preparations. When administered daily, the LHRH analogue, D-His, prevented ovulation and corpus luteum formation, as manifested by absent menses and persistently low levels of progesterone. Levels of luteinizing hormone, follicle-stimulating hormone, and estradiol decreased as well. The attacks of porphyria, which had occurred cyclically before treatment, also ceased (Fig. 1). We did not observe a decrease in porphyrin-precursor excretion, which was somewhat surprising, because clinical improvement in patients with acute intermittent porphyria is commonly associated with decreases in the output of these precursors. However, increased porphyrin-precursor excretion can occur in asymptomatic patients with the disease,[1,2] and in two women treated successfully with hematin to prevent regular premenstrual attacks of porphyria, urinary excretion of δ-aminolevulinic acid and porphobilinogen remained above normal.[1,9] Further studies will be needed to ascertain whether interruption of ovulatory cycles by administration of LHRH agonists can regularly produce decreases in porphyrin-precursor excretion.

The only side effects noted by our patient during treatment with D-His were hot flushes and a decrease in breast size. These symptoms have previously been described during LHRH analogue therapy,[26,27] and it is possible that a decrease in the dose might alleviate them. The possibility of other consequences of decreased secretion of endogenous estrogens, such as bone demineralization, may also merit consideration, if long-term treatment is anticipated.

It is difficult to generalize from a single clinical experience; nevertheless, in a disorder as uncommon, yet as disabling, as acute intermittent porphyria with recurrent and frequent premenstrual attacks, a single case in which the clinical course is clearly altered by a therapeutic procedure may be valuable to report.[9] The present case suggests that LHRH analogues may be useful in the prevention of such exacerbations of this disease, as well as in the treatment of similar symptoms due to variegate porphyria and hereditary coproporphyria. Moreover, women with these disorders who take steroid-containing contraceptives are at some risk for exacerbation of symptoms,[1] and LHRH analogues may be an appropriate alternative for birth control as well.

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A Gonadotropin Releasing Hormone Analogue Prevents Cyclical Attacks of Porphyria

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• **Acute intermittent porphyria is a genetic disease in which endogenous hormones affect clinical expression. Premenstrual exacerbations can occur, sometimes often, in women with this disease. Gonadotropin releasing hormone analogues can prevent ovulation by reducing secretion of luteinizing hormone and follicle-stimulating hormone. In six patients with well-documented acute intermittent porphyria and frequent cyclical exacerbations, daily administration of an agonistic gonadotropin releasing hormone analogue, ([ImBzl]-D-His^6,Pro^9-NET)gonadotropin releasing hormone, intranasally or subcutaneously for as long as 26 months reduced or eliminated premenstrual attacks and caused only minor side effects. Adjustments in dosage or route of administration were sometimes needed. We conclude that endocrine manipulation by treatment with a gonadotropin releasing hormone agonist will prevent neurovisceral attacks of acute intermittent porphyria due to cyclical changes in endogenous hormones and is a safe alternative to exogenous steroids, which may induce attacks of this disease.**
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Acute intermittent porphyria is due to an inherited (autosomal dominant) deficiency of porphobilinogen deaminase, the third enzyme of the heme biosynthetic pathway.[1,2] Clinical expression of the disease, characterized by recurrent attacks of abdominal pain and other neurovisceral symptoms, occurs with additional factors, such as sex steroid hormones and certain of their metabolites,[3-5] dietary factors, and a wide variety of drugs.[1] Some women experience exacerbations of the disease in relation to the menstrual cycle, and, in a minority, disabling attacks occur with almost every cycle. Preliminary reports indicate that agonistic analogues of gonadotropin releasing hormone (Gn-RH) might benefit such patients.[6-10] We treated six patients with a Gn-RH analogue for up to 26 months and found that it represents a useful new approach to treatment of selected patients with acute intermittent porphyria.

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PATIENTS AND METHODS

The Gn-RH analogue ([ImBzl]-D-His^6,Pro^9-NET)Gn-RH (D-His) was synthesized by the Peptide Biology Laboratory of the Salk Institute, La Jolla, Calif. Solutions of D-His were prepared for intranasal administration or subcutaneous injection as previously described.[7] The study was approved by the Institutional Review Boards of the Rockefeller University Hospital and The Population Council, New York, NY, and written informed consent was obtained from each patient.

Six women, from 25 to 36 years of age, in whom the diagnosis of acute intermittent porphyria was documented by decreased activity of porphobilinogen deaminase in erythrocytes[11] and markedly increased excretion of δ-aminolevulinic acid and porphobilinogen in urine[12] participated in the study (Table 1). Each had had frequent attacks of porphyria associated with the menstrual cycle for at least 6 months before entry to the study. Although D-His might also benefit women with less frequent premenstrual attacks, women with attacks of porphyria with almost every cycle were selected because fewer patients would be required to assess efficacy. The recurrent attacks were characteristic of acute intermittent porphyria. In five patients there was no evidence of endometriosis or other pelvic or abdominal disease to account for the symptoms. One patient (patient 4) had coexistent regional enteritis producing mild diarrhea (one to three stools daily); however, the cyclical porphyric attacks were clearly distinguishable. Dietary calcium intake was assessed by history, and patients were prescribed an oral calcium supplement when needed to maintain a calcium intake of at least 1 g daily during the study. Patients used birth control methods other than treatment with the Gn-RH analogue or contraceptive steroids during the study. Initial results of D-His administration in patients 1 through 4 have been briefly described.[6,7]

The D-His was administered as a single daily dose in the morning. Patient 1 was initially treated by the subcutaneous (SC) route, but later all patients were treated by the intranasal (IN) route because it was likely to be more convenient and practical for long-term treatment. In three patients, treatment was later changed to the SC route to obtain a better response and conserve a limited supply of the intranasal preparation. After the study was completed, five of the patients entered a trial of another Gn-RH analogue.

Plasma levels of progesterone and estradiol were measured as described[6,7] at intervals of 4 to 14 days. For patient 1 and initially for patient 2 (see below), plasma samples were obtained at unspecified times of the day. Plasma was obtained only in the morning before D-His administration during the remainder of the treatment course of

patient 2 and throughout the treatment periods for patients 3 through 6. Hormone levels for each patient were tabulated as means, SDs, and ranges for various study periods. Because of possible carryover effects and initial agonistic effects of the Gn-RH analogue, hormone results were tabulated beginning 1 month after an initiation or change in treatment. Urinary porphyrin precursor results during pretreatment and treatment periods were compared by the Mann-Whitney *U* test with the use of a computer (MacIntosh SE; Apple Computer Inc, Cupertino, Calif) and software (StatView; Brainpower Inc, Calabasas, Calif).

Table 1.—Laboratory Data Documenting Diagnosis of Acute Intermittent Porphyria in Six Women at Study Entry

Patient/ Age, y	Erythrocyte Porphobilinogen Deaminase, nmol·h⁻¹·mL⁻¹	Urinary δ-Aminolevulinic Acid, μmol/d	Urinary Porphobilinogen, μmol/d
1/36	17	107	168.0
2/32	16.5	297	322.7
3/34	15.9	99	141.4
4/29	14.2	183	154.7
5/28	16.3	259	291.7
6/25	21.9	76	114.9
Normal values	30.8±5.6*	0-53†	0-17.7‡

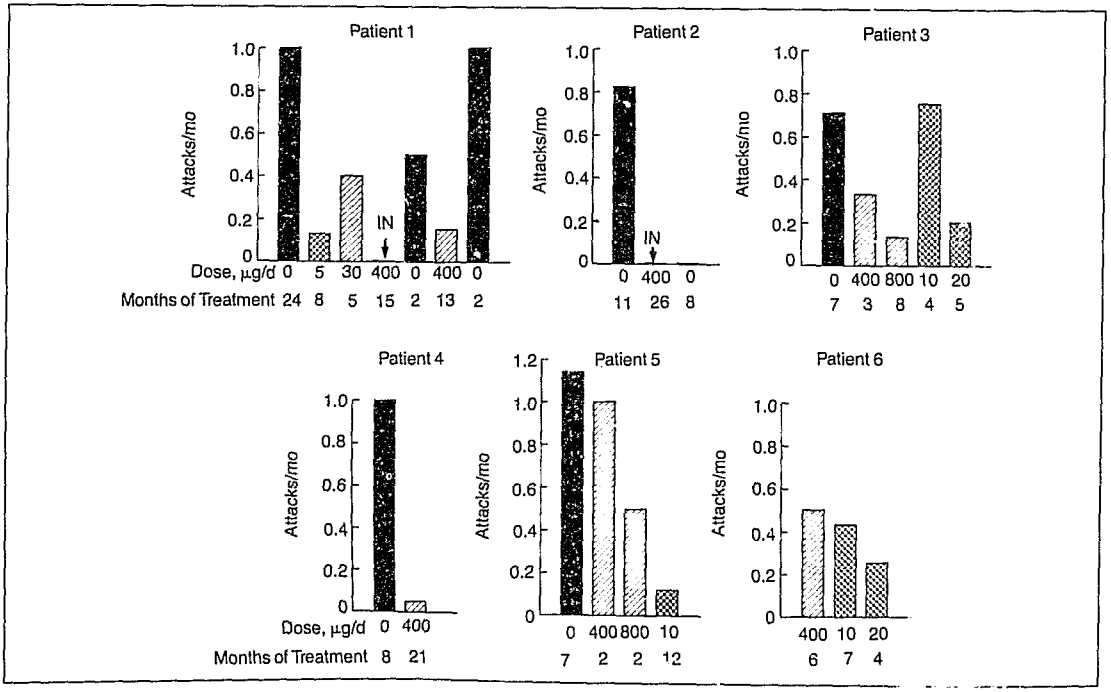
*Mean±SD.
†Normal range in conventional units is 0 to 7 mg/d.
‡Normal range in conventional units is 0 to 4 mg/d.

RESULTS

The frequency of menses-associated attacks of porphyria in each patient during the SC or IN administration of D-His was compared with the frequency of such attacks before treatment (accurately determined by history in patients 1 through 5) and the frequency of attacks during periods when D-His was discontinued (as observed three times in two patients: twice in patient 1 and once in patient 2). As shown in Fig 1, the frequency of cyclical attacks was markedly lower during periods of D-His administration than during the control periods in patients 1 through 5, with the single exception of the post-treatment period in patient 2. Furthermore, changes in the dosage or route of administration, which were necessary to improve the effectiveness of treatment in four patients (patients 1, 3, 5, and 6), resulted in decreased frequencies of menses-associated porphyric attacks in all instances.

Urinary porphyrin precursor excretion could be assessed during some periods of D-His treatment in four patients. As shown in Table 2, urinary δ-aminolevulinic acid excretion was lower during D-His treatment in two patients, and urinary porphobilinogen level was lower in all four patients. The number of pretreatment urine samples for patient 1 was insufficient to provide an adequate comparison; therefore, comparisons were made between a time when the dosage of D-His (30 μg/d IN) was too low to prevent premenstrual attacks and two successful D-His treatment periods (Table 2).

Fig 1.—Menses-associated attacks of acute intermittent porphyria per month during periods of daily administration of ([ImBzl]-D-His[6],Pro[9]-NET)gonadotropin releasing hormone (D-His) by either the intranasal (hatched bars, or IN, where bars are too short to show hatching) or subcutaneous route (crosshatched bars). Solid bars indicate the frequency of perimenstrual attacks during control periods when no gonadotropin releasing hormone analogue was administered. Patients are shown in the order of entry into the study.



In the other two patients, any effects of D-His were obscured by hematin, which was administered for symptoms of porphyria mostly during control periods; hematin is known to reduce porphyrin precursor excretion substantially.[1,2]

Side effects during D-His administration were hot flushes in four patients (mild in two and moderate or moderately severe in two), decreased breast size (three patients), transient breast swelling or tenderness (two patients), decreased libido (two patients), and vaginal dryness and irritation (two patients). Less frequently observed were acneiform skin lesions; mild loss of pubic hair; increased hair on arms, legs, and face; and worsening of psoriasis (one patient each). None of these side effects required alterations in treatment. Monitoring of standard blood counts, urinalyses, and serum chemistry studies disclosed no abnormalities related to D-His administration.

REPORT OF CASES

Case 1.—A 36-year-old woman had had premenstrual attacks of porphyria, usually lasting for 3 to 7 days, for at least 2 years before entry. Therapy with D-His (5 μg SC daily) was started on day 11 of a menstrual cycle, when a cyclical increase in plasma estradiol concentrations had already occurred, and was followed by a luteinizing hormone surge, ovulation manifested by increased plasma progesterone levels, symptoms of porphyria, and menstrual bleeding.

For 8 months no further menses or porphyria attacks occurred. After 1 month of therapy, progesterone levels remained suppressed, although estradiol levels were incompletely suppressed (Table 3). Administration of D-His was then changed to a dosage of 30 μg IN daily. When menses and premenstrual attacks recurred, accompanied by increased progesterone and estradiol levels, the dosage was increased to 400 μg IN daily. For 15 months of continued treatment, there were no further attacks of porphyria (Fig 1); after the first month, progesterone levels were suppressed, and estradiol levels were reduced (Table 3).

When D-His administration was again discontinued for 2 months, one premenstrual attack occurred. When D-His therapy, 400 μg daily IN, was reinstated for 13 months, only two menses-associated attacks occurred. Estradiol levels after the first month of treatment were somewhat higher than during the previous daily dosage of 400 μg of D-His IN (Table 3); two menses and several episodes of light vaginal bleeding unassociated with symptoms also occurred. When treatment was again discontinued for 2 months, premenstrual exacerbations recurred (Fig 1).

Case 2.—A 32-year-old woman had had monthly attacks of porphyria for at least 11 months. She also had temporal lobe epilepsy (primarily psychomotor), the onset of which (at age 21 years) had preceded the onset of porphyria symptoms (at age 31 years). Treatment with carbamazepine, 600 μg daily, was maintained for 1 year before D-His treatment was started and was continued throughout the study, with good control of seizures. For 3 months before D-His administration, ovulatory cycles were documented by serial progesterone and estradiol levels; prophylactic infusions of hematin were successful in preventing premenstrual attacks with two of these three cycles (Fig 2).

Administration of D-His, 400 μg IN daily, was started on day 3 of a cycle. Menses were absent, progesterone levels remained low, estradiol levels decreased (Table 3), and only a single noncyclical attack of porphyria occurred during the subsequent 26 months of intranasal D-His administration (Fig 2). Initially, plasma was obtained twice weekly, the first usually in the morning and the second in the evening. A pattern of lower (morning) estradiol levels alternating with higher (evening) levels was noted during the first 3 months of treatment (Fig 2). This pattern did not continue when sampling was performed only in the morning. Frequent blood sampling for up to 24 hours on several occasions showed that estradiol levels increased during the day in this patient (data not shown).

When treatment was discontinued, seven menstrual periods with associated cyclical hormone changes (Table 3) and no attacks of porphyria developed during follow-up for 8 months. Because antiseizure medication was unchanged during the study, other factors, which we could not identify, reduced her susceptibility to premenstrual attacks of porphyria.

Case 3.—A 34-year-old woman had had frequent premenstrual attacks of porphyria for at least 7 months (six menses and five associated attacks) before entry. Symptoms resolved slowly after these attacks. Although menses were irregular, cyclical hormone changes were documented for one cycle before D-His administration (Table 3). However, symptoms of porphyria persisted, ovulation did not occur the following month, and hematin administrations decreased porphyrin precursor excretion but did not produce a clear clinical benefit. Therapy with D-His, 400 μg daily IN, was initiated 45 days after the last menses and caused a suboptimal response; during 3 months there were two increases in estradiol and progesterone levels, menses occurred twice, and there was one premenstrual exacerbation of porphyria (Table 3, Fig 1). A satisfactory response was obtained when D-His therapy was increased to 800 μg IN and continued for 8 months (Fig 1); after the first month of this dosage, progesterone levels remained suppressed and estradiol levels were lower (Table 3). Because the 800-μg regimen required large amounts of D-His and the supply was limited, SC administration was instituted. An initial dose of 10 μg daily SC did not induce a satisfactorily response (two menses and three associated attacks occurred in 4 months), but a good response was obtained when the dosage was increased to 20 μg daily (two menses and one perimenstrual attack occurred during 5 months) (Fig 1).

Table 2.—Effects of Administration of D-His on Urinary Excretion of Porphyrin Precursors in Four Women With Acute Intermittent Porphyria*

Patient	Treatment	No. of Samples	δ-Aminolevulinic Acid, μmol/d	P†	Porphobilinogen, μmol/d	P†
1	D-His, 5 μg/d, SC‡	44	92±47	NS	112.9±60.6	<.05
	D-His, 30 μg/d, IN	21	104±50	NS	167.4±116.7	<.02
	D-His, 400 μg/d, IN‡	40	95±56		121.1±80.9	
2	Pretreatment	27	343±172	NS	341.7±159.6	<.02
	D-His, 400 μg/d, IN‡	39	284±101		253.7±118.9	
3	Pretreatment	49	67±39	NS	81.3±65.4	NS
	D-His, 400 μg/d, IN	26	76±28	<.003	95.1±48.2	<.015
	D-His, 800 μg/d, IN‡	23	52±18		60.1±30.1	
4	Pretreatment	17	278±56	<.0002	355.8±86.6	<.0001
	D-His, 400 μg/d, IN‡	38	199±70		227.6±86.2	

*D-His indicates ([ImBzl]-D-His⁶,Pro⁹-NET)gonadotropin releasing hormone; SC, subcutaneously; IN, intranasally; and NS, not significant. Values shown are means±SDs. Sequences of courses of treatment are as in Fig 1.

†P values determined by Mann-Whitney U test.

‡Courses of treatment associated with clinical improvement.

Table 3.—Progesterone and Estradiol Plasma Levels During *D-His Administration in Six Women* With Acute Intermittent Porphyria*

Patient	Treatment	Duration, d	No. of Samples	Progesterone, nmol/L	Estradiol, pmol/L
1	D-His, 5 μg/d, SC†	208	37	0.3±0.2 (<0.3-1.0)	261±290 (37-1362)
	D-His, 30 μg/d, IN	141	17	0.3±0.3 (<0.3-1.6)	268±389 (48-1443)
	D-His, 400 μg/d, IN†	439	50	0.3±0.2 (<0.3-1.0)	92±81 (37-503)
	None	56	12	1.9±3.8 (0.6-14.3)	334±466 (29-1634)
	D-His, 400 μg/d, IN†	401	13	1.6±2.5 (<0.3-9.9)	356±356 (70-1035)
2	Pretreatment	86	17	18.4±22.3 (<0.6-61.1)	833±418 (349-1711)
	D-His, 400 μg/d, IN†	798	100	1.0±0.4 (<0.3-2.5)	246±242 (18-1600)
	Posttreatment	221	12	10.2±25.8 (<0.6-91.3)	422±595 (37-1920)
3	Pretreatment	51	24	42.0±9.9 (<0.3-42.0)	253±20 (81±536)
	D-His, 400 μg/d, IN	68	6	1.6±1.0 (<0.6-3.2)	463±290 (173-863)
	D-His, 800 μg/d, IN†	203	15	0.6±0.3 (<0.3-1.6)	312±77 (220-437)
4	Pretreatment	54	16	11.4±21.0 (<0.6-61.7)	664±547 (33-2059)
	D-His, 400 μg/d, IN†	646	48	1.0±0.6 (<0.3-3.2)	198±143 (66-907)
5	Pretreatment	62	18	7.6±10.5 (1.6-39.1)	624±40 (95-1138)
	D-His, 400 μg/d, IN	65	7	5.1±1.9 (2.2-7.3)	488±202 (272-852)
	D-His, 800 μg/d, IN†	66	7	2.9±1.3 (1.6-5.4)	341±77 (239-455)
	D-His, 10 μg/d, SC†	315	43	2.5±1.3 (1.0-5.7)	367±176 (84-742)
6	Pretreatment	11	2	22.6±24.8 (5.1-40.1)	1278±11 (1270-1285)
	D-His, 400 μg/d, IN	196	13	17.2±16.2 (1.6-43.9)	481±231 (191-947)
	D-His, 10 μg/d, SC	208	5	2.9±1.0 (2.2-4.5)	595±294 (151-954)
	D-His, 20 μg/d, SC†	136	4	1.4±0.2 (1.3-1.6)	184±26 (158-220)

*D-His indicates ([ImBzl]-D-His⁶,Pro⁹-NET)gonadotropin releasing hormone; SC, subcutaneously; and IN, intranasally. Values shown are means±SDs with ranges in parentheses. During treatment and posttreatment periods, values shown were obtained after the first 30 days. Sequences of courses of treatment are as in Fig 1.

†Courses of treatment associated with clinical improvement.

Case 4.—A 29-year-old woman had had monthly premenstrual attacks of porphyria for at least 8 months before study. During the two cycles preceding treatment, two peaks in plasma levels of both progesterone (to 62 and 13 nmol/L) and estradiol (to 2059 and 1417 pmol/L) were documented (Table 3). Administration of D-His, 400 μg daily IN, was begun on day 9 of a cycle. During treatment for 21 months, the patient had only three episodes of vaginal bleeding and one associated exacerbation of porphyria (Fig 1). Two additional attacks were not temporally related to menses. During D-His administration, progesterone and estradiol levels were lower than during the pretreatment period (Table 3).

Case 5.—A 28-year-old woman had had frequent premenstrual attacks for 7 months before entry. She also had regional enteritis (previously diagnosed during tubal ligation); her symptoms of mild diarrhea and anorexia did not change during this study. Cyclical changes in progesterone and estradiol were documented during a 2-month period before treatment. An unsatisfactory response to D-His therapy, 400 μg daily IN (initiated on day 2 of a cycle, and associated with three menses and two premenstrual attacks in 2 months), was somewhat improved by increasing the dosage to 800 μg daily (two menses and one premenstrual attack in 2 months; Fig 1). Treatment was then changed to 10 μg daily SC, and during the ensuing 12 months only one premenstrual attack occurred. She had another attack, not related to menses, during this treatment period and had nine episodes of mild vaginal bleeding. Progesterone and estradiol levels were lower after the first month of the D-His, 800 μg daily IN, regimen and after the first month of the D-His, 10 μg daily SC, regimen (Table 3).

Case 6.—A 25-year-old woman had a history of more than 2 years of repeated attacks of porphyria, often requiring hospitalization. The patient also had petit mal epilepsy, occasionally accompanied by grand mal seizures, which had begun at age 11 years and predated the first symptoms of porphyria by 8 years. Seizures were reasonably well controlled with clonazepam and ethosuximide (other antiseizure drugs either exacerbated porphyria or induced allergic reactions in the patient). Porphyric attacks occurred near the time of menstruation. However, the frequency of premenstrual attacks could not be determined precisely because dates of menses and porphyric exacerbations had not been recorded. Some attacks were prolonged and overlapped more than one cycle, menses were irregular, and there were periods of amenorrhea. Therapy with D-His was started 3 months after menses, when there was partial persistence of porphyric symptoms. Treatment with D-His, 400 μg daily IN, for 6 months and then with 10 μg SC daily for 7 months induced little or no reduction in the frequency of premenstrual attacks, and there were frequent episodes of irregular, light vaginal bleeding. However, the frequency of attacks was reduced when the dosage was increased to 20 μg SC daily for 4 months (Fig 1). Progesterone and estradiol levels were lower during the 20-μg SC regimen (Table 3), and vaginal spotting decreased.

COMMENT

The results of this study of six patients confirm previous, more limited reports[6-10] that preventing ovulation by daily administration of a Gn-RH analogue can prevent cyclical attacks of acute intermittent porphyria. During treatment with D-His for as long as 26 months, the patients had substantially fewer menses-associated attacks during optimal treatment compared with pretreatment periods or with periods of suboptimal dosage (Figs 1 and 2). Changes in dosage or route of administration were sometimes needed to obtain a satisfactory response. In four patients (patients 1 through 4; Fig 1) IN administration of D-His was effective, whereas in two others (patients 5 and 6), D-His was more effective when the route was SC (Fig 1). This experience is consistent with reports that IN insufflation is useful for long-term Gn-RH analogue administration, although the extent of absorption is low and more variable (1% to 5% of the dose) than with SC administration.[13,14] Higher initial dosages by either route might have necessitated fewer dosage adjustments but might also have caused more frequent side effects.

The side effects we recorded have been described in other studies with Gn-RH analogues[13-15] and included hot flushes, breast changes, vaginal dryness, and decreased libido. An exception was a worsening in psoriasis in one of our patients, which has not been previously reported, to our knowledge. The side effects were minor compared with previous attacks of porphyria in these patients. Other possible consequences of decreased endogenous estrogen secretion, such as bone demineralization, are being evaluated by others in larger groups of women undergoing treatment with Gn-RH analogues for more common conditions, such as endometriosis.[16]

Our results also suggest that if Gn-RH analogue administration prevents ovulation and resultant progesterone production (as indicated by scant or absent menses and progesterone levels approximately <32 nmol/L), it may not always

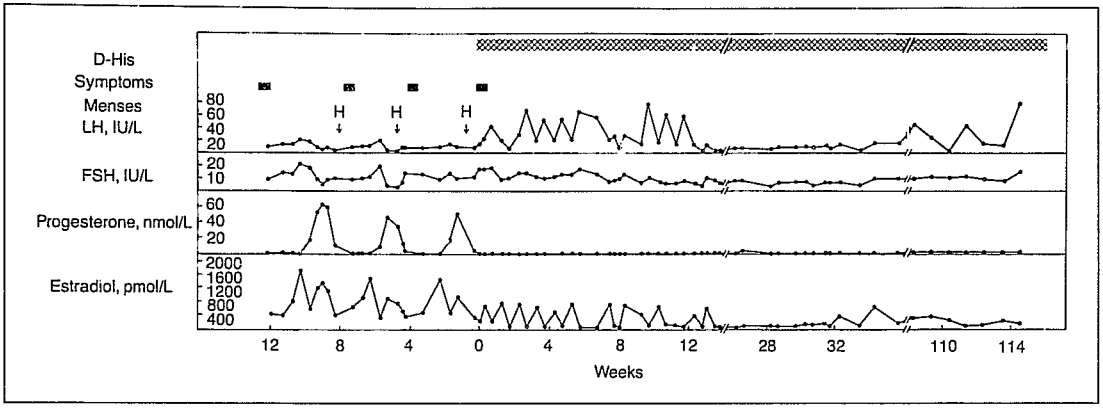


Fig 2.—Premenstrual symptoms of porphyria, menses, and plasma hormone levels for 13 weeks before and during a 115-week course of treatment with ([ImBzl]-D-His^6,Pro^9-NET)gonadotropin releasing hormone (D-His) (400 μg/day intranasally) in patient 2. Prophylactic hematin infusions (H) prevented attacks twice during the 3-month period before D-His treatment was initiated. LH indicates luteinizing hormone; FSH, follicle-stimulating hormone.

be necessary to suppress circulating hormones to castrate levels (ie, for estradiol, approximately <73 pmol/L) to prevent cyclical attacks of porphyria. The degree of suppression of circulating estrogens by Gn-RH analogues has been noted to be dose dependent.[16] Studies in patients with endometriosis also show that substantial clinical improvement may be achieved with dosages that interrupt ovulation but do not reduce estrogens to castrate levels.[16-20] Lower dosages also appear to be associated with fewer menopausal side effects and may induce less depletion of bone calcium.

Porphyrin precursor excretion in this study was reduced by D-His administration but not to normal levels (Table 2). Reduced excretion of δ-aminolevulinic acid and porphobilinogen would be expected with any treatment that reduces the frequency of acute attacks because their excretion increases during exacerbations of acute intermittent porphyria and decreases partially with clinical improvement.[1] In one reported case, a Gn-RH analogue did not normalize porphyrin precursor excretion, although levels were lower during asymptomatic periods than during attacks.[8] In two other cases, during Gn-RH administration urinary δ-aminolevulinic acid and porphobilinogen excretion did not differ significantly from that before treatment (when measured between attacks).[9,10] Thus, marked reduction or normalization in porphyrin precursor excretion is unlikely to occur in patients with acute intermittent porphyria treated with a Gn-RH analogue. This is not surprising, because increased excretion of porphyrin precursors can occur in asymptomatic persons with this disease[1,21] and can remain increased even in women treated with hematin to prevent recurrent premenstrual attacks.[21,22]

The optimal duration of Gn-RH analogue administration, or of prophylactic hematin treatment,[22] is uncertain, partly because the natural course and long-term prognosis of women with cyclical attacks of porphyria have not been clearly described. However, it is clear that only a minority of adults with porphobilinogen deaminase deficiency have symptoms of porphyria, and such symptoms are most likely to develop in the late 20s or early 30s rather than soon after puberty.[1] In our patients, for example, menarche (at 12 to 14 years of age) was not followed immediately by the onset of symptoms (at 19 to 31 years of age). One of our patients no longer had premenstrual attacks after completion of a 26-month course of D-His therapy (patient 2). These observations suggest that susceptibility to cyclical attacks is likely to vary over time and that it may be useful to reassess the need for endocrine manipulation after 2 to 3 years of Gn-RH analogue administration.[23]

The present study, in which ovulation and the cyclical increases in ovarian steroids were suppressed by a Gn-RH analogue, provides further evidence of the important role of reproductive hormones in acute intermittent porphyria. Treatment with D-His seems to be a safe, effective method of reproductive hormone manipulation in this disease. In women with porphyria, Gn-RH analogues might also be safe for other indications, such as birth control. The administration of oral contraceptives, progestins, or androgens, which can also interrupt ovulation and have been beneficial in preventing perimenstrual attacks in some patients,[24,25] have also precipitated attacks of porphyria.[5,25-29] Danazol, a synthetic weak androgenic steroid used in the treatment of endometriosis, is among the exogenous steroids that have exacerbated this disease when used in an attempt to prevent cyclical attacks.[26] Danazol is also a potent inducer of δ-aminolevulinate synthase in the chick embryo liver.[30] In the present study, none of the six patients treated with D-His had more frequent attacks during treatment. Moreover, we have shown that this compound does not accentuate porphyrin formation in the chick embryo liver,[7] which is highly sensitive for the detection of chemicals and drugs that are inducers of heme synthesis and may be harmful in acute intermittent porphyria.[30-34] These clinical and laboratory observations are consistent with the idea that Gn-RH analogues act mainly on the pituitary[13] and have little or no direct influence on metabolic pathways on other tissues, such as the heme biosynthetic pathway in the liver.

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Whereas the previous two papers relate to a GnRH agonist acting at the hypothalamic-pituitary level, the final series of publications are devoted to an evaluation of mifepristone, a synthetic steroid which blocks progesterone and glucocorticoid receptors and acts as a potent antiprogestin and antiglucocorticoid.

DRUG THERAPY

ALASTAIR J.J. WOOD, M.D., *Editor*

MIFEPRISTONE (RU 486) — A MODULATOR OF PROGESTIN AND GLUCOCORTICOID ACTION

IRVING M. SPITZ, M.D., AND C.W. BARDIN, M.D.

ANTIPROGESTINS, agents that inhibit the action of progesterone, are among the most controversial and yet the more interesting therapeutic compounds developed in the past 20 years. These agents provide the most effective and safest means of medical abortion, and in addition they may be used for the treatment of patients with cancer, Cushing's syndrome, and gynecologic disorders and for contraception. The first effective antiprogestin was mifepristone (also known as RU 486),[1] a derivative of the progestin norethindrone (Fig. 1). By analogy with the antiestrogens, it is likely that the substitution at the 11 beta position is responsible for the antiprogestin action of mifepristone.[2,3] Mifepristone also has antiglucocorticoid activity.[4]

PHARMACOLOGY AND METABOLISM

The metabolic clearance rate of mifepristone is 30 liters per day.[5] Because of its slow removal rate from the circulation, mifepristone can be administered in a single oral dose (600 mg) for the termination of pregnancy. Serum drug concentrations increase progressively after oral doses ranging from 50 to 100 mg, but no further increases occur after doses of 100 to 800 mg.[6] In contrast, serum concentrations of the metabolites of mifepristone do increase in a dose-dependent fashion when large doses are given, so that serum metabolite concentrations exceed those of the parent compound.[6] The metabolites have some antiprogestin and antiglucocorticoid properties, indicating that some of the action of mifepristone may be mediated by its metabolites.[5]

ANTIPROGESTATIONAL ACTIVITY

Physiology and Mechanism of Action

Endometrium

The administration of mifepristone to normal women in the early and midluteal phases of the menstrual cycle induces profound changes in endometrial histologic features, and menstrual bleeding invariably ensues within 72 hours.[7-11] These endometrial changes are characterized by retardation of endometrial development, which begins within 12 hours after mifepristone administration,[7] inhibition of glandular secretory activity, accelerated degenerative changes, vascular changes, and increased stromal, but not glandular, mitotic activity.[8] All these changes are consistent with the withdrawal of progesterone. In postmenopausal women (and ovariectomized monkeys) treated with estradiol and progesterone, mifepristone also causes morphologic changes in the endometrium similar to those associated with the withdrawal of progesterone.[12-14] Menstrual bleeding after the administration of mifepristone results from the action of the drug on the endometrium rather than on the hypothalamo–pituitary–ovarian axis.[15-17]

In postmenopausal women (and ovariectomized monkeys) receiving estrogen alone, mifepristone has morphologic and biochemical effects on the endometrium similar to those of progesterone,[12-14] suggesting that in some circumstances mifepristone acts as a progestin agonist. The administration of higher doses blocks the proliferative action of estradiol on the endometrium.[14] This antiestrogenic action of mifepristone is independent of estrogen receptors, since mifepristone does not bind to these receptors.[4,14] Because high doses of progestins have similar antimitogenic effects[18] and lead to endometrial atrophy, this antiproliferative action of mifepristone should also be regarded as agonistic.

These results suggest that mifepristone inhibits the actions of progesterone when the latter is present. This progestin-antagonist action forms the basis of most contraceptive and gynecologic uses of mifepristone. By contrast, in the absence of progesterone, mifepristone acts as a partial progestin agonist. The beneficial actions of antiprogestins on tumor tissue may depend on such progestin-agonist effects.

An attempt to rationalize the diverse effects of mifepristone with those of progesterone is shown in Figure 2. When progesterone binds to its intracellular receptor, the conformation of the receptor is altered, leading to loss of the associated heat-shock proteins and the formation of dimers of hormone–receptor complexes. The progesterone–receptor dimer binds to progesterone-response elements in the promoter region of progesterone-responsive genes and increases their rates of transcription, causing progesterone effects at the cellular and tissue levels. When mifepristone binds to the progesterone receptor, the conformation of the receptor changes in a different way, but the same loss of heat-shock proteins and dimerization both occur.[19,20] The mifepristone–receptor dimer also binds to progesterone-response elements and activates some genes if progesterone is absent. In this respect, mifepristone acts as a partial agonist. When both progesterone and mifepristone are present, the dimers

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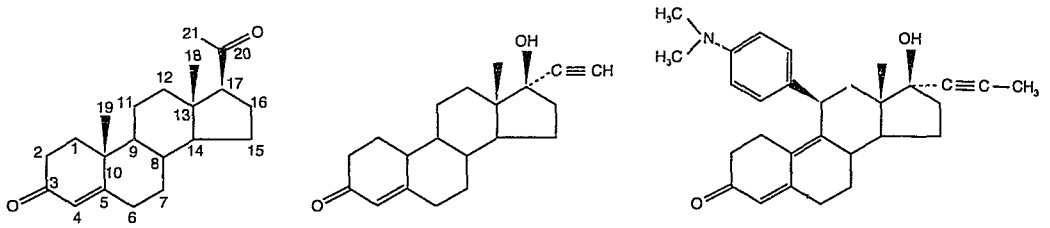


Figure 1. Chemical Structure of Progesterone, Norethindrone (17-Hydroxy-[17α]-19-norpregn-4-en-20-yn-3-one), and Mifepristone (11-[4-(Dimethylamino)phenyl]-17-hydroxy-17-[1-propynyl]-[11β,17β]-estra-4,9-dien-3-one).
Norethindrone is a synthetic 19-nor-testosterone progestin.

that are formed contain a drug–receptor complex and a progestin–receptor complex and are transcriptionally inactive, so that the action of progesterone is antagonized at the cellular and therefore the tissue level. This model is consistent with the diverse biologic effects of mifepristone, but it has not been fully proved.

Gonadotropin and Steroid Secretion

The short-term administration of mifepristone decreases the secretion of luteinizing hormone in both the follicular and the luteal phases of the cycle.[17,21,22] By contrast, long-term administration (i.e., for three months) increases the secretion of luteinizing hormone.[23] Because mifepristone acts on both the hypothalamus and the pituitary[24] and because it has antagonistic as well as agonistic actions, it is not surprising that its effects on gonadotropin secretion are diverse.

Mifepristone inhibits the production of progesterone by human granulosa cells in vitro.[25] Its administration to monkeys in the early follicular phase blocks ovulation unless exogenous gonadotropins are given. Therefore, if there is an important effect on ovarian steroidogenesis, it can be overcome by gonadotropins.[26] Thus, the dominant, if not the only, effect on the ovary is through the drug's effects on the secretion of luteinizing hormone.

Pregnant Uterus

The administration of mifepristone during pregnancy results in the withdrawal of progesterone support to the endometrium, menstrual bleeding, and the disruption of placental function (Fig. 3). Crucial to the process of termination of pregnancy is an increase in prostaglandin action. Myometrial contractile activity is normally regulated by the balance between the inhibitory action of progesterone and the stimulatory action of prostaglandin $F_{2\alpha}$.[28] During pregnancy, myometrial activity is suppressed; during labor, the production of prostaglandin $F_{2\alpha}$ increases, stimulating myometrial contractility. In cultures of decidual cells, antiprogestins stimulate the secretion of prostaglandin $F_{2\alpha}$[29] and reduce its metabolism.[30]

Mifepristone also increases the myometrial response to exogenous prostaglandins. Whereas prostaglandins alone increase uterine tone, the administration of mifepristone several days before that of prostaglandins promotes coordinated contractions, with an increase in amplitude and frequency (Fig. 4).[31] Thus, mifepristone increases the sensitivity of the myometrium to prostaglandins in addition to increasing their synthesis and decreasing their metabolism.[29-31] The increase in uterine contractility after the administration of mifepristone persists in women in whom prostaglandin synthesis is inhibited by the administration of indomethacin,[32] suggesting that mechanisms other than prostaglandins must also be involved in the increase in uterine contractility.

Cervix

Women given mifepristone who had incomplete abortions have marked softening and dilatation of the cervix at the time of uterine evacuation (Fig. 3). The same changes occurred in women treated with mifepristone who underwent surgically induced abortions late in the first trimester and in the second trimester.[33-35] The cervical-softening action of mifepristone is independent of local prostaglandin release.[33] This is a highly beneficial effect of antiprogestins, since it allows easy access to the lumen of the uterus for a variety of procedures.

Clinical Usefulness

Abortion Induced by Mifepristone Alone

The total doses of mifepristone administered to induce abortion have ranged from 140 to 1600 mg, given for a period of one to seven days to women who had amenorrhea for up to nine weeks.[36-41] When mifepristone was used alone, the success rate in women who had amenorrhea for less than seven weeks ranged from 64 to 85 percent,[36-39] no vaginal bleeding occurred in 1 to 10 percent, and 10 to 30 percent had incomplete abortions.[3] The success rate was lower in women who had been pregnant longer. Thus, there were only three successes in nine women who had had amenorrhea for 8 to 10 weeks.[41]

There are several reasons why some women do not respond to mifepristone alone. The dose may be too small, although in one study unresponsiveness did not appear to be related to the dose.[37] Genetic variations in the progesterone receptor could also result in variations in the ability of the receptors to bind mifepristone.[42] There also might be variations in drug metabolism[43]; however, differences in the pharmacokinetics

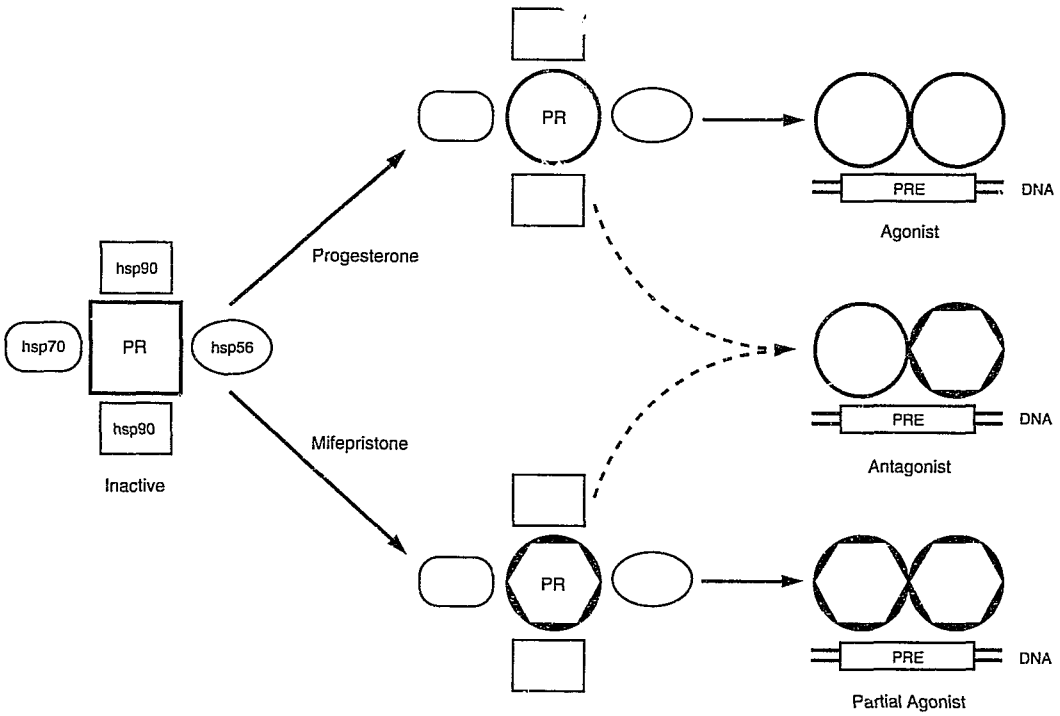


Figure 2. Mechanisms of Action of Progesterone and Mifepristone, Showing Their Agonist and Partial-Agonist Effects When Administered Alone and Their Antagonist Effect When Administered Together.

In the absence of a ligand, the progesterone receptor (PR) is associated with different heat-shock proteins (hsp) and is inactive. Association of the receptor with either progesterone or mifepristone induces different conformational changes in the receptor, both of which result in the dissociation of heat-shock proteins and the dimerization of progesterone–receptor or drug–receptor complexes. Association of the progesterone–receptor dimer with the progesterone-response elements (PRE) in the promoter regions of progesterone-responsive genes activates the transcription of these genes, resulting in agonist effects on cells and tissues (right, top). In the presence of both progesterone and mifepristone, the dimers contain both a progesterone–receptor complex and a drug–receptor complex. This dimer (right, middle) is transcriptionally inactive, and the effect of progesterone is antagonized. In the absence of progesterone, however, mifepristone can form a complex with the progesterone receptor that dimerizes and is transcriptionally active on some genes, with partial-agonist effects (right, bottom).[19,20] Alternatively, differential interaction of receptor dimers with other transcription factors and DNA response elements or activation by other ligands could account for these varied responses to antiprogestins.

of mifepristone and its metabolites and in the serum concentrations of the α1-acid glycoprotein that binds mifepristone[3] have not been detected in women who did not respond to the drug.[44] Finally, and most likely, the lack of response could be due to an inadequate increase either in endogenous accumulation of prostaglandin $F_{2\alpha}$ or in uterine contractility, resulting in insufficient uterine contractions to expel the fetus.[31]

Abortion Induced by Mifepristone plus Prostaglandins

The combination of mifepristone (usually in a single 600-mg dose) with a prostaglandin given 48 hours later by intramuscular injection (sulprostone),[45] by vaginal suppository (gemeprost),[45-51] or orally (misoprostol)[52,53] has resulted in a rate of complete abortion approaching 100 percent.

More than 120,000 women in more than 20 countries have now received mifepristone together with a prostaglandin, resulting in the termination of 92.7 to 99.0 percent of the pregnancies (Ulmann A: personal communication). The combination remained highly effective in women who had amenorrhea for up to nine weeks.[46]

In cross-sectional studies, the administration of larger doses of prostaglandin was associated with more abdominal pain and vaginal bleeding.[49] Despite the side effects, 88 percent of women in one series responded affirmatively when asked if they would choose the method again to terminate a pregnancy.[48] In another study, an attempt was made to relate the amount of blood loss and the dose of prostaglandin. In women given 600 mg of mifepristone followed by 0.5 or 1 mg of gemeprost,[47] the median blood loss was 74 ml, with no difference in blood loss between the doses and no difference as compared with mifepristone alone, vaginal gemeprost alone, or vacuum aspiration.[47]

The effect of vacuum extraction was compared with that of mifepristone and gemeprost, administered either alone or in combination, in women who had

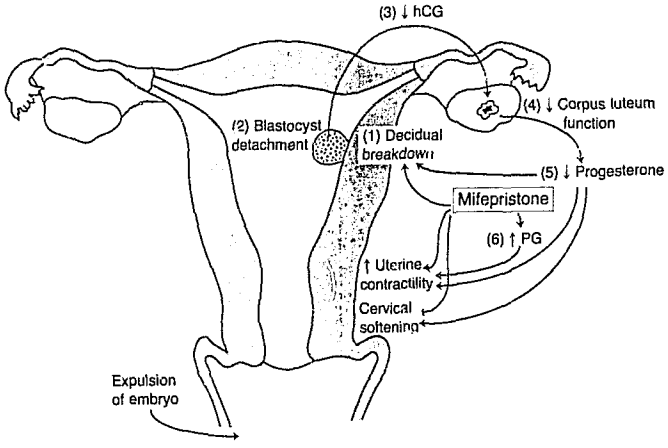


Figure 3. Action of Mifepristone in the Uterus of a Pregnant Woman.

Initially, mifepristone promotes decidual breakdown, leading to detachment of the blastocyst. With the resulting decrease in secretion of human chorionic gonadotropin (hCG), there is a decrease in progesterone secretion by the corpus luteum. The decrease in progesterone secretion further accentuates decidual breakdown. Mifepristone also stimulates the accumulation of prostaglandin (PG) and enhances uterine sensitivity to prostaglandin, disturbing the balance between prostaglandin and progesterone and thereby increasing uterine contractility. An independent action of mifepristone results in cervical softening, which facilitates expulsion of the blastocyst. The direct effects of mifepristone on syncytiotrophoblast cells[27] and ovarian granulosa cells[25] to reduce the secretion of hCG and progesterone, respectively, are probably of little clinical importance.

amenorrhea for up to eight weeks.[50] The dose of gemeprost when mifepristone was also used was one fifth of the dose when gemeprost was administered alone. In this small study of 97 women, the incidence of complete abortion with vacuum extraction, gemeprost alone, and the combination of gemeprost and mifepristone was 96 percent, 97 percent, and 95 percent, respectively, as compared with 60 percent in the women who received mifepristone alone. The frequency of side effects and of the need for analgesic drugs was lower in the women who received mifepristone and gemeprost than in those who received gemeprost alone. In another study,[51] 1 to 3 mg of gemeprost alone was compared with the combination of mifepristone and 1 mg of gemeprost in 301 women. The incidence of complete abortion was significantly higher with the combination (98 percent vs. 87 percent), and side effects were fewer.

Contraception

The administration of 200 to 800 mg of mifepristone after a dominant follicle has been demonstrated by ultrasonography inhibits the surge of luteinizing hormone as well as further follicular growth and ovulation. Thereafter, follicular growth resumes and ovulation occurs.[10,21,54,55] Continuous administration of mifepristone in a dose of 2 mg per day (but not 1 mg) for 30 days inhibits ovulation and delays menstruation.[56,57] More prolonged administration results in low serum estradiol concentrations; the periodic addition of a progestin (norethindrone) leads to secretory transformation of the endometrium.[58] This regimen produces well-controlled bleeding but does not always block ovulation. Similarly, the administration of mifepristone once weekly does not consistently inhibit ovulation.[59]

An alternative approach to the use of mifepristone as a contraceptive is based on the fact that it has a greater effect on the endometrium than on the pituitary.[8] The administration of 10 mg of mifepristone five and eight days after the surge of luteinizing hormone results in the impaired development of a secretory endometrium (endometrial desynchronization) without disturbing the hormonal events of the menstrual cycle.[60] The administration of a single 200-mg dose on the second day after the midcycle surge of luteinizing hormone also retards the development of a secretory endometrium but does not alter cycle length or serum concentrations of follicle-stimulating hormone, estradiol, and progesterone.[7] Preliminary results indicate that the administration of mifepristone at this time does prevent pregnancy.[61] For this approach to be effective, however, a simple method of detecting the midcycle surge in the secretion of luteinizing hormone is required.

Another way in which mifepristone could be used as a contraceptive is through its administration each month during the late luteal phase to induce menses, whether or not pregnancy has occurred.[10,11,15,17,62] Clinical trials were conducted in women who had had unprotected intercourse and were given mifepristone at the end of the luteal phase. Serum concentrations of the beta subunit of chorionic gonadotropin were determined serially to document the number of women who conceived.[63-67] The failure rate in these studies, expressed as the number of continuing pregnancies in relation to the number of proved pregnancies, ranged from 17 to 19 percent. These results are similar to the percentage of failures that occur when mifepristone alone is given to terminate pregnancies in women with amenorrhea of less than seven weeks' duration, thereby implying that responsiveness is determined at the earliest stage of gestation. In addition to the low efficacy, there was dissatisfaction with the method because it disrupted the menstrual rhythm[63] and caused a failure to bleed after anovulatory cycles.[67] Because of these limitations, mifepristone cannot now be recommended for regular use as a contraceptive.

Mifepristone has also been used as a postcoital contraceptive within 72 hours of unprotected intercourse.[68,69] In a randomized study, 402 women received a single dose of 600 mg of mifepristone, and 398

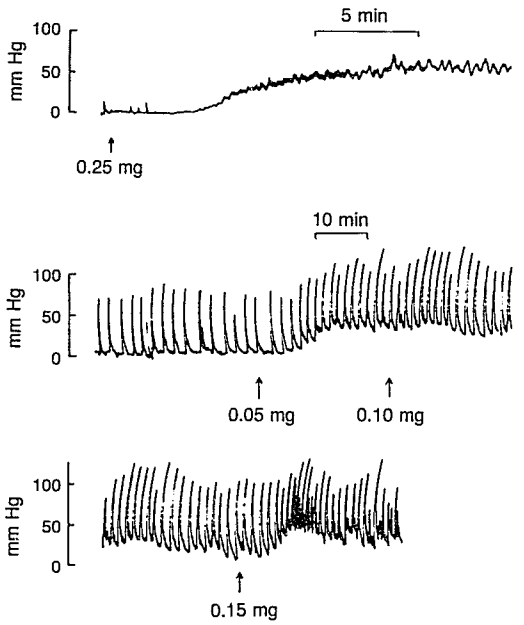


Figure 4. Tracings of Uterine Contractility and the Response to Sulprostone.

The upper tracing, obtained in the basal state, shows almost no spontaneous uterine activity. After the intramuscular administration of sulprostone (0.25 mg), there was a slow increase in uterine tone, with irregular contractions of low amplitude. The middle and lower tracings were obtained in another woman who had received 25 mg of mifepristone for four days. After this treatment, there were spontaneous regular uterine contractions. After the administration of 0.05, 0.10, and 0.15 mg of sulprostone, there was an increase in the frequency and amplitude of contractions. As compared with the upper tracing, there was increased sensitivity to sulprostone. Reprinted from Bygdeman and Swahn[51] with the permission of the publisher.

women received 100 μg of ethinyl estradiol and 1 mg of norgestrel given twice 12 hours apart. None of the women who received mifepristone became pregnant, as compared with four of those who received the other therapy,[68] and the frequency of side effects, including nausea, vomiting, headache, and breast tenderness, was lower in the mifepristone group. In addition, mifepristone may remain effective longer after intercourse than the combination of estrogen and progesterone, which is effective for only 72 hours.[63-65,68,69]

Cervical Dilatation

Because of its marked effects on cervical dilatation and myometrial contractility, mifepristone is useful for the preoperative preparation of women for surgical abortion late in the first trimester. It is as effective as prostaglandins, with fewer side effects.[34,35,70] In women undergoing second-trimester surgical abortion, pretreatment with mifepristone softens the cervix and reduces the interval between the administration of prostaglandin and the expulsion of the uterine contents.[71] The effect on the cervix is maintained at a time when the endometrium has lost its responsiveness. In view of these promising results, mifepristone followed by prostaglandin could become the method of choice for preparing women for the surgical termination of second-trimester pregnancies.

Induction of Labor

Mifepristone has been used to induce labor after intrauterine fetal death.[72] This response suggests that fetal death restores the sensitivity of the uterus to mifepristone.

Another proposed use is for the induction of labor at the end of the third trimester. In a randomized, double-blind study of women at term, 50 percent of those who received mifepristone had spontaneous labor, as compared with 25 percent of those who received placebo.[73] The use of mifepristone at term prompted concern about its effect on the infant, since it crosses the fetal–placental barrier.[74-76] No untoward effects have been observed to date, but further studies are needed to establish possible risks and benefits.

Other Gynecologic Indications

Mifepristone has been administered to menstruating women with endometriosis for up to three months. Pelvic pain improved in all the women, but there was no change in the extent of the disease as determined by follow-up laparoscopy.[23] When mifepristone therapy was used in women with uterine leiomyomas, there was a 49 percent reduction in tumor volume after three months.[77]

Breast Cancer

In view of the antiproliferative activity of mifepristone on the endometrium, its effect on breast tumors that have progesterone receptors has been explored. Mifepristone has different dose-dependent effects on several human breast cancers cultured in vitro, both mimicking and antagonizing the action of progestins.[78,79] Such results emphasize that the drug has both partial agonist and antagonist actions (Fig. 2). In animals with breast cancer, combined treatment with mifepristone and an antiestrogen or a gonadotropin-releasing–hormone agonist produced high rates of tumor remission.[80] Preliminary clinical trials suggest that some women with breast cancer may respond to mifepristone.[80,81]

Meningioma

Most meningiomas have no estrogen receptors but have substantial concentrations of progesterone receptors.[82] In one trial of 14 patients with unresectable meningiomas, 5 had an objective response and 3 had subjective improvement.[83] Further trials to document the usefulness of mifepristone for the treatment of meningioma should be undertaken.

Antiglucocorticoid Activity

Physiology

In addition to its antiprogesterone properties, mifepristone has antiglucocorticoid activity in vivo and in vitro.[4,84] In humans, it blocks the feedback effect of cortisol on corticotropin secretion in a dose-dependent

fashion.[11,39,40,85] A single dose of 1 to 2 mg per kilogram of body weight had no effect, but a dose of 4 to 6 mg per kilogram led to an increase in serum corticotropin and cortisol[85] that was particularly evident during the early morning hours, when the concentrations normally are increasing. Thus, the administration of mifepristone (6 mg per kilogram) at midnight augmented the increase in serum corticotropin and cortisol during the subsequent morning, but not when the same dose was given at 10 a.m.[85] Although long-term administration of mifepristone causes persistent elevations in serum concentrations of corticotropin and cortisol, the response to corticotropin-releasing hormone is unchanged and the diurnal rhythm of corticotropin and cortisol is maintained,[23,86] implying that the central regulatory mechanisms remain intact. Some patients receiving 4 to 10 mg per kilogram per day have had weakness, nausea, and vomiting that were attributed to cortisol deficiency and that responded to dexamethasone,[80,83,86,87] but they did not have more objective signs of cortisol deficiency. Since measurements of serum cortisol cannot be used to establish the diagnosis of functional hypocortisolism during receptor blockade, clinicians will have to resort to seemingly antiquated manifestations of cortisol deficiency, such as eosinophilia, hypoglycemia, and inability to excrete a water load, to determine whether hypocortisolism is present. It is clear that most patients with a normal pituitary–adrenal axis compensate for glucocorticoid-receptor blockade by increasing the secretion of corticotropin and cortisol. An important point is that higher doses of mifepristone are needed to produce an antiglucocorticoid effect, as opposed to an antiprogestin effect.[11,85]

Antiglucocorticoid Application

Treatment with mifepristone ameliorates the clinical manifestations of hypercortisolism in over 50 percent of patients with Cushing's syndrome caused by ectopic corticotropin secretion or adrenocortical carcinoma.[88,89] By contrast, in patients with Cushing's disease, in whom corticotropin release is sensitive to the reduced secretion or action of cortisol, mifepristone is not effective. Its use in these patients will increase the secretion of corticotropin and therefore of cortisol, obviating any effect of blockade of glucocorticoid receptors.

On the basis of studies in animals, other applications for the antiglucocorticoid effects of mifepristone have been suggested. These include the local application of eye drops containing mifepristone in order to lower intraocular pressure in patients with glaucoma[90] and oral administration of the drug to prevent progression of viral disease in humans.[91]

Untoward Effects

The side effects of long-term high-dose administration of mifepristone in rats and monkeys result from the antihormonal properties of the drug.[92] Few women who receive single doses of mifepristone to interrupt pregnancy have any side effects. When such effects do occur, they include heavy bleeding, nausea, vomiting, abdominal pain, and fatigue.[36-40] It is often difficult to dissociate many of these symptoms from those that result from normal pregnancy and spontaneous abortion.

The long-term administration of mifepristone in doses of 100 to 200 mg daily is generally well tolerated; the most common side effect is fatigue, which develops in the majority of subjects.[83] Nausea, anorexia, and vomiting may also occur.[80,83,86] Other side effects reported during long-term administration include a slight decrease in the serum potassium concentration, weight loss, cessation of menses in premenopausal women, intermittent hot flashes, transient thinning of the hair, development of Hashimoto's thyroiditis, and an occasional decrease in libido and gynecomastia in men.[80,81,83,89] The latter is presumably caused by the binding of mifepristone to androgen receptors.[4]

Because a few women do not abort and instead continue with their pregnancies after the administration of mifepristone and prostaglandin, it was important to determine whether these agents have teratogenic effects. No such effects were observed in monkeys and rats receiving mifepristone.[92,93] Rabbits, however, had skull deformities that were attributed to mechanical effects due to uterine contractions that resulted from the decrease in progesterone activity.[94] Unlike mifepristone, prostaglandins have been reported to be teratogenic.[95,96] There are isolated case reports of normal pregnancies and offspring when women have taken mifepristone alone or in combination with a prostaglandin, have not aborted, and have elected to continue their pregnancies.[97,98] One woman's pregnancy was terminated at 18 weeks because ultrasonography revealed that the fetus had multiple severe congenital defects not thought to have been caused by mifepristone.[98] Nonetheless, at the current state of knowledge, women who do not abort after the administration of mifepristone plus a prostaglandin should be warned about possible teratogenic effects and should be offered surgical abortion.

There have been three instances of cardiovascular complications in women given a single dose of mifepristone and sulprostone, including a fatal myocardial infarction. All three women were smokers.[99] As a consequence, an intramuscular prostaglandin should be used cautiously in women with heart disease and is not recommended in women over the age of 35 years or in regular smokers. Misoprostol, a stable prostaglandin E_1 analogue used to prevent gastric ulcer, is an effective substitute.[52,53]

It is evident that most of the side effects associated with the medical methods of terminating pregnancy are related to the high dose of prostaglandin. There are fewer side effects with the mifepristone–prostaglandin combination than with prostaglandin alone.[50,51] The comparison of mifepristone plus prostaglandin with surgical abortion is complicated by the fact that the safety of the surgery depends on the skill of the operator and the availability of backup facilities. Thus, complications vary widely from clinic to clinic. The best U.S. data suggest that the death rate per 100,000 surgical abortions performed in wom-

en with pregnancies of less than eight weeks is 0.4 (95 percent confidence interval, 0.2 to 0.7).[100] Since there has been only one death from treatment with mifepristone and prostaglandin, there is no difference between this method and surgical abortion performed under the safest conditions. In India, where the death rate associated with surgical abortion is 10 times higher,[100] the combination of mifepristone and prostaglandin would be much safer.

Conclusions

Approximately 55 million pregnancies are terminated each year by abortion. In many countries where abortions are illegal, numerous women still seek assistance from untrained persons in terminating their pregnancies. Even in countries where abortion is legal, these services are not always readily available because of the shortage of trained personnel and facilities. Consequently, abortion is performed in less than ideal situations that result in levels of maternal mortality and morbidity approaching those in countries where abortion is illegal. In developing countries where abortion is legal but not widely available, mifepristone could provide women their only option for safe abortion.

In France, the combination of mifepristone plus a prostaglandin has been approved for ending pregnancies of up to 49 days in duration. One third of women now choose this approach over the standard surgical procedures (Ulmann A: personal communication). The percentage of women choosing medical abortion in developing countries is likely to be much higher. The use of mifepristone plus an oral prostaglandin, presumably with fewer side effects, will probably improve the acceptability of this method for early first-trimester abortion.

Mifepristone has also been approved in France for the induction of labor in the event of fetal death and as an adjunct to a prostaglandin for the therapeutic termination of pregnancies in the first or second trimester. Indeed, adequate clinical studies have demonstrated the safety and effectiveness of the drug for these indications, and these studies support applications to regulatory authorities in other countries. The evidence indicates that mifepristone can also be used for postcoital contraception and for the treatment of patients with meningioma or Cushing's syndrome that is due to ectopic corticotropin production or adrenal carcinoma. The evidence also suggests that mifepristone could be used as a contraceptive, and preliminary results suggest that it may be helpful in patients with breast cancer, endometriosis, and uterine leiomyomas, but clinical trials will be required to confirm these suggestions.

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ANTIPROGESTINS: Mechanism of Action and Contraceptive Potential

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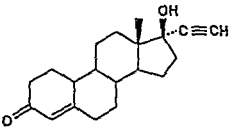
Ann Robbins
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KEY WORDS: mifepristone, onapristone, progesterone, estradiol, receptors

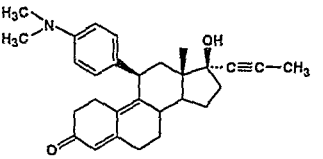
ABSTRACT

Antiprogestins are characterized by substitutions at the 11β and 17α positions of the steroid ring system and bind strongly to both progesterone and glucocorticoid receptors. Although they function predominantly as antiprogestins and antiglucocorticoids, on occasion they display progestin agonistic and even antiestrogenic properties. The most common clinical use of the antiprogestin mifepristone is to induce a medical abortion in the early stages of pregnancy. Progesterone maintains the endometrium, transforming it from a proliferative to a secretory state. It also facilitates the luteinizing hormone surge, which initiates ovulation. As a consequence, antiprogestins may also have contraceptive potential. Although antiprogestins do delay ovulation, this effect is inconsistent unless high doses are given, and under these circumstances, the antiprogestin effect is associated with unopposed estrogen action on the endometrium. Very low doses of antiprogestins do not affect hormonal secretion or ovulation or alter bleeding patterns, but they do have contraceptive potential by inducing profound alterations in endometrial morphology. Mifepristone is also a very effective and safe postcoital agent. This new class of pharmacological agents has numerous other gynecological and obstetrical indications, such as endometriosis, uterine myoma, and expulsion of the fetus in the case of fetal death in utero. Antiprogestins may also be used in the treatment of steroid-dependent tumors. There are also therapeutic implications consequent to their antiglucocorticoid properties.

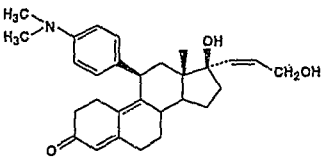
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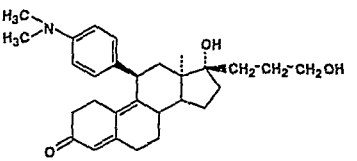
NORETHINDRONE



MIFEPRISTONE (RU 486)

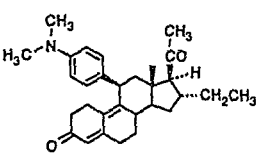


LILOPRISTONE (ZK 98734)

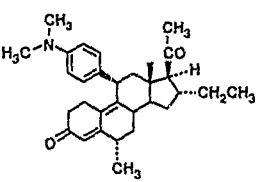


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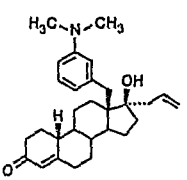
Figure 1 Structural formulae of norethindrone, mifepristone (RU 486), lilopristone (ZK 98734), and onapristone (ZK 98299).



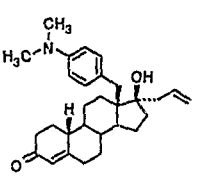
RTI 3021-022



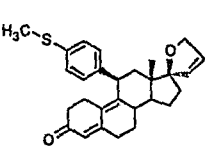
RTI 3021-020



ORG 31167



ORG 31343



RU 46556

Figure 2 Structural formulae of RTI 3021-022, RTI 3021-020, ORG 31167, ORG 31343, and RU 46556.

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ANTIPROGESTINS: Mechanism of Action and Contraceptive Potential

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ABSTRACT

Antiprogestins are characterized by substitutions at the 11β and 17α positions of the steroid ring system and bind strongly to both progesterone and glucocorticoid receptors. Although they function predominantly as antiprogestins and antiglucocorticoids, on occasion they display progestin agonistic and even antiestrogenic properties. The most common clinical use of the antiprogestin mifepristone is to induce a medical abortion in the early stages of pregnancy. Progesterone maintains the endometrium, transforming it from a proliferative to a secretory state. It also facilitates the luteinizing hormone surge, which initiates ovulation. As a consequence, antiprogestins may also have contraceptive potential. Although antiprogestins do delay ovulation, this effect is inconsistent unless high doses are given, and under these circumstances, the antiprogestin effect is associated with unopposed estrogen action on the endometrium. Very low doses of antiprogestins do not affect hormonal secretion or ovulation or alter bleeding patterns, but they do have contraceptive potential by inducing profound alterations in endometrial morphology. Mifepristone is also a very effective and safe postcoital agent. This new class of pharmacological agents has numerous other gynecological and obstetrical indications, such as endometriosis, uterine myoma, and expulsion of the fetus in the case of fetal death in utero. Antiprogestins may also be used in the treatment of steroid-dependent tumors. There are also therapeutic implications consequent to their antiglucocorticoid properties.

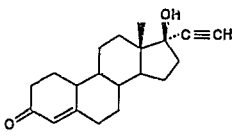
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Progesterone plays a critical role in mammalian reproduction in that it is essential for the initiation and maintenance of pregnancy. After the discovery of the progesterone receptor in 1970 (1), researchers realized that a progesterone receptor antagonist would have a major impact on female reproductive health. The search for such an antiprogesterone extended over more than a decade. In 1981, Philibert, Deraedt, and Teutsch from the French pharmaceutical company, Roussel Uclaf, reported on a newly synthesized glucocorticoid receptor antagonist known as RU 38486 (2). It soon became evident that this antiglucocorticoid also displayed marked antiprogestin activity. RU 38486 was subsequently abbreviated to RU 486 and is now currently known by the generic name mifepristone. The original studies showed that mifepristone had a relative binding affinity five times that of progesterone and three times that of dexamethasone at their respective receptors. When compared to testosterone, it had a relative binding affinity of 25% for the androgen receptor but did not bind to either estrogen or mineralocorticoid receptors (3).

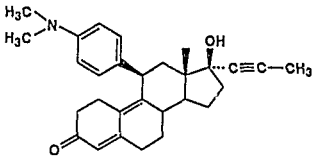
CHEMISTRY

The chemical name of mifepristone is 11-[4-(dimethylamino)phenyl-17-hydroxy-17-(1-propynyl)-(11β,17β)-estra-4,9-dien-3-one. It is a derivative of norethindrone (Figure 1) and possesses an additional (4-dimethylamino)phenyl group at the 11β position and 1-propynyl chain at the 17α position. The conjugated C9-C10 double bond in mifepristone should also be noted (Figure 1). Since the initial report of mifepristone, over 400 additional antiprogestins have been synthesized (4). The 17α substitution is responsible for promoting higher binding affinity to the receptor (5). The vast majority of these antagonists possess a substituent at the 11β position. It is likely that the 11β substituent is responsible for the antagonistic action (5). Cook et al (6) have developed a series of 11β-substituted compounds such as RTI 3021-022 and RTI 3021-020 with 16α-ethyl-17β-acetyl substitutions in the D ring (Figure 2). In their test system, RTI 3021-022 and RTI 3021-020 act as potent progestins and possess no antagonistic activity (6). In addition, scientists at Organon, in the Netherlands, have developed antiprogestins such as ORG 31167 and ORG 31343 (Figure 2) in which the dimethylaminophenyl group is situated at C18 (7). Thus both the nature and position of substitutions in the steroid structure appear to be critical for the antagonistic activity. Although a selective antiprogestin that does not bind to glucocorticoid receptors (GRs) has not been reported, recently, a number of compounds were described with minimum antiglucocorticoid activity (8). One such example is RU 46556 (Figure 2).

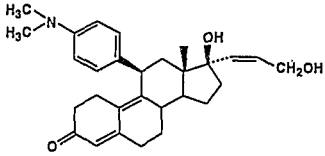
In the past, all of the clinical studies reported were conducted with mifepristone (5, 9–12), but recently results of a few studies in humans have been published with onapristone (ZK 98299) (13) and lilopristone (ZK 98734) (14),



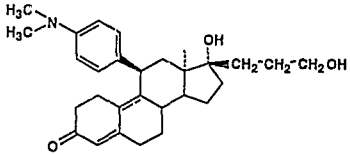
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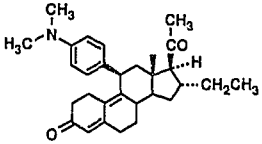


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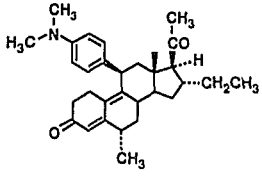


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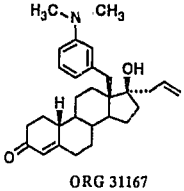
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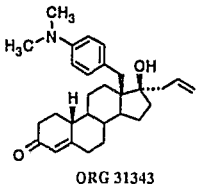
RTI 3021-022



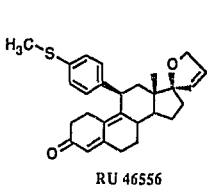
RTI 3021-020



ORG 31167



ORG 31343



RU 46556

Figure 2 Structural formulae of RTI 3021-022, RTI 3021-020, ORG 31167, ORG 31343, and RU 46556.

antiprogestins synthesized at Schering AG Berlin. Both of these are structurally similar to mifepristone (15). Lilopristone has a [Z]-configuration in the 3-hydroxy-1-propenyl side chain at the 17α position (Figure 1). The two-dimensional structure of onapristone is similar to mifepristone and lilopristone (Figure 1), but onapristone has a different molecular shape due to configurational inversions at the C13 and C17 positions (15).

MECHANISM OF ACTION

The actions of progesterone and antiprogestins in target tissues are mediated by the progesterone receptor (PR), which belongs to a family of nuclear receptors. This family includes receptors not only for the steroid hormones (glucocorticoid, mineralocorticoid, androgen, estrogen, and vitamin D) but also for the thyroid hormones and the retinoids. These receptors are ligand-activated transcription factors with domains for DNA binding, hormone binding, and transactivation (16).

The antiprogestin mifepristone does not bind to the chicken or hamster PRs. A glycine in the hormone binding domain of the human PR at position 722 (Gly^{722}) and at the comparable position of the PR of most other species is critical for mifepristone binding and action (17). Due to a cysteine residue at this position in the hamster and chicken PR (17), these receptors bind progesterone but not mifepristone. As a consequence, these species are insensitive to this antagonist (5). Substitution of this cysteine by glycine converts the hormone-binding domain of the chicken PR to one that binds mifepristone and facilitates its antagonistic action (17). Substitution of Gly^{722} with cysteine in the human PR generates a receptor that behaves like the chicken and hamster PRs. The human GR also has a glycine in this corresponding position, and introduction of a cysteine substitution at this position in the human GR resulted in a loss of binding not only to mifepristone but also to dexamethasone. Mifepristone also displays some binding to the androgen receptor, which also has a glycine in this corresponding position. Neither the estradiol nor the mineralocorticoid receptors have a glycine in this position, and antiprogestins do not bind to these receptors. Because glycine is the only amino acid without a side chain, these results suggest that Gly^{722} in the human PR is at a critical position, and the presence of amino acid side chains in this position may sterically impede mifepristone binding (17). These observations have important clinical implications because genetic mutations in the PR in humans may result in variations in the ability of the receptor to bind to antiprogestins. This may explain why some women are nonresponsive to mifepristone (10).

The precise molecular mechanisms whereby progestins and antiprogestins produce agonist or antagonist activities via the PR are currently unknown but are under active evaluation (18, 19). Both progestins and antiprogestins pro-

duce a dramatic change in conformation of the PR that is associated with transforming (or activating) PR from a non-DNA-binding form to a form that will bind to DNA (18, 19). This transformation is accompanied by a loss of associated heat shock proteins and dimerization. The 42–amino acid sequence at the extreme C-terminal region of the hormone-binding domain is required for the receptor to bind to progesterone; antiprogestins on the other hand, bind to a site situated further toward the N-terminal region of the hormone-binding domain (20). The activated progestin-receptor dimer binds to progesterone response elements in the promoter region of progesterone-responsive genes and, in the presence of other nuclear transcription factors, increases the rate of transcription of these genes producing agonist effects at the cellular and tissue levels. The steps occurring subsequent to DNA binding are detailed elsewhere (18, 19, 21, 22).

Some researchers have suggested that there are two types or classes of progesterone antagonists (23, 24). With the first type, such as mifepristone, the antiprogestin-receptor complex binds to progesterone response elements, but these DNA-bound receptors are transcriptionally inactive (23, 24). The C-terminal region of the hormone-binding domain of the PR contains an inhibitory function that silences receptor transactivation if the agonist is absent or if an antagonist is present (19, 20). With the second type of antagonist, which includes onapristone and some other 13α-configurated progesterone antagonists, the antiprogestin-receptor complex fails to bind to progesterone response elements (23–29). Some workers deny the existence of different types of antagonists and maintain that the PR complexed with onapristone also binds to progesterone response elements but with a 10-fold lower binding affinity than that of mifepristone (30). A more recent study has suggested that under in vivo conditions no PR-antiprogestin complexes bind to progesterone response elements (31).

These receptor antagonists use different mechanisms to produce antiprogestin and antiglucocorticoid activity. Sucrose gradient experiments have demonstrated that when mifepristone binds to the GR, it stabilizes the GR–heat shock protein complex (which sediments at 8S), thereby reducing the amount of GR converted from an 8S to a 4S form. That part of the mifepristone receptor complex converted to the 4S form does bind to the glucocorticoid response element with the same affinity as the nuclear transformed glucocorticoid agonist-receptor complex, but unlike the latter, it fails to induce transcription. This indicates that the antiglucocorticoid activity occurs at two levels: prevention of complete GR transformation and alteration of a step subsequent to DNA binding (32).

Although antiprogestins function predominantly as progestin antagonists, under certain circumstances both in vivo and in vitro, they may display agonistic actions (33–36). The precise molecular mechanisms underlying these ob-

servations are unknown but may be related to the fact that the progesterone receptor exists as two separate isoforms: a PR-A isoform and a PR-B isoform. The structural configurations of both isoforms are similar, although the PR-B isoform contains an N-terminal fragment of 164 amino acids, which is absent from the PR-A isoform. Both isoforms may arise as a result of alternative initiation of translation from the same mRNA or by transcription from alternate promoters within the same gene (37, 38). When analyzed in reconstituted progesterone response systems in heterologous cells, both isoforms display similar DNA- and hormone-binding affinities but they have different promoter specificities (38, 39). Two isoforms of PR have been identified in most species, with the exception of the rabbit where PR exists only as a B isoform (20).

A close relationship exists between the second-messenger signal transduction pathways and the steroid receptors. This results in cross talk between these two systems (25, 28, 29, 40). In the presence of cGMP and cAMP, both progesterone and mifepristone (but not onapristone) activate transcription with the PR-B isoform (26). This cross talk stimulates the PR-mifepristone complex to interact in a more efficient way with the transcriptional machinery. The end result is *trans*-activation by the PR-B isoform. Although the precise mechanism for this is unknown, it could be due to a direct modification of the receptor or it may occur indirectly through alterations of an adaptor protein or other transcription factor involved in PR-mediated *trans*-activation (40). This cross talk between the second messenger and the PR-B isoform signal transduction pathways overrides the antagonistic effects of mifepristone and is one possible explanation for its agonistic action (40). Under these in vitro conditions, mifepristone does not cause any enhancement of transcription with the PR-A isoform (26).

The two PR isoforms themselves have variable effects on transcription even in the absence of cAMP. In experiments where expression vectors that encoded exclusively for PR-A or PR-B were transiently transfected into cells together with progesterone-responsive mouse mammary tumor virus reporters, in the presence of progesterone and the progesterone antagonists mifepristone and onapristone, activation of transcription always occurred with PR-B but less frequently with PR-A (22, 27, 41). In promoter and cell contexts where PR-A was inactive, it acted as a potent transdominant repressor of PR-B–mediated transcription (27, 41). When both isoforms were transfected into the same cell, the effect of PR-A dominated, and there was failure of transcription (41). These results imply that an antagonist may display agonistic actions depending on which PR isoform predominates.

Cell transfection studies have also demonstrated that PR-A but not PR-B is capable of inhibiting glucocorticoid, androgen, estrogen, and mineralocorticoid receptor–mediated gene transcription (41–43). This implies that the PR-A isoform has a specific role in steroid receptor–mediated transcription. In all

these receptor systems, this transdominant effect of PR-A was also induced by antiprogestins and certain progestins. This PR-A inhibition of transcription is not a generalized phenomenon observed with all receptors of this steroid receptor superfamily because vitamin D receptor transcription is not inhibited (41, 42). The inhibition of estrogen-mediated gene transcription (43) may explain the antiestrogenic activity of antiprogestins, which has been described as noncompetitive (34, 44) because antiprogestins do not bind to estrogen receptors (3). Thus PR-A has a central role in regulation of the transcriptional activity of steroid hormone receptors by functioning as a hormone-dependent transdominant inhibitory protein. Thus PR-A determines the pharmacological response to both progestins and antiprogestins (45, 46).

The modulating effect of the PR-A isoform on transcription suggests a possible mechanism whereby cells can generate dissimilar responses to a single hormone. These in vitro studies indicate that depending on which PR isoform is predominant, antiprogestins may demonstrate progestin antagonistic, agonistic, or antiestrogenic effects. The expression levels of the A and B isoforms may differ with respect to each other in different target tissues (41, 46). Recently it was shown that the ratio of the A and B isoforms vary in the follicular and luteal phases of the menstrual cycle and that estrogens selectively induce an increase in tissue concentration of the B isoform (47, 48). In addition, Brandon et al have shown that the expression of both PR isoforms is elevated in human uterine leimyomas relative to the adjacent myometrium (49).

CLINICAL APPLICATIONS

One of the major effects of progesterone is to maintain pregnancy. It also promotes uterine quiescence by inhibiting myometrial contractions. This is the physiological basis for the use of antip ·gestins as medical abortifacients. The initial trial conducted by Hermann et al (50) showed that mifepristone successfully terminated pregnancy in 9 of 11 subjects with amenorrhea of less than 6–8 weeks duration. Since this landmark study, numerous other reports have been published demonstrating the abortifacient effect of antiprogestins (5, 9–12, 14, 51). Regardless of the dose or duration of mifepristone or lilopristone administration, the success rate for complete abortion usually ranges from 64 to 85% when an antiprogestin is administered to women with pregnancies of less than 49 days (5, 9–12, 14, 50, 51).

The efficacy rate can be significantly improved by the addition of an exogenous prostaglandin. Prostaglandins act to enhance uterine contractions. Bygdeman & Swahn have shown that mifepristone sensitizes the myometrium to exogenous prostaglandins (52). In addition, antiprogestins increase endometrial prostaglandin concentrations by inhibiting prostaglandin dehydrogenase, the progesterone-dependent enzyme that metabolizes the active prosta-

glandins PGE_2 and $PGF_{2\alpha}$. As a consequence, these active prostaglandins accumulate (53, 54). Antiprogestins have also been shown to increase the myometrial response to prostaglandins (52). An increase in gap junctions is believed to be one of the major mechanisms for this enhanced myometrial response. Progesterone-induced relaxation of muscle cells is associated with hyperpolarization of the cell membrane, prevention of calcium ion influx, and suppression of cell coupling by gap junctions. When antiprogestins are administered, myometrial cell excitability increases in association with establishment of gap junctions between cells and influx of calcium ions. This results in coordinated uterine contractions (55, 56).

It has been well documented that prostaglandins (such as oral misoprostol or the vaginal pessary gemeprost) administered 36–48 h after mifepristone provide an effective method of medical termination of pregnancy. In women with amenorrhea of under 49 days duration, the success rate reaches 95% (57–59), and this new method has been approved for use in France, Sweden, Great Britain, and China. A multicenter Phase 3 study using mifepristone (600 mg) followed in 48 h by misoprostol (400 μg) is currently under way in the United States in pregnant women with amenorrhea of up to 63 days. The aim is to register this combination in the United States, thus making this medical method of pregnancy termination available to American women.

Antiprogestins have numerous other obstetrical and gynecological applications. In addition to their ability to enhance myometrial contractility, antiprogestins also dilate and soften the uterine cervix. The available data indicates that antiprogestins do not act on the cervix by stimulating endogenous prostaglandin production (60, 61). Rather it is believed that this process originates from inflammatory cells and chemotactic agents such as cytokines [e.g. interleukin-8 (IL-8), IL-1β] (61). Indeed Kelly et al have shown that progesterone inhibits and mifepristone stimulates IL-8 release in human choriodecidual cells in vitro (62). Because of its action on the uterine cervix, antiprogestins are useful in the preoperative preparation of women for first trimester vacuum aspiration. Mifepristone is usually administered 48 h prior to surgical abortion, is as effective as prostaglandins, and has significantly fewer side effects than prostaglandins (63). In second trimester abortions, pretreatment with mifepristone reduced the interval between prostaglandin administration and expulsion. Furthermore, the dose of prostaglandin required was reduced, and the women experienced considerably less pain (64). Pretreatment with mifepristone also reduces the force required to dilate and soften the cervix, particularly in nonpregnant women (65). This could prove to be useful for planned outpatient procedures such as insertion or removal of an IUD, hysterography, dilatation and curettage, or any other procedures requiring access to the uterine lumen (11).

Mifepristone is very effective in inducing labor following intrauterine fetal death, and it is used routinely in France for this purpose (66). Mifepristone

has also been used to induce labor at the end of the third trimester (67). However, because this agent crosses the fetal placental barrier (68), further studies are required to ensure that no untoward effects are observed on the fetus.

As already noted above, in addition to their antagonistic actions, antiprogestins may on occasion also display agonistic or antiestrogenic effects. Thus, antiprogestins may have a role in the treatment of estrogen-dependent gynecological disorders such as endometriosis and uterine fibromyoma (69–72). In endometriosis there was an improvement in pelvic pain and a decrease in the extent of disease, as determined by laparoscopy (70). With uterine fibromyoma, myoma volume decreased significantly after two months of treatment (69–72).

In light of their diverse effects, antiprogestins have also been proposed in the treatment of tumors that contain steroid receptors. Two preliminary nonconclusive clinical trials have been reported in advanced breast carcinoma (73, 74); thus further controlled studies are required to determine whether antiprogestins might be useful therapeutic agents of this condition. Meningiomas contain progesterone receptors, and antiprogestins inhibited growth of meningioma cells in culture (75). Antiprogestins also reduced the size of a human meningioma implanted into nude mice (76). In one clinical trial, mifepristone (200 mg) was given daily for up to 62 months to a total of 28 patients with unresectable meningiomas. Eight subjects demonstrated objective responses as shown by reduced tumor size on computerized tomography (CT) or magnetic resonsance imaging (MRI) scanning and improvement in visual field examination (77). A randomized double-blind placebo-controlled Phase 3 trial is currently underway to confirm the activity of mifepristone in unresectable meningioma (77). Studies in animals have suggested that antiprogestins could be used in other tumors such as endometrial cancer and gliomas (12, 78).

Mifepristone has also been used to treat Cushing syndrome due to ectopic adrenocorticotropic hormone (ACTH) secretion. High-dose, prolonged mifepristone administration has been shown to normalize the Cushinoid phenotype, ameliorate depression, decrease hypertension, eliminate abnormal carbohydrate metabolism, and correct glucocorticoid-induced gonadal and thyroid hormone suppression (79, 80). However, this drug cannot be used in Cushing's disease where the hypothalamic-pituitary-adrenal axis is intact but regulated at a higher set point. Under these circumstances the mifepristone-induced increase in ACTH and cortisol secretion may overcome the glucocorticoid receptor blockade. Other situations in which the antiglucocorticoid properties of this class of compound may prove useful include local application in eye drops to lower intraoccular pressure in glaucoma (81) and in the prevention of the progression of viral diseases in humans (82). There is also some evidence

for their use in the treatment of burns, certain forms of hypertension, depression, arthritis, and cataracts (12, 82–84).

Thus this new class of compounds has numerous proven and possible clinical applications. Another important therapeutic approach of antiprogestins relates to their use as possible contraceptive agents. This is reviewed in detail below.

CONTRACEPTIVE POTENTIAL

In addition to its ability to maintain endometrial integrity after implantation of the embryo, progesterone acts at several discrete anatomical sites to regulate a number of steps in the female reproductive process that precede implantation. Thus, besides disrupting an ongoing implantation, blockade of progesterone action may conceivably prevent pregnancy by other mechanisms. Rather than reviewing all known progesterone actions, the discussion focuses only on those that have been blocked by antiprogestins in women.

The best-known functions of circulating progesterone before implantation include the following: (*a*) facilitation and reinforcement of the positive feedback of estrogen on the luteinizing hormone (LH) surge near the end of the follicular phase, (*b*) synergism with estradiol during the luteal phase to maintain negative feedback control on gonadotropin secretion, (*c*) transformation of the endometrium from a proliferative to a secretory state for reception of the fertilized egg, and (*d*) maintenance of endometrial integrity. From a theoretical standpoint, antagonizing some or all of these actio of progesterone could result in a contraceptive effect. Antiprogestins could thus function as contraceptive agents by (*a*) inhibiting ovulation, thereby preventing fertilization; (*b*) interfering with the secretory transformation of the endometrium, rendering it nonreceptive for implantation, and (*c*) inducing shedding of the endometrium and dislodging the implanting embryo. All these potential contraceptive strategies are reviewed below.

Inhibition of Ovulation

Administration of antiprogestins during the follicular phase of the menstrual cycle disrupts development of the leading follicle (85–89). This suggests that the presence of the low levels of circulating progesterone typical of the follicular phase or of the high levels of intrafollicular progesterone or both are essential for the growth of the dominant follicle, the last stage in the development of a preovulatory follicle in primates. There are two ways by which antiprogestins prevent ovulation: firstly, by interfering with the growth of the dominant follicle, the so-called antifolliculotropic or folliculostatic effect; and secondly, by inhibiting the LH surge. This antifolliculotropic effect was suspected when investigators observed that administration of large doses of mife-

pristone (25–100 mg/day) during the mid to late follicular phase prolonged the follicular phase and delayed the LH surge. This occurred in association with lower estradiol levels than were normally observed at corresponding times of the normal cycle (86–89). Ultrasonography clearly documented cessation of growth of the dominant follicle (86, 89).

This delay in the LH surge consequent to antiprogestin administration was also evident with considerably lower doses of mifepristone and was observed following 1 mg given for 10 days during the follicular phase after the dominant follicle had achieved a size of 14–16 mm (90). Five mg of mifepristone administered for up to three days in the follicular phase impaired follicular development, as documented by ultrasound, and was associated with a reduction in serum estradiol and inhibin levels (91). This folliculostatic effect of antiprogestins is exquisitely sensitive and a single 5-mg dose of mifepristone transiently arrests follicular growth (Figure 3), an effect that is evident 12 h after pill ingestion (91). The antifolliculotropic activity is also displayed by onapristone (13), and it has also been observed with mifepristone, onapristone, and lilopristone in nonhuman primates (92, 93). This antifolliculotropic effect of antiprogestins is not evident during the earliest part of the menstrual cycle. Thus, administration of mifepristone during the first three days of the follicular phase, at a time when no dominant follicle is present, has no effect on follicular

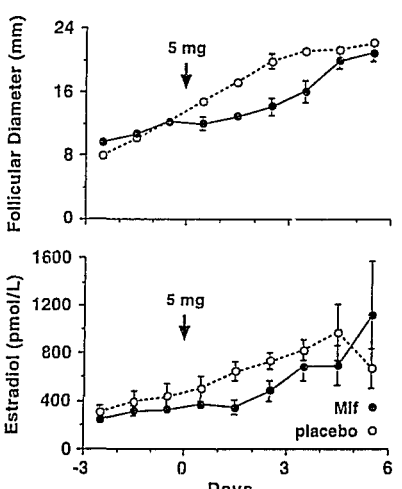


Figure 3 Effect of mifepristone (5 mg) on follicular diameter (as determined by ultrasound) and serum estradiol levels.

growth and fails to alter subsequent events of the cycle (94). This effect of antiprogestins is best demonstrated when the dominant follicle reaches a diameter of 12 mm. This suggests that growth beyond 12 mm is associated with functional changes in the follicle, such as acquisition of progesterone receptors by granulosa cells of the dominant follicle as it approaches maturity (91, 95).

Continuous administration of 5 or 10 mg of mifepristone per day throughout one menstrual cycle prevented the leading follicle from achieving maturity and from producing adequate circulatory estradiol levels necessary to trigger the LH surge (Figure 4) (96, 97). With lower doses, e.g. 1 or 2 mg/day, full follicular growth and normal estrogen levels were attained in several women, but normal ovulation failed to occur in many instances during treatment (96, 97). With these low doses, the occurrence of unruptured luteinized follicles

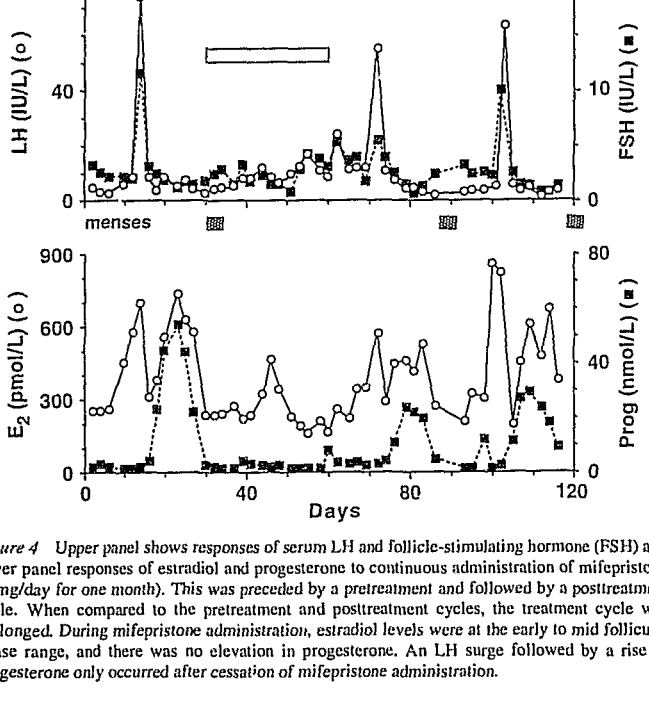


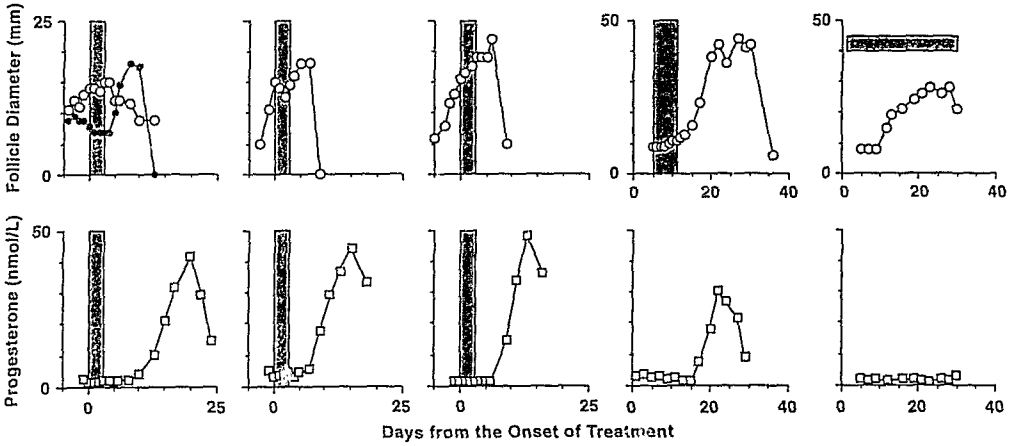
Figure 4 Upper panel shows responses of serum LH and follicle-stimulating hormone (FSH) and lower panel responses of estradiol and progesterone to continuous administration of mifepristone (5 mg/day for one month). This was preceded by a pretreatment and followed by a posttreatment cycle. When compared to the pretreatment and posttreatment cycles, the treatment cycle was prolonged. During mifepristone administration, estradiol levels were at the early to mid follicular phase range, and there was no elevation in progesterone. An LH surge followed by a rise of progesterone only occurred after cessation of mifepristone administration.

was inferred by elevation of serum progesterone without evidence of ovulation on ultrasound (Figure 5) (97). Thus, with high doses of mifepristone, ovulation suppression during treatment can be accounted for primarily by the absence of estradiol positive feedback. Even though adequate estrogen signaling does occur with lower doses, ovulation still fails due to either an inadequate LH surge or an inadequate follicular responsiveness (97).

After cessation of mifepristone administration at doses that arrest follicular growth, there is either resumption of growth by the same arrested follicle (13) or new follicular recruitment (Figure 5) (86). In either case, an estrogen surge evoking a gonadotropin discharge occurs that is followed by a normal or inadequate luteal phase (85, 96, 97). As a consequence, the cycle is lengthened, owing to prolongation of the follicular phase. Bleeding fails to occur until luteolysis takes place (86, 97). Thus, for that particular intermenstrual interval, ovulation is delayed rather than suppressed. When mifepristone is administered in the follicular phase in moderate (10 mg/day) or high (100 mg/day) doses, the delay in the LH surge is independent of the amount of mifepristone administered and is also uninfluenced by the duration of antiprogestin administration (89). The time interval from ingestion of the last pill until the LH surge remained a remarkably constant 12.3 ± 3.0 days (mean ± SD), which approximates the duration of the normal follicular phase (89). When low mifepristone doses (1, 2, or 5 mg/day) were used, the interval from the last pill to the LH surge tended to be 6 to 10 days shorter than a normal follicular phase of the pretreatment cycle. This is probably because with higher doses a new follicle must be recruited more often than with the lower doses, when the existing follicle can resume its growth (86, 97, 98).

The mechanism of the antifolliculotropic effect remains to be elucidated. It could represent an effect of antiprogestins at the hypothalamic-pituitary level, because animal studies demonstrate that antiprogestins bind to PR located in brain areas that control reproductive processes (99). Alternatively, it could represent a primary effect on the ovary. In addition, antiprogestins may operate at both levels.

Several in vitro studies suggest that antiprogestins can act directly on ovarian cells, although this has not been confirmed in all studies (100). Mifepristone in doses of 4–40 ng/ml suppressed progesterone production from cultured human granulosa cells (101). High concentrations of mifepristone (100 μg/ml) are required to inhibit LH-induced stimulation of progesterone from cultured human granulosa cells. This suggests that mifepristone probably acts directly on steroidogenic enzymes rather than by a receptor-mediated response (102). Other studies utilizing an isolated rat ovary model have also shown that mifepristone inhibits ovulation in the rat by acting directly at the level of the ovary. However, this effect was also only observed with high, nonphysiological doses (103). The inhibition of ovulation induced by antiprogestins in monkeys



igure 5 Variable responses of dominant follicle to antiprogestin administration in five individual subjects. Upper panels show follicle diameter as determined by ultrasound, and lower panels show serum progesterone levels. Horizontal bars indicate antiprogestin treatment. The first three subjects received mifepristone 5 mg daily for 3 days; the fourth subject, onapristone 5 mg daily for 7 days; and the final subject, mifepristone 1 mg daily for 30 days. The first subject showed cessation of growth of the dominant follicle (*open circles*). Ovulation occurred when a new follicle was recruited from the opposite ovary (*closed circles*). In the second subject, there was temporary cessation of follicular growth during mifepristone administration followed by regrowth with ultimate ovulation. In the third subject, mifepristone failed to retard follicle growth. In the fourth subject, following cessation of onapristone, the follicle resumed rapid growth and achieved a size of 45 mm. There was no ovulation, but the rise of progesterone indicates that this was a luteinized follicle. The final subject also demonstrated continual growth of the follicle, but there was no rise in progesterone.

can be overcome by exogenous gonadotropins (93, 104), suggesting that if follicular responsiveness is affected by antiprogestins, the combined action of endogenous plus exogenous gonadotropins is able to overcome this effect.

Other observations are more in line with an antiprogestin effect operating at the hypothalamic or pituitary levels. One report (105) describes an acute decrease in plasma gonadotropin levels following administration of a single oral dose of mifepristone (10 or 100 mg) in the mid or late follicular phase. The decrease in mean LH was consequent to a reduction in amplitude without any change in frequency of the LH pulses and was evident only in those women with high estradiol concentrations. However, most authors fail to observe a decrease in immunoreactive gonadotropin levels during the follicular phase associated with the antifolliculotropic effect of mifepristone in women (86, 94). In addition, recent studies show that circulating bioactive LH levels are also unaltered (91). On the other hand, during the mid luteal phase, mifepristone decreased mean LH secretion and LH pulse amplitude and blunted the pituitary response to gonadotropin hormone-releasing hormone (GnRH). In the late luteal phase, both LH pulse frequency and amplitude decreased (106).

In contrast to these short-term studies that demonstrate an inhibitory effect of antiprogestins on gonadotropin secretion, long-term mifepristone treatment (50 to 100 mg/day for three months) to women with endometriosis or uterine myoma transiently increased mean LH levels and LH pulse amplitude but not LH pulse frequency (69, 71, 72). In view of its antagonistic and agonistic properties, it is not surprising that antiprogestins exert diverse effects in different conditions. At the moment, neither a direct action of the antiprogestin on the follicle rendering it less responsive to FSH nor an effect on the pituitary resulting in the secretion of a less bioactive FSH molecule can be excluded as the mechanism underlying the folliculostatic effect (91).

Antiprogestins may also directly inhibit the LH surge normally elicited by high endogenous estrogen levels (85, 96–98). Furthermore, mifepristone is able to block the gonadotropin surge elicited by exogenous estradiol. This blockade of the estradiol-induced positive feedback on the LH surge has been demonstrated in two studies (107; HB Croxatto, in preparation). Inhibition of the LH surge most likely results from an action of the antiprogestin exerted directly at the hypothalamic-pituitary level. Some in vitro studies (108) indicate that mifepristone inhibits LH and FSH secretion by acting directly at the pituitary. In a rat pituitary cell culture system primed with estradiol, mifepristone inhibited GnRH-induced LH and FSH secretion in a dose-dependent manner. This inhibition was antagonized by the addition of progesterone (108). On the other hand, by using rat pituitary cells obtained without regard to the estrous cycle, Rojas et al demonstrated that mifepristone had no effect on GnRH-induced gonadotropin secretion (100). There is also some evidence that antiprogestins may act directly at the level of the hypothalamus. In rats,

progesterone induces an increase in GnRH mRNA levels, an effect that is blocked by antiprogestins (109). From the results of studies conducted in women with hypothalamic amenorrhea receiving exogenous GnRH, it would appear that the dominant inhibitory effect of antiprogestins resides at the level of the pituitary. This is because the delay in LH surge occasioned by mifepristone persisted despite exogenous GnRH replacement (110).

In view of its ability to arrest follicular development and inhibit the LH surge, attempts have been made to develop antiprogestins as contraceptive agents that act by blocking ovulation. To be successful, any potential regimen must be given at repeated intervals every 8 to 10 days to prevent a dominant follicle from becoming a preovulatory follicle. Alternatively, the antiprogestin could be administered continuously. Both these possibilities have been evaluated.

INTERMITTENT ADMINISTRATION This type of regimen has been used to suppress ovulation in monkeys, and a clear dose-response effect emerged (111). Weekly oral administration of 25 mg of mifepristone blocked the expected mid-cycle LH and FSH surge, and progesterone concentrations remained low. Progesterone inhibition however was not complete when half the dose of the mifepristone was used (111). We have evaluated a similar regimen in women (112). Mifepristone given in a dose of 10 mg or 50 mg at weekly intervals for five weeks could not consistently inhibit the LH surge and ovulation, and no dose-response effect was observed. In a further study, 3 subjects received 50 mg for 3 successive days repeated at 10-day intervals for a total of 80 days. In two of these subjects ovulation was inhibited; however, in one subject there was an LH surge during the course of treatment, with elevation of progesterone levels compatible with ovulation (Figure 6). The possibility of failure of ingestion of the tablets was excluded because circulatory mifepristone concentrations were detected in the serum (Figure 6). From these studies it was concluded that in humans, unlike in monkeys, there was no consistent inhibition of the LH surge and ovulation from intermittent mifepristone administration.

CONTINUOUS ADMINISTRATION Croxatto and colleagues have carried out studies using continuous daily administration of 1, 5, or 10 mg of mifepristone to volunteers for one month (97). With the two higher doses, there was no evidence of ovulation, as indicated by ultrasonography and failure of progesterone levels to increase during treatment: Other workers have also observed that continuous administration of 2 or 5 mg of mifepristone daily for one month suppresses ovulation (96), whereas 1 mg daily fails to do so consistently (97, 98).

During both intermittent and continuous administration of mifepristone,

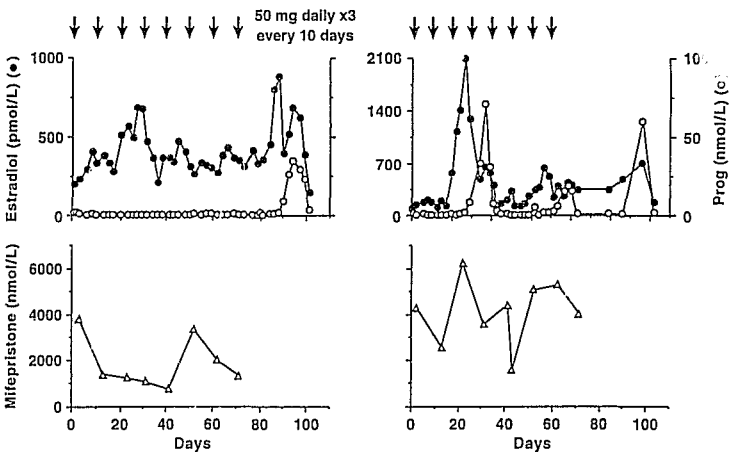


Figure 6 Upper panels show serum estradiol and progesterone, and lower panels show serum mifepristone levels in response to intermittent mifepristone administration (50 mg for 3 consecutive days, repeated every 10 days for 80 days). During treatment in the subject on the left, ovulation did not occur, and progesterone levels were low. Estradiol levels were at the early to mid follicular phase range. The subject depicted on the right demonstrated an LH surge with ovulation and corpus luteum function.

although progesterone levels were low in those subjects who did not ovulate, estradiol concentrations were in the range of the early or mid follicular phase (96, 97, 112). This raises the possibility of potential adverse effects of unopposed estrogen levels on the endometrium.

Considerable controversy exists in the literature regarding the estrogenic or antiestrogenic effects of antiprogestins on the endometrium. In estradiol- plus progesterone-treated ovariectomized monkeys, mifepristone behaved as a classical progestin antagonist by preventing the transformation of the endometrium to a secretory pattern (34, 35, 44). By contrast, in estradiol-treated ovariectomized monkeys not receiving progesterone, doses of mifepristone of 1 mg/kg per day acted as a progestin agonist and induced endometrial secretory transformation; a higher dose (5 mg/kg per day) however was antiestrogenic and inhibited both endometrial proliferation and secretory activity (34, 44). Slayden & Brenner (113) administered mifepristone alone or with progesterone after two weeks of estradiol treatment to ovariectomized rhesus monkeys. Treatment with mifepristone without progesterone resulted in an antiproliferative effect, with a marked reduction in endometrial wet weight and endometrial thickness.

There was some mitotic activity in the glandular epithelium, with a marked increase in apoptosis and significant stromal compaction (113). Chwalisz and coworkers reported similar antiestrogenic effects in onapristone-treated rabbits (114). Because antiprogestins do not bind to estrogen receptors (3), this has been described as a noncompetitive antiestrogenic effect (34, 44). This effect is possibly due to suppression of estrogen receptor (ER)–mediated transcription by mifepristone occupied PR-A, as previously discussed.

In contrast, other animal studies have failed to document an antiestrogenic effect during antiprogestin administration. Histological evidence of estrogenic stimulation of the endometrium has been observed in intact adult rats receiving long-term onapristone administration. This was interpreted as an effect of unopposed endogenous estrogen (115). Bigsby & Young (116) observed that onapristone exhibited mild estrogenic activity and acted as a weak estrogen agonist in the immature ovariectomized rodent uterus. This was attributed to a direct low binding affinity reaction of onapristone with the estrogen receptor. In addition, the effect of onapristone was antagonized by tamoxifen (116). However, recent unpublished studies in ovariectomized and ovariectomized plus adrenalectomized rats indicate that neither onapristone nor mifepristone exhibits estrogenic activity in the uterus and vagina in vivo (61).

There is only minimum information in the literature on possible estrogenic or antiestrogenic effects of antiprogestins in humans. In estradiol- plus progesterone-treated postmenopausal women, mifepristone acted as a classical progesterone antagonist. However, in these same postmenopausal women receiving estrogen replacement without progesterone, mifepristone produced progestone agonistic effects, including induction of secretory changes, increased estradiol dehydrogenase, and reduced DNA polymerase activity—effects that were also produced when progesterone was administered to estrogen-treated women (36).

In a study of 15 women with endometriosis or myoma treated with mifepristone (50 mg a day for six months) serum estradiol levels were in the range of the early follicular phase, and the endometrial morphology was dyssynchronous. The data support the hypothesis that mifepristone could act as a weak progestin agonist in the relative absence of progesterone. The endometrial morphology was more reminiscent of an unopposed estrogen effect and there was no suggestion of an antiestrogenic effect (72, 117). On the other hand, there was also no unequivocal evidence of endometrial hyperplasia, probably due to low endogenous estrogen levels. There have been some isolated reports of unopposed estrogen effects with endometrial hyperplasia in premenopausal women receiving long-term high-dose mifepristone (200 mg daily) for the treatment of inoperable meningioma (SM Grunberg, unpublished data); however, this observation requires systematic study. As discussed below, no evidence of endometrial hyperplasia was reported following low doses of mife-

pristone (1 mg daily for 5 months) (HB Croxatto & L Kovacs, unpublished data). In view of the conflicting and unresolved effect of antiprogestins on the endometrium and until further studies are forthcoming, the wisdom of ovulation inhibition as a strategy for contraception with antiprogestins must be questioned.

ADDITION OF EXOGENOUS PROGESTINS An attempt has been made to obviate the potential effect of unopposed estrogen activity on the endometrium by the addition of an exogenous progestin. This would also ensure the occurrence of regular withdrawal bleeding. Many different regimens have been used and all are associated with normal bleeding patterns. However, in many subjects LH surges were evident during progestin administration, and they were frequently followed by an increase in progesterone that was compatible with normal corpus luteum activity (118, 119). On occasion, the increases in progesterone observed during these cycles were less than those in control subjects, and serial ultrasonography did not always document the occurrence of ovulation (118, 119). This implies luteinization of an unruptured follicle. This LH surge appears to be induced by progestins because Batista et al (90) demonstrated that intramuscular progesterone triggered an LH surge in women in whom the follicular phase had been prolonged by low-dose mifepristone administration. The addition of progestin to an antiprogestin regimen reduces the effectiveness of ovulation inhibition below the level where it can be recommended as a contraceptive method.

Effect of Antiprogestins on Endometrial Development

Several observations suggest that the endometrium is more easily disrupted by antiprogestins than are the hormonal events of the menstrual cycle. Batista and coworkers (98) treated 11 normally cycling women with mifepristone (1 mg daily) for one month in a randomized double-blind study. In this study there was retardation of endometrial maturation without any change in gonadal steroid production. However, even with this low dose, the mid cycle LH surge was delayed, and the follicular phase prolonged by 1 to 11 days in 9 of these women (98). Croxatto et al (97) have demonstrated that when 1 mg of mifepristone was administered daily for one month to five normal women, endometrial histology was disturbed in all subjects. Suppression of ovulation only occurred in one of the five subjects and the hormonal profile was suggestive of ovulation in the remaining four (97). Ishwad et al (120) showed that in bonnet monkeys given weekly onapristone, low doses (5–10 mg) impaired endometrial development without altering cycle length. A dose of 20 mg significantly prolonged the length of the treatment cycle (120).

These findings have been confirmed by other studies in humans (121, 122).

A single dose of mifepristone (10 mg) administered five and eight days after the LH surge disrupted endometrial maturation producing a marked assynchrony between glandular and stromal elements. Stromal edema was reduced, and glandular development was delayed, whereas the bleeding patterns and hormonal events of the cycle remained undisturbed (123).

These observations suggest an alternative approach to contraception. Endometrial and stromal synchrony are essential for successful implantation. Antiprogestins could function as contraceptives by delaying endometrial development, making it impossible for the endometrium to sustain implantation of the blastocyst. Navot et al (124) studied women with ovarian failure treated with sequential estrogen and progesterone replacement therapy administered in a pattern to mimic the natural 28-day cycle. This therapy was to ensure an endometrial milieu suitable for embryo implantation. The temporal window of endometrial receptivity was measured by replacing 2- to 12-cell embryos between days 16 and 24 of these hormonally and histologically defined idealized cycles. Only transfers on days 17 to 19 resulted in conception (124). This suggests that there is a critical time period during which the endometrium is receptive for successful implantation. This is known as the implantation window.

Studies in animals lend support to the role of a temporally defined implantation window. In pseudopregnant rabbits in which ovulation is induced by hCG, the administration of onapristone and estradiol retards endometrial development. The transfer of day 4 donor blastocysts into these recipient rabbits on day 12, following hCG administration, resulted in normal implantation and embryonic development (125). Thus the antiprogestin treatment delayed endometrial development. Transfer of day 4 donor blastocysts to untreated recipients on day 6 failed to result in any successful implantations (126). The delay in endometrial development consequent to antiprogestins would preclude successful implantation of the normally fertilized ovum.

That this strategy has contraceptive potential has been shown in studies conducted in both monkeys and humans. Ghosh & Sengupta (127) administered mifepristone in a single dose of 2 mg/kg or 10 mg/kg two days after ovulation for two to three consecutive cycles to rhesus monkeys. There were no pregnancies in 18 treatment cycles compared to 4 out of 5 pregnancies in control cycles. Studies in cycling bonnet monkeys have shown that low-dose onapristone administration (2.5 or 5 mg every third day by subcutaneous injection) had little effect on the menstrual cycle and estradiol and progesterone concentrations. Four animals treated with 2.5 mg for 17 consecutive cycles and another receiving 5 mg for 21 cycles did not conceive. This treatment failed in one additional monkey with a short pretreatment and treatment cycle, and there was conception in the first treatment cycle. Each of the five controls receiving vehicle alone became pregnant within the first, second, or third cycle.

In all treated animals, endometrial growth and development was retarded and out of phase (128).

Similar observations have also been made in women. Swahn et al (122) administered a single 200 mg dose of mifepristone on the second day after the LH surge and noted that this resulted in endometrial retardation that was evident 12 h after mifepristone, becoming more prominent after 36 and 84 h. This regimen did not alter the length of the cycle or serum FSH or progesterone levels. When the strategy was used as the only contraceptive method in 21 women for a total of 169 treatment cycles for up to 12 months, only one clinical pregnancy resulted (129). For this approach to receive wide application, however, a simple method of detecting the LH surge is required.

Instead of a single administration of antiprogestin, this method could be simplified by a continuous low-dose regimen. This avenue has been explored in a two-center study (HB Croxatto & L Kovacs, unpublished data). A daily dose of 1 mg mifepristone was given continuously for five months. Assessment of ovarian function during treatment revealed two types of response. Nearly one half of the cycles were ovulatory (Figure 7) with or without prolongation of the follicular phase but with normal luteal phase progesterone levels. However the endometrial morphology assessed on the seventh to ninth day after the LH surge demonstrated delayed or irregular development, indicating again that the endometrium is more sensitive than the ovary to the action of mifepristone. The remaining subjects presented monophasic cycles (Figure 8) with normal or low serum estradiol levels, absence of ovulation, and no luteal phase. These subjects remained amenorrheic during treatment. Despite continuous exposure to unopposed estrogen and no endometrial shedding for a period of five months, endometrial hyperplasia was not observed.

These results indicate that mifepristone in a dose of 1 mg per day is still too high to attain a selective endometrial effect in the majority of women. Further studies need to be conducted to determine a practical approach to administer antiprogestins in doses low enough to alter endometrial maturation but without affecting hormonal patterns or events in the menstrual cycle. Presumably such an approach would not result in unopposed estrogen effects on the endometrium.

Several endometrial factors or markers appear to be essential for the implantation process, and their measurements may give an indication of implantation (130, 131). Such markers should be specific to the endometrium, have known functions, be unaffected by embryonic signaling, and have specific probes available to detect their presence. These markers should be maximally present during the window of implantation (132). Potential markers include the $\alpha v\beta 3$ vitronectin receptor, which is an integrin (133), or one of the cytokines, which include leukemia inhibiting factor (LIF) (134), colony stimulating factor (CSF-1) (135), and interleukin-1 (IL-1) (136), although there are nu-

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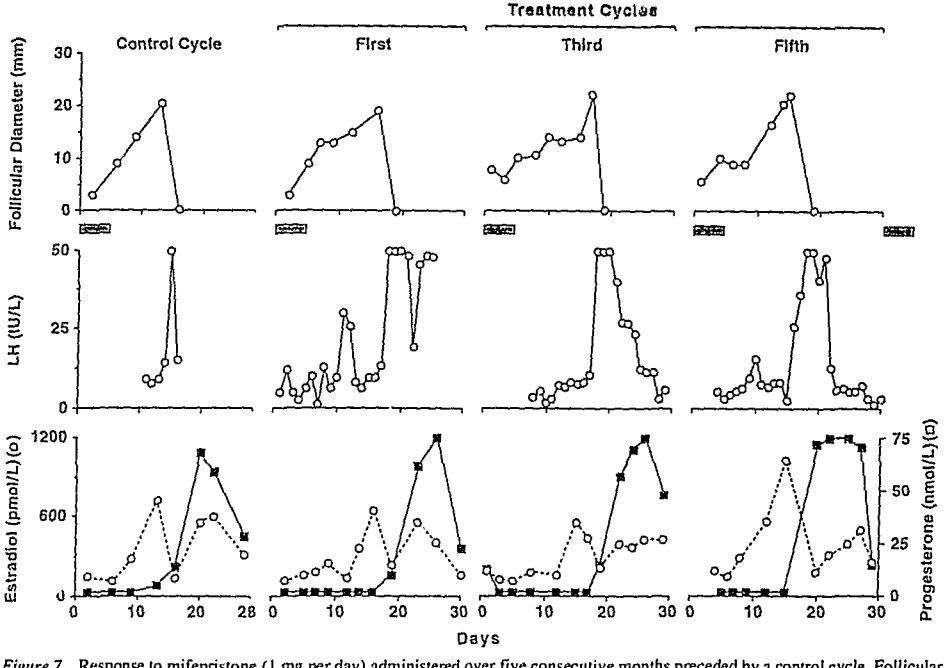


Figure 7 Response to mifepristone (1 mg per day) administered over five consecutive months preceded by a control cycle. Follicular diameter, as determined by ultrasound, is depicted in the upper panel, serum LH is in the middle panel, and serum estradiol and progesterone levels are in the lower panel. Bleeding is indicated by the horizontal bars. In the three treatment cycles depicted, ovulation and menstrual bleeding occurred regularly.

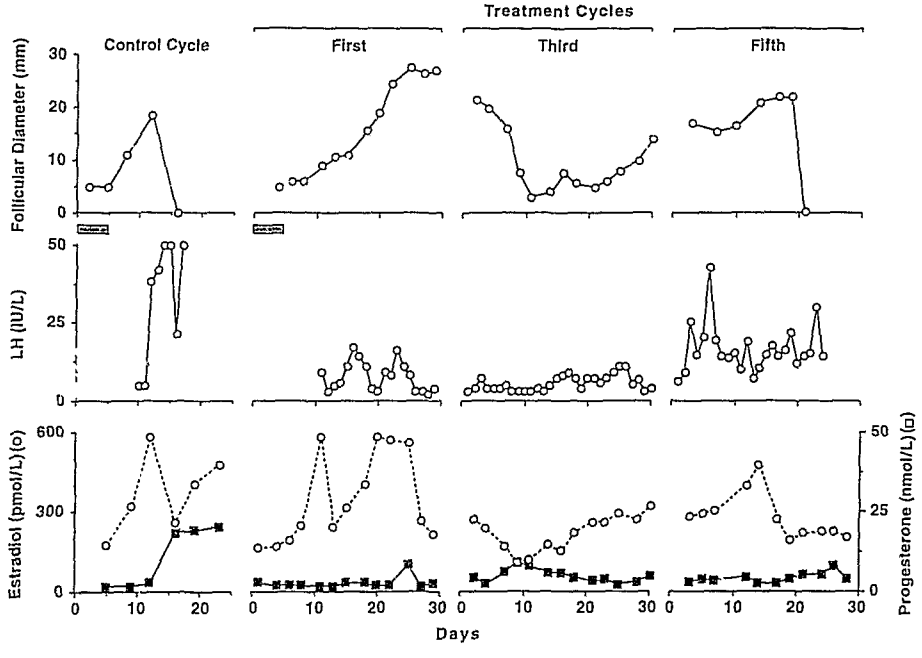


Figure 8 Response to mifepristone (1 mg per day) administered over five consecutive months preceded by a control cycle. See Figure 7 for details. Follicle rupture did occur in the fifth treatment cycle. However, in none of the three treatment cycles depicted was there evidence of ovulation. Moreover, serum progesterone levels remained low, estradiol levels were in the early to mid follicular phase range, and menstrual bleeding did not occur.

merous additional potential candidates (130, 131). Progesterone-dependent endometrial markers that have been shown to be down-regulated by antiprogestins include certain histones (H2A, H2B, and H3) (137), 17β hydroxysteroid dehydrogenase (138), metalloproteinase inhibitor (139), keratinocyte growth factor (KGF) (140), heparin-binding epidermal growth factor–like growth factor (HB-EGF) (141), and an endometrial secretory glycan (142). Other markers such as estrogen and progesterone receptors (138) and creatine kinase (143) are estrogen dependent. They are suppressed by progesterone, and this effect is blocked by antiprogestins (138, 143). Some markers, e.g. calcitonin, are under dual control of both estrogens and progestins (144). Only a few of these markers have been described in the human endometrium (137, 139, 142). Moreover, many of the changes induced by antiprogestins are too qualitative and are of limited value in the individual subject. Nevertheless finding such a marker will be of inestimable value in precisely defining subtle endometrial effects of antiprogestins, thus facilitating clinical studies aimed at developing antiprogestins as contraceptive agents acting by delaying endometrial development without altering ovulation or bleeding patterns.

Effect of Antiprogestins on Endometrial Integrity

With the fall in estradiol and progesterone at the end of the luteal phase, endometrial bleeding occurs. One of the major effects of progesterone is to maintain endometrial integrity. Thus an antiprogestin could display contraceptive potential by inducing endometrial shedding. When a single dose of mifepristone (50 to 800 mg) was administered 6 to 8 days after the LH surge to normal women, menses was induced within 72 h (145). In one third of these subjects, this represented the only bleeding episode and was associated with a decline in estradiol and progesterone concentrations, suggesting luteolysis. However in two thirds of the subjects, mifepristone did not induce complete luteolysis, and after a few days there was a further bleeding episode. Daily blood samples from subjects in which luteolysis did not occur immediately after antiprogestin treatment showed the initial decline in estradiol was followed within three days by a rebound increase in LH, estradiol, and progesterone levels (106). The onset of the second episode of uterine bleeding coincided with the decline in estradiol and progesterone when spontaneous luteolysis occurred. Thus mid luteal mifepristone administration is associated with early onset of bleeding, variable bleeding patterns, and inconsistent luteolysis. In addition, luteolysis appears to be unrelated to the dose of mifepristone administered (145). Thus mid luteal phase antiprogestin does not appear to offer a practical contraceptive potential.

In contrast to the occurrence of variable bleeding patterns observed when mifepristone was administered in the mid luteal phase, late luteal phase ad-

ministration resulted in only one bleeding episode, which usually commenced within 24 to 48 h of drug administration (106, 146). Such treatment often shortened the luteal phase and prolonged the follicular phase of the following untreated cycle (106, 146). In one study, mifepristone (100 mg per day) was administered to 10 women for 4 consecutive days prior to the expected menses in 3 successive cycles. This was preceded by and followed by two placebo treatment cycles. Results showed that there was no disturbance in bleeding patterns and hormonal parameters. Daily measurement of urinary estrone glucuronide and pregnanediol glucuronide over the duration of the study in all of these women revealed the occurrence of normal ovulation and appropriate corpus luteum function during treatment and posttreatment cycles (146). Thus mifepristone had no major effect on menstrual cycle events when administered at the time of the natural progesterone withdrawal occurring before menses in normal women.

ONCE MONTHLY ADMINISTRATION Because late luteal phase mifepristone administration did not disturb the cycle, studies have been undertaken in women to ascertain whether antiprogestins, administered each month at the expected time of menses or alternatively at the time of missed menses, could prevent pregnancy. These women were not using any other form of contraception. With the first alternative, antiprogestins are used regularly even though there may be no fertilization; with the second modality, antiprogestins are only used in those cycles where there is a delay in menses.

When used monthly, mifepristone was usually administered in a single dose of 600 or 400 mg at the end of the luteal phase; however, in some studies, 100 mg was given daily for four successive days (147–151). In all of these studies βhCG concentrations were determined in order to precisely document the number of subjects who conceived. This ranged from 12.5 to 60%. Mifepristone was successful in terminating pregnancy in the majority of subjects. The percentage of those subjects with a continuing pregnancy as a function of the total number of women studied ranged from 2.9 to 8.3%. The 3 largest studies each involved more than 20 confirmed pregnancies (147, 150, 151). By expressing the results as the number of continuing pregnancies in relation to the number of confirmed pregnancies, the actual failure rate in these three studies ranged from 17 to 19%. This is similar to the failure rate of mifepristone when used to terminate early pregnancy without a prostaglandin (5, 9–12, 14, 50, 51).

When used as a monthly menses regulator, many subjects were dissatisfied with the method. This was related to the fact that on occasion, the treatment cycle was prolonged, causing alteration in the menstrual rhythm (151). Furthermore, mifepristone did not induce bleeding in anovulatory cycles. Thus Couzinet et al (151) had intended to examine 12 subjects for 18 consecutive

cycles; however, only four subjects completed the study. Compliance was poor, and there was general dissatisfaction with the method. Indeed acceptability studies conducted in women from different ethnic groups and varying social backgrounds indicate that the majority of women prefer ovulation inhibition rather than prevention of implantation or dislodgement of an implanted embryo as their method of contraception (152).

When used after missed menses, mifepristone together with a prostaglandin has been shown to prevent pregnancy in women with menstrual delay of up to 11 days (153). In this study 193 of the 228 women enrolled were shown retrospectively to be pregnant. They all received 600 mg mifepristone followed within 48 h by 1 mg of the vaginal suppository gemeprost. The overall success rate was 97.9% (153). Further studies need to be conducted to determine whether a similar antiprogestin-prostaglandin combination could function as a once-a-month contraceptive.

POSTCOITAL ADMINISTRATION All the above-mentioned effects—such as ovulation inhibition, delayed endometrial development, and endometrial sloughing—may be operating when antiprogestins act as postcoital agents. Indeed, mifepristone has been successfully used to prevent pregnancy when given within 72 h of unprotected intercourse. Glasier et al (154) administered 600 mg of mifepristone to 402 women and compared the response to the standard high-dose estrogen progestin treatment that was given to 398 women. A total of 23 pregnancies was expected in each group. None of the subjects receiving mifepristone became pregnant, as compared to four who received the standard regimen (154). Webb et al (155) reported results of a comparative study in which women received either 600 mg of mifepristone (195 women), high-dose estrogen-progestin (191 women), or danazol (193 women), within 72 h of unprotected intercourse. There were no pregnancies in the mifepristone group, five in the high-dose estrogen-progestin group, and six in the danazol group. Thus in these two studies, there have been a total of 9 pregnancies with high-dose estrogen-progestin and none with mifepristone. This low failure rate indicates that mifepristone is an effective postcoital agent. In both studies, side effects such as nausea, vomiting, and breast tenderness were significantly less with mifepristone than the standard high-dose estrogen-progesterone methods. However, menstrual cycle delay was more common in the group receiving the antiprogestin (154, 155). Another possible advantage of mifepristone as a postcoital agent is that it may be effective for longer than 72 h after unprotected intercourse. It is clear that this is a very promising new strategy for family planning, and further large-scale dose-finding studies are currently being conducted.

When mifepristone is used as a postcoital agent, careful follow-up is mandatory because some women may continue with their pregnancy despite mife-

pristone administration. Although no teratogenic effects have been observed to date in rats and mice (156), skull deformities were detected in a rabbit study (157). These were attributed to mechanical effects secondary to uterine contractions consequent to a decrease in progesterone levels. Experiments conducted in monkey embryos exposed in vivo and in vitro in the perinidatory interval show them to be tolerant of mifepristone (158). There are isolated case reports of normal pregnancies and offspring when women have taken mifepristone alone or in combination with a prostaglandin, have not aborted, and have elected to continue their pregnancies (159, 160). One woman's pregnancy was terminated at 18 weeks because ultrasonography revealed that the fetus had multiple severe congenital defects not thought to have been caused by mifepristone (160).

CONCLUSION

Antiprogestins have numerous proven and potential clinical applications. To regard antiprogestins exclusively as abortifacients is to grossly underestimate their usefulness. Few of these clinical applications were envisaged when antiprogestins were first described 14 years ago. One major area of clinical research and development on antiprogestins is their use as contraceptives. Although ovulation inhibition and monthly menses regulation do not presently appear to be feasible, there are several promising leads. Use of mifepristone as a postcoital contraceptive administered within 72 h of unprotected intercourse appears very effective. Another potentially useful method would be to delay endometrial development so that the fertilized ovum fails to implant. The antiprogestin should be given in a low enough dose to delay endometrial development without altering bleeding patterns or hormonal events of the cycle. In particular, there should be no progesterone suppression with unopposed estrogen activity, and evidence from studies in both animals and women indicate that this may be achieved. Further research on development of these uses of antiprogestins is required to extend their clinical application.

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Effects of the Antiglucocorticoid RU 486 on Adrenal Function in Dogs*

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ABSTRACT. We studied the antiglucocorticoid effects of RU 486 given orally in doses of 5 (low), 20 (intermediate), and 50 mg/kg (high) daily for 10 days to seven female mongrel dogs. No changes in plasma ACTH or cortisol levels were produced by the 5 mg/kg dose. Plasma ACTH levels increased 3-fold with both intermediate and high dosages. Plasma cortisol levels rose 4-fold ($P < 0.05$) within 2 days of commencing the high dose schedule and within 3 days with the intermediate dosage. Plasma aldosterone concentrations increased significantly only with the highest dose schedule. Plasma RU 486 levels rose progressively during the 10 days of RU 486 administration and with the highest dose remained elevated for 7 days after it was stopped. Plasma RU 486 levels measured by RIA and high pressure liquid chromatography were comparable. The monodemethylated metabolite of RU 486 changed in parallel to the parent compound. During the high dose of RU 486, there was a 4% increase in body weight, with a reduction in hematocrit and plasma protein concentration. Plasma electrolyte levels and osmolality did not change. We conclude that in dogs a daily RU 486 dose of 5 mg/kg does not alter adrenal function, whereas higher doses (20 and 50 mg/kg) induce increases in plasma ACTH and cortisol concentrations. Despite the blockade of glucocorticoid receptors by RU 486, the presence of isotonic hypervolemia suggests that there was no functional deficiency of cortisol at the renal tubule or it was overshadowed by augmented mineralocorticoid production and action. (*J Clin Endocrinol Metab* 66: 473, 1988)

RU 486 is a synthetic 19-nor-steroid with substitutions at positions C11 and C17 [17β-hydroxy-11β-(4-dimethylaminophenyl)17α-(prop-1-ynyl)estra-4,9-dien-3-one] that binds to both glucocorticoid and progesterone receptors (1–4). The *in vitro* binding affinity of RU 486 for glucocorticoid receptors is 3 times greater than that of dexamethasone (4–7). After *in vivo* administration of RU 486, there are rapid rises in plasma ACTH and cortisol concentrations in several species, suggesting that this agent is a glucocorticoid antagonist (4, 8–12). This conclusion is strengthened by the observation that in humans and rats the ACTH rise is reversed by the administration of dexamethasone in a dose-dependent fashion (4, 8, 9). Moreover, by blocking the actions of cortisol, RU 486 can alleviate the clinical and biochemical consequences of Cushing's syndrome resulting from excess ACTH secretion (13, 14). There is, however, no competition for mineralocorticoid receptors in *in vitro* systems (1).

In view of the antiglucocorticoid properties of RU 486, we studied whether it produces functional adrenal insufficiency by assessing adrenal function in conscious dogs that received RU 486 in doses of 5, 20, and 50 mg/kg for 10 days. Dogs were selected because they can be trained to withstand repeated blood sampling without "stress" (15–20). RU 486 was administered for 10 days, because adrenalectomized dogs develop objective evidence of adrenal insufficiency 5–9 days after withdrawal of dexamethasone treatment (21, 22). In addition to measuring hormone and drug concentrations in the dogs, we also evaluated their fluid and electrolyte status during and after treatment.

Materials and Methods

Animals

Seven female mongrel dogs, weighing 21–30 kg, were studied. The dogs were individually housed at a room temperature of 21

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* In conducting the research described in this report the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animals Resources, National Research Council. Presented in part at the International Congress of Endocrinology, Quebec City, Quebec, Canada, July 1–7, 1984. This work was supported in part by the Ford and Mellon Foundations. The views expressed in this article are those of the authors and do not reflect the official policy or position of the Department of the Army, the Department of Defense, or the U.S. Government.

C, with relative humidity of 50% and lights on from 0600–1800 h. The animals were fed (Purina dog chow, Ralston-Purina, St. Louis, MO) at 1400 h daily, given water *ad libitum*, and trained to stand quietly in a modified Pavlov sling (Alice King Chatham Medical Arts, Los Angeles, CA). Each dog underwent all of the experimental manipulations, with a minimum of 21 days between each drug experiment.

RU 486 administration

RU 486 was given for 10 consecutive days in the following regimens in random order: 5 (low dose), 20 (intermediate dose), and 50 mg/kg·day (high dose). The drug was administered orally in gelatin capsules in divided doses at 0600 and 1800 h each day. All animals also were included in a control study in which the gelatin capsules were administered twice daily for 5 days.

Before receiving RU 486, each dog was weighed, brought into the laboratory, and placed in the sling, and a leg vein was cannulated (20-gauge Angiocath, Deseret, Sandy, UT). After a 10-min equilibration period, a 20-mL venous blood sample was obtained, and the volume was replaced with 150 mmol/L saline. The catheter was removed, and the dog was given RU 486 and returned to its home cage. During the course of the low and high dose RU 486 treatments, blood samples were taken in the morning immediately before administration of drug on days 1–10; with the intermediate dose, blood samples were taken on days 1–5 and then on days 8–10. In the control study, blood samples were taken for the first 5 days and on day 10. To determine when basal values were reestablished after termination of RU 486, blood was drawn each morning for a further 7 days in those dogs receiving the high and low doses of RU 486.

Sample collection and assays

Blood samples were collected in chilled tubes containing heparin or EDTA, and placed on ice. Samples were centrifuged at 5 C, and the plasma was immediately frozen at −20 C.

Plasma ACTH, cortisol, and aldosterone levels were measured in all blood samples. Plasma protein, sodium, and potassium levels, plasma osmolality, and hematocrit were measured in all samples from the dogs receiving the low and high doses, but only on days 1, 5, and 10 during the intermediate dose and control studies. Plasma sodium and potassium levels were determined using an internal standard flame photometer (model 433, Instrumentation Laboratories, Wilmington, MA), and osmolality by freezing point depression (model DIII, Advanced Instruments, Needham, MA). Plasma protein concentrations were measured by refractometry (American Optical Corp., Buffalo, NY), and hematocrit by the microcapillary method.

For hormone analysis, all samples from one dog were analyzed in duplicate in the same assay. Plasma cortisol levels were measured by RIA (New England Nuclear, Boston, MA). The intraassay coefficient of variation was 8% (n = 24), and the lower limit of detectability was 0.3 μg/dL (8.3 nmol/L). The cross-reactivity of this antiserum for corticosterone is 15%. Plasma aldosterone was determined by RIA using kits purchased from Diagnostic Products (Los Angeles, CA). The sensitivity of the aldosterone assay was 1 ng/dL (27.7 pmol/L), and the intraassay coefficient of variation was 8% (n = 17). ACTH was measured by RIA in unextracted plasma. Rabbit anti-ACTH serum (IgG-ACTH-1) was obtained from IgG Corp. (Nashville, TN), and synthetic ACTH-(1–39) was purchased from Peninsula Laboratories (Burlingame, CA). Free and bound ACTH were separated using a goat antibody to rabbit γ-globulin obtained from Calbiochem-Behring Corp. (La Jolla, CA). The sensitivity of the ACTH assay was 5 pg/mL (1.1 pmol/L), and the intraassay coefficient of variation was 12% (n = 20).

For RIA of RU 486, standard RU 486 and [6,7-^{3}H]RU 486 (SA, 37 Ci/mmol) were obtained from Roussel-UCLAF (Romainville, France). The labeled material was purified before use by thin layer chromatography [Kieselgel 60 F 254 (Merck, Darmstadt, West Germany), dissolved in chloroform-acetone (9:1) and redissolved in 0.1% gelatin-phosphate-buffered saline (PBS), pH 7.0, containing 0.025% Triton X-100]. Serum samples (0.1 mL) were diluted with 0.4 mL 150 mmol/L saline containing 0.025% Triton X-100. The diluted serum was extracted once with 5 mL diethyl ether. The ether extract was dried and redissolved in 0.1% gelatin-PBS buffer containing 20% methanol. The RIA was performed according to the method of Salmon and Mouren (23). The standard curve ranged from 10–1000 pg RU 486/tube, prepared in the serum gelatin-PBS-methanol solution. Plasma containing 10, 200, and 2000 ng/mL RU 486 was included in each assay. The interassay coefficient of variation varied from 20.7–25.9%.

Plasma RU 486 concentrations and those of its monodemethylated metabolite also were measured by high pressure liquid chromatography (HPLC), as follows. Plasma samples (0.3 mL) were added to Chromosorb W-NAW 60/80 Mesh 20% ethylene glycol chromatography columns (3 mL in 5-mL disposable pipettes), left for 30 min, and then eluted with 5 mL *n*-hexan ethyl acetate (95:5). The eluates were evaporated to dryness, dissolved in methanol, and vortex mixed. Fifty-microliter samples were injected into the HPLC system (Water Associates, Inc., Milford, MA) using a Hibar LiChrosorb RP-18 column. The eluent was methanol-water (9:1), with a flow rate of 1 mL/min. The retention times were 4 min, 20 s for RU 486 and 3 min, 10 s for the monodemethylated metabolite. The UV absorption was measured at 304 nm. The sensitivity of the HPLC assay was 10 ng RU 486, the precision ranged from 3.4–12.1%, and the recovery of RU 486 with the column chromatography-HPLC system ranged from 71–87%. Samples were not corrected for recovery.

Statistical analysis

A two-way analysis of variance for repeated measures was used to determine significant differences over time and between doses. Differences between means were assessed by a Newman-Keuls test after analysis of variance. When appropriate, Student's *t* test was used to assess differences between paired data. $P < 0.05$ was considered significant. The results are given as the mean ±SE.

Results

Response to multiple doses of RU 486

Mean plasma ACTH levels did not change during the control study or during the administration of 5 mg/kg

RU 486 (Fig. 1, *top panel*). With the intermediate and high doses, plasma ACTH levels rose and were significantly greater ($P < 0.05$) than the basal value by day 8. Because of the large variances, there were no differences in ACTH levels between the 20 and 50 mg/kg doses, and on the 10th day, mean ACTH values were 34 ± 9 and 67 ± 30 pg/mL (7.5 ± 2.0 and 14.7 ± 6.6 pmol/L), respectively. After the highest RU 486 dose was stopped, ACTH values remained significantly greater than those on day 1 for a further 3 days. Sampling was not continued after the intermediate dose.

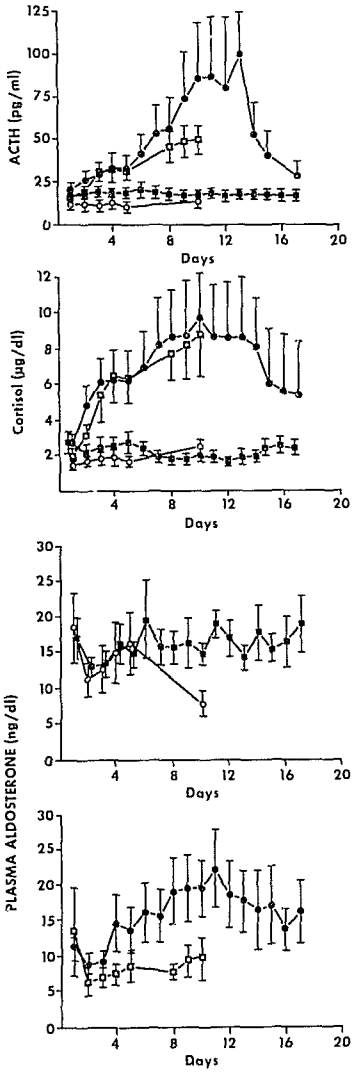


FIG. 1. Plasma ACTH (*top panel*), cortisol (*second panel*), and aldosterone (*third and fourth panels*) levels in response to 10 days of treatment with RU 486. The doses of RU 486 administered were 50 (●—●), 20 (□—□), and 5 (■—■) mg/kg·day. The control study is also shown (○—○). Values are the mean ± SEM. (Conversion to SI units: ACTH, pg/mL × 0.2202 = pmol/L; cortisol, µg/dL × 27.59 = nmol/L; aldosterone, ng/dL × 27.74 = pmol/L.)

Individual basal plasma cortisol levels on day 1 ranged from 0.7–3.2 µg/dL (19.3–88.3 nmol/L), and mean plasma cortisol levels did not change during the control study or during the low dose of RU 486 (Fig. 1, *second panel*). Plasma cortisol increased after 3 days of administration of 20 mg/kg·day ($P < 0.05$); during the highest dose the plasma cortisol level increased after 2 days. There was no significant difference between the cortisol levels during the RU 486 doses of 20 and 50 mg/kg [8.8 ± 2.4 and 9.8 ± 3.1 µg/dL (242.8 ± 66.2 and 270.4 ± 85.5 nmol/L), respectively] on day 10. Cortisol values remained significantly greater than basal values for 2 days after the high dose of RU 486 was stopped.

Individual basal plasma aldosterone values on day 1 in the untreated dogs varied from 3–43 ng/dL (83–1193 pmol/L). There were, however, no significant differences between the study groups on day 1 (Fig. 1, *third and fourth panels*). The mean plasma aldosterone levels fell slightly but not significantly from the first to the second day in all four experimental groups. During the entire control study and throughout the 5 or 20 mg/kg RU 486 studies, plasma aldosterone levels did not change significantly. By day 8 during the highest dose of RU 486 (50 mg/kg), the mean aldosterone level was significantly increased compared to that on day 1, and it rose to a peak of 22 ng/dL (610 pmol/L) on day 11 (Fig. 1, *bottom panel*). Aldosterone remained significantly elevated for 2 days after discontinuation of the high dose of RU 486, but thereafter aldosterone levels were not significantly different from basal values.

Plasma RU 486 levels

During low dose RU 486 administration, the mean plasma RU 486 level, as measured by RIA, attained a peak of 0.21 ± 0.06 mg/L by day 4 (Fig. 2), and RU 486 remained detectable (0.004 ± 0.001 mg/L) for 7 days after it was discontinued. The half-disappearance time of RU 486, thus, was on the order of 24 h. During the 50 mg/kg dose, the progressive rise in RU 486 levels indicated accumulation in the dogs. The RU 486 level (9.96 ± 1.69 mg/L) was higher on day 10 (Fig. 2). Thereafter, plasma RU 486 levels decreased, with a half-disappearance time exceeding 4 days. RU 486 levels were still quite high (4.48 ± 2.38 mg/L) 6 days after discontinuation of RU 486. Plasma RU 486 levels, measured by HPLC during administration of 50 mg/kg·day, were similar to those measured by RIA (Fig. 2). In addition, the pattern of monodemethylated RU 486 levels was similar. The

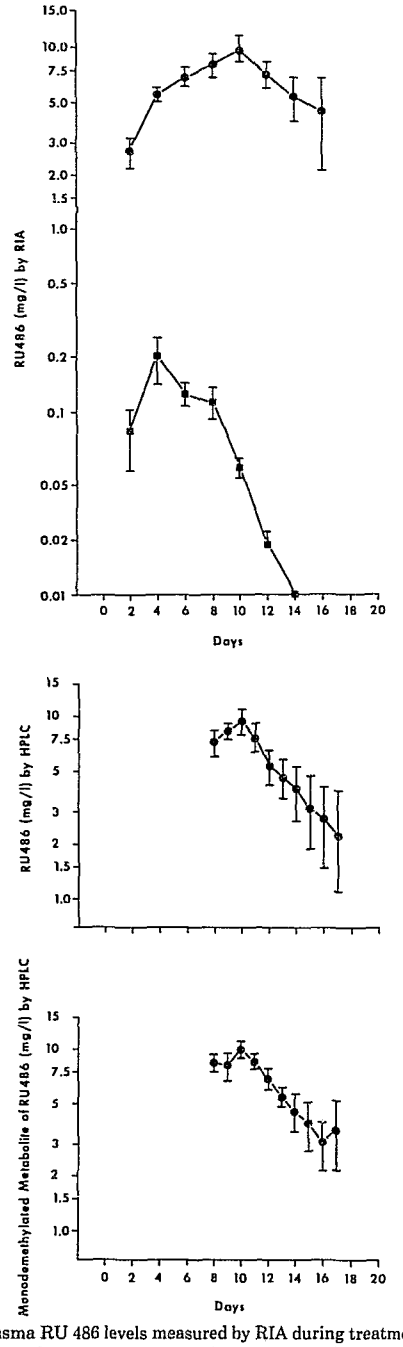


FIG. 2. Plasma RU 486 levels measured by RIA during treatment with doses of 5 (■—■) and 50 (●—●) mg/kg·day for 10 days. Plasma RU 486 and the monodemethylated metabolite levels were measured by HPLC, after 10 days of RU 486 treatment (50 mg/kg·day). Values are the mean ±SEM. (Conversion to SI units: μg/mL × 1.0 = mg/L.)

half-disappearance time of RU 486 (measured by HPLC or RIA) and that of the metabolite were similar.

Metabolic changes

Body weight. Because the seven dogs differed in body weight (range, 21–30 kg; mean, 26.8 ± 2.7 kg), the results are expressed as change in body weight. Mean body weight did not change significantly during the control study or during low dose RU 486 administration (Fig. 3). During administration of the two higher RU 486 doses, mean body weight significantly increased. There were no differences between these doses in terms of the weight gained by the fifth and tenth days (only these points were available with the intermediate dose). On day 10, body weight was increased by 0.9 ± 0.1 and 0.8 ± 0.3 kg with the intermediate and high doses, respectively (Fig. 3). The increase in body weight during the highest dose of RU 486 was significant ($P < 0.05$) by day 4, and it remained elevated for 3 days after RU 486 was stopped. With the highest dose, one dog had a progressive increase in body weight for 6 days, after which the dog developed diarrhea and periodic vomiting that resulted in a weight loss of over 6 kg in the next 9 days. This dog recovered several days after treatment when RU 486 was stopped.

Hematocrit, and plasma protein and *electrolyte concen-*

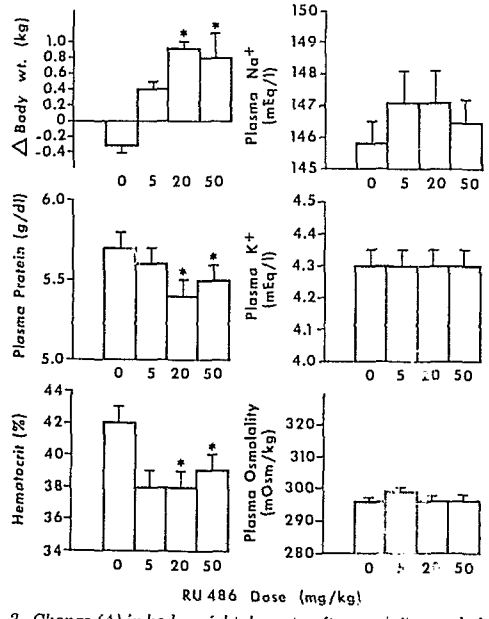


FIG. 3. Change (Δ) in body weight, hematocrit, osmolality, and plasma concentrations of protein, sodium, and potassium on the last day (day 10) for all doses of RU 486. *, Significantly different ($P < 0.05$) from day 1 value for that treatment. (Protein may be converted to SI units by: g/dL × 10 = g/L.)

trations. Mean plasma protein concentration decreased only during the intermediate and high doses of RU 486. The reduction in plasma protein with the intermediate dose was significant on day 10 (Fig. 3), whereas with the high dose the reduction was significant by day 3. The magnitude of the decrease at these times was similar. After RU 486 was stopped, plasma protein values tended to rise, but were still significantly decreased compared to basal levels 7 days later.

The increases in body weight of dogs receiving the 20 and 50 mg/kg RU 486 doses were accompanied by reductions in hematocrit (Fig. 3); there was, however, no significant hematocrit change during the control study or during low dose RU 486 administration. In dogs that received the intermediate RU 486 dose, hematocrit was reduced by day 10 (Fig. 3). The decreases in hematocrit were significant by day 6 in dogs that received the highest RU 486 dose, and hematocrit remained reduced until day 16, *i.e.* 6 days after cessation of treatment. The magnitude of the reduction in hematocrit was similar in the two groups.

Plasma sodium and potassium levels and osmolality were not different among the various treatment groups (Fig. 3). Further, no changes in plasma sodium and potassium levels or in osmolality occurred during the lowest or highest dose during daily measurements.

Discussion

RU 486 increases plasma ACTH and cortisol levels in humans and several animal species (8–11, 13). In humans, oral administration of a single 4.5 mg/kg dose raised plasma cortisol levels, and 6 mg/kg produced no further cortisol increase, but sustained the rise for a longer period (8). Further, 6 mg/kg RU 486 abolished the inhibition of cortisol secretion produced by administration of 1 mg dexamethasone (8). In rats, an oral dose of 10 mg/kg raised plasma ACTH and corticosterone levels and overcame dexamethasone-induced suppression of ACTH (4). A single im injection of 1–10 mg/kg RU 486 in monkeys produced a rapid, but transient, dose-dependent rise in plasma ACTH and cortisol values (10, 11). It is thus evident that there are quantitative differences in the cortisol response to RU 486 in dogs compared to those in other species. A single oral or sc dose of RU 486 (10 mg/kg·day) did not influence cortisol levels in dogs (24), and in this study, 5 mg/kg·day for 10 days given orally did not result in any change in plasma ACTH or cortisol levels. However, with the intermediate (20 mg/kg) and high (50 mg/kg) doses of RU 486, ACTH and cortisol levels increased. Thus, a 2-fold higher or greater dose of RU 486 was required to produce an increase in cortisol in dogs compared to that required in humans, monkeys, or rats.

With the intermediate and high RU 486 doses, plasma cortisol levels increased from control values of 0.7–3.2 μg/mL (19.3–88.3 nmol/L) to maximum levels of 8–10 μg/dL (221–226 nmol/L). These values are similar to those in dogs after exogenous ACTH or insulin-induced hypoglycemia (15, 16, 19). Doses of exogenous ACTH producing plasma levels exceeding those attained in this study do not produce further increases in cortisol levels, although they do sustain the rise for a longer period of time (25). Thus, the intermediate ACTH response to the dose of RU 486 produced a maximal increase in cortisol levels.

Although plasma cortisol levels increased 2 days after the start of RU 486 administration, plasma ACTH concentrations did not rise significantly until day 8. The unexpected delay in the increase in ACTH concentration during RU 486 administration may have been due to several factors. 1) The ACTH RIA may not have been capable of detecting small increases in ACTH sufficient to stimulate cortisol secretion (17, 18). 2) Since ACTH is released episodically, the single-sampling technique used may have missed transient ACTH increases (19).

The quantitative differences in ACTH and, thus, cortisol responses to RU 486 in dogs compared to those in other animals may be explained by species differences in the affinity of the glucocorticoid receptors for RU 486. However, such differences also could be related to variability in the bioavailability of RU 486 in the dog. Plasma immunoreactive RU 486 levels after 10 days of administration of 5 mg/kg·day RU 486 ranged from 0.08–0.20 mg/L, and after 50 mg/kg·day the corresponding levels were 2.7–5.0 mg/L. With the same RIA, plasma RU 486 levels in women after a single oral dose of 4 mg/kg ranged from 2–3 mg/L (24). These results indicate that gastrointestinal absorption of the compound may be less efficient in dogs compared to humans.

The differences in both circulating RU 486 levels and half-disappearance times with the low and high RU 486 doses reflect differences in the metabolism of the compound. After discontinuation of the low dose of RU 486, the half-disappearance time was approximately 24 h, whereas after discontinuation of the high dose of RU 486 it was at least 4 days. These data suggest that the capacity of the enzyme that degrades RU 486 is small, and the high dose of RU 486 saturates the system so that accumulation of the drug occurs, as indicated by the progressive rise in plasma RU 486 levels. In rats there is a high rate of diffusion of radiolabeled RU 486 into the extravascular space (26). This compartment may contain a large amount of RU 486 after chronic administration of the high doses in the dogs. Hence, levels of circulating RU 486 may remain high for a long period of time. Further, the long disappearance time could be related to binding to a plasma protein(s). Preliminary studies in-

dicate that in man this component might be an α-globulin (3).

The antibody used in the RU 486 RIA was not specific for the parent compound; therefore, metabolic breakdown products, including the mono- and didemethylated derivatives, are also measured (23). The latter, in particular, has significantly reduced biological activity (23). However, the high levels in these dogs did represent parent compound, since specific RU 486 levels measured by HPLC were comparable to those measured by RIA, and the half-disappearance times were similar. Of interest is the fact that the circulating levels and kinetics of the monodemethylated metabolite were similar to those of the parent compound. This similarity indicates that a step other than monodemethylation limits the rate of metabolism of RU 486 in the dog. The persistence of detectable RU 486 after its administration was stopped indicates that biologically active material remained in the circulation. That plasma ACTH, cortisol, and protein concentrations, hematocrit, and body weight did not return to basal levels after stopping RU 486 administration is also evidence of persistence of the biological activity of the drug and the hormonal response to it.

During administration of the high dose of RU 486, plasma aldosterone levels increased. Known stimuli for aldosterone secretion include the renin-angiotensin system, hyperkalemia, hyponatremia, ACTH, and possibly β-endorphin. The major stimulus for the renin-angiotensin system is a reduction in circulatory volume. In our dogs, the rise in body weight coupled with the reduction in plasma protein and hematocrit suggested an expanded plasma volume. Neither hyperkalemia nor hyponatremia occurred, suggesting that there was isotonic expansion of extracellular fluid volume. This isotonic expansion probably resulted from sodium (and water) retention due to the elevation of plasma mineralocorticoid levels. The increase in ACTH levels after RU 486 administration could account for the rise in aldosterone concentrations. β-Endorphin levels parallel those of ACTH during RU 486 administration (8, 10); thus, an increase in β-endorphin levels may have contributed to the increase in aldosterone levels during RU 486 administration (20, 27, 28). *In vitro* studies have shown that RU 486 does not compete with aldosterone for mineralocorticoid receptors (1, 5), so that any increase in aldosterone would have its expected renal action. While cortisol binds to mineralocorticoid receptors, it does so with considerably less affinity than does aldosterone (29, 30), and its action is as an agonist as well. Had the volume expansion been solely due to glucocorticoid deficiency, the hypervolemia should have been accompanied by hyponatremia.

In conclusion, dogs given an oral dose of 5 mg/kg RU 486 for 10 days had no changes in plasma ACTH or cortisol levels; ACTH and cortisol concentrations increased when RU 486 was given in oral doses of 20 or 50 mg/kg·day for 10 days. Several days after RU 486 was stopped, ACTH and cortisol levels were still elevated due to the prolonged retention of RU 486. Plasma aldosterone levels only increased during the high dose of RU 486. The increases in body weight and reductions in hematocrit and plasma protein levels during RU 486 administration were not associated with changes in plasma sodium and potassium levels or osmolality. These findings are compatible with the presence of isosmotic fluid retention and hypervolemia.

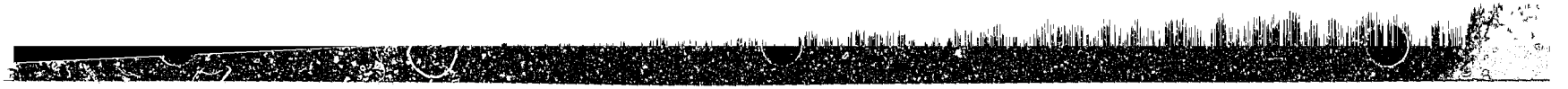
Acknowledgments

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The divergent effect of RU 486 on adrenal function in the dog is related to differences in its pharmacokinetics

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This study has evaluated the relationship between the antiglucocorticoid effect of RU 486 and its pharmacokinetics in seven female mongrel dogs (weight 21–30 kg), all of which had appropriate responses to insulin-induced hypoglycemia and metyrapone. They were given RU 486 (50 $mg \cdot kg^{-1} \cdot d^{-1}$) for ten days. Three dogs (responders) demonstrated increased secretion of ACTH and cortisol which reached a maximum at the termination of RU 486 administration and returned to baseline by days 22 and 18, respectively. The remaining four dogs (non-responders) showed only transient non-sustained increases in ACTH and cortisol. Serum concentrations of RU 486 and its monodemethylated and di-demethylated (but not its hydroxylated) metabolites were significantly higher in the responder dogs. The ratios of the hydroxylated metabolite to RU 486 and its demethylated derivatives were higher in the non-responders, whereas the ratio of the di-demethylated to monodemethylated compound was higher in the responders. Since variations in the hydroxylation and reductive catabolism of steroids have been well documented in different strains of a species, it is possible that the differences in levels of RU 486 and its metabolites in the two groups are accounted for by variations in RU 486 metabolism. In the responder group the metabolism of RU 486 is directed to demethylation, and in the non-responder group to hydroxylation. Thus, differences in pharmacokinetics of RU 486 in the two groups of dogs may explain the variable biological response to this antiglucocorticoid.

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RU 486, 11-[4-(dimethylamino)phenyl]-17-hydroxy-17-(1-propynyl)-(11β,17β)-estra-4, 9-dien-3-one or mifepristone is an antiglucocorticoid and antiprogestin synthesized by Roussel Uclaf, Paris, France (1). RU 486 has been widely used for terminating early human pregnancy. Various dose schedules have been utilized with similar success rates (2–4). Unlike the antiprogesterone effects, the antiglucocorticoid responses are dose-dependent and are only seen after high doses in man (5–7), in the monkey (8), and in the dog (9, 10).

Owing to saturation of the serum binding capacity, the circulating concentrations of RU 486 in humans are similar following administration of single doses ranging from 200 to 800 mg (5, 11). However, RU 486 is extensively metabolized in humans and in other species, leading to high concentrations of demethylated and hydroxylated metabolites (11–14). In rats, the monodemethylated, di-demethylated, and hydroxylated derivatives retain antiglucocorticoid activity (14). Receptor binding assays have indicated that in addition to RU 486, the metabolites might also have strong antiglucocorticoid effects in man (13).

We have previously shown that mongrel dogs when challenged with RU 486 in a dose of 20 or 50 $mg \cdot kg^{-1} \cdot d^{-1}$ for 10 days respond with elevation in ACTH and cortisol (9, 10). Individual variability in response to RU 486 was noted (10). In the current study we have determined how the metabolism of RU 486 relates to its antiglucocorticoid action. The same dogs used in our previous studies were rechallenged with 50 $mg \cdot kg^{-1} \cdot d^{-1}$ of RU 486 for 10 days. In addition, the previous data were reanalyzed. We have also assessed the response of these dogs to other stimuli of the ACTH-cortisol axis (insulin-induced hypoglycemia, metyrapone administration and ACTH infusion). Our results have shown that all seven dogs respond appropriately to the latter three stimuli; however, three dogs (responders) demonstrated pronounced increases in ACTH and cortisol following RU 486 administration, and four dogs (non-responders) consistently failed to show appropriate ACTH and cortisol increments following RU 486. It is most likely that differences in the circulating levels of RU 486, as well as its mono- and di-demethylated derivatives, account for these varying biological responses.

Materials and methods

Animals and test procedures

The same seven female mongrel dogs previously des-

cribed were used in the current study (9). The integrity of the hypothalamic-pituitary-adrenal axis was assessed by insulin-induced hypoglycemia, ACTH infusion, and metyrapone administration. Insulin (0·5 IU·kg^{-1}) was given intravenously and blood samples were taken at 5, 10, 15, 20, 30, 40, 50, 60, 80, 100, and 120 min after insulin administration. On another occasion, exogenous ACTH was infused in a dose of 20 IU/min for 5 min. Blood samples were taken at 5, 10, 15, and 20 min after commencement of the infusion. All dogs also received 500 mg metyrapone orally every 4 h for six consecutive doses. Blood samples were taken 4 h following the last dose of metyrapone. All dogs were also challenged with RU 486 given in a dose of 50 mg·kg^{-1} for 10 consecutive days. The drug was administered orally in gelatin capsules in divided doses twice daily at 06.00 and 18.00. Blood samples were taken at 05.30 for three days prior to commencement of RU 486 therapy (pretreatment phase), daily during RU 486 administration (treatment phase) and thereafter on days 11, 12, 13, 14, 18, 22, 24, 29, 33, and 39 after cessation of RU 486 (post-treatment or recovery phase). An interval of 6–8 weeks separated all test procedures.

Sample collection and assays

Blood samples were collected in chilled tubes containing heparin or EDTA and placed on ice. Samples were centrifuged at 4°C and the plasma was immediately frozen at −20°C. Plasma cortisol was determined by our previously described method (9). Plasma ACTH was measured by RIA (Nichols Institute, San Juan Capistrano, CA). RU 486 as well as its mono-, di-demethylated, and hydroxylated derivatives were measured by HPLC followed Chromosorb column chromatography as previously described (11–13). In brief, Pasteur pipettes were filled with Chromosorb W-NAW 60–80 mesh/20% ethylene glycol (w/w). Serum samples of 0.2–0.4 ml were applied to the column and left for 30 min. RU 486 was separated in the first eluate of 5 ml n-hexane:ethylacetate [95:5]. A second eluate of 5 ml n-hexane:ethylacetate [40:60] was used for the elution of the mono-, di-demethylated, and hydroxylated metabolites of RU 486. For the assay of RU 486, the first eluate was evaporated until dry, and then dissolved into the HPLC-eluant of methanol-water-triethanolamine [90:10:0.05]. For the assay of the metabolites, the eluant of methanol-water-acetonitrile [40:25:35] was used. A LiChrosorb (250×4) RP-18-column (Merck) was used for both RU 486 and the metabolites. The detection was carried out at the wavelength of 304 nm and the eluate was pumped at the rate of 1 ml/min. The results of the assays were corrected according to the recovery of the serum pools containing known amounts of the synthetic RU 486 and the metabolites. Plasma glucose was measured with a glucometer (Ames, Elkhart, IN). Plasma protein concentrations were measured by refractometry (American Optical Corp., Buffalo, NY).

Statistical analysis

A two-way analysis of variance for repeated measures was used to determine significant differences over time and between groups. Differences between means were assessed by a Newman Keuls test after analysis of variance. When appropriate, a log transformation was used to normalize the data. Student's *t*-test was used to assess differences between paired data. $P<0.05$ was considered significant. The results are given as the mean ± SEM.

Results

Plasma ACTH and cortisol response to RU 486

When challenged with RU 486 (50 mg·kg^{-1}·d^{-1}), three dogs (responders) demonstrated increases in plasma ACTH which reached a peak of 400 ± 100 ng/l (mean ± SEM) on the last day of RU 486 administration (Fig. 1). This represented an eightfold rise. By day 22, i.e. 12 days after the last dose of RU 486, ACTH values had returned to baseline. These same dogs also had a threefold rise in plasma cortisol which reached a peak of 313.9 ± 58.3 nmol/l two days following the end of RU 486 administration. Basal levels were attained by day 18. The other four dogs (non-responders) had a transient non-sustained but nevertheless significant twofold in-

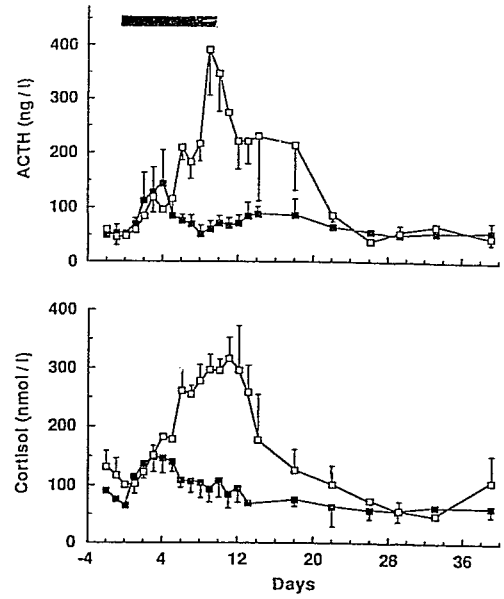


Fig. 1. Mean ± SEM ACTH and cortisol responses to RU 486 (50 mg·kg^{-1}·d^{-1} × 10 days) in the responder and non-responder groups. In this and subsequent Figures, RU 486 administration is shown by the black bar (□—□ responder; ■—■ non-responder).

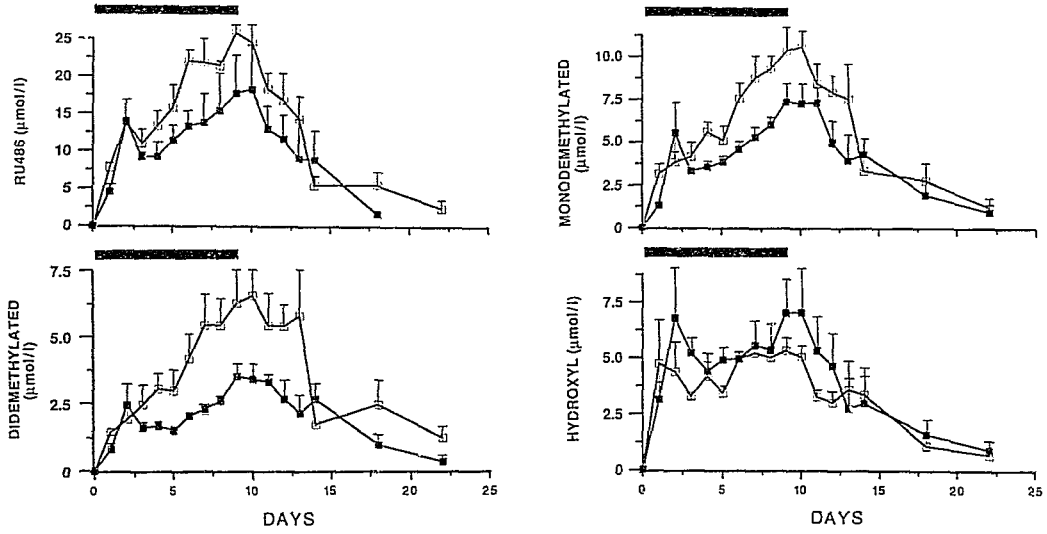


Fig. 2. Mean ± SEM values of RU 486 and the di-demethylated derivative (left panel), and the mono-demethylated and hydroxylated derivatives (right panel) in the responder and non-responder animals (see legend to Fig. 1).

crease in ACTH and cortisol which reached a maximum on the fourth or fifth day after commencement of RU 486. Thereafter, despite further drug administration, ACTH and cortisol levels decreased, returning to baseline values (Fig. 1). The previously published data (9, 10) following 10-day administration of 20 and 50 $mg \cdot kg^{-1} \cdot d^{-1}$ of RU 486 were reanalyzed and the same three responder dogs showed ACTH and cortisol responses of similar magnitude, whereas the four non-responders failed to show appropriate ACTH and cortisol increments.

RU 486 levels

When considering both the RU 486 treatment and post-treatment phases together, ANOVA showed significantly higher levels of the mono-demethylated ($p<0.003$) and the di-demethylated ($p<0.001$) derivatives in the responder as compared to the non-responder animals (Fig. 2). For the di-demethylated derivative, these differences were significant during both the treatment ($p<0.001$) and the recovery phases ($p<0.01$); with the mono-demethylated derivative, this was only evident in the treatment ($p<0.02$) but not recovery phase. Although the levels of RU 486 also appeared to be higher in the responders as compared to the non-responders (Fig. 2) this was only significant ($p<0.001$) when a logarithmic transfer was performed to normalize the distribution.

Although levels of the hydroxylated derivative were not different in the two groups, during RU 486 treatment the ratios of hydroxylated derivative to RU 486, the mono-demethylated compound, and the di-demethylated compound were significantly higher ($p<0.002$) in the non-responders than in the responders (Fig. 3). There were no significant differences in the ratio of the mono-demethylated or di-demethylated derivative to RU 486; however, the ratio of the di-demethylated to mono-demethylated compound was higher ($p<0.001$) in the responder than the non-responder animals (Fig. 4).

Body weight and serum protein changes following RU 486

Mean body weight at the commencement of this study was 33.7 ± 1.4 kg in the non-responders and 30.9 ± 1.1 kg in the responders ($p<0.05$). In neither group was there a significant change in body weight over the duration of the study (Fig. 5b). During RU 486 administration, serum protein decreased in both groups, but the decrease was significantly greater ($p<0.05$) in the responder as compared to the non-responder animals (Fig. 5a).

Correlations following RU 486 administration

As shown in Table 1, when all seven dogs were considered together, ACTH correlated significantly with cortisol, levels of RU 486, as well as the mono-demethylated and di-demethylated derivatives. Cortisol correlated with these same parameters. Serum protein was negatively correlated with ACTH, cortisol, RU 486, and its mono- and di-demethylated derivatives.

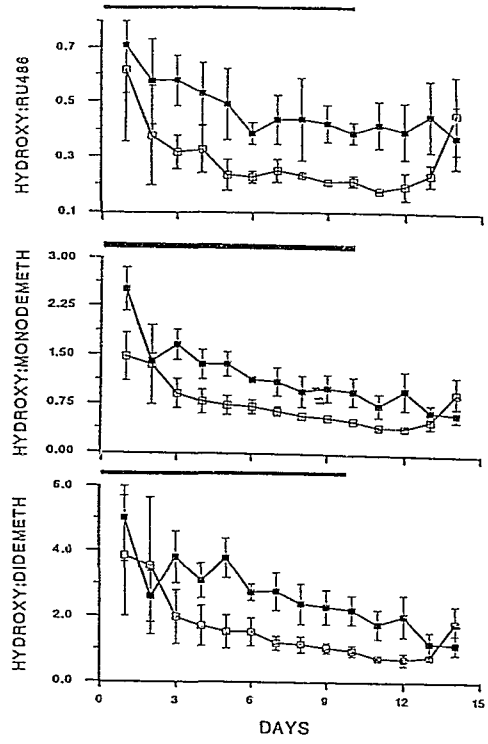


Fig. 3. Ratio of hydroxylated derivative to RU 486 (upper panel), mono-demethylated derivative (middle panel) and the di-demethylated derivative (lower panel) in the two groups of responder and non-responder animals (see legend to Fig. 1).

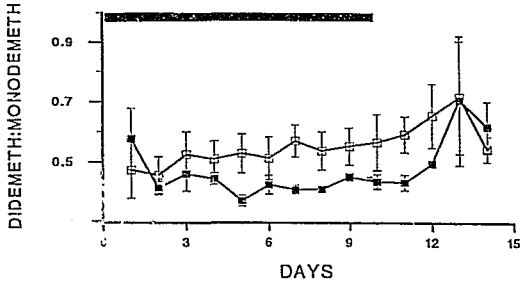


Fig. 4. Ratio of the di-demethylated to mono-demethylated compound in the two groups (see legend to Fig. 1).

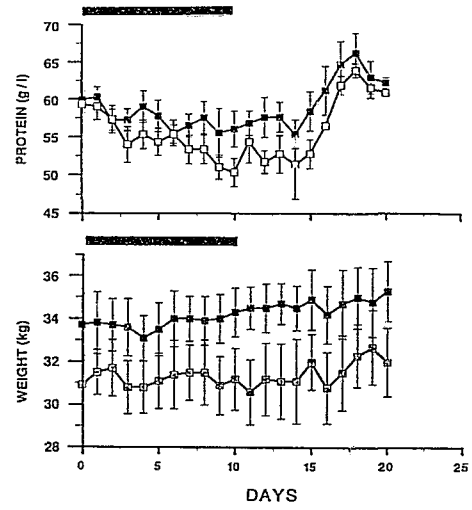


Fig. 5. Mean±SEM changes in serum proteins (upper panel) and body weight (lower panel) in the two groups (see legend to Fig. 1).

Tests to assess the integrity of the hypothalamic-pituitary-adrenal axis

Following insulin administration, hypoglycemia of the same degree (1.39 mmol/l) was observed in both responders and non-responders. Both groups had similar increases in ACTH and cortisol (Fig. 6). Following exogenous ACTH administration, cortisol rose to 233.3±8.3 nmol/l in the responders and 294.4±30.6 nmol/l in the non-responders. There was no statistical difference between these two responses.

Twenty-four hours following metyrapone administration, ACTH levels increased from 60±30 ng/l in the basal study to 118±40 ng/l ($p = <0.05$). Cortisol levels during this time decreased from 63.9±38.9 nmol/l in the basal state to 27.8±2.8 nmol/l ($p<0.05$). There was no difference between groups in either ACTH or cortisol levels following metyrapone treatment.

Discussion

RU 486 is an antiglucocorticoid steroid which increases the secretion of ACTH and other proopiomelanocortin-derived peptides as well as cortisol in a dose-dependent manner in man (5–7), monkey (8), and dog (10). In man, RU 486 in a dose of 4 $mg \cdot kg^{-1} \cdot d^{-1}$ increased ACTH and cortisol secretion significantly (7). On the other hand, in dogs, a dose of 5 $mg \cdot kg^{-1} \cdot d^{-1}$ was ineffective, and 20 $mg \cdot kg^{-1} \cdot d^{-1}$ was required for activation of the pituitary-adrenal-axis (9). In addition to its antiglucocorticoid activity, RU 486 also has glucocorticoid agonistic properties (15). However, RU

Table 1. Correlation coefficients (R values).

	ACTH	Cortisol	Protein	RU 486	Di-demethyl	Mono-demethyl
Cortisol	0.69					
Protein	−0.52	−0.57				
RU 486	0.52	0.67	−0.59			
Di-demethyl	0.62	0.74	−0.54	[illegible]		
Mono-demethyl	0.58	0.69	−0.60	[illegible]	0.94	
Hydroxyl				[illegible]	0.61	0.77

Only statistically significant correlation coefficients are shown.
Di-demethyl = di-demethylated derivative; mono-demethyl = mono-demethylated derivative; hydroxyl = hydroxylated derivative.

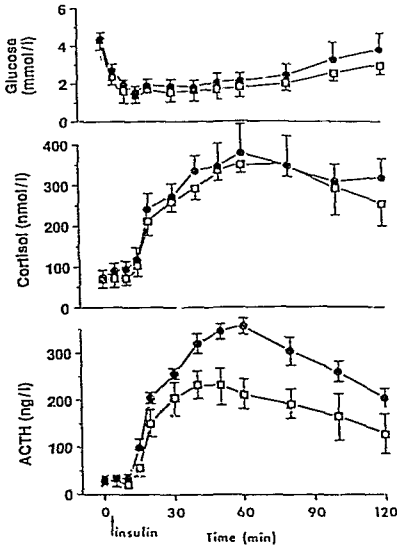


Fig. 6. Mean±SEM glucose, cortisol and ACTH response to insulin-induced hypoglycemia in the two groups of animals (□—□ responder; ■—■ non-responder).

486 was unable to support life in adrenalectomized monkeys (16).

All seven dogs responded with appropriate changes in cortisol and ACTH following exogenous ACTH infusion, insulin-induced hypoglycemia, and metyrapone administration. From this it can be concluded that in all dogs, the ACTH-cortisol axis is intact and responded in a similar manner to classical stimuli. However, when challenged for 10 days with RU 486 at a dose of 20 $mg \cdot kg^{-1}$ and subsequently with 50 $mg \cdot kg^{-1}$, on two separate occasions, three dogs (responders) repeatedly demonstrated elevation in ACTH and cortisol; the other four dogs failed to demonstrate sustained appropriate increases in ACTH and cortisol (non-responders).

Although both groups demonstrated a reduction in serum proteins, this was more pronounced in the responders than in the non-responders. We have previously shown that this dose of RU 486 does not induce changes in electrolytes (9, 10). This suggests the presence of fluid retention with isosmotic expansion of the extracellular volume, which was more pronounced in the responders and probably relates to the mineralocorticoid-like effects of cortisol.

The disparity in ACTH and cortisol responses in the two groups is related to the fact that circulatory levels of RU 486, but more particularly its mono- and di-demethylated derivatives, were higher in the responders than in the non-responders. These differences could be accounted for by variations in absorption, distribution, or metabolism of RU 486. RU 486 absorption exceeds 71% in those species in which this has been studied and the bioavailability ranges from 40% in man and rat to only about 15% in monkeys (14). Detailed pharmacodynamics have yet to be performed in the canine species.

It cannot be excluded that the lower levels of RU 486 noted in the non-responder animals are due to a decrease in the absorption of RU 486. It is, however, unlikely that this is the mechanism underlying the lack of response, since high concentrations of RU 486 and the three metabolites were measured in both groups. Also, a similar divergence in ACTH and cortisol response was noted between the responder and non-responder groups when these dogs were challenged with 20 $mg \cdot kg^{-1} \cdot d^{-1}$ of RU 486. Neither group responded when the dose of RU 486 was 5 $mg \cdot kg^{-1} \cdot d^{-1}$ (9).

In humans, RU 486 is bound to orosomucoid, an α_1-acid glycoprotein (17), and the volume of distribution of RU 486 is only 10% of the ideal body weight (14). In contrast, the volume of distribution is equivalent to twice the body weight in rats and monkeys (14). Although it is not known whether RU 486 is bound to serum proteins in the dog, the high serum RU 486 concentrations do suggest that it is, in fact, probably protein bound.

The non-responder dogs had an increased initial body weight and, for this reason, RU 486 was administered as a function of body weight. Since they were mongrel animals it cannot be concluded that they had greater adipose tissue mass. Nevertheless, it is conceivable that the lower levels of RU 486 and its mono- and di-demethylated derivatives may be due to sequestration into adipose tissue. RU 486 is lipophilic and it is known that fat concentrates lipid-soluble androgens, estrogens as well as progesterone. In addition, adipose tissue is

active in the metabolism of steroids and obese females have been shown to have an increased metabolic clearance of cortisone, as well as testosterone and other androgens which are not bound to sex-hormone binding globulin (18, 19). Thus, increased clearance of RU 486 and its demethylated derivatives could also account for the lower levels in the non-responder group.

RU 486 is metabolized by mono- and di-demethylation of the di-methylaminophenyl group in position 11β, and by hydroxylation of the 17α-propynyl chain in both human and rat (13, 14). These same three metabolites could also be identified in the dog. Serum concentrations of RU 486 were higher in the responder group, although the divergence between the two groups was only statistically significant after logarithmic transfer of the RU 486 levels. However, the levels of the mono-demethylated and di-demethylated derivatives were significantly higher in the responder than in the non-responder group. On the other hand, concentrations of the hydroxylated metabolite were similar in the two groups of dogs. Since the predominant differences between the two groups occurred in the levels of mono-demethylated and di-demethylated derivatives, it is considered likely that variations in biological responses are accounted for by divergence in the RU 486 metabolism between the two groups.

Strain differences in the hydroxylation and reductive catabolism of steroids have been documented in guinea pigs, rats, and mice (20). Since our animals were mongrel dogs, strain differences may also account for the variable response to RU 486 observed. The ratios of the hydroxylated metabolite to RU 486 as well as to the mono-demethylated and di-demethylated compounds were higher in the non-responders. This suggests that in the latter group there is enhanced hydroxylation of RU 486. In contrast, in the responder group the metabolic pathway is directed to mono- and especially di-demethylation, since the responders had a greater ratio of di-demethylated to mono-demethylated compound. The differential metabolism of RU 486 may thus be secondary to genetic differences in steroid metabolism. It is of great interest that the differences in response of the hypothalamic-pituitary adrenal axis in the two groups was associated with alterations in metabolism of RU 486.

The mono-, di-demethylated, and hydroxylated metabolites of RU 486 retain high affinities for the human glucocorticoid receptor. This ranges from 45 to 61% when compared to RU 486 (100%) and dexamethasone (23%) (13). These metabolites also bind with high affinity to the rat glucocorticoid receptor (14). When administered orally to rats, each of the three metabolites behaved as an antiglucocorticoid, the hydroxylated and mono-demethylated metabolites being the most potent (14). The binding affinities of these steroids to the canine steroid receptors have not been investigated; however, the positive correlations between the mono- and di-demethylated metabolites, and the magnitude of the ACTH-cortisol response, suggest that the metabolites retain antiglucocorticoid activity in the dog.

This study has been directed to the antiglucocorticoid effects of RU 486. RU 486 induces abortion when given to women with pregnancies under 49 days' duration, although failure occurs in approximately 20–25% of subjects (2–4, 21). Responsiveness can be improved by the administration of prostaglandins 48 to 72 h after RU 486 administration (21–24). However, despite prostaglandin administration, approximately 5% of subjects still fail to abort (21–24). Could abnormalities of metabolism of RU 486 in the human account for non-responsiveness to the drug? This appears unlikely, since it has recently been shown that no differences in pharmacokinetics of RU 486 exist between subjects who abort and those who fail to respond to RU 486 (25). However, in this study RU 486 and its metabolites were only determined two or three days following RU 486 administration. It is thus conceivable that these differences may have been missed. It should also be noted that differences in drug metabolism do exist between individuals (26), and recent studies have shown that obese women respond less well to RU 486 with regard to abortion induction (24, 27). The results in dogs indicate that detailed studies of RU 486 metabolism in women who abort or fail to abort after RU 486 administration should be performed.

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In situ hybridization of arginine vasopressin (AVP) heteronuclear ribonucleic acid reveals increased AVP gene transcription in the rat hypothalamic paraventricular nucleus in response to emotional stress

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The regulation of anterior pituitary adrenocorticotropin hormone (ACTH) secretion during stress involves several hypothalamic neurohormones, including arginine vasopressin (AVP). In situ hybridization techniques have been used to study the regulation of neuropeptide messenger ribonucleic acids in the hypothalamus. Owing to the relatively slow time course of the changes in cytoplasmic messenger ribonucleic acid concentrations, rapid alterations in the level of neuropeptide gene transcription could not be detected. Because of its rapid processing, the nuclear level of the heteronuclear ribonucleic acid should reflect the rate of its synthesis, namely the transcription of the gene. We have used in situ hybridization with a probe complementary to a portion of an intronic sequence of the rat AVP gene to study rapid changes in the level of AVP gene transcription during emotional stress. The specificity of our technique was demonstrated by the localization of the hybridization signals in the paraventricular nucleus, the supraoptic nucleus and the suprachiasmatic nucleus, and was confirmed by the nuclear localization of the labeling. Isolation and exposure of male rats to a novel environment induced an activation of the pituitary–adrenal axis and an increase in AVP heteronuclear ribonucleic acid concentrations in the paraventricular nucleus 2 h after the onset of the stress, suggesting that an increased AVP gene transcription may play a role in the activation of the pituitary–adrenal axis in response to emotional stress.

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The regulation of anterior pituitary ACTH secretion during stress is a multifactorial process that involves mainly two hypothalamic neurohormones: corticotropin-releasing factor (CRF) and arginine vasopressin (AVP) (1). Corticotropin-releasing factor is synthesized in the parvocellular portion of the paraventricular nucleus (PVN) and is secreted into the hypophysial portal blood (HPB). Arginine vasopressin is synthesized in two classes of neurons in the hypothalamus (2): magnocellular neurons of the supraoptic nucleus (SON) and the PVN, and parvocellular neurons of the suprachiasmatic nucleus (SCN) and the PVN. Arginine vasopressin synthesized by magnocellular neurons is released into the general circulation at the level of the posterior pituitary and acts on the kidney and the blood vessels, while AVP from parvocellular neurons of the PVN is released into the HPB and plays a role in the activation of ACTH release by anterior pituitary corticotrope cells. Several reports have shown that AVP and CRF are co-localized in parvocellular neurons of the PVN (3) and that CRF immunoreactive neurons also contain the messenger ribonucleic acid (mRNA) coding for the AVP precursor (4). The relative participation of CRF and AVP in the regulation of ACTH secretion during stress is still a matter of debate (5). Indeed, in the rat, both CRF and AVP are necessary for the ACTH response to restraint or formalin stress (6). Plotsky et al. (7) have reported that insulin-induced hypoglycemia resulted in no change in hypophysial portal venous CRF but caused significant increase in AVP levels. In addition, passive immunization against CRF blocked ACTH secretion induced by insulin-induced hypoglycemia. This suggests that during hypoglycemia CRF is a tonic factor, while AVP plays a dynamic role in the stimulation of ACTH secretion. However, Guillaume et al. (8) have found an increased release of CRF into the rat HPB under similar experimental condition. Recent studies performed in conscious, unrestrained sheep indicate that both CRF and AVP secretion into HPB are stimulated during stress. During profound hypoglycemia, AVP secretion is stimulated to a greater extent than that of CRF, supporting the above-mentioned hypothesis (9). In addition to the measurements of neuropeptide secretion, in situ hybridization techniques have been used to study the

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PLASMA CONCENTRATIONS AND RECEPTOR BINDING OF RU 486 AND ITS METABOLITES IN HUMANS

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Summary—Using Chromosorb® chromatography and HPLC, we measured the plasma concentrations of RU 486, and its monodemethylated (RU 42633), didemethylated (RU 42848) and alcoholic non-demethylated (RU 42698) metabolites up to 72 h following oral ingestion of 100 mg of RU 486 by five female volunteers. The peak plasma level of RU 486 (4.5 μmol/l) occurred within 1 h after ingestion of the compound; at this point significant amounts of the metabolites were also present in the plasma. After the initial redistribution within 6 h the plasma concentrations of RU 486 and three of its metabolites measured remained stable for 24 h. Concentrations of the monodemethylated metabolite exceeded those of the parent steroid during the time period measured, whereas the concentrations of the didemethylated and alcoholic metabolites were lower than those of RU 486, but still notable. At 72 h the concentrations of all the four steroids were still in the micromolar range. The relative binding affinities of these metabolites to human endometrial and myometrial progesterone receptors as well as to human placental glucocorticoid receptors were determined *in vitro*. The affinity of RU 486 for the human uterine progesterone receptor ($K_d = 1.3 \times 10^{-9}$ M for RU 486) was higher than that of progesterone but lower than that of ORG-2058, a potent synthetic progestin. The relative binding affinities of the monodemethylated, alcoholic and didemethylated metabolites to the progesterone receptor were 21, 15 and 9%, respectively, compared with the parent compound RU 486; each was lower than that of progesterone (43%). RU 486 had an approx. 4-fold higher relative binding affinity to the glucocorticoid receptor than dexamethasone. Interestingly, the relative binding affinities of the metabolites studied to the human glucocorticoid receptor exceeded those of dexamethasone or cortisol. Compared with the parent compound RU 486, they were 61, 48 and 45% for the monodemethylated, alcoholic and didemethylated metabolites, respectively; each was higher than that of dexamethasone (23%). The affinity of dexamethasone to the human glucocorticoid receptor was 1.6×10^{-9} M. These data indicate that the [illegible] of certain metabolites of RU 486 may contribute to a significant extent to the antiprogestagenic (23–33%) and even greater extent to the antiglucocorticoid (47–61%) effects of RU 486.

INTRODUCTION

RU 486 is a recently described 19-nor-steroid derivative with considerable antiprogestagenic and antiglucocorticoidal properties [1, 2]. When given during the luteal phase of the menstrual cycle, RU 486 is able to induce uterine bleeding [1]. In preliminary clinical studies RU 486 induced abortion in approx. 80% of the subjects when given between weeks 5–8 of pregnancy, at a daily dose of 200 mg for 4 days [3, 4]. Recently, using RU 486, Nieman *et al.* reported successful symptomatic treatment of Cushing's syndrome [5].

The dimethylaminophenyl side-chain at carbon 11 of RU 486 is important for antiprogestagenic action [6]. For all mammalian progesterone receptors investigated, RU 486 has a higher affinity than progesterone [4, 7, 8]. The relative binding affinity of RU 486 for the glucocorticoid receptor is either equal to [7] or greater than [4] that of dexamethasone. Synthetic steroids may have biologically active metabolites. Recently, Deraedt *et al.*[9] identified micromolar plasma concentrations of a monodemethylated metabolite after oral ingestion of RU 486. Our earlier studies indicate the presence of additional immunoreactive metabolites [10].

Deraedt *et al.* studied the metabolism of RU 486 in rats and found that the monodemethylated, didemethylated and alcoholic metabolites all retain antiglucocorticoidal and antiprogestagenic activity that correlated with the binding affinity to both progesterone and glucocorticoid receptors [9].

Since RU 486 has a high potential for clinical use, the biological activity of its major metabolites is of

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interest. Hence, plasma concentrations of RU 486 and its monodemethylated (RU 42633), didemethylated (RU 42848) and alcoholic non-demethylated (RU 42698) metabolites were measured specifically by high pressure liquid chromatography (HPLC) up to 72 h following oral ingestion of 100 mg of RU 486. Furthermore, their relative binding affinities for human placental glucocorticoid and uterine (myometrial and endometrial) progesterone receptors *in vitro* were compared with those of reference steroids.

EXPERIMENTAL

Chemicals

RU 486 (17β-hydroxy-11β-(4-dimethylaminophenyl)-17α-(1-propynyl)-estra-4,9-dien-3-one), the monodemethylated metabolite RU 42633 (17β-hydroxy-11β-(4-monomethylaminophenyl)-17α-(1-propynyl)-estra-4,9-dien-3-one), the didemethylated metabolite RU 42848 (17β-hydroxy-11β-(4-aminophenyl)-17α-(1-propynyl)-estra-4,9-dien-3-one), the alcoholic metabolite RU 42698 (17β-hydroxy-11β-(4-dimethylaminophenyl)-17α-(1-propynol)-estra-4,9-dien-3-one) and [6,7-^{3}H]RU 486 (sp. act. 37 Ci/mmol) were kindly donated by the Roussel-Uclaf Research Center, Romainville, France. The molecular structures of the compounds are presented in Fig. 1.

Progesterone (4-pregnene-3,20-dione), dexamethasone (9-fluoro-16α-methyl-11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione) and cortisol (11β,17,21-trihydroxy-4-pregnene-3,20-dione) were purchased from Steraloids Inc., Wilton, NH, U.S.A. ORG-2058 (16α-ethyl-21-hydroxy-19-nor-4-pregnene-3,20-dione) was obtained from Organon Int., Oss, The Netherlands. [6,7-^{3}H] dexamethasone [DXM] (sp. act. 45.8 Ci/mmol) was from New England Nuclear, Boston, MA, U.S.A., and [6,7-^{3}H]ORG-2058 (sp.act. 54 Ci/mmol) from Amersham Int. Ltd. Amersham, U.K.

Acetic acid, diethyl ether, ethyl acetate, ethylene glycol, *n*-hexane, gelatin, glycerol, methanol, triethanolamine, titriplex III (EDTA), and HPLC column Hibar LiChrosorb RP-18 (250 × 4 mm int. dia) were purchased from Merck, Darmstadt, West Germany. Tris–HCl, dithiothreitol and Chromosorb® W-NAW 60/80 Mesh were from Sigma, St Louis, MI, U.S.A. Norit A was purchased from Amend, Irvington, NJ, U.S.A., and dextran T70 from Pharmacia, Uppsala, Sweden. Ammonium sulfate was purchased from Schwartz/Mann and scintillation fluid YA-riatuike (70% pseudochumene) was obtained from Yliopiston Apteekki, Helsinki, Finland.

Human samples

Plasma samples were collected from five healthy female volunteers after oral ingestion of 100 mg RU 486 in mid-luteal phase of their cycle. Uteri were obtained from patients undergoing hysterectomy for uterine fibroids. The last menstrual period of the patients had occurred approx. 2 weeks prior to operation. Only non-myomatous uterine tissue was used for the experiments described below. Placentas were obtained from women undergoing elective Caesarean section.

HPLC studies

The Chromosorb® column—HPLC-method described before [10] was modified. Disposable Pasteur pipettes were packed with 3 ml of Chromosorb® W-NAW 60/80 Mesh/20% ethylene glycol. A plasma sample was applied to the column, left for 30 min

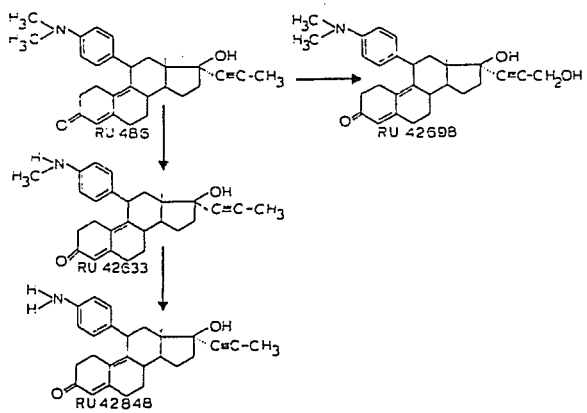


Fig. 1. Molecular structures of RU 486 and its monodemethylated (RU 42633), didemethylated (RU 42848) and alcoholic non-demethylated (RU 42698) metabolites.

and then eluted as follows: (I) 5 ml of ethyl acetate–*n*-hexane 5:95, and (II) 5 ml of ethyl acetate–*n*-hexane 60:40. The eluates were evaporated to dryness, redissolved in the HPLC-eluent used and vortex-mixed. A sample (100 μl) was injected into the HPLC system. The eluent used in HPLC for the assay of RU 486 was methanol–water–triethanolamine, 90:10:0.05, pumped at a rate of 1.5 ml/min; and for the assay of the three metabolites, methanol–water–acetic acid–diethyl ether–triethanolamine, 75:45:30:7.5:0.05, pumped at a rate of 2.2 ml/min.

Preparation of tissue samples

The uterine samples were processed as described by Haukkamaa [11] and placental tissues as described by Kontula *et al.* for adrenal cortical tissue [12]. Cytosol samples were prepared by high-speed centrifugation of tissue homogenates. To remove endogenous steroids from the cytosol samples a Dextran-coated charcoal (DCC) suspension containing 0.5% Norit A, 0.005% Dextran T70 and 0.1% gelatin in 50 mM Tris–HCl-buffer, pH 7.4, was prepared. An aliquot of DCC suspension (the volume corresponding to the cytosolic preparation to be stripped) was centrifuged at 3000 *g* for 10 min. The supernatant was discarded and the cytosol preparation was added to the charcoal pellet. The tubes were vortex-mixed and incubated for 10 min at +4°C. After centrifugation at 3000 *g* for 10 min, the stripped cytosol samples were used for the competitive protein binding assays.

Competitive receptor binding assays

All assays were performed in duplicate or triplicate in disposable glass test tubes and were repeated at least 3 times. For progesterone receptor studies, varying amounts (final concentrations, 10^{-10} to 10^{-5} M) of the steroids investigated (RU 486, RU 42633, RU 42848, RU 42698, ORG-2058 and progesterone) together with 10^{-7} M cortisol (to block binding to corticosteroid-binding globulin and to the glucocorticoid receptor), were pipetted into the tubes and evaporated to dryness. One-hundred microliters of cytosol (diluted to such an extent that approx 50% of the tritiated ligand was bound in the absence of any competitor) and 0.03 μCi of [^{3}H]ORG-2058 (pipetted in 100 μl of 50 mM Tris containing 1% ethanol; final concentration 2.8 nM) were added, the tubes were vortexed-mixed and then incubated overnight at +4°C. After incubation, 200 μl of DCC suspension was added to each tube and the contents vortex-mixed. After 10 min at +4°C, The tubes were centrifuged for 5 min at 3000 *g*. The supernatants (containing the bound fraction of the tritiated ligand) were transferred to polyethylene counting vials together with 3 ml of scintillation fluid and were counted for 5 min in a liquid scintillation 1212 Minibeta counter (Wallac, Turku, Finland). The relative binding affinities of the different compounds to the progesterone receptor were calculated at the 50% competition level according to Korenman [13].

For glucocorticoid receptor studies, similar incubations were carried out, with the following modifications: undiluted placental cytosol was used instead of uterine cytosol; no cortisol was added to the tubes; and [^{3}H]DXM (0.03 μCi/tube; final concentration 3.3 nM) served as tracer instead of [^{3}H]ORG-2058.

Scatchard-plot analysis

To verify the glucocorticoid receptor-nature of the steroid-binding component in placental cytosol, the dissociation constant (K_d) of its interaction with [^{3}H]DXM was measured. Aliquots (0.1 ml) of charcoal-stripped placental cytosol were incubated, in a total volume of 0.2 ml, with varying concentrations (0.3–300 nM) of [^{3}H]DXM dissolved in 50 mM Tris-buffer. The extent of non-specific binding of [^{3}H]DXM was estimated from a parallel set of tubes also containing 10^{-5} M non-radioactive DXM. The tubes were incubated overnight at +4°C. 0.25 ml of DCC was added to separate bound and unbound steroids. Further steps were carried out as described above for the competitive receptor binding assays. The binding data (corrected for non-specific binding) were analyzed according to Scatchard [14].

To measure the K_d of RU 486 for the human uterine progesterone receptor, a partially purified progesterone receptor preparation from human myometrial cytosol was first prepared as described by Kontula *et al.*[15]. Before use, [^{3}H]RU 486 was purified using the Chromosorb® technique [10]. The rest of the analysis was essentially as described above, except that partially purified progesterone receptor preparation and [^{3}H]RU 486 were used instead of placental cytosol and [^{3}H]DXM, respectively, and non-radioactive RU 486 was used instead of DXM for the correction for non-specific binding. No excess of cortisol was used.

RESULTS

The u.v.-absorption spectra of the synthetic metabolites and their behavior in our HPLC system were analyzed. All the synthetic metabolites shared a common u.v.-absorption maximum at 304 nm. Each also had a characteristic u.v.-absorption maximum: RU 42633 at 250 nm, RU 42848 at 240 nm and RU 42698 at 258 nm. Their retention times in our HPLC system were 4 min 36 s, 3 min 56 s and 2 min 49 s, respectively.

Plasma concentrations (mean + SEM) of RU 486 and of its monodemethylated (RU 42633), didemethylated (RU 42848) and non-demethylated alcoholic (RU 42698) metabolites, after oral ingestion of 100 mg of RU 486 by five female volunteers, are depicted in Fig. 2. Peak plasma concentrations of RU 486 (4.5 μmol/l) were reached within 1 h after ingestion of the drug. The concentrations of the monodemethylated metabolite (RU 42633) and hydroxylated alcoholic metabolite (RU 42698) also reached

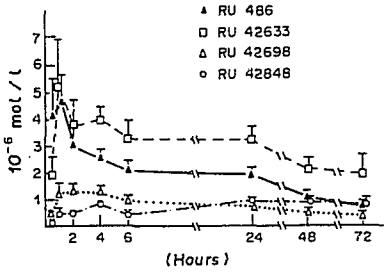


Fig. 2. Plasma concentrations (mean + SEM) of RU 486, RU 42633, RU 42848 and RU 42698, after oral ingestion of 100 mg of RU 486 by five female volunteers.

peak concentrations within 1–2 h suggesting rapid first pass metabolism of RU 486. Plasma concentrations of the didemethylated metabolite (RU 42848) increased slowly between 6 and 24 h, maximum concentrations were measured 24 h after ingestion of RU 486. After initial redistribution of 6 h the plasma concentrations of RU 486 and three of the metabolites assayed plateaued for 24 h or more. Concentrations of the monodemethylated metabolite exceeded those of the parent RU 486. Plasma concentrations of the didemethylated and the alcoholic metabolite were lower than those of RU 486 but still notable. Importantly, both RU 486 and the three metabolites were still present in micromolar concentrations at 72 h.

The binding of RU 486 and its metabolites to human progesterone receptor *in vitro* was studied

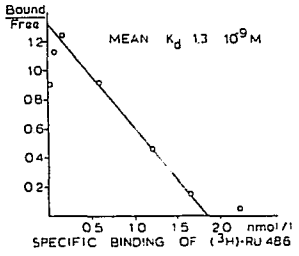


Fig. 3. [³H]RU 486 Scatchard plot analysis of human myometrial progesterone receptor. Mean K_d 1.3 × 10^{-9} M.

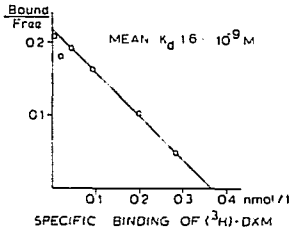


Fig. 4. [³H]dexamethasone Scatchard plot analysis of human placental glucocorticoid receptor. Mean K_d 1.6 × 10^{-9} M.

Table 1. The relative binding affinities of the steroids investigated for human myometrial and endometrial progesterone receptor

Compound*	Relative† affinity %
ORG-2058	373
RU 486	100
Progesterone	43
RU 42633	21
RU 42698	15
RU 42848	9

*For systematic names see Experimental.
†Relative to RU 486 (= 100%).

using both human endometrial and myometrical cytosol. The relative binding affinities were identical and therefore combined. The K_d (mean of three separate experiments) of the binding of RU 486 to the human myometrial progesterone receptor was 1.3 × 10^{-9} M (Fig. 3). The relative binding affinity of RU 486 to the human progesterone receptor was higher than that of progesterone but lower than that of the potent synthetic progestin ORG-2058. All the metabolites of RU 486 studied had a lower affinity to the progesterone receptor than progesterone itself. The relative binding affinities of ORG-2058, progesterone and the three metabolites of RU 486 to the progesterone receptor are given in Table 1.

The binding of RU 486 and its metabolites to the human glucocorticoid receptor *in vitro* was studied using human placental cytosol. Figure 4 shows a representative Scatchard-plot of the interaction between the placental glucocorticoid receptor and tritiated DXM. The mean K_d in four experiments was 1.6 × 10^{-9} M. Competition studies revealed that all three major metabolites of RU 486, along with the parent compound, had higher affinities for the glucocorticoid receptor than the potent glucocorticoids dexamethasone and cortisol. Table 2 gives the relative affinities of the steroids tested for the human placental glucocorticoid receptor (mean values of 5 separate experiments).

DISCUSSION

Synthetic steroid derivatives may have biologically active metabolites. Radioimmunoassays often lack

Table 2. The relative binding affinities of the steroids investigated for human glucocorticoid receptor

Compound*	Relative affinity %
RU 486	100
RU 42633	61
RU 42698	48
RU 42848	45
Dexamethasone	23
Cortisol	9

*For systematic names, see Experimental.
†Relative to RU 486 (= 100%).

the specificity to discriminate between the parent compounds and their metabolites. Furthermore, a metabolite cross-reacting in the radioimmunoassay may lack biological activity.

Earlier studies on plasma RU 486 concentrations were carried out using direct radioimmunoassay [16, 17]. We have developed methods to specifically measure plasma concentrations of RU 486 and its three most proximal metabolic products using Chromosorb®-column chromatography and HPLC. The HPLC method described previously [9] had to be improved since it did not separate the monodemethylated metabolite from the alcoholic metabolite. Our results show that after ingestion of 100 mg of RU 486 by human female volunteers, at least three metabolites of RU 486, the monodemethylated (RU 42633), didemethylated (RU 42848) and alcoholic non-demethylated (RU 42698) forms, are circulating in micromolar concentrations, i.e. close to that of the parent compound for 72 h. When measured by a specific Chromosorb®-HPLC-method the plasma concentrations of RU 486 did not differ significantly when the single oral dose of RU 486 was increased from 100 to 800 mg [10]. This suggests rapid distribution of RU 486 into the tissues, and rapid first-pass metabolism of RU 486. Oral administration of [^{3}H]RU 486 resulted in remarkable extravascular diffusion in rats as reported by Deraedt *et al.*[9]. Studies employing specific HPLC method will reveal whether there is a change in the ratios between RU 486 and its metabolites after the administration of different oral and parenteral doses of RU 486. In general the receptor binding ability of a steroid gives an indication, although not proof, of its biological activity. Deraedt *et al.* determined the relative binding affinities of RU 486, RU 42633, RU 42848 and RU 42698 to cytosolic progesterone and glucocorticoid receptors. Oral administration of RU 486, RU 42633, RU 42848 or RU 42698 in rats resulted in abortion or inhibited the thymolytic effect of dexamethasone thus demonstrating their antiprogestational and antiglucocorticoidal nature, respectively [9]. Their results indicate that the alcoholic metabolite might have a higher biological activity in relation to receptor binding as compared with the monodemethylated metabolite. The relative binding affinities of RU 486 and its three metabolites to the human glucocorticoid and progesterone receptors were determined, using dexamethasone and ORG-2058, respectively, as reference steroids. Before accepting the previously characterized progesterone [11] and glucocorticoid receptor [12] systems as models, the saturability and high affinity of the binding was confirmed in each case (Figs 3 and 4). In previous studies, RU 486 has been shown to display a binding affinity greater than that of progesterone in all the mammalian progesterone receptors investigated [4, 7]. Variations in the reported affinities [4, 7, 8] may be explained by species differences in the characteristics of steroid receptors [15]. The lower binding affinity of progesterone to the human progesterone receptor, as compared to RU 486 (43%, Table 1), is in accord with the value of 67%, which was reported previously by Gravanis *et al.*[8].

The hydrophobic molecular structure of RU 486 reveals features suggesting high affinity binding to progesterone receptor [18]. The antiprogestagenic properties of RU 486 are thought to be due to the dimethylaminophenyl side chain at carbon 11 [6]. Demethylation of this side chain decreases its hydrophobicity, and also decreases the binding affinity of mono- and didemethylated metabolites to 21 and 9%, respectively (Table 1). Hydroxylation of the side chain at carbon 17 decreases the binding affinity of the compound from 100% (RU 486) to 15% (RU 42698) (Table 1).

Based on the relative receptor binding affinities of the metabolites (Table 1) and their plasma concentrations (Fig. 2), it is possible to estimate the contribution of the metabolite pool in the antiprogestational action of RU 486. The theoretical contribution of the prevailing metabolite pool to the antiprogestational activity of RU 486 after ingestion of 100 mg of RU 486 amounts to about 23% at 1 h but as high as 33% at 24 h.

Comparatively little is known about the relative affinity of RU 486 for human glucocorticoid receptors. However, in comparison with published clinical and experimental studies [2, 4, 7], the high affinities of RU 486 and of its metabolites to the human glucocorticoid receptor (Table 2) are not surprising. However, it must be kept in mind that competition studies performed at 0–+4°C in cell-free conditions do not necessarily correctly reflect the situation at +37°C and in the whole organism [19]. The theoretical contribution of the metabolites of RU 486 to the antiglucocorticoidal action of RU 486 was calculated. This was based on the relative receptor binding affinities (Table 2) and plasma concentrations (Fig. 2) of the metabolites. These results suggests that 1 and 24 h after the intake of 100 mg of RU 486, the three metabolites would represent 47 and 61%, respectively, of the total antiglucocorticoid activity of RU 486.

Despite the high affinity binding of RU 486 and its metabolites to the human glucocorticoid receptor *in vitro*, previous clinical experience suggests that large single doses of RU 486 ($\geq$400 mg) are needed to promote antiglucocorticoid effects *in vivo* [2, 20]. Chronic treatment with 25–200 mg/day of RU 486, doses sufficient to produce uterine bleeding in 80% or more cases, did not result in any apparent antiglucocorticoidal effects [1, 3]. This may be partly explained by the fact that the concentrations of plasma cortisol are at least one order of magnitude higher than that of plasma progesterone, even during the luteal phase of the menstrual cycle. The commonly used clinical parameters of antiglucocorticoid activity, i.e. plasma ACTH and cortisol concentrations,

necessitate transport of the antiglucocorticoid molecule to the hypothalamus or the pituitary in order to affect ACTH secretion. Thus, the fact that up to 400 mg of RU 486 was needed to equal the suppressive effects of 1 mg of dexamethasone on ACTH and cortisol *in vivo* [2], might be explained by the higher bioavailability of DXM (32% non-protein bound in plasma, ref. 21) or higher hypothalamic/pituitary uptake of DMX compared to RU 486. In view of the fact that plasma concentrations of RU 486 are not elevated by increasing the oral dose of RU 486 from 100 to 800 mg, all associated with micromolar concentrations of antiglucocorticoid steroids (Fig. 2, ref.10), it still remains an enigma why systemic antiglucocorticoidal effects are virtually never seen at RU 486 doses below 400 mg.

In conclusion, the remarkable binding affinities of the metabolites of RU 486 to human progesterone and glucocorticoid receptors suggest an important role of these metabolites, along with the parent compound, as regards the antisteroidal action of RU 486. This also justifies further metabolic studies after administration of varying oral or parenteral doses of RU 486.

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Successful Treatment of Cushing's Syndrome with the Glucocorticoid Antagonist RU 486*

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ABSTRACT. A patient with Cushing's syndrome due to ectopic ACTH secretion was treated successfully with the new glucocorticoid antagonist RU 486 [17β-hydroxy-11β-(4-dimethylamino phenyl)17α-(1-propynyl)estra-4,9-dien-3-one]. This compound is a 19-nor steroid with substitutions at positions C11 and C17 which antagonizes cortisol action competitively at the receptor level. Oral RU 486 was given in increasing doses of 5, 10, 15, and 20 mg/kg·day for a 9-week period. Treatment efficacy was monitored by assessment of clinical status and by measuring several glucocorticoid-sensitive variables, including fasting blood sugar, blood sugar 120 min after oral glucose administration, and plasma concentrations of TSH, corticosteroid-binding globulin, LH, testosterone-estradiol-binding globulin, and total and free testosterone. With therapy, the somatic features of Cushing's syndrome (buffalo hump, central obesity, and moon facies) ameliorated, mean arterial blood pressure normalized, suicidal depression resolved, and libido returned. All biochemical glucocorticoid-sensitive parameters normalized. No side-effects of drug toxicity were observed. We conclude that RU 486 may provide a safe, well tolerated, and effective medical treatment for hypercortisolism. (*J Clin Endocrinol Metab* 61: 536, 1985)

THE CURRENTLY available treatments for Cushing's syndrome caused by metastatic ACTH-producing tumors or adrenal cancer are often unsatisfactory. Surgical resection of the tumor, when feasible, may be only partially or temporarily effective in controlling Cushing's syndrome. Medical therapy with adrenolytic agents (*o,p'*-DDD) or steroidogenic enzyme inhibitors (aminoglutethimide or metyrapone) is frequently associated with toxic side-effects (1–5).

A clinically applicable glucocorticoid antagonist is, in theory, an attractive alternative treatment for hypercortisolism and has been sought for many years (6). The recently discovered compound RU 486 [17β-hydroxy-11β-(4-dimethylamino phenyl)17α-(1-propynyl)estra-4,9-dien-3-one], a 19-nor steroid with a high affinity for the rat glucocorticoid receptor with no agonist effects *in vitro* or *in vivo*, is a potent competitive glucocorticoid antagonist in rodents (7), nonhuman primates (8, 9), and man (10–12).

We report here the successful treatment with RU 486 of a 25-yr-old man with Cushing's syndrome caused by the ectopic secretion of ACTH. During therapy, the somatic features of Cushing's syndrome (cervical fat pad, central obesity, and moon facies) improved, suicidal depression cleared, and glucocorticoid-sensitive measures, such as elevated fasting and postabsorptive blood sugar, normalized. The drug was tolerated well, and no side-effects were noted during therapy or after its discontinuation.

Case Report

The patient was in excellent health until the fall of 1981 when he noted loss of strength, short term memory, and attention span. In the spring of 1982, because these symptoms worsened, he discontinued his weight-lifting regimen. He complained of increasing anxiety and depression. In September 1982, he stopped working because of these cognitive and psychological changes. Treatment with antidepressants was initiated. His depression deepened, however, and led to two suicide attempts. At that time, he had moon facies, hypertension, and diabetes, and was evaluated for Cushing's syndrome. Both serum and urinary cortisol levels were elevated, and 17-hydroxycorticosteroid excretion increased during a standard 2- and 8-mg dexamethasone suppression test (13).

An intrathoracic mass lesion was found and was resected in

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March 1983. The lesion was not contiguous with a bronchus. Microscopic and immunohistochemical examination of the specimen showed a carcinoid tumor with granules that stained with anti-ACTH serum. Immediately after surgery, plasma cortisol levels were normal. Insulin and antihypertensive and antidepressant medications were discontinued, and the patient's symptoms improved. By May 1983, however, his symptoms recurred, and his urinary cortisol excretion rate was about 500 μg/day. He was given metyrapone, but had only transient clinical improvement.

In August 1983, he was admitted to the NIH. He complained of disorientation, diminished memory and cognitive ability, impotence, a 20-lb weight gain over 3 yr, and long-standing muscle weakness. He had a ruddy round face. His blood pressure was 180/120 mm Hg, and his pulse was 90 beats/min. He was anxious and depressed. He performed calculations slowly. The thoracotomy scar was hyperpigmented. Computerized axial tomograms of the chest revealed multiple lung nodules. He had hypokalemic alkalosis (serum potassium, 1.9 meq/liter; bicarbonate, 38 meq/liter; chloride, 94 meq/liter; sodium, 147 meq/liter).

His medications, including maprotiline hydrochloride (Ludiomil), trifloroperazine (Stelazine), benztropin mesylate (Cogentin), and metyrapone (1 g/day) were stopped before laboratory evaluation. He became withdrawn, severely depressed, and complained that he felt unable to think clearly. Ludiomil was reinitiated because of suicidal ideation, and his depressive symptoms and cognition improved. Potassium supplements were given (20–120 meq/day). Treatment with increasing doses of RU 486 for 9 weeks caused marked improvement in all biochemical and clinical parameters of hypercortisolism (see *Results*).

Materials and Methods

Protocol

The protocol for the therapeutic use of RU 486 was approved under an investigational exemption for new drugs by the National Center for Drugs and Biologics, DHHS, and by the NICHHD Clinical Research Committee (83-CH-87). The patient participated in the study after giving informed consent. All tests were performed at the NIH Clinical Center.

RU 486 was formulated into 50-mg tablets by Roussel-UCLAF (Paris, France). A single oral dose of 6 mg/kg RU 486 given at midnight has been found to prevent morning adrenal suppression caused by 1 mg dexamethasone (11). Accordingly, the initial oral daily dose was 5 mg/kg and increased in 5 mg/kg increments every 1 or 2 weeks to a maximum of 20 mg/kg·day (see Fig. 1).

A number of clinical and biochemical glucocorticoid-sensitive measures were monitored to evaluate treatment efficacy. Clinical measures included blood pressure and body weight. The patient's mood was assessed daily by a self-report questionnaire and three times a week by psychiatric interviews (14). Metabolic and hormone measures included urinary excretion of nitrogen and fasting and postabsorptive blood sugar, which are elevated by hypercortisolism, and plasma concentrations of corticosteroid-binding globulin (CBG) (15), testosterone-estradiol-binding globulin (TeBG), testosterone (16, 17), LH (16, 17), and TSH (18, 19), which are suppressed by hypercortisolism.

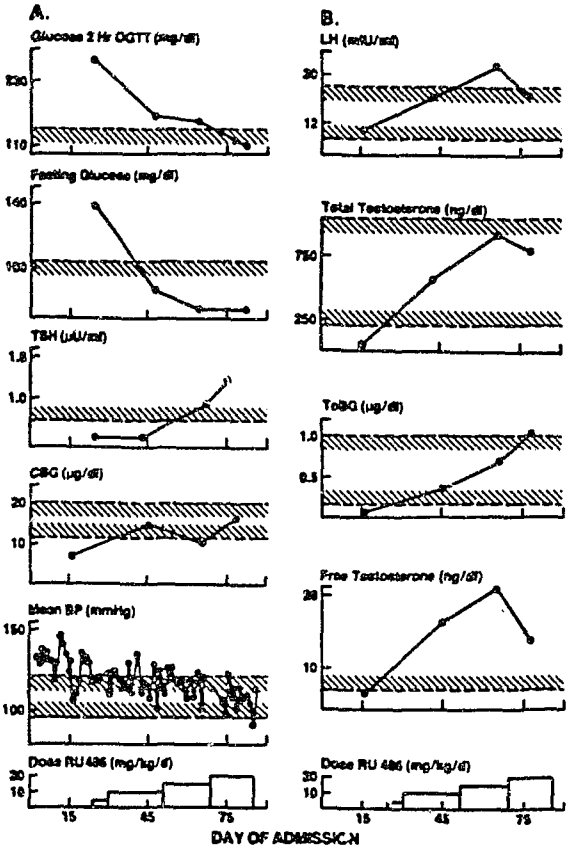


FIG. 1. The effect of RU 486 treatment on glucocorticoid-sensitive variables. A, Two hour post-OGTT (oral glucose tolerance test) and fasting blood sugar levels were elevated before RU 486 therapy and fell to normal levels during treatment. The serum TSH concentration was initially subnormal and rose progressively. CBG concentrations also rose into the normal range. Mean daily blood pressure decreased during RU 486 therapy. B, Plasma concentrations of LH, total testosterone, and free testosterone were initally depressed; all normalized with RU 486 therapy. TeBG capacity showed similar increases. *Shaded areas* represent the upper (▨) or lower (▨) normal range.

Plasma ACTH and plasma and urinary cortisol levels also were measured frequently. Metabolic and hormonal measurements were made on one to three morning blood samples drawn before therapy and during the final week of each dose interval. Standard oral glucose tolerance tests were performed after 3 days of ingestion of a 100-g carbohydrate diet using a 100-g glucose challenge. Creatinine, blood urea nitrogen (BUN), serum glutamic oxaloacetic acid-transaminase (SGOT), and serum glutamic pyruvic acid-transaminase (SGPT) measurements were monitored throughout treatment as indices of drug toxicity. Serial electrocardiograms and chest x-rays were done for a similar purpose.

Assays

Plasma testosterone (20), LH (21), ACTH (22), steroid biosynthetic intermediates (pregnenolone, 17-hydroxypregnenolone, 17-hydroxyprogesterone, and 11-deoxycortisol) (20, 23), plasma and urinary cortisol (23), and serum TSH (24) were measured by RIA as previously described. CBG and TeBG were measured using a solid phase Concanavalin A-Sepharose assay (25). The free testosterone concentration was calculated from the measured levels of total hormone and binding proteins (albumin and TeBG) (25). Plasma glucose concentrations were measured with a Cobas bioanalyzer; SGOT, SGPT, BUN, creatinine and albumin concentrations were measured with an Autoanalyzer (Beckman, Palo Alto, CA).

Using a previously described method for separation of bound from free hormone (25), competitive binding assays were done to exclude displacement by RU 486 of testosterone or cortisol from their plasma binding proteins, an action that might result in spurious changes in hormone concentrations. Increasing concentrations of RU 486 or unlabled hormone were added to samples with known amounts of radioactively labeled hormone and binding globulin. RU 486 did not displace cortisol from CBG or testosterone from TeBG in concentrations ranging from 10^{-10}–10^{-5} M.

Results

All glucocorticoid-sensitive clinical and biochemical parameters were initially abnormal in this patient, and each became normal during treatment with RU 486 despite continued marked hypercortisolism.

The physical stigmata of Cushing's syndrome, including supraclavicular and dorsocervical fat pads and central obesity, regressed considerably by the conclusion of therapy. This change in fat distribution was not associated, however, with a change in total body weight, which varied between 85 and 89 kg both before and during RU 486 treatment. Maximum daily systolic and diastolic blood pressures decreased steadily during treatment with RU 486, from 200/120 mm Hg before therapy, to 140/90 mm Hg at its conclusion (Fig. 1A). The hypokalemic alkalosis resolved, serum potassium ranged from 3.9–4.6 meq/liter, and serum bicarbonate ranged from 25–29 meq/liter following discontinuation of potassium after the sixth week of RU 486 therapy.

Both subjective and objective psychological measures improved during RU 486 therapy. When the daily dose of RU 486 was increased to 15 mg/kg, Ludiomil therapy was stopped (fourth week of therapy). The patient's depression continued to improve, and he reported increasing attention span, libido, and sense of wellbeing. This subjective improvement was corroborated by self-rating questionnaires and psychiatric interviews.

Plasma glucose levels were initially 140 mg/dl in the fasting state (normal, <105 mg/dl) and 268 mg/dl 2 h after ingestion of 100 g glucose (normal, <140 mg/dl; Fig. 1A). The fasting blood sugar level became normal while the patient was taking RU 486 in a dose of 10 mg/kg·day, and the 2 h postoral glucose tolerance test blood sugar level normalized when he was taking 20 mg/kg (Fig. 1A). Serum TSH concentration was initially subnormal (<0.18 μU/ml) and rose progressively to 1.5 μU/ml during treatment (normal, 0.5–4.5 μU/ml; Fig. 1A). CBG-binding capacity increased from 7.4 μg/dl (normal, 12.2–20 μg/dl) to 16.8 μg/dl (Fig. 1B).

Plasma LH levels rose during treatment with RU 486 from 9.4 to 23.2 mIU/ml (normal, 6–26 mIU/ml; Fig. 1B). Similarly, plasma total and free testosterone concentrations and TeBG-binding capacity increased from subnormal to normal levels during therapy with RU 486 (Fig. 1B). The total testosterone concentration was initially 73 ng/dl (normal, 200–1000 ng/dl) and rose to 842 ng/dl when the patient was taking 20 mg/kg·day RU 486. TeBG capacity increased from 0.063 μg/dl (normal, 0.2–1.0 μg/dl) to 1.02 μg/dl at the conclusion of therapy. Free testosterone increased from 3.5 ng/dl (normal, 5–30 ng/dl) to 17.4 ng/dl.

Twenty-four hour urinary nitrogen excretion fell from 22 g/day (normal, 12–20 g/day) before treatment to 5 g/day at its conclusion. No abnormalities in serum creatinine, BUN, SGOT, or SGPT, urinalysis, electrocardiogram, chest radiography, or physical examination were found during or after therapy. The patient experienced no adverse subjective effects.

In contrast to the marked improvement in these glucocorticoid-sensitive parameters, urinary cortisol, plasma cortisol, and ACTH levels remained significantly elevated throughout the treatment with RU 486. G-50 gel chromatography revealed that 85% of ACTH immunoreactivity was in the same fractions as ACTH-(1–39). Before initiation of RU therapy, the mean plasma ACTH concentration was 165 ± 7.6 (±SE) pg/ml (n = 5); during treatment, it was 241 ± 14 pg/ml (n = 14; normal, 8–15 pg/ml). The range of plasma cortisol concentration was 29–49.5 μg/dl (mean ± SE, 43.5 ± 3.3 μg/dl; n = 7) before and 13.8–56.5 μg/dl (mean ± SE, 31.8 ± 2.0 μg/dl; n = 27) during RU 486 administration (normal, 8–18 μg/dl). Mean daily urinary cortisol excretion rates also were elevated, ranging between 514 and 11,592 μg/day (mean ± SE, 4865 ± 1159 μg/24 h; n = 11) before therapy. During therapy, urinary cortisol excretion was similar and ranged between 106 and 8,072 μg/day (mean ± SE, 1175 ± 327 μg/24 h; n = 27; normal, 20–95 μg/24 h).

Plasma steroid precursor concentrations during therapy were within the normal range or mildly elevated. Pregnenolone was 124 ng/dl (normal, <250), 17-hydroxypregnenolone was 135 ng/dl (normal, <250), 17-hydroxyprogesterone was 706 ng/dl (normal, <200), and 11-deoxycortisol was 431 ng/dl (normal, <200).

No side-effects occurred during RU 486 treatment. In the 10th week, because limited availability of RU 486

prevented further treatment, the patient underwent a bilateral adrenalectomy 48 h after discontinuation of therapy and during supplemental glucocorticoid therapy. Tissue vascularity was normal at the time of surgery, and his postoperative course and wound healing were satisfactory.

Discussion

The glucocorticoid antagonist RU 486 ameliorated the clinical and biochemical features of hypercortisolism in this patient. Treatment with RU 486 was associated with redistribution of body fat and resolution of severe depression, hyperglycemia, and hypertension, obviating the need for a variety of medications which he previously required. Several hormonal disorders typical of hypercortisolism (suppressed plasma levels of TSH, LH, testosterone, CBG, and TeBG) also reverted to normal during RU 486 therapy (15, 19).

The satisfactory response to RU 486 administration despite persistent marked elevation of serum and urinary cortisol levels is consistent with studies of its mechanism of action *in vitro* and in animals. RU 486 interacts with the glucocorticoid receptor and thereby blocks the effects of cortisol (7). The mild decline in plasma and urinary cortisol during therapy might be due to an additional effect of RU 486 to diminish adrenal steroidogenesis directly via enzyme inhibition or a result of spontaneous fluctuation in the severity of the syndrome. No major block occurred, however, in the enzymes 3β-hydroxysteroid dehydrogenase-Δ^5,Δ^4-isomerase,21-hydroxylase, 17-hydroxylase, or 11-hydroxylase, as suggested from the levels of measured steroid precursors in the patient's plasma.

Although RU 486 was an effective therapy in our patient with Cushing's syndrome due to ectopic ACTH secretion, control may be more difficult to achieve in patients with hypercortisolism of pituitary origin (Cushing's disease). Previous studies in nonhuman primates and normal subjects suggest that the dose of RU 486 necessary to achieve normal glucocorticoid status in Cushing's syndrome will depend on the plasma free cortisol concentration and the presence of cortisol feedback. In nonhuman primates and normal men and women, doses of RU 486 greater than 5 mg/kg cause an increase in both plasma cortisol and ACTH levels, presumably by antagonizing cortisol feedback at the pituitary or hypothalamus (8–12). In patients with Cushing's disease in whom cortisol feedback is present, ACTH levels may increase, perhaps in an exaggerated manner, as is often the case with ACTH responses to CRH (22) or metyrapone (26). Nevertheless, high doses of a glucocorticoid antagonist may overcome the reserve of the pituitary adrenal axis in patients with Cushing's disease and thus alleviate the toxic effects of hypercorticolism on tissues. If this were true, then an antiglucocorticoid could be used for preparation of patients for surgery.

The lack of side-effects or toxicity associated with RU 486 administration in our patient contrasts markedly with the morbidity that characterizes the other medical treatments for hypercortisolism. Although the incidence of side-effects cannot be established until additional patients are studied, the present experience suggests that RU 486 therapy may be tolerated better than other currently available medical treatments of hypercortisolism. Greater tolerance may yield greater efficacy, since the available medical treatments often cannot be given in fully effective doses because of their side-effects.

One potential problem with RU 486 is that overtreatment might cause glucocorticoid insufficiency. Since glucocorticoid insufficiency cannot be assessed through measurement of adrenal steroids during RU 486 therapy, we suggest that patients be given RU 486 in gradually increasing doses in concert with careful evaluation for signs and symptoms of adrenal insufficiency.

Currently, the major drawback of RU 486 is that it is costly to synthesize and not available in quantities sufficient for extensive clinical study. Despite these problems, RU 486 holds promise as a safe, well tolerated, and effective medical therapy for hypercortisolism that merits further clinical evaluation.

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Treatment of unresectable meningiomas with the antiprogesterone agent mifepristone

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✓ The possibility that meningioma growth may be related to female sex hormone levels is suggested by several lines of evidence. Meningiomas are twice as common in women as in men, have been observed to wax and wane with pregnancy, and are positively associated with breast cancer. A physiological explanation for these phenomena is provided by the finding of steroid hormone receptors in meningiomas. However, unlike breast cancer, meningiomas are much more commonly positive for progesterone receptors than for estrogen receptors.

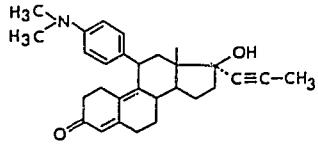
The authors initiated a study on long-term oral therapy of unresectable meningiomas with the antiprogesterone mifepristone (RU486). Fourteen patients received mifepristone in daily doses of 200 mg for periods ranging from 2 to 31+ months (≥ 6 months in 12 patients). Five patients have shown signs of objective response (reduced tumor measurement on computerized tomography scan or magnetic resonance image, or improved visual field examination). Three have also experienced subjective improvement (improved extraocular muscle function or relief from headache). The side effects of long-term mifepristone therapy have been mild. Fatigue was noted in 11 of the 14 patients. Other side effects included hot flashes in five patients, gynecomastia in three, partial alopecia in two, and cessation of menses in two. Long-term therapy with mifepristone is a new therapeutic option that may have efficacy in cases of unresectable benign meningioma.

KEY WORDS • meningioma • mifepristone • progesterone • hormone therapy

THE possibility that growth of meningiomas could be influenced by female sex hormones was originally suggested by epidemiological observations. Intracranial meningiomas are found twice as often in women as in men.[14] Several reports[3,9,19,22,36] have described an increase in meningioma size or symptoms during pregnancy, with resolution after completion or termination of pregnancy, and reappearance during successive pregnancies. An association between meningioma and breast cancer has also been noted.[11,33] Schoenberg, *et al.*,[33] studied data from the Connecticut Tumor Registry and demonstrated a significant correlation between breast cancer and meningioma (eight observed cases vs. 3.37 expected cases, $p < 0.05$). Based on data from the Los Angeles County Tumor Registry, Emry[11] confirmed a positive association for meningioma following breast cancer (37 observed cases vs. 10.5 expected cases, $p < 0.001$).

A physiological explanation for these correlations was first suggested by Donnell, *et al.*,[10] who detected estrogen receptors in four of six meningioma specimens. However, numerous later studies,[2,4,7,12,13,16,17,20,21,27,29,30,32,34,35,37–39] which examined both estrogen and progesterone receptors, determined that the hormone receptor pattern of meningioma differed markedly from that of breast cancer. While positivity for estrogen receptor is more common than for progesterone receptor in breast cancer, the opposite situation exists in meningioma. Overall, 72% of meningioma specimens have been found positive for progesterone receptor while only 31% were positive for estrogen receptor.[2,4,7,10,12,13,16,17,20,21,27,29,30,32,34,35,37–39]

Identification of the putative progesterone-receptor protein as a true receptor has been confirmed by several methods. Markwalder, *et al.*,[20] Blankenstein, *et al.*,[4] and Ironside, *et al.*,[13] have demonstrated appropriate specificity of the progesterone-binding protein through competitive binding assays. Blankenstein, *et al.*,[5] and Press and Greene[28] have also demonstrated positive immunostaining of meningioma specimens with monoclonal antibodies directed against the progesterone receptor. Blankenstein, *et al.*,[5] has further demonstrated an excellent correlation between intensity of monoclonal antibody staining and levels of progesterone



RU 486

FIG. 1. Illustration of the molecular structure of mifepristone (RU486).

receptor. In view of the frequent presence of progesterone receptors and the epidemiological correlations discussed above, modulation of progesterone levels or of the progesterone-receptor protein would seem to be a promising strategy for inhibiting meningioma growth.

Mifepristone (RU486; 11β-(4-dimethyl-amino phenyl)-17β-hydroxy-17α-(prop-1-ynyl)-estra-4,9, dien-3-one; Fig. 1) is a 19-nor steroid with high affinity for both the progesterone and glucocorticoid receptors.[1] Antiprogesterone activity requires a lower dose of mifepristone than for antiglucocorticoid effects.[1] This compound is therefore of great interest in clinical situations where a specific blockade of progesterone receptors is desired. It has been used for termination of early pregnancy[8] and may be useful for contraceptive purposes.[23]

Several *in vitro* studies have supported the possible use of mifepristone as a treatment for meningioma. Olson, *et al.*,[25] using a cell culture assay, demonstrated 18% to 36% growth inhibition by mifepristone in all three meningiomas tested. The same group,[26] using a nude mouse model, demonstrated the disappearance of implanted meningioma nodules in two of three mice. More recently, Blankenstein, *et al.*,[6] demonstrated a significant decrease in thymidine labeling index in 13 meningioma specimens treated with increasing concentrations of mifepristone.

We therefore initiated a trial of the administration of mifepristone for the treatment of unresectable meningioma. The daily oral dose chosen was similar to that used in other studies[8,23] where an antiglucocorticoid effect without a clinically significant antiprogesterone effect was required. However, in contrast to those studies, which required treatment for only a few days, the present study anticipated long-term treatment with mifepristone.

Clinical Material and Methods

Patient Eligibility

Patients with a persistent or recurrent unresectable meningioma with measurable or evaluable disease were eligible for this study. Documentation of the histological diagnosis of meningioma was obtained whenever possible; however, patients in whom biopsy might have resulted in excessive morbidity due to tumor location (optic nerve or cavernous sinus meningiomas) were eligible for the study after a review of records and diagnostic scans by one of the investigators (A.S.), a neuro-ophthalmologist experienced in the diagnosis and treatment of meningiomas in these locations. Adequate hematological reserve (white blood count $\geq$ 3000/cu mm, platelet count $\geq$ 100,000/cu mm), renal reserve (creatinine $\leq$ 2 mg%), and hepatic reserve (bilirubin $\leq$ 2 mg%) were required. All patients were ambulatory adults with a life expectancy of 12 weeks or more. Signed informed consent was obtained from all patients. This study was approved by the Institutional Review Board of the Los Angeles County-University of Southern California Medical Center.

Patients were considered ineligible if curative surgery was possible. Premenopausal females were required to have a negative pregnancy test immediately before beginning therapy and were strongly urged to use effective contraceptive methods. Patients were also ineligible if there was evidence of a second active neoplasm requiring cytotoxic chemotherapy, a serious intercurrent illness, or a history of thrombophlebitis. Patients who had undergone some other additive or ablative hormonal therapy within the preceding 8 weeks were excluded. Patients with prior cranial irradiation were eligible only if the tumor had shown definitive progression following irradiation.

Treatment Plan

All patients received a daily oral dose of 200 mg mifepristone (supplied as 200-mg tablets) throughout the course of the study. This dose was estimated to provide antiprogesterone activity without clinically significant antiglucocorticoid activity.[8,23] However, in view of the possibility of antiglucocorticoid activity resulting from this compound, all patients also received a daily oral supplement of 1 mg dexamethasone for the first 14 days of therapy. Treatment with mifepristone was planned to continue for at least 1 year.

Study Parameters and Follow-Up Period

Patients were seen every month during the 1st year of treatment and every 3 months thereafter. Complete physical and neurological examination including evaluation for subjective side effects or improvement was performed at each clinic visit. A complete blood count and serum chemistry panel were performed every 3 months, and objective tumor measurements were made every 6 months based on computerized tomography (CT) scanning, magnetic resonance (MR) imaging, or visual field examination.

Definition of Responses to Therapy

Responses to treatment were defined as follows: "complete response," complete disappearance of tumor on CT or MR studies; "tumor regression," any reduction in objective tumor measurements (preferably accompanied by subjective improvement or lessening of neurological symptoms); "stable disease," no significant change in objective parameters with no change in symptoms or neurological findings; and "progressive dis-

TABLE 1

Clinical summary in 14 patients with unresectable meningioma

Case No.	Age (yrs) & Sex	Menopausal Status	Karnofsky Performance Scale Score	Tumor Histology	Site of Tumor	Prior Therapy	Duration of Mifepristone Therapy (mos)	Best Response
1	38, M	—	90%	meningothelial	sphenoid wing	surgery	31+	regression
2	49, M	—	80%	meningothelial	cervical spinal cord	surgery, tamoxifen	8	progression
3	38, F	premenopausal	100%	cellular	cervical spinal cord	surgery	24	regression
4	66, F	postmenopausal	90%	meningothelial	petrous axis/ cavernous sinus	surgery	25+	stable
5	56, F	postmenopausal	100%	fibrous	petrous apex	surgery	27+	stable
6	61, F	postmenopausal	60%	malignant	frontoparietal/lung metastases	surgery	2	progression
7	43, M	—	80%	malignant	temporal lobe	surgery, radiotherapy	6	progression
8	63, M	—	90%	meningothelial	cerebellopontine angle/ petrous apex	surgery, tamoxifen	20	stable
9	78, F	postmenopausal	80%	cellular	cervical spinal cord	surgery, tamoxifen	3	refused further therapy
10	41, F	postmenopausal	90%	meningothelial	cavernous sinus	surgery, megestrol acetate	23+	regression
11	52, M	—	100%	unbiopsied	cavernous sinus/sphenoid ridge	none	19+	regression
12	69, F	postmenopausal	90%	fibrous	cavernous sinus/petrous apex	surgery	12+	stable
13	80, M	—	90%	unbiopsied	cavernous sinus	none	12	stable
14	23, F	premenopausal	100%	cellular	cavernous sinus	surgery	9	regression

ease," an increase of more than 25% in tumor size seen on CT or MR studies or any worsening of symptoms or neurological signs.

Results

Patient Characteristics

Fourteen patients were entered into this study between November, 1987, and May, 1989 (Table 1). Although the study population included a wide range of patient ages (range 23 to 80 years, median 54 years) most patients had an excellent Karnofsky Performance Scale score (range 60% to 100%, median 90%). Eight women (two premenopausal and six postmenopausal) and six men comprised the study group. The most common tumor histology was meningothelial or cellular (eight cases). Two patients had fibrous meningiomas and two had malignant meningiomas. Two patients were entered without biopsy; in both cases, a clinical history strongly consistent with meningioma was present and biopsy for the sole purpose of obtaining histology was considered to carry an undue risk of morbidity. The most common location of tumor was in the cavernous sinus or at the base of the brain. Three patients had meningiomas of the cervical spinal cord, and two had cerebral meningiomas. One of the two with a cerebral lesion had a malignant meningioma metastatic to the lung; the other had undergone multiple attempted resections of recurrent meningioma in a cerebral location over a period of 13 years, with progression from meningothelial to malignant meningioma. In 12 patients, prior surgical procedures had been performed. One patient had previously received radiotherapy, one had been given megestrol acetate therapy, and three patients had been administered tamoxifen.

Two patients were receiving chronic glucocorticoid supplementation at the initiation of mifepristone therapy. A third patient began glucocorticoid therapy during mifepristone treatment due to the development of a symptomatic spinal cyst. In two patients chronic thyroid supplementation had been started before the initiation of mifepristone therapy, while a third patient began thyroid supplementation for fatigue and abnormal results of thyroid function tests during mifepristone therapy. Eight patients were receiving chronic treatment with antiseizure medications including Dilantin (phenytoin sodium), Tegretol (carbamazepine), valproate, phenobarbital, and Mysoline (primidone).

Study Therapy

In this study group, daily mifepristone therapy has been delivered for periods ranging from 2 to more than 31 months. Twelve of the 14 patients have received mifepristone for at least 6 months and nine of these have received mifepristone for at least 1 year.

Responses to Therapy

Of 13 patients considered assessable for response (one patient refused further therapy), 12 had received therapy for at least 6 months and one discontinued therapy before 6 months due to progressive disease. Five of these patients experienced tumor regression (Table 2). One male patient with meningothelial meningioma of the sphenoid wing achieved minor decrease in tumor mass, as observed on a serial CT scan, accompanied

TABLE 2

Summary of patients with tumor regression in response to mifepristone therapy

Case No.	Menstrual Status or Gender	Tumor Location	Response*	
			Objective	Subjective
1	male	sphenoid wing	minor decrease on CT scan	improved extraocular muscle function
3	premenopausal	cervical spinal cord	minor decrease on MRI	disappearance of occipital headache
10	postmenopausal	cavernous sinus	minor decrease on CT scan	none
11	male	cavernous sinus/sphenoid ridge	improved visual field examination	improved extraocular muscle function
14	premenopausal	cavernous sinus	minor decrease on MRI	none

* CT = computerized tomography; MRI = magnetic resonance image.

by improved extraocular muscle function. A second male patient with an unbiopsied meningioma in the cavernous sinus/sphenoid wing experienced objective improvement in his visual field examination accompanied by improved extraocular muscle function. One premenopausal patient with a cellular meningioma of the cervical spinal cord achieved minor decrease in tumor mass, as demonstrated by MR imaging, accompanied by resolution of a tumor-related occipital headache. Regrowth of her meningioma occurred after 24 months of mifepristone therapy. One postmenopausal patient with meningothelial meningioma of the cavernous sinus demonstrated a minor decrease in tumor mass on CT scanning. An MR study in another premenopausal patient with cellular meningioma of the cavernous sinus showed a minor decrease in tumor mass. Subjective improvement tended to appear within 2 to 3 months of the initiation of mifepristone therapy while minor objective regression (decrease in cross-sectional tumor size by approximately 10%) was generally noted after 6 to 12 months.

Only three of the 14 patients had direct disease progression while undergoing therapy. One patient with a meningothelial meningioma of the cervical spinal cord had tumor progression after 8 months of therapy. The other two patients were those with the most malignant histologies. Malignant meningioma and lung metastases in one patient progressed 2 months after beginning therapy. The second patient, who had undergone multiple attempted resections of a cerebral meningioma over a 13-year period with increasing histological malignancy, had tumor progression after 6 months of therapy.

Side Effects

All 14 patients were evaluable for side effects. Long-term therapy with mifepristone was well tolerated (Table 3). The most common side effect was mild to moderate fatigue which developed in 11 patients. Both premenopausal patients experienced cessation of menses which continued during the course of treatment. Menses returned in both cases after discontinuation of treatment. Three of the six male patients developed tender palpable gynecomastia during the first several months of therapy and three of them noted intermittent hot flashes. One premenopausal and one postmenopausal patient also noted hot flashes. Two female patients noted mild transient thinning of the hair. No patient noted sexual dysfunction or a change in libido. Increase in serum cortisol and decrease in thyroxine levels were noted and may explain some of the other observations (SM Grunberg, unpublished data). Detailed analyses of hormonal parameters are presently underway.

Two patients chose to discontinue mifepristone therapy. In one patient this decision was due to increasing pedal edema after 3 months of therapy. However, review of medical records revealed that pedal edema had begun to increase prior to initiation of mifepristone therapy. One premenopausal patient chose to discontinue therapy after 9 months due to concern over cessation of menses.

Discussion

Mifepristone

Although mifepristone was originally designed and has been most extensively studied for termination of pregnancy and potential contraceptive purposes,[1,8,23] the potent activity of this agent as a progesterone antagonist and its significant activity as a glucocorticoid antagonist have raised the possibility of numerous other therapeutic applications. Nieman, *et al.*,[24] reported the case of a patient with Cushing's syndrome treated with daily oral administration of mifepristone for 9 weeks at doses ranging from 5 to 20 mg/kg/day. Improvement in clinical symptoms as well as in glucocorticoid-related endocrinological variables were noted during therapy.

Romieu, *et al.*,[31] and Klijn, *et al.*,[15] reported trials of mifepristone as second-line hormonal therapy after tamoxifen in postmenopausal patients with metastatic

TABLE 3

Side effects of long-term treatment with mifepristone

Side Effect	No. of Patients*		
	Premenopausal	Postmenopausal	Male
cessation of menses	2/2	NA	NA
hot flashes	1/2	1/6	3/6
gynecomastia	0	0	3/6
partial alopecia	0	2/6	0
fatigue	2/2	4/6	5/6

* Numbers of patients are presented as affected patients/total number of patients in that group. NA = not applicable.

breast cancer. Romieu, *et al.*, treated 22 patients with mifepristone, 200 mg/day, for periods of 1 to 3 months. Three patients achieved 50% regression of skin lesions while eight patients experienced a decrease in levels of carcinoembryonic antigen. Klijn, *et al.*, treated 11 patients and noted a partial response of lymph node metastases in one. The present study is the first report of mifepristone therapy for meningioma, a tumor for which the potential of progesterone to act as a growth factor has only recently been appreciated.

Progesterone Receptors

Progesterone modulation would be most promising for tumors in which the progesterone receptor level is high. Progesterone receptor levels could not be measured in most of our patients. Some patients were referred to our institution with recurrence or persistence of meningioma months or years after the original surgical procedure. In other patients it was impossible to obtain sufficient tissue for hormone receptor assays due to the unresectable location of the tumor itself. However, due to the high frequency (72%) of progesterone receptor positivity noted in meningiomas in multiple series over the past decade,[2,4,7,12,13,16,17,20,21,27,29,30,32,34,35,37–39] we felt that a trial of mifepristone for all patients with unresectable meningioma was reasonable.

Analysis of Results

We are encouraged by the tumor regression noted in five of the 13 evaluable patients. All five had objective signs of regression after 6 to 12 months of therapy, while three patients also experienced subjective improvement within 2 to 3 months. In this study, the criteria were not as strict as those generally used for partial response of malignant neoplasms (50% shrinkage); however, a relatively benign tumor such as meningioma might not be expected to demonstrate the rapid 50% shrinkage characteristic of a responding malignant neoplasm. In addition, tumors within the closed space of the skull or spinal cord may cause significant neurological deterioration with progression of less than 25% and may be associated with marked clinical improvement when there is regression of a similar magnitude.

Two of the three patients who directly developed progressive disease while on mifepristone therapy were those with the most malignant histologies. Lesch, *et al.*,[17] suggested that a lower incidence of progesterone receptor positivity may be seen in anaplastic meningioma. Thus, malignant meningioma may not be an appropriate histology for trials of hormonal modulation. Previous clinical studies with mifepristone have concentrated on medium-term therapy in postmenopausal patients[15,31] and short-term therapy in premenopausal patients.[8,23] We have demonstrated that extended cessation of menses in premenopausal women and gynecomastia and hot flashes in men may also be observed after long-term therapy of these patient groups.

Long-Term Therapy

The experience with our patient population indicates that long-term therapy with daily oral mifepristone for periods of 2 years or more is feasible and tolerable. All of our patients who were employed at the initiation of therapy were able to continue in their work, although some decreased their workload due to fatigue. One patient who was disabled at the initiation of therapy was able to return to work as extraocular muscle function improved and diplopia decreased. Experience with tamoxifen in breast cancer indicates that long-term therapy may be necessary for maximum effect.[18] The ability to deliver an antiprogestational agent for a period of several years may have similar implications for progesterone-dependent tumors.

Although the daily dose of mifepristone was selected so as to fall in a range that would achieve potent antiprogestational activity without severe antiglucocorticoid activity, we were concerned about the possibility of clinical glucocorticoid deficiency after chronic treatment. Klijn, *et al.*,[15] observed an increase in adrenocorticotropic hormone and serum cortisol levels in patients receiving medium-term therapy with mifepristone. No patient in our study required initiation of glucocorticoid supplementation for signs of clinical glucocorticoid deficiency. However, one patient who was glucocorticoid-dependent prior to initiation of mifepristone therapy required an increase in daily baseline glucocorticoid supplementation for relief of significant treatment-related fatigue.

Future Studies

Objective tumor regression observed during this study was minor; meningiomas may remain stable for years even without therapeutic intervention. These results must therefore be considered preliminary and will require confirmation in larger controlled studies. However, it should be noted that 11 of the patients in this trial had demonstrated objective or symptomatic progression of disease prior to study entry. Our observations of the activity of mifepristone in treating unresectable meningioma represent a fascinating correlation of *in vitro* data on tumor biology with the clinical application of an appropriately designed pharmacological agent. Increased appreciation of the role of steroidal hormones as potential growth factors in specific situations may lead to new therapeutic avenues.

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PREGNANCY TERMINATION WITH A HIGH AND MEDIUM DOSAGE REGIMEN OF RU 486

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ABSTRACT

Sixty healthy pregnant women who wished to terminate their pregnancy and who were no more than 49 days pregnant were treated with one of three different dose regimens of a synthetic progesterone receptor blocker, RU 486. Serum cortisol was measured to determine the antiglucocorticoid effects of this compound.

The high dose but shorter treatment regimen (400 mg/day RU 486 X 4 days or 200 mg/day X 4 days) was associated with a high (>80%) rate of side effects, especially nausea, vomiting, weakness and heavy bleeding and a low rate of success (10 %). A group of 50 subjects received the medium dose but longer treatment regimen (100 mg/day X 7 days). This group had less side effects (40-60%) and a 72.3% success rate of complete abortion.

The AM cortisol values were significantly elevated in all treatment groups but higher in those receiving the high dose. These values returned to normal one week following cessation of treatment.

Medium dose but longer duration (100 mg/day X 7 days) of RU 486 treatment is associated with a higher success rate and less side effects than higher dose therapy administered over a shorter period. There were no predictive indices to determine which subjects would respond successfully. The reason for the failure of the drug in 30% of the subjects on the medium dose is not known at this time.

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INTRODUCTION

With the increase in demand for pregnancy termination, the search for a non-surgical but effective abortifacient has incited new interest. To date, prostaglandin analogues have been the only compounds used in large clinical trials. They have an average success rate of about 64% (1,2), but the frequency of side effects, especialy diarrhea and vomiting, have been high (3,4).

Since progesterone plays an indispensable role in implantion and support of early pregnancy, efforts have been made to develop a compound that either interferes with progesterone action or synthesis. RU 486 (17 β-hydroxy-11 β,4-dimethyl-aminophenyl-17 β-propynl estra-4,9-dien-3-one) is a new synthetic steroid which acts as an antagonist to progesterone and glucocorticoid at the receptor level (5-7). Preliminary results indicate that RU 486 can be used effectively to terminate early pregnancy, but the frequency of incomplete abortion has been high (8-11).

In the present study, the efficacy and tolerance of different doses of this compound in terminating early pregnancy were investigated. In addition, since RU 486 is also a glucocorticoid antagonist, the anti-glucocorticoid effect of these different dosages were examined.

MATERIAL AND METHODS

SUBJECTS STUDIED

A group of 60 healthy women desiring early termination who were no more than 49 days (7 weeks) pregnant as calculated from the first day of their last normal menstrual cycle were selected for this study. All subjects were volunteers and a written informed consent was signed prior to entering the study. Inclusion criteria included a positive pregnancy test with a pelvic examination revealing a uterine size consistent with the gestational age as determined by date of onset of last menses. Medical history, physical examination and electrolyte, complete blood count and serum chemistries were all normal. A real time ultrasound examination (ADR 4000 SL) was done prior to treatment as another measure of gestational age. Subjects were excluded from the study if they had any signs or symptoms of threatened abortion, clinical evidence of cervical incompetence or recent use of glucocorticoids.

On the day treatment was initiated, food and water were withheld for two hours before and following drug administration. RU 486 tablets (supplied by Roussel-Uclaf, Romainville, France, as 50 mg tablets) were ingested at 8:00 AM and 6:00 PM according to the following regimens. High dose: 400 mg/day X 4 days (N=5) or 200 mg/day X 4 days (N=5). Medium dose: 100 mg/day X 7 days (N=50).

A successful abortion was defined as sustained vaginal bleeding and a steady decline in β-HCG. Incidence of side effects, time of onset of bleeding, duration and estimate of amount of bleeding were all recorded.

METHODS

Blood samples for β-HCG, estradiol (E_2), progesterone (P), cortisol, hematology and SMA-18 were drawn at 8:00 AM just prior to ingestion of the first tablet, 4, 8 and 15 days after initial treatment and then weekly until normal. Previously reported (12-14) double antibody radioimmunoassays were used for β-HCG, E_2 and P. Plasma cortisol levels were determined by

Core Endocrine Laboratory, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania. Results are expressed as mean ± SE and the student's t-test, paired-t and chi square test were used for statistical analysis.

RESULTS

HIGH DOSE

Three of 10 patients receiving the high dose complained of mild to moderate headaches. Eight patients complained of moderate to severe nausea and vomiting, and 8 of weakness and tiredness. One patient on the 400 mg/day required hospitalization for hyperemesis.

All subjects experienced uterine bleeding which was extremely heavy in 8 of the 10 women. All but one began bleeding by the fourth day after the initiation of medication (mean onset 3.2 days). Three women receiving the 400 mg/day dosage and 2 receiving the 200 mg/day dosage required an emergency curettage for heavy bleeding. Although the hematocrit had fallen to < 30% (mean 28.2%) in these 5 subjects, no transfusions were necessary.

Only one subject who received the 200 mg/day regimen and none of the 5 subjects ingesting the 400 mg dose had a successful termination of pregnancy with the use of RU 486 alone. The 9 other subjects had a surgical termination of their pregnancy either as an emergency procedure (n=5) or as elective suction curettage on day 14 after ingestion of the last pill (n=4). β-HCG levels did not decline in these 4 subjects despite uterine bleeding.

Mean cortisol levels were significantly increased 4 and 8 days after initial treatment in both the 400 and 200 mg treatment groups (Figure 1). The initial AM cortisol levels (mean 15.4 ± 2.3 ug/dl) rose two-fold by day 4 (mean 30.4 ± 2.3 ug/dl, $p < 0.05$) after the 200 mg dose. Only one patient, however, was above the normal range (10-30 ug/dl). The AM cortisol increased on day 4 to a mean of 36.6 ± 4 ug/dl after the 400 mg dose ($p<0.01$ compared to initial baseline). Of the 5 subjects, 4 had values above the normal levels. Cortisol levels on day 8 after treatment remained significantly elevated in subjects receiving the 200 mg/day ($p<0.05$) and 400 mg/day ($p<0.01$) dosage but returned to baseline by 15 days. Although the day 4 values were greater in those receiving the 400 mg dose, there were no significant differences between the 2 treatment groups.

MEDIUM DOSE

Of the 50 patients receiving 100 mg/day X 7 days, 3 subjects were unable to complete the protocol because of personal problems and were dropped from the study. Of the remaining 47, 34 (72.3%) had a successful termination of pregnancy and 13 were failures.

Side effects included heavy bleeding (59%), nausea or vomiting (40%), uterine cramps (42%) and headaches (9%). These side effects were successfully treated by analgesics and antiemetics. Some of the symptoms such as nausea, occur in normal pregnancy and it was difficult to determine if these side effects were drug related.

Bleeding/spotting began 2.4 ± 0.1 days after starting treatment in the group who aborted and on day 3.2 ± 0.4 days ($p<0.01$) in the group who failed to abort. The total number of days of bleeding/spotting was 13.5

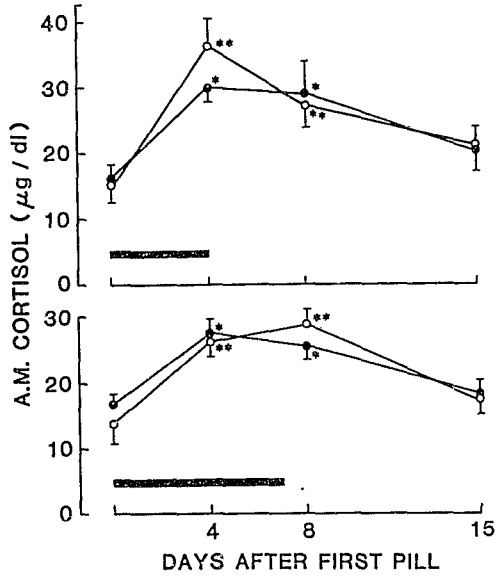


Figure 1. Upper panel: Mean AM cortisol during and after treatment with 200 mg/day X 4 days (●) or 400 mg/day X 4 days (○).

Lower panel: Mean AM cortisol during and after treatment with 100 mg/day X 7 days; (●) successful abortions, (○) failures.
*= p<0.05, **= p<0.01 compared to initial pretreatment level.

Bar represents treatment days with RU 486.

± 1.4 in the group who aborted and 8.0 ± 1.5 in the group which failed (p<0.05). There were 4 patients whose hemoglobin levels fell more than 3 gm% to less than 10 gm%. The lowest value was 8.7 gm%. However, no patient required transfusion, and in all subjects hematocrit and hemoglobin returned to pretreatment levels by day 21. The hemoglobin level in 31 patients fell less than 1 gm% and the remaining 12 patients had a fall of hemoglobin from 1 to 3 gm%.

Mean pretreatment ß-HCG levels tended to be lower in the group who aborted (28,117 ± 3464 mIU/ml) as compared to those who failed (42,918 ± 8144 mIU/ml) but were not significantly different (p=0.056). Baseline P

levels in the group that aborted were lower than in the group that failed (22.1 ± 1.7 vs 25.5 ± 2.3 ng/ml) but this difference was also not significant. Baseline estradiol levels were similar in both groups (848.7 ± 82.8 vs 894.0 ± 154.4 pg/ml). Of the 19 patients with an initial β-HCG < 20,000, 16 (84.7%) aborted. Of the 16 patients with an initial P < 18 ng/ml, 13 (81%) aborted.

There was no significant difference between the 2 groups when comparing days from last menstrual period (45.1 vs 44.9 days) or weeks of gestation as determined by ultrasound (5.9 vs 4.9 wks).

There was a significant increase in AM cortisol values in both the successful and failure groups 4 and 8 days after the start of treatment ($p<0.01$ and $p<0.05$ compared to baseline values) (Figure 1). There were however, no significant differences between the groups who aborted and those who did not. The AM cortisols returned to normal by day 15 in all subjects. There were 18 patients (38%) that had elevations above the normal range for AM cortisol. The AM cortisol values in the high dose group were significantly greater ($p<0.05$) than the subjects using the medium dose regimen on treatment day 4.

DISCUSSION

A higher rate of success and lower incidence of side effects occurred with the longer but medium dose treatment regimen (100 mg X 7 days) ($p<0.001$) as compared to the high dose regimens. In most studies, RU 486 has been shown to be a progesterone antagonist with no agonist properties. However, in one study in postmenopausal women receiving estradiol benzoate pretreatment, RU 486 did display an agonistic effect on DNA polymerase and estradiol dehydrogenase (15) when measured in tissue obtained by endometrial biopsies. If RU 486 does have some agonist effects, this may explain why the high doses were less successful.

On the other hand, RU 486 in higher dose may be more destructive to the endometrium and prevent prostaglandin activity, thus reducing uterine contractility and hemostasis. This action may account for the higher incidence of heavy bleeding requiring emergency curettage in women using this dosage regimen.

It is also conceivable that the increased antiglucocorticoid effects of the higher dose regimen may play a role. The AM cortisol levels in the 2 higher dosage groups were higher on day 4 as compared to the subjects receiving the lower dose and cortisol may have progestational effects at high levels.

Haspels (16) reported a complete abortion rate of 79% in 24 pregnancies which were less than 55 days, when treated with a dose of RU 486 of 200 mg X 4 days. The difference between this study and ours cannot be explained.

The results of this study indicate that RU 486 is a highly promising medication for the non-surgical termination of pregnancy. However, the optimal dosage regimen has not yet been established.

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THE USE OF THE ANTIPROGESTIN RU486 (MIFEPRISTONE)

AS AN ABORTIFACIENT IN EARLY PREGNANCY - CLINICAL AND PATHOLOGICAL FINDINGS; PREDICTIVE FACTORS FOR EFFICACY

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ABSTRACT

RU486, a potent antiprogesterone steroid was administered to 124 women requesting therapeutic abortion. All were less than 49 days from their last menstrual period. Ten of these subjects (Group I) received high doses of RU486 in a decremental dose regimen (400, 300, 200 and 100mg/day) over 4 successive days and 14 received 50mg/day for 7 days (Group II). A further 50 subjects (Group III) received 100mg/day for seven days and the remaining 50 subjects (Group IV) received 450mg in a single dose. In the first three groups, half the daily dose was given in the morning and the remainder in the evening. Blood was collected before, and on Days 4 and 7 and then once a week after commencing therapy until disappearance of circulating ßHCG. In addition to ßHCG, estradiol-17ß (E2), progesterone (P), cortisol, and various metabolic and hematological parameters were measured. Plasma RU486 concentrations were also assayed in Group II, III and IV subjects

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on Day 7 of therapy and in some cases on Days 14 and 21. Ultrasonography was performed in all cases on Day 1 and on Day 14. All the patients bled within five days following RU486 administration, for 1 to 21 days. A complete abortion occurred in 60% in Group I, 50% in Group II, 86% in Group III, and 80% in Group IV. The difference between the last two groups and the first two was significant at $p < 0.01$. The non-responders were submitted to a uterine vacuum aspiration.

A stepwise discriminant analysis was performed and indicated that the best predictors of the outcome of therapy were ßHCG values and the gestational sac diameter. With these criteria,the prediction was accurate in 86.4% of the cases. The best results were obtained in the cases where the ultrasonic measurement of gestational sac was under 10mm in diameter and the initial ßHCG values under 15,000mIU/ml.

Among the observed side effects were moderate pelvic cramps (20.9%), nausea (27%), fainting (4.8%); 61.3% of the women complained of fatigue. Heavy bleeding occurred in 15.3% of the women but only one of them required blood transfusion.

In the patients with complete abortion, ßHCG values decreased to below 500mIU/ml by Day 14 (but in 11 cases values fell below 2,000mIU/ml only by Day 21). Plasma estradiol and progesterone also fell. Cortisol levels increased during therapy especially in subjects of Group I, but returned to basal values after termination of treatment.

Pathological findings showed only partial necrosis of the decidua compacta whilst the deeper layer of the endometrium (decidua spongiosa) remained unaltered. Chorionic villi also remained intact after therapy.

It is concluded that RU 486 is an interesting and novel medical alternative to vacuum aspiration. In this limited study, there was no evident dose-dependent effect, but a time-related effect, as better results were obtained when conceptus size was under 10mm at ultrasonography. The effects of RU486 appear to be limited to the superficial layer of the endometrium. This may explain why it is more effective in the very early stages of the pregnancy.

INTRODUCTION

Progesterone plays a key role in ovum implantation and pregnancy maintenance (1) and its withdrawal induces abortion in animals (2).

RU486 [17ß-hydroxy, 11ß-(4-dimethylaminophenyl) - 17α-(1 propynyl), estra-4, 9-dien-3-one), a steroid synthesized by Roussel Uclaf, Paris, France, has recently been shown to be a potent antiprogestational compound able to interrupt early pregnancy in humans (3-5). RU486 binds to both progesterone as

well as to glucocorticoid receptors and competes with these natural steroids at their receptor sites (6-8).

Different studies have shown that the rate of pregnancy interruption with RU486 is about 60 to 85%, depending on the therapeutic regimens used and duration of pregnancy (3-5).

The present dose-finding studies were conducted to determine which dose is most effective in inducing abortion. The response of a decremental dose regimen of 400, 300, 200 and 100mg/day administered over 4 consecutive days was compared to that of 50mg daily for 7 days, 100mg daily for 7 days, and a single dose of 450mg. The products of abortion were collected to assess morphology of the decidual endometrium.

METHODS

PROTOCOL DESCRIPTION

The study comprised 124 healthy women applying for legal abortion. All had previously documented regular cycles of 26 to 31 days. The age of the pregnancy did not exceed 49 days of amenorrhea from their last menstrual period. In none of the subjects was there evidence of threatened abortion and a pelvic examination showed a uterine size consistent with less than 7 weeks of amenorrhea. Subjects were excluded if there was recent use of corticosteroids. The nature of the study was carefully explained to all subjects who signed an informed consent. The protocol and the use of this drug had been approved by the Hospital Ethics Committee.

RU486 was administered orally according to four different therapeutic regimens. Initially, it was planned to enroll 50 patients in each of these 4 groups. However, because of poor effectiveness and side effects, the trial was terminated earlier in the first two groups. Group I (10 patients) received 400mg on Day 1, and then 300, 200 and 100mg on Days 2,3 and 4, respectively (decremental dose regimen). Group II (14 patients) received 50mg/day for 7 days, Group III (50 patients) 100mg/day for 7 days and Group IV (50 patients) 450mg in a single dose. In Groups I, II and III, RU486 was given in two divided doses between 8 and 10 a.m. and 6 and 8 p.m. No food was permitted one hour before and one hour after drug administration.

On Day 1 of the treatment, ultrasonography was performed to assess the precise duration of pregnancy and to facilitate comparison with a second ultrasonographic examination which was performed on Day 14 of follow-up. The patients recorded all bleeding days, basal body temperature and other side effects. They were taught to examine for passage of products of conception and, when possible, to save this material in a fixative solution. When the abortion occurred at night, they were instructed to refrigerate the material until the next morning.

The subjects returned to the hospital on Days 4 and 7 (between 9 and 10 a.m.), then once a week until ßHCG levels became undetectable. Their blood pressure was recorded prior to each blood collection. ßHCG, progesterone (P), estradiol (E2) and cortisol as well as SMA12 and hematology were determined at each visit. Plasma levels of RU486 were measured in Groups II, III and IV by radioimmunoassay in 108 patients on days 7, 14 and 21 as described previously (8). On Day 7 the outcome of the therapy was evaluated on the basis of uterine size, the degree of cervical opening, amount of bleeding, pathological examination of eliminated products and ßHCG levels.

On Day 14, a second ultrasonography was performed to assess uterine size and contents. Based on the results of the ultrasonography and ßHCG levels, it was decided whether to terminate the pregnancy by vacuum aspiration. If partial elimination had occurred, further ultrasonography and ßHCG determinations were performed one week later. Vacuum aspiration was undertaken if retention was obvious.

After completion of the abortion, the women received counselling for effective contraception. Three months later, a questionnaire was mailed to all patients asking them about bleeding patterns since the abortion date, their final choice of contraception and their feelings about their experience with RU486.

HORMONAL MEASUREMENTS

ßHCG, E2, P, cortisol and RU486 were determined by previously described radioimmunoassay methods (5,8,9,10).

STATISTICAL ANALYSIS

Mean values and standard errors were calculated in responders and non-responders of all groups. Significant differences between groups were evaluated using classic methods. We then performed a stepwise discriminant analysis in order to find the more predictive parameter using the BMDP statistical software (11). This method performed a discriminant analysis between all variables measured on Day 1 before administering the therapy (i.e. ßHCG, P, E2, cortisol, days of amenorrhea and ovular sac diameter). At each step, the variable that adds the most to the separation of the groups is entered into the discriminant function. Output includes at each step, F statistics for entering variables (11).

PATHOLOGICAL STUDIES

In most of the cases the abortive material and the decidua samples were collected in a fixative solution. In Groups I, II and III, alcoholic Bouin solution was used and picric acid-formaldehyde (PAF) solution was used in Group IV. The products of abortion were collected by the patients themselves when abortion occurred. They were told to collect them in a flask containing Bouin or PAF

fixative which was given to them on the first day. In the failure cases, the products of conception resulting from the extraction were immediately collected in PAF solution.

RESULTS

CLINICAL RESULTS: EFFICACY AND TOLERABILITY

A complete abortion occurred in 6 out of 10 patients (60%) in Group I, 7 out of 14 patients (50%) in Group II, 43 out of 50 patients (86%) in Group III and 40 out of 50 patients (80%) in Group IV. The difference between Groups I and II and III and IV was significant at $p < 0.01$. Of the total cohort of 124 patients, 96 aborted (77.4%), whereas 28 failed to respond (22.6%).

When the results were evaluated according to days of amenorrhea, the poorest results were found in cases with amenorrhea of 43-49 days (27.2% in Groups I and II) and the best were observed in those with amenorrhea of less than 42 days (76.9% in Groups I and II) (Table I). In Groups III and IV, there was no difference according to days of amenorrhea.

With the exception of 2 subjects who bled on Day 6 and Day 14, all started bleeding within the first five days after commencing therapy. The duration of bleeding was 2 to 3 weeks in the responders and the blood loss was subjectively, approximately the same to twice the amount of the normal menstrual flow. The non-responders bled less and for a shorter duration (between 1 and 3 days) than did the responders. The responders usually aborted between Day 3 and Day 5 although some as early as day 2 or as late as Day 15. A total of 19 patients (15.3%) had prolonged heavy bleeding of approximately four or five times their normal menstrual flow, and four of them experienced a transient decrease in blood pressure and fainting. In those patients, hemoglobin values decreased up to 9.3% but remained within the normal range. The mean ± SD values were 12.9 ± 1.2g/dl at start and decreased to 11.7 ± 1.5 on day 7. In 3 of these cases (1 in Group II and 2 in Group IV) hospitalization was required as the systolic blood pressure (BPs) was under 90mmHg in 2. In the third subject (in Group II), therapy was initiated at 49 days of amenorrhea. Bleeding initially occurred on Day 14 after commencing the medication. In view of the bleeding it was decided to wait one further week. Though heavy bleeding occurred during the next 7 days, the patient failed to return to the clinic for the next 7 days. Three weeks after commencing therapy her hemoglobin was 5.4g/dl on day 21 and she received a 500ml blood transfusion.

No difference was observed between the women who experienced heavy bleeding and the others as far as age of pregnancy and initial hormonal values are concerned.

Other side effects observed included pelvic cramps (20.2%), nausea (27%) and fainting (4.8%), decrease in blood pressure (BP)(0.03%).

TABLE I

Responders to RU486 according to number of days of amenorrhea or to gestational sac diameter in different therapeutic groups, comparing Groups I & II and Groups III & IV

	Groups I & II n = 24	Groups III & IV n = 100	Total n = 124
Amenorrhea (days)			
< 42	76.9% (10/13)	85.3% (58/68)	83.0% (68/81)
43-49	27.2% (3/11)	86.6% (26/30)	70.7% (29/41)
Sac diameter (mm)			
< 10	60% (6/10)	97.6% (41/42)	90.3% (47/52)
10-20	54.5% (6/11)	78.7% (37/47)	74.1% (43/58)
> 20	0% (0/3)	16.6% (1/6)	11.1% (1/9)

However, when means are considered, no significant difference was observed between pre- and post-treatment BP evaluations, except in 4 of the 19 cases with heavy bleeding. In these 4 cases the mean ± SD for BP was 120 ± 14.1 (systolic BP) and 75 ± 5.7 (diastolic BP) before therapy and after 2 weeks 100 ± 7.0 (systolic BP) and 60 ± 14.1 (diastolic BP). Fatigue was the most common side effect reported by the patients (61.3%). Five subjects, all in Group I, experienced transient mild pyrexia up to 38°C. In 19 of the 28 non-responders, pregnancy was partially interrupted. Partial retention of the products accompanied with fever occurred in 7 of these 28 cases. This required antibiotic therapy both before and after vacuum aspiration. 34 women (27.4%) complained about nausea after starting therapy.

No significant changes in SMA12 were observed after RU486 intake in any subjects. In all groups mean hemoglobin (Hb) and hematocrit values were decreased on Day 7 to about -6 to -8%, compared with the initial values. Normal values were evident by Day 28.

In those 19 subjects who had severe bleeding, the mean reduction in Hb was only -9.3%. The mean ± SD value for Hb was 12 ± 1.2g/dl on Day 1 and 11.7 ± 1.5g/dl on Day 7, indicating a slight decrease. The maximum decrease observed on day 7 was in one patient of 28.9%, from 12.8 to 9.1g/dl. However, as already discussed, in one subject in Group II, Hb decreased from 11.5 to 5.4g/dl on Day 21 and blood transfusion was required.

ULTRASONOGRAPHY

Ultrasonography on Day 1 showed that the gestational sac size varied from undetectable to 40 mm in diameter. In 5 cases, ultrasonography indicated that the pregnancy was more advanced than expected from the last menstrual period. There was a definite relationship between the outcome of therapy and the size of the conceptus as determined by ultrasonography. Failures were more common in those subjects with the largest gestational sacs. When the diameter of the gestational sac was under 10 mm, the incidence of successful outcome in Groups I and II, and III and IV was 60% and 97.6%, respectively. When the diameter of the gestational sac was above 20 mm, most of the cases failed to abort (8 out of 9) (Table I). In the intermediate range of 10 to 20 mm, a high rate of success was still observed in Group III and IV (78.7%) but only 54.5% was noted in groups I and II (Table I).

On Day 14, ultrasonography showed complete uterine evacuation in 66 of the 96 responders. In the remaining 30 responders, there was still evidence of material in the uterine cavity. In those cases who bled initially and eliminated abortive material confirmed by the pathologist to be villi and decidual fragments, a conservative approach was adopted with weekly ßHCG and ultrasound evaluation. If ßHCG showed a progressive decrease and only incomplete evacuation was present at the next ultrasound, we decided to wait and see whether spontaneous evacuation would occur.

Indeed, in 29 out of 30 of these subjects, the abortion was subsequently completed between Day 15 and Day 45 after commencing therapy, as shown by complete uterine evacuation and return of menses.

HORMONAL VALUES (Fig. 1 and 2 and Table II)

1) ßHCG (Fig. 1 and Table II)

Mean ± SD ßHCG levels for the 4 groups are presented in Fig. 1. With one exception, the mean ßHCG values were higher in the non-responders than in the responder group. On Day 1, this was significant ($p < 0.00001$).

In those subjects who aborted, mean ßHCG values decreased rapidly and on day 7 after therapy were 13%, 45%, 21.8% and 25.9% of basal values in Groups I, II, III, IV, respectively. Of 96 responders, 85 ßHCG values were below 500 mUI/ml 14 days after commencement of therapy and values were undetectable within 3-4 weeks. For the 11 other responders, ßHCG values were below 2,000 mUI/ml on day 21 after therapy. In 27 of the 28 cases who failed to abort, the anticipated increase in serum ßHCG of a normal pregnancy was not observed in the time period. For the non-responders, values of ßHCG expressed as per cent of initial values on Day 7 were 109.3%, 128.3%, 132.3% and 121.5% in Groups I, II, III and IV, respectively. In a single subject from Group III (patient J.U.), therapy was initiated at 35 days of amenorrhea with a ßHCG value of 65 mUI/ml (Fig.1). Although the patient bled slightly, ßHCG values increased progressively and the pregnancy continued. The patient did take the medication since blood levels of RU486 were comparable to the other subjects. Moreover immunocytochemical studies showed the presence of RU486 in the endometrial tissue obtained at vacuum aspiration 35 days after commencement of therapy. No explanation for this failure was found.

2) Estradiol (E2) and Progesterone (P) (Fig. 2 and Table II)

Initial pretreatment E2 and P levels in the responder and non-responder groups are represented in Table II. For all subjects, the initial mean ± SEM for E2 was 517.6 ± 28.0 and 793.5 ± 84.7 pg/ml in the responders and non-responders, respectively ($p<0.0001$). Initial mean ± SEM, P values were 23.9 ± 1.3 and 24 ± 2 ng/ml in the responders and the non-responders, respectively (p=NS).

P decreased progressively and more rapidly in the responders than in the non-responders. In the non-responders the initial decrease of P values observed on Day 7 was followed by a secondary increase in groups I and III. There was a transient but non-sustained decrease in P in Groups II and IV.

In the responders, P values on Day 7 were 17.5%, 24.2%, 27.3% and 17.8% of basal values in Groups I, II, III and IV, respectively.

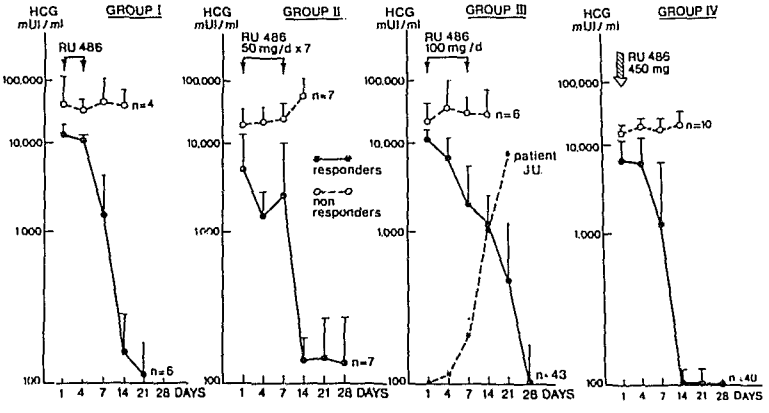


FIGURE 1

Plasma ßHCG values in patients treated with RU486. From the left to the right are shown Group I, II, III and IV. They received, respectively, the decremental regimen (400, 300, 200, and 100 mg/day over 4 successive days), 50 mg/day for 7 days, 100 mg/day for 7 days and 450 mg in a single dose. The subjects who aborted with therapy are designated as responders (●———●) The remaining subjects who failed to abort are designed non-responders (o- - -o). Values shown are mean ± SD. Note ßHCG values are expressed on a logarithmic scale. In Group III patient J.U. is represented alone and not included in the mean of the non-responders of that group since the initial values were very low.

TABLE II

Mean ± SEM values on Day 1 for hormonal parameters, duration of amenorrhea, and diameter of the gestational sac in RU486 responders and non-responders independent of the dose regimen

Basal values Day 0	Successes n = 90	Failures n = 28	p
HCG mUI/ml	10,558 ± 1,338	31,366 ± 5,521	< 0.00001
E2 pg/ml	517.6 ± 28.0	793.5 ± 84.7	< 0.00001
P ng/ml	23.9 ± 1.3	24.0 ± 2.0	NS
Cortisol ng/dl	12.6 ± 0.7	11.3 ± 1.2	NS
Amenorrhea (days)	39.9 ± 0.5	42.5 ± 1.0	< 0.01
Sac Diameter (mm)	8.9 ± 0.7	16.9 ± 1.5	< 0.00001

(Total numer (n) of patients 118; 6 cases have been excluded from the final analysis as some values in the follow-up were missing)

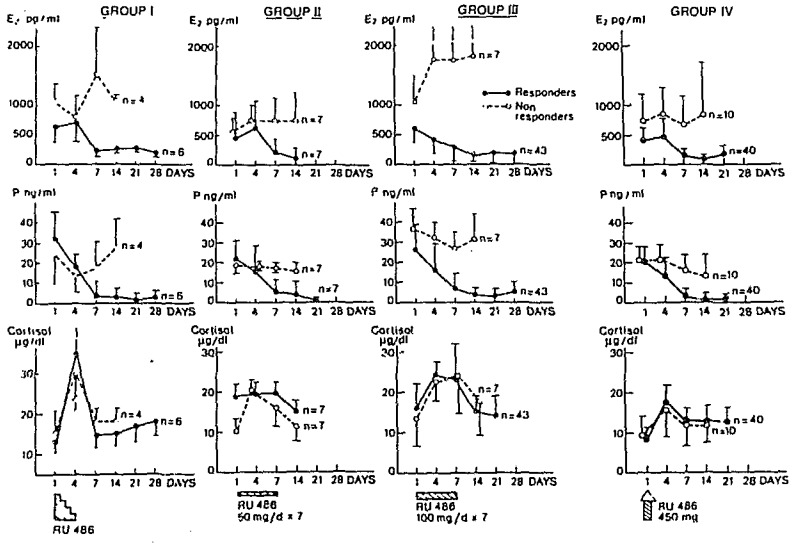


FIGURE 2

Plasma levels of estradiol (E2), progesterone (P) and cortisol in the four groups of patients. See Figure 1 for details.

In the same groups, corresponding P values in the non-responders were 116%, 97.5%, 72.3% and 75.5% of basal values, respectively.

Although mean E2 levels did decrease in the responders, this change was not significant because of the wide range of individual values. E2 either increased or showed no change in non-responders in all groups.

3) Cortisol (Fig. 2 and Table II)

Mean basal morning cortisol levels in this laboratory are 16 ± 7 µg/100 ml. There was an increase in mean cortisol level with all dose regimens and the same trend was evident in both responders and non-responders.

However, only with the decremental dose schedule (Group I) did the plasma levels on Day 4 significantly exceed the normal range. By Day 7, basal levels were attained in this group as well as in Group IV which received RU486 as a single dose. When RU486 was given for seven successive days (Groups II and III), basal values were only attained by Day 14.

4) RU486

Plasma levels of RU486 are presented in Table III. High values of plasma RU486 were seen when the blood sample was collected on the last day of drug intake. Circulating levels were still detectable fourteen days after the last drug intake. When responders were compared with non-responders, no significant difference in RU486 levels was found.

There was no correlation between immunoreactive RU486 levels and P levels either on Day 1 or on Day 7. In one failure case of Group III, plasma RU486 levels were very high on Day 7 and Day 14 but the drug was not found in the decidual tissue obtained by aspiration.

RELATIONSHIP BETWEEN THE DIFFERENT PARAMETERS; PREDICTIVE FACTORS OF EFFICACY

The final stepwise discriminant analysis of the four groups considered together included 107 patients from the 124 who were enrolled. The 17 excluded cases were patients in whom some values were lacking during the follow-up. The only variables useful to predict the outcome were HCG (F value was 31.8 $p < 0.001$) and sac diameter ($F:9.7$ $p<0.001$).

No other parameter could significantly predict the response. The initial values of βHCG and sac diameter were able to predict the outcome of therapy in 86.5% of the successes and 85.7% of the failures. For all cases, 86.4% accurate prediction was possible with these two variables. Significant correlations were found between βHCG levels and sac diameter ($r=0.4$, $p<0.01$) and also E2

TABLE III

Plasma RU486 levels in 108 subjects applying for medical termination of early pregnancy. The samples were collected on Days 7, 14 and 21 after first intake of the drug. In Group II and III, Day 7 was the last day of RU486 intake. In Group IV the total dose was ingested on Day 1, and Day 7 values reflected the drug levels after 6 days of interruption of therapy. In the non-responders, no sampling was performed after Day 14 when surgical termination of the pregnancy was decided.

RU486 LEVELS ng/ml, m ± SD

		GROUP II (50mg/d x 7d) n = 7	GROUP III (100mg/d x 7d) n = 39	GROUP IV (450mg single dose) n = 39
SUCCESSES	Day 7	3,349 ± 2,217	3,611 ± 1,759	340 ± 215
n =	Day 14	37 ± 52	249 ± 296	7 ± 8
	Day 21	1.5 ± 0.6	18 ± 17	2 ± 1.5
FAILURES	Day 7	3,242 ± 1,220	5,061 ± 2,769	317 ± 209
n =	Day 14	113 ± 159	372 ± 374	6 ± 4

Note: Only 108 subjects had an additional plasma sample which allowed us to measure RU486 in addition to the laboratory values originally planned in the protocol.

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