

THE PHARMACOLOGICAL EFFECTS
OF CLIVIA MINIATA ON
ISOLATED RAT UTERUS
AND ILEUM

Denise Joy Hall Veale

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ABSTRACT

Black South African women take an infusion of *Clivia miniata* leaves as a traditional medicine to induce or augment labour. Some of the chemistry of *Clivia* has been investigated and it has been shown to possess antiviral and antimicrobial activity. However, its pharmacological effects on smooth muscle have not as yet been described. There is therefore no explanation for its ethnic use to induce and augment labour and its potential toxicity when used during childbirth cannot be assessed. The pharmacological effects of an aqueous extract of *Clivia* were therefore studied on isolated rat uterus and ileum.

Virgin female Sprague-Dawley rats (150-200 g) were pretreated with diethylstilboestrol and killed by inhalation of CO₂ 24 hours later. Uteri and pieces of ileum were dissected out and were maintained in a 50 ml organ bath containing Tyrode's solution gassed with 5% CO₂ in oxygen. Isotonic contractions against a load of 1 g were recorded electronically. The direct effects of *Clivia* on smooth muscle and its possible interaction with cholinergic, oxytocic, serotonergic and adrenergic receptors as well as prostaglandin synthesis were investigated.

Clivia exhibited smooth muscle stimulant activity, causing concentration dependant contractions of the uterus and ileum and also increasing the frequency of spontaneous uterine contractions. Pretreatment with *Clivia* extract (2×10^{-3} g/ml) augmented the effects of subthreshold and threshold concentrations of acetylcholine (ACh) and oxytocin on the uterus. The extract also increased the effects of subthreshold and threshold concentrations of ACh on the ileum, but at higher concentrations of ACh, *Clivia* inhibited the maximal response of the ileum to ACh. Atropine inhibited the effects of the extract on the response of the uterus and ileum to ACh. Atropine did not affect the effects of *Clivia* on the uterine response to oxytocin. Indomethacin blocked the initial augmentation by *Clivia* of the response of the uterus to ACh but appeared not to alter the effects of the extract on the uterine response to oxytocin or the response of the ileum to ACh. The extract produced a dose-dependant, reversible competitive blockade of serotonin induced uterine contractions. *Clivia* did not seem to interact with the adrenergic system in the uterus.

The uterotonic effects of the extract of *Clivia miniata* would give some foundation for its ethnic use as an oxytocic agent. *Clivia* appears to act directly on

muscarinic receptors in the uterus and ileum and as a competitive antagonist on serotonergic receptors in the uterus. The results of this study indicate that **Clivia** acts dualistically by interaction on multiple receptor types. A functional model is proposed for the pharmacological activity of **Clivia**.

These results indicate a similarity between the pharmacological effects of **Clivia** and ergometrine on the uterus and therefore the possibility exists that large volumes of **Clivia** infusion taken to induce or augment labour could cause toxic uterine hypertonia.

DECLARATION

I hereby declare that the work reported in this dissertation was carried out by me in the Department of Experimental and Clinical Pharmacology, University of the Witwatersrand, Johannesburg. Where help was received, it has been acknowledged. This work is being submitted for the degree of Master of Science in Medicine and has not been submitted before for any degree or examination in any other University.



Denise Joy Hall Veale

26 day of April, 1991.

DEDICATION

This dissertation is dedicated to my parents, Bernice and Hector Norton, for all the love, encouragement and support they have given me over the years.

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LIST OF ABBREVIATIONS

α	Relative intrinsic activity in a system where stimulus and effect are directly proportional
α^S	Relative intrinsic activity in a system where the stimulus and effect relationship is non-linear
[A]	Concentration of agonist drug A
[A']	Concentration of agonist drug after alkylation of the receptors
ACh	Acetylcholine
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
CO ₂	Carbon dioxide
c-AMP	Cyclic adenosine monophosphate
DAG	Diacylglycerol
ϵ	Intrinsic efficacy
e	Efficacy
E	Pharmacological effect
E _A	Effect obtained in the presence of drug A
E _{Am}	Maximal effect obtainable with drug A in the receptor-effector system
E _M	Maximal effect obtainable in the receptor effector system

EC50	Concentration of agonist that produces 50% of its own maximum effect
fig.	Figure
$[G_t]$	Total concentration of coupling G-protein
GDP	Guanyl diphosphate
GTP	Guanyl triphosphate
5-HT	5-hydroxytryptamine or serotonin
IP ₃	Inositol triphosphate
k_1	Association rate constant
k_2	Dissociation rate constant
K_A	Dissociation constant of drug A - receptor complex
K_B	Dissociation rate constant of competitive antagonist (B) - receptor complex
K_G	Dissociation constant of the ternary complex
M	molar concentration
MLCK	Myosin light chain kinase
MLCK-P	Phosphorylated myosin light chain kinase
myosin-P	Phosphorylated myosin
pA_2	Affinity of the competitive antagonist for its specific receptor
pD_2	Affinity of an agonist for its specific receptor
pD_2'	Affinity of a metactoid drug for its non-specific receptor

PGE ₂	Prostaglandin E ₂
PGF _{2α}	Prostaglandin F _{2α}
PI	Phosphoinositide
PIP ₂	Phosphoinositol 4,5 biphosphate
q	Fraction of receptors remaining after alkylation
R	receptor
R'	non-specific receptor
R _C	conformationally changed receptor
R _u	conformationally unchanged receptor
[R]	concentration of free receptors
[RA]	concentration of drug-receptor complex
[R _t]	concentration of the total receptor population
S	Stimulus
S _A	Stimulus caused by the action of an agonist, A, on the receptor
S _{Am}	Maximal stimulus caused by agonist A
S _{pm}	Maximal stimulus that can be generated by a partial agonist of the same family which can maximally cause an effect equal to 1/2 E _m
S.E.M.	Standard error of the mean
SR	Sarcoplasmic reticulum

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INTRODUCTION

Traditional medicine and the use of herbal remedies is an integral part of the lives of most Black South Africans. *Clivia miniata* Regel (fire lily, bush lily) is a member of the Amaryllidaceae family and is indigenous to South Africa. Rural Black South African women take an infusion of the leaves of *Clivia* ("imayime") as a traditional medicine to induce or augment labour (Gerstner, 1941; Watt and Breyer-Brandwijk, 1962; Batten and Bokelmann, 1966; Bryant, 1966; Palmer, 1987; Hutchings, 1989a). Although some of the chemistry of *Clivia* has been investigated (Briggs, Hight and Wildman, 1956; Kobayashi, Ishikawa, Sasakawa, Kihara, Shingu and Kato, 1980; Ieven, Vlietinck, Van den Berge and Totte, 1982; Ali, Ross, El-Moghazy and El-Moghazy, 1983) and it has been shown to possess antimicrobial (Ieven, Van den Berghe, Mertens, Vlietinck and Lammens, 1979) and antiviral activity (Ieven et al., 1982; Ieven, Van den Berghe and Vlietinck, 1983), no attention has been paid to its pharmacological activity on smooth muscle. The potential toxicity of its ethnic use as an oxytocic agent during parturition can therefore not be predicted. The adverse effects of traditional medicines used during childbirth have been described in

the literature (Broster, 1981; Bullough, 1981; Larsen, Msane and Monkhe, 1983; Mbura, Mgaya and Heggenhougen, 1985; Mitri, Hofmeyr and Van Gelderen, 1987). The aim of this study was therefore to investigate the pharmacology of *Clivia* on isolated rat uterus and ileum preparations in order to gain insight into its effects on smooth muscle and assess its potential toxicity when used as an oxytotic agent.

This study of the pharmacology of *Clivia* on the smooth muscle of the uterus and ileum was structured as follows:

- i. The direct effects of an aqueous extract of *Clivia miniata* leaves on smooth muscle required investigation and a study was therefore made of its effects on isolated rat uterus and ileum.
- ii. The interaction of *Clivia* with smooth muscle receptor systems required clarification. The effects of a combination of the extract and various agonists on the cholinergic, oxytotic, serotonergic and adrenergic receptors were therefore investigated.
- iii. Combinations of *Clivia* extract with various agonists and antagonists of these receptor systems was examined in order to determine a possible mechanism of action for *Clivia*.

iv. The influence of the extract on prostaglandin synthesis was investigated to determine a possible indirect effect of *Clivia* in inducing smooth muscle contractions.

CHAPTER 1

Literature Review - Medicinal plants and Ethnopharmacology

1.1 Medicinal plants and traditional medicine - an overview

1.1.1 Medicinal plants

The research into medicinal plants is a fascinating study that goes back to ancient times. Early man, in his search for food, soon discovered the therapeutic properties of plants. By trial and error, he discovered which plants were poisonous and which had healing properties and this was passed on from one generation to another. In the early 1900's 80% of all medicines were obtained from plants (Kreig, 1964). Since then, science has replaced most of the old plant drugs with synthetics. In today's modern world, although herbal medicine plays a very small role in "westernized" society it is still a very important facet of the lives of people living in the developing countries.

In the past few decades there has been an international revival of interest in medicinal plants. If one looks at the number of drugs originally derived from plants which have made important contributions to modern medicine, the potential for discovering new and valuable drugs from plants is very real. Examples here are ephedrine (*Ephedra* species),

digitalis (*Digitalis purpurea*), cocaine (*Erythroxylon coca*), quinine (*Cinchona* sp.), reserpine (*Rauwolfia serpentina*) and dicumarol (Sweet Clover), to name but a few. The most sensible way of discovering new drugs would be the study of herbal remedies used in traditional or "folk" medicine by rural populations who live closely associated with their natural environment.

1.1.2 The international status of traditional medicine

A survey of the current status of traditional medicine and the use of medicinal plants in the world was made by Sofowora (1982) and his findings were as follows:

North and South America - Traditional medicine was still practiced in South America and the south western states of the U.S.A. In Mexico a drug research institute, IMEPLAM, undertook multi-disciplinary research on local medicinal plants and the University of Illinois operated a similar program. Although not common knowledge, an interesting fact was that 25% of all prescriptions dispensed in public pharmacies in the U.S.A. in 1973 contained drugs extracted from higher plants.

Europe and the United Kingdom - Traditional medicine

had been replaced by "alternative" medicine which was very much in the vogue. (Herbal medicines are now quite popular in Europe and the U.K. and the Pharmacognosy Department of the London College of Pharmacy is at present compiling a pharmacopoeia of herbal remedies sold in the United Kingdom (Phillipson, 1989)).

South East Asia - In India, traditional medicine had been integrated into the official health care system and multi-disciplinary research on medicinal plants was carried out by many institutes. China was a good example of the successful integration of traditional and modern medicine and medicinal plants were extensively used and researched.

Africa - Most governments in Africa accepted traditional medicine. Research into African medicinal plants was being financed and co-ordinated by the Organisation for African Unity through its Scientific, Technical and Research Commission.

Farnsworth (1980) describes the highly successful development of the "Task Force on Indigenous Plants for Fertility Regulation" organised by the WHO special Programme of Research, Development and Research Training in Human Reproduction. Six centres, the

University Federal (Recife, Brazil), the University Sri Lanka (Peradeniya, Sri Lanka), the City University (London, England), the University of Illinois (Chicago, USA), the Natural Products Research Centre, Seoul National University (Seoul, South Korea) and the Chinese University of Hong Kong have established a close working collaboration between botanists, natural product chemists, reproductive biologists and pharmacologists. These centres are funded to carry out plant selection, literature surveillance, plant collection, bioassays, isolation of active ingredients and structure-elucidation studies on active principles.

1.1.3 Traditional medicine in South Africa

In South Africa, most of the Black population use traditional herbal medicines. This applies particularly to the rural areas where modern health care facilities are lacking.

Traditional medicine is not integrated into the South African health services. The South African Medical and Dental Council made their rejection of traditional healers official in the Medical, Dental and Supplementary Health Service Professions Act (No.56 of 1974), which forbids non-registered healers to practise or perform any act pertaining to the profession of a

medical practitioner and also forbids medical doctors from working in collaboration with traditional healers. The Associated Health Service Professions Act (No.63 of 1982), however, makes provision for a Council of Associated Health Professions which can register and control healers (Edwards, 1986).

The rural Blacks are in many cases able to identify and harvest their own herbal remedies from the veld. The Zulu, Xhosa and Sotho appear to use many similar remedies. In urban areas, the Black people are able to purchase traditional medicines from the herbalist or "Muti" shops. The plants in the herbalist shops are generally purchased from a central supplier or from individual collectors (Cunningham, 1989; Hutchings, 1989a). Herbal medicine has become an important trade in South Africa and some indigenous plants are being threatened with extinction as a result. In 1987 the Herbal Traders Association had over 100 shops spread throughout Natal/Kwazulu and the National Nyangas Association owned additional outlets. The Institute of Natural Resources is currently working on the establishment of medicinal plant nurseries such as the Silverglen Nursery project developed by the Durban Municipality, which could cultivate medicinal plants cheaply and on a large scale for supply to the herbalist shops and traditional healers (Cunningham,

1989).

Traditional healers still play a very prominent role in Black South African society and they possess the most comprehensive knowledge of medicinal plants. The Zulus call their traditional "doctor" who specializes in herbal medicine "izinyanga" (inyanga) and the Xhosa equivalent is "amaxwhele" (Hutchings, 1989c). The diviners, (or "witch doctors") are traditionally female and are called "izangomas" by the Zulu and "amagqirhas" by the Xhosa. They are qualified "doctors" who act as diagnosticians, soothsayers and interpreters of the ancestors. They also specialize in herbal medicine (Conco, 1972; Broster, 1981; Edwards, 1986). The inyangas in Kwazulu have formed their own professional society, "The National Nyangas' Association", and are required to register with the Kwazulu Department of Health. Other associations of traditional healers have been established but there is no legislation regarding the official registration of healers. A traditional healer usually undergoes a training period of 2 to 5 years under the guidance of a "qualified" healer.

The inyangas are still regarded as "witch doctors" by the majority of the White population and are treated with fear and distrust. Medical doctors and hospitals tend to see only the tragic failures of traditional

medicine and therefore regard herbal remedies with scepticism, anger and contempt. Traditional medicines were found to be responsible for 12,1% of poisonings in Black people in the Garankuwa area (Joubert and Mathibe, 1989). Karlsson and Moloantoa (1984) describe some of the disastrous results of traditional healer treatment - rectal mucosal sloughing following enemas, acute hepatic failure (especially in children), methaemoglobinaemia in all age groups, irreversible eye-damage and perforated ear-drums. The Universities of Kwazulu and the Transkei and Medunsa are at present actively engaged in promoting a working relationship between the traditional healers and the medical services in these areas. In most cases the medical services to the outlying areas are limited and the traditional healers should be able to play an important role in primary health care. The value of the traditional healers in the field of psychiatry has been recognized (Wessels, 1985; Edwards, 1986). Bryant (1966), Conco (1972), Ngubane (1977) and Broster (1981) all found that the traditional healers were of high moral standing and had a profound professional concern for the welfare of their patients. The problem with traditional medicine and herbal remedies in the South African context is, however, the ignorance of some of the traditional healers as to the seasonal toxicity of plants, the standardisation of dosage and

the potential longterm toxicity of some plants. In many cases they are unable to diagnose conditions that require "conventional" medical attention and they are reluctant to refer their patients to hospitals.

Hutchings (1989b) analysed the usage of 794 traditional medicinal plants by the Zulu, Xhosa and Sotho into the following treatment categories: Stress-related ailments; nervous or psychological ailments; pain-producing ailments; general debility; swellings or growths; respiratory ailments; febrile complaints; leprosy; dental problems; sorcery; snake bite; dermatological ailments; fractures, sprains and bruises; ear, nose and throat ailments and procreation-related ailments. Procreation-related ailments included sexual problems, sterility and impotence, venereal diseases, neonatal problems and gynecological problems. The remedies for gynecological problems included special herbal medicines taken regularly during pregnancy and those used during childbirth.

1.2 South African traditional medicine in pregnancy and childbirth

Fertility is a dominant theme in the culture of Black South Africans as it ensures the preservation and propagation of the tribe. Children are regarded as

an insurance against loneliness and poverty in old age and therefore a sterile woman is treated with contempt and pity (Krige, 1957; Gumedi, 1978; Brindley, 1985). Pregnancy is therefore an event of great importance and many traditions and taboos have to be upheld in order to ensure a successful confinement and the birth of a healthy child.

The Zulus believed that certain precautions had to be taken to prevent wizards from harming the unborn child. A pregnant woman had to take care not to walk across the tracks of certain animals. For instance, if she crossed the tracks of an eland her baby could be born with hydrocephalus (Krige, 1957). To avoid these "pathway-poisons" she would tie a bunch of the small umKhondo plant around her ankle as a protective charm (Bryant, 1967). She also had to be careful in her choice of food. The eating of the flesh of certain animals could pass on certain physical characteristics of that animal to her unborn child eg. eating a rock rabbit could cause her child to have long front teeth (Krige, 1957).

Traditional medicine recognized the value of antenatal medication and herbal remedies used in pregnancy and childbirth are therefore numerous. The most common antenatal medication is one known as "isihlambezo"

(that which cleanses) which is taken by both the Xhosa and Zulu women. (Gerstner, 1941; Krige, 1957; Gumedi, 1978; Broster, 1981; Brindley, 1985). This medicine is taken regularly for the last three months of pregnancy in order to ensure an easy confinement and sound foetal growth.

Isihlambezo consists of a number of ingredients, some of them, "inembe", specifically used to facilitate labour (Gerstner, 1941; Bryant, 1967; Conco, 1972). There are many different recipes for isihlambezo and the ingredients are usually a closely guarded secret. Pregnant woman can obtain the recipe from certain old women in the community or an inyanga can be consulted or the isihlambezo ingredients can be purchased from a herbalist. Isihlambezo is generally prepared by boiling the herbal ingredients in water for a long time in order to extract the active principles. This infusion is then kept in a covered pot and a spoonful is taken regularly by the pregnant woman until the day of her confinement. Brindley (1985) reports that amongst the Zulu, the isihlambezo is sipped by the pregnant woman whenever she is thirsty and is also used as a vaginal douche. The pot containing the isihlambezo has to be kept covered and the medicine may not be taken by any other woman or looked upon by anyone else except the inyanga. It is

believed that if anyone looks into the pot or leaves a shadow on the isihlambezo, it may become bewitched or the child could be born mentally deranged. (Gerstner, 1941; Brindley, 1985).

The concentration of the isihlambezo can be increased at term to induce or augment labour. The inembe may also be used individually or in combinations for this purpose (Bryant, 1966; Brindley, 1985). Another remedy called "imbelekisane" may be taken during pregnancy or to facilitate labour (Gumedi, 1978; Broster, 1981; Larsen, Msane, and Monkhe, 1983; Brindley, 1985). Infusions called "umsekelo" (a "propper-up") may also be taken during pregnancy to prevent miscarriage and premature birth (Bryant, 1966; 1967).

The thalidomide tragedy (1960-61) demonstrated the susceptibility of an unborn child to the teratogenic effects of drugs taken by a pregnant woman. Astronomical amounts of money are now spent by the pharmaceutical industry in investigating the safety of drugs in pregnancy. Health care professionals and the public alike are educated and continuously reminded about the dangers of taking drugs in pregnancy. It is therefore both ironical and frightening to see that so little research is being done regarding the

potential toxicity of the herbal remedies used during pregnancy by so many women in South Africa.

Mitri, Hofmeyr and Van Gelderen (1987) found that foetal meconium passage was more common in Black women who had recently taken isihlambezo. Caesarean section and low Apgar scores were significantly more common in pregnancies complicated by meconium passage. Foetal meconium passage could indicate foetal distress due to uterine hypertonia. Broster (1981) quotes Dr P.J. Key, former medical superintendent of All Saints Hospital, Engcobo, Transkei, who implicated that taking large quantities of imbelekisane could be the cause of several cases of unexplained foetal death as a result of premature labour. Larsen et al., (1983) tested an imbelekisane infusion (used by Zulu traditional birth attendants to treat prolonged labour) on isolated rat uterus and found that it repeatedly produced tonic contractions. Hutchings (1989a) lists some toxic plants used in pregnancy by the Zulu and Xhosa of which four are known to have caused fatal human poisoning and eight are regarded as potentially dangerous. Similar cases of toxicity have been reported elsewhere in Africa - Bullough (1981) analysed the causes of maternal deaths in Central Malawi during 1977 and the toxic effects of herbal medicines was diagnosed in a significant number of cases. Mbura, Mgaya and

Heggenhougen (1985) reported incidences of ruptured uteri and hepatorenal failure due to the use of herbal medicines by pregnant women in Tanzania. These examples illustrate the importance of investigating the use of traditional herbal remedies by these Black women.

An isihlambezo mixture purchased from a herbalist shop in Diagonal Street, Johannesburg consisted of a collection of dried leaves, roots, bark, sections of stem, lumps of sulphur, magnesium sulphate crystals and lumps of dried dassie urine (hydraceum), all wrapped up in an old piece of newspaper (fig. 1). Hydraceum is known to be used by the Xhosa as an isihlambezo or imbelekisane ingredient (Broster, 1981) and was identified in the mixture by a Sangoma. It was impossible to identify the herbal ingredients except for some leaves which were tentatively identified by the Botanical Research Institute as *Senecio serratuloides*. The owner of the herbalist shop was not prepared to identify the herbal ingredients and the sales staff refused to sell any more isihlambezo once they discovered that the purchaser was a member of staff at a Medical School. Aqueous infusions were made of individual constituents of the isihlambezo and the hydraceum and ingredient G (unidentified root) both produced tonic contractions when tested on isolated rat

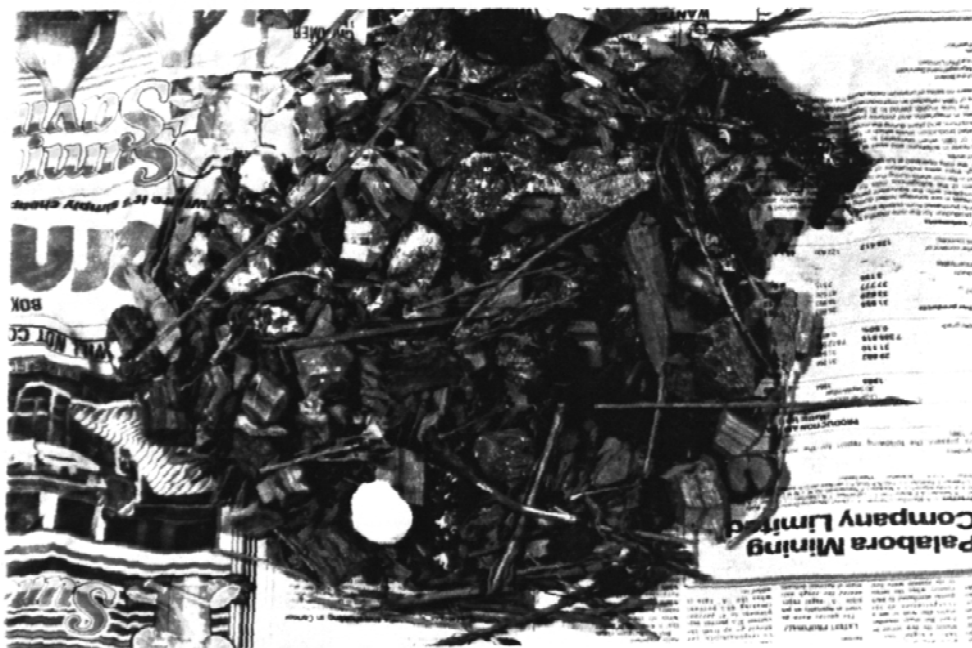
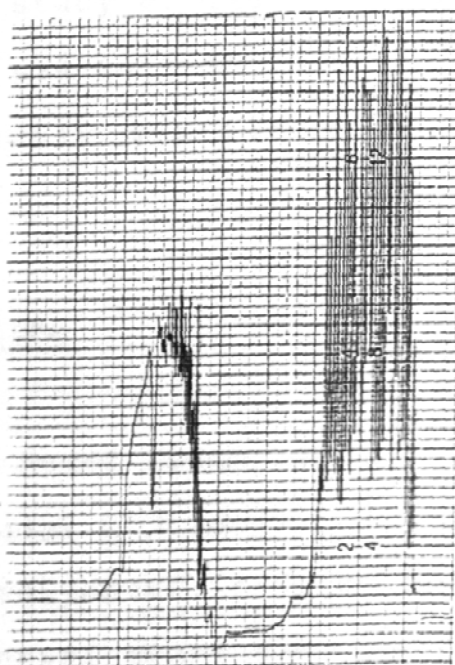


Fig. 1. An isihlambezo mixture purchased from a herbalist shop in Johannesburg.



HYDRACEUM UNIDENTIFIED ROOT

Fig. 2. The effect of aqueous solutions of hydraceum and of root G on isolated rat uterus.

uterus (fig. 2).

An investigation into the potential toxicity of traditional herbal remedies used during pregnancy therefore necessitated a thorough literature survey of plants reported to be used in pregnancy by Black South African women. The results of this survey are contained in Table 1. Many of these plants are poisonous and details of their toxicity is given in Table 2.

In order to initiate a study into the pharmacology and toxicology of these plants used as traditional herbal remedies during pregnancy and childbirth it was decided to start with a study of *Clivia miniata* as its use during pregnancy was well documented in the literature and it was readily available from nurseries in Johannesburg.

1.3 *Clivia miniata*

Clivia miniata Regel (Bush Lily, Fire Lily, Kaffir Lily, St John's Lily) is a member of the Amaryllidaceae family and is indigenous to South Africa. It is an evergreen, perennial plant that is found in shady, moist places (Batten and Bokelmann, 1966; Palmer, 1987). It is also cultivated in gardens for its

magnificent show of flame-coloured blooms in Spring (fig. 3).



Fig. 3. *Clivia miniata* in bloom.

An infusion of *Clivia* leaves is mentioned in the literature as being taken by Xhosa and Zulu women during pregnancy to augment or induce labour (Gerstner, 1941; Watt and Breyer-Brandwijk, 1962; Batten and Bokelmann, 1966; Bryant, 1966; Palmer, 1987; Hutchings, 1989a). *Clivia miniata* is also a South African remedy for impotence and barrenness (Bryant, 1966; Broster, 1981) and the roots are used as a snake-bite remedy and an antipyretic (Watt and Breyer-Brandwijk, 1962).

Some of the chemistry of *Clivia miniata* has been investigated (Briggs, Highet and Wildman, 1956;

Kobayashi, Ishikawa, Sasakawa, Kihara, Shingu and Kato, 1980; Ieven, Vlietinck, Van den Berghe and Totte, 1982; Ali, Ross, El-Moghazy and El-Moghazy, 1983) and it has been shown to possess antimicrobial activity (Ieven, Van den Berghe, Mertens, Vlietinck and Lammens, 1979) as well as antiviral activity (Ieven et al., 1982; Ieven, Van den Berghe and Vlietinck, 1983). To date little attention has been paid to its pharmacological activity and, in particular, to its effects on smooth muscle that can be related to its ethnic use during pregnancy and childbirth.

Isolated tissues have been used extensively by pharmacologists to obtain information about the direct effects of drugs and unidentified compounds on specific organs and to elucidate their mechanisms of receptor interaction (Kenakin, 1984). The objective of this study was to conduct an investigation into the pharmacological effects of an aqueous extract of *Clivia miniata* leaves on isolated rat uterus and ileum preparations in order to gain insight into its ethnic use as an oxytocic agent.

TABLE 1

PLANTS USED IN TRADITIONAL HERBAL REMEDIES BY BLACK SOUTH AFRICAN
WOMEN DURING PREGNANCY AND CHILDBIRTH

Botanical Family and Species ^a	Local Name ^b	Part Used	Remedial Use	Reference ^c
AMARYLLIDACEAE				
<u>Clivia miniata</u> Regel	Umayime(Z)	Leaf	Isihlambezo, Inembe	1,4,5,8 11,12, 15,17.
<u>Crinum bulbispermum</u> (Burm.F) Milne-Redh. and Schweick	Umduzi(Z)		Isihlambezo, Inembe	7,12
<u>Cyrtanthus obliquus</u> (L.F.) Ait.	Umathunga(Z)		Umsekelo	5
<u>C. sanguineus</u> (Lindl) Walp.		Bulb, roots soaked in water	Isihlambezo	14
<u>Scadoxus puniceus</u> (L.) Friis and Nordal (<u>Haemanthus natalensis</u> Pappe ex Hook)	Umphompo(Z), Idumbilikha nhloyile(Z)		Isihlambezo, Inembe	8,9, 11,12
APOCYNACEAE				
<u>Rauvolfia caffra</u> Sond. (<u>R. natalensis</u> Sond.)	Umhlambamanzi (Z), Umjela(X)		Isihlambezo, Inembe	8,12
ASCLEPIADACEAE				
<u>Asclepias fruticosa</u> L.	Usinga lwesal- ukazi(Z)		Isihlambezo, Inembe	8,12
ASTERACEAE (COMPOSITAE)				
<u>Bracylaena elliptica</u> (Thunb.) DC.	Iphahle(Z), Isiduti(X)		Unspecified	12
<u>Callilepis laureola</u> DC.	Impila(Z)	Root	Isihlambezo, Inembe	8,9,12

Botanical Family and Species ^a	Local Name ^b	Part Used	Remedial Use	Reference ^c
<u>Saxania pectinata</u> (Thunb.) Spreng. <u>(G. pinnata</u> (Thunb.)	Isaphete Umkwinti (X)	Whole plant	Prevention of 17 miscarriage	
<u>Senecio bupleuroides</u> DC.			Unspecified	12
<u>S. lanceus</u> Ait.	Ubinihi (Z)		Inembe	6.12
<u>Vernonia natalensis</u> Sen. Bip.	Isibhara (Z)	Root	Isihlambezo	9
<u>V. neocorymbosa</u> Hilliard <u>(V. corymbosa</u> (L.f.) Less)	Ulunguhlungu (Z)		Isihlambezo, Inembe	4.5.8. 9.12
COMBRETACEAE				
<u>Combretum erythro-</u> <u>phyllum</u> (Burch.) Sond.	Umdubu (Z) Umdubo (X)		Isihlambezo	9
CRASSULACEAE				
<u>Cotyledon cuneata</u> Thunb. (C. <u>decussata</u> Sims.)	Seredile (SS)		Difficult confinements	17
<u>Kalanchoe thrysiflora</u> Harv.	Seredile (SS)	Root	Unspecified	1.12 17
CUCURBITACEAE				
<u>Cucumis hirsutis</u> Sond.	Uthangazana (Z)	Root	Isibelekisane, Inembe	3.8
CYATHEACEAE				
<u>Cyathea dregei</u> Kunze	Isikhomakhoma (Z)		Isihlambezo, Inembe	8.12
CYPERACEAE				
<u>Cyperus</u> species	Ibhuma (Z) Indawa (X, Z)	Rush	Inembe	4

Botanical Family and Species ^a	Local Name ^b	Part Used	Remedial Use	Reference ^c
DIOSCOREACEAE				
<u>Dioscorea dregeana</u> (Kunth) Dur. and Schinz	Isidakwa (Z)		Isihlambezo, Inembe	8,12
EBENACEAE				
<u>Euclea undulata</u> Thunb.	Umshekisane (Z) Umgwali (X)		Isihlambezo, Inembe	8,12
FLACOURTIACEAE				
<u>Gerrardina foliosa</u> Oliv.	Umlulama (Z)		Isihlambezo, Inembe	8,12
GENTIANACEAE				
<u>Chironia krebsii</u> Griseb.	Tjatane (S)		Unspecified	1,17
HALORAGACEAE (HALORRHAGIDACEAE)				
<u>Gunnera perpensa</u> L.	Ugobo, Uklenya (Z) Iphuzilomlambo (X)	Root	Isihlambezo, Inembe, Expulsion of placenta	1,2,4,5 8,9,12, 17
ICACINACEAE (HYDNORACEAE)				
<u>Pyrenacantha scandens</u> Planch. ex Harv.	Umsekelo (Z)	Root	Inembe, Umsekelo	4,5,8, 17
IRIDACEAE				
<u>Gladiolus sericeo- villosus</u> Hook.F. (<u>G. ludwigii</u> Pappe ex Bak.)	Isidwa (Z)	Corm	Expulsion of placenta	4,17
LABIATIAE				
<u>Mentha longifolia</u> (L.)	Inzinziniba, Inxina (X), Ufuthanelon- hlanga (Z)	Leaf	Inembe	1,17

Botanical Family and Species ^a	Local Name ^b	Part Used	Remedial Use	Reference ^c
LILIACEAE				
<u>Agapanthus africanus</u> (L.) Hoffmg.	Uhlakahla, Umhlambezo (Z) Isilakati (X)	Root	Isihlambezo, Inembe	3,4,5,7 12,13, 15,17
<u>Aloe arborescens</u> Mill.	Inkalane (Z)	Leaf	Unspecified	17
<u>A. cooperi</u> Bak.	Isiphuthumana, Isiphukutwane (Z)		Isihlambezo, Inembe	8,12,
<u>A. davyana</u> Schonl.	Kxophane (K,T)		Unspecified	17
<u>Bowiea volubilis</u> Harv. ex Hook. F.	Ugibisisila (Z) Umgaqaqana (X)	Tuber	Isihlambezo, Inembe	8,11,12
<u>Chlorophytum comosum</u> (Thunb.) Jacq.		Roots - soaked in water	Isihlambezo (Isikakathi)	10
<u>Eucomis autumnalis</u> (Mill.) Chitt. (<u>E. undulata</u> Ait.)	Umathunga (Z)		Isihlambezo, Inembe	8,11,12
<u>E. regia</u> (L.) L'Herit	Mathunga (Z), Khapumpu (SS)	Root	Prevention of premature birth	17
<u>Ledebouria cooperi</u> (Hook. F.) Jessop (<u>Scilla cooperi</u> Hook.f.)	phetola (S)		Unspecified	12,17
<u>S. natalensis</u> Planch.	Inguduza (Z)		Isihlambezo, Inembe	8,11,12
<u>Urginea physodes</u> (Jacq.) Bak.	Umahlokoloza (Z)		Isihlambezo, Inembe	8,11,12
MENISPERMACEAE				
<u>Cissampelos torulosa</u> E. Mey. ex Harv.	Umthombo (Z)	Root	Isihlambezo, Inembe	8,12

Botanical Family and Species ^a	Local Name ^b	Part Used	Remedial Use	Reference ^c
MORACEAE				
<u>Ficus natalensis</u> Hochst.	Umthombe (Z,X) Uluzi (X)		Isihlambezo, Inembe	8,12
ORCHIDACEAE				
<u>Eulophia clavicornis</u> Lindl. (<u>E. hians</u> sensu Rolfe)	Lekzolela, Moisa-teleha, Maholahanya (S)	Tuber	Unspecified	12,17
<u>E. tenella</u> Reichb. f.			Unspecified	12
PLANTAGINACEAE				
<u>Plantago major</u> L.	Indlebe kathe- kwane (Z)	Root	Isihlambezo, Inembe	2,8,12, 17
POLYGALACEAE				
<u>Polygala fruticosa</u> Berg. (<u>P. oppositifolia</u> L.)	Ithethe (Z)		Isihlambezo, Inembe	8,12
RUBIACEAE				
<u>Pentanisia prunelloides</u> (Eckl. and Zeyh.) Walp. (<u>P. variabilis</u> Harv.)	Icimamlilo (Z,X)	Root	Isihlambezo, Inembe, Expulsion of placenta	1,3,8,9 12,17
RUTACEAE				
<u>Ruta graveolens</u> L.			Inembe	17
SCROFULARIACEAE				
<u>Cycnium racemosum</u> Benth.	Injanga (X)	Root	Inembe	1

Botanical Family and Species ^a	Local Name ^b	Part Used	Remedial Use	Reference ^c
THYMELAEACEAE				
<u>Gnidia capitata</u> L.f. (<u>Lasiosiphon capitatus</u> (L.f.) Burt Davy)	Mofubetswana, Setele(SS), "Radix jovis"		Unspecified	17
<u>G. kraussiana</u> Meisn. (<u>Lasiosiphon kraussii</u> Meisn.)	Imfuzane(Z)		Isihlambezo, Inembe	8,12
TILIACEAE				
<u>Grewia occidentalis</u> L.	Iklolo, Ilalanyathi(Z)	Root	Isihlambezo	4,9,17
<u>Triumfetta rhomboidea</u> Jacq.	Inothwane, Indola encane (Z)	Root	Inembe	4,12,16 17
TYPHACEAE				
<u>Typha capensis</u> (Rohrb.) N.E. Br. (<u>T. latifolia</u> L.)	Umkhanzi(X) Ibhuma(Z) Motsitla(S)	Root	Isihlambezo, Inembe, Expulsion of placenta	1,8,12, 17
VERBENACEAE				
<u>Clerodendrum triphyllum</u> (Harv) H.Pearson	Umathanjana(Z)		Isihlambezo, Inembe	8,12
VITACEAE				
<u>Cyphostemma natalitium</u> (Szyszyl.) J.v.d.Merwe (<u>Cissus connivens</u> Lam.)	Igolo lenkon- yane, Ingquza yenkonyane(Z)		Isihlambezo, Inembe	8,12
<u>Rhoicissus tridentata</u> (L.f.) Wild and Drum (<u>R. cuneifolia</u> (Eckl. and Zeyh.) Planch., <u>R. cirrhiflora</u> sensu Gilg and Brandt, <u>Cissus cuneifolia</u> Eckl. and Zeyh.)	Isinwazi(Z)	Root	Isihlambezo, Inembe	4,8,9, 12,17

Footnote

^a The plant nomenclature has been updated according to Gibbs Russell et al. (1985, 1987) and all plant names given in parentheses are original names as they appear in the literature.

^b Abbreviations: K = Kwená; S = Sotho; SS = South Sotho; T = Tswana; X = Xhosa; Z = Zulu

^c Reference:

1 = Batten and Bokelman (1966)	10 = Hutchings (1986)
2 = Brindley (1985)	11 = Hutchings (1989a)
3 = Broster (1981)	12 = Hutchings (1989b)
4 = Bryant (1966)	13 = Krige (1957)
5 = Bryant (1967)	14 = Mbuthama (1989)
6 = Gerstner (1938)	15 = Palmer (1985)
7 = Gerstner (1939)	16 = Osore (1982)
8 = Gerstner (1941)	17 = Watt and Breyer-Brandwijk (1962)
9 = Gumedi (1978)	

TABLE 2

POTENTIAL TOXICITY OF PLANTS USED IN TRADITIONAL HERBAL REMEDIES
TAKEN DURING PREGNANCY AND CHILDBIRTH^a

Botanical Family and Species	Extent of Toxicity ^b	Main Features of Poisoning	Toxic Principles
AMARYLLIDACEAE			
<u>Clivia miniata</u>	++ (bulb)	Salivation, vomiting, diarrhoea, depression of CNS in severe cases.	lycorine
<u>Scadoxus puniceus</u>	++ (bulb, leaf)	Dizziness, visual disturbances, CNS excitation or depression.	lycorine, haemanth- idine.
APOCYNACEAE			
<u>Rauvolfia caffra</u>	+		rauvolfine
ASCLEPIADACEAE			
<u>Asclepias fruticosa</u>	+	Severe gastrointestinal irritation.	cardiac glycosides
ASTERACEAE			
<u>Callilepis laureola</u>	+++ (tuber)	Confusion, vomiting, diarrhoea, convulsions, hypoglycaemia, hepatic failure, renal failure.	atractyl- oside
<u>Senecio bupleuroides</u>	++	Abdominal pain, nausea, vomiting, ascitis, cirrhosis.	pyrroliz- idine alkaloids
COMBRETACEAE			
<u>Combretum erythro- phyllum</u>	+		

Botanical Family and Species	Extent of Toxicity ^b	Main Features of Poisoning	Toxic Principles
CRASSULACEAE			
<u>Cotyledon cuneata</u>	+	Poisonous to stock	cotyled- ontoxin
<u>Kalanchoe thyrsiflora</u>	+	Poisonous to stock	piperid- ine alka- loids
LILIACEAE			
<u>Agapanthus africanus</u>	+	Sap causes severe ulceration of mouth.	
<u>Bowiea volubilis</u>	++ (tuber)	Severe vomiting and diarrhoea, salivation, cardiac failure.	bufadien- olides
<u>Eucomis autumnalis</u>	++ (bulb)	Abdominal pain, diarrhoea, renal failure.	haemolytic saponin
<u>Scilla natalensis</u>	++ (bulb)	Death of experimental sheep.	
<u>Urginea physodes</u>	++ (whole plant)	Death of stock	bufadien- olides
THYMELAEACEAE			
<u>Gnidia kraussiana</u>	++ (young leaf, flowering tip)	Severe gastrointestinal irritation, death of stock.	mezerine
VITACEAE			
<u>Rhoicissus tridentata</u>	++ (rootstock)	CNS paralysis, respiratory arrest.	

Footnote

^a References: Frohne and Pfander, 1983; Hutchings, 1989 a, b;
Munday, 1988; Veale, 1987; Watt and Breyer-Brandwijk
1962.

^b Extent of Toxicity Code:

+	=	Potentially poisonous
++	=	Potentially fatal if taken in large quantities
+++	=	Potentially fatal if taken in small quantities

CHAPTER 2

Literature Review - Receptor theory and the interpretation of results obtained from isolated organ experiments

2.1 The isolated organ preparation

An aqueous extract of *Clivia miniata* is taken by Black women to induce or augment labour. This suggests that the extract may act as an oxytocic, affecting the strength of uterine contractions, or it may have a laxative effect. The possible mechanism(s) of action of *Clivia* can be studied "in vitro" by investigating pharmacodynamic interactions using the well described "isolated organ" technique. Van den Brink (1977) states: " All facts considered and in spite of its limitations, the isolated organ experiment is an indispensable tool of molecular pharmacology, although not the only one".

Isolated smooth muscle preparations are commonly used for fundamental pharmacodynamic research and for pharmacological screening purposes. The isolation of the organ frees it from complicating factors such as nervous and humoral influences. In addition, the isolated organ system minimizes the influence of factors such as absorption, distribution and biotransformation on the drug or chemical administered. The pharmacological effect of a drug on an organ is proportional to its concentration in the biophase (the immediate environment of the receptor). The isolated organ is removed from the experimental animal and is suspended in a physiological fluid in an organ bath.

The concentration of the drug in the biophase in the isolated organ preparation is taken to be equal or proportional to that in the bath fluid (Ariëns, Simonis and Van Rossum, 1964a). The fluid in the organ bath is kept at a constant temperature (usually 37°C) and is continuously aerated with a suitable gas.

The results of the isolated organ experiments are expressed in the form of concentration-response curves which can depict and explain the nature of the pharmacodynamic interactions between drugs or unknown chemicals and receptors in the isolated organ.

2.2 Classical receptor theory

A specific receptor can be defined as a molecular site on the macromolecular structures of the biological system with which chemicals interact to result in a stimulus that leads to a pharmacological effect. In order for a drug or chemical molecule to interact with a receptor, there must be a mutual physiochemical attraction between them. The sum of all these intermolecular attractive forces is defined as affinity (Ariëns and Simonis, 1964) and this is the first prerequisite for receptor interaction. The ability of a chemical to stimulate a receptor for which it has affinity in order to bring about a pharmacological

effect is known as intrinsic activity (Ariëns, 1954).

A chemical or drug possessing both affinity and intrinsic activity is known as an agonist. Chemicals possessing affinity for a specific receptor but not full intrinsic activity are defined as partial agonists or dualists (Stephenson, 1956) and those that have affinity and no intrinsic activity are defined as competitive antagonists. It is not yet possible to measure absolute intrinsic activity as such and therefore, if we assume that the stimulus and effect are directly proportional to one another, it is expressed as the relative intrinsic activity (α). α is the ratio of the actual intrinsic activity to the maximal intrinsic activity possible on a particular receptor-effector system (Van den Brink, 1977). The value of α for a full agonist is 1, for a competitive antagonist it is 0, and for a partial agonist it lies between 0 and 1.

The simplest assumption about the formation of a drug-receptor complex is that it may be expressed as a chemical reaction :



where $[R]$ is the concentration of free receptors, $[A]$

is the drug concentration in the biophase and [RA] is the concentration of the drug-receptor complex or bound receptors. The association rate constant and dissociation rate constant are represented by k_1 and k_2 respectively. The relationship between drug-receptor interactions and response are explained by a number of different theories: the "occupation theory" (Clark), the "rate theory" (Paton), the "macromolecular perturbation theory" (Belleau), the "induced fit theory" (Koshland), the "carrier flux theory" (Mackay) and the "competitive antagonist theory" (Rocha and Silva) (Review - Venter, 1982). The occupation theory is the simplest of these - response is a function of the occupation of the receptors by an agonist. This theory therefore assumes that a linear relationship exists between receptor occupation and response and that maximal response is obtained when all the receptors are occupied.

The following equation for an agonist can be derived from equation (1) by applying the Law of Mass Action :

$$\frac{S_A}{S_{Am}} = \frac{1}{1 + \frac{K_A}{[A]}} \quad \dots\dots\dots 2$$

S_A = stimulus caused by the action of agonist A on the receptor

S_{Am} = maximal stimulus caused by A

K_A = dissociation constant of A (the affinity of A for

R is proportional to the reciprocal of K_A)

A stimulus, however, cannot be measured directly in an isolated organ experiment, whereas the effect (response) it causes can be measured. If the simplest case (the occupation theory) is accepted, the stimulus is directly proportional to the effect (E) it causes and the equation becomes :

$$\frac{E_A}{E_{Am}} = \frac{1}{1 + \frac{K_A}{[A]}} \dots\dots\dots 3$$

E_A = effect obtained in the presence of A

E_{Am} = maximal effect obtainable with drug A in the receptor-effector system

E_A , E_{Am} , and $[A]$ can be measured in isolated organ experiments and by means of this mathematical model, the theory can be related to experimental evidence.

If stimulus and effect are assumed to be directly proportional, relative intrinsic activity (α) can be expressed as

$$\alpha = \frac{E_{Am}}{E_M}$$

where E_M is the maximal effect obtainable in the receptor-effector system.

Equation (3) then becomes :

$$\frac{E_A}{E_M} = \frac{\alpha}{1 + \frac{K_A}{[A]}} \quad \dots\dots\dots 4$$

and the formula represents affinity as well as intrinsic activity.

If the stimulus-effect relation is not linear, then a more general assumption is that the magnitude of the effect only depends on the magnitude of the stimulus and not on the way in which the stimulus was created. In this case another intrinsic activity constant, α^S , is used (Van den Brink, 1977) where

$$\alpha^S = \frac{S_{Am}}{2S_{pm}} \quad \dots\dots\dots 5$$

and S_{pm} is the maximal stimulus that can be generated by a partial agonist of the same family, which maximally can cause an effect equal to $1/2 E_M$ (Van den Brink, 1977).

Stephenson (1956) recognized that response was not necessarily always directly proportional to receptor occupancy and he assumed that response was some undefined function of stimulus:

$$\text{Response} = f(S) = \frac{E_A}{E_M} \quad \dots\dots\dots 6$$

Responses in a tissue could therefore be produced by two agonists that produced equal stimuli regardless of

two agonists that produced equal stimuli regardless of their receptor occupancy. Efficacy (e) was the parameter that related stimulus to occupancy and it was assumed that, if a nonlinear relationship existed between stimulus and response, not all the receptors needed to be occupied to produce a maximal response. Efficacy is a tissue-dependent parameter and according to Stephenson (1956) and Kenakin (1984),

$$S = \frac{e}{1 + \frac{K_A}{[A]}} \dots\dots\dots 7$$

(Note: α^S is analogous to e and $e = 2\alpha^S$ (Van den Brink, 1977)).

Furchgott (1966) introduced the property "intrinsic efficacy" (ϵ) as a strictly receptor related parameter and related it to efficacy as follows:

$$e = \epsilon [R_t]$$

where $[R_t]$ is the concentration of the total receptor population.

2.3 Evaluation of drug-receptor interactions

2.3.1 Concentration-response curves

If the responses of the isolated organ to various concentrations of the drug are measured and are

the drug and these values are plotted against the log concentration of the drug, a sigmoid curve is obtained. Figure 4 depicts theoretical log concentration-response curves of compounds with varying values for affinity ($1/K_A$) and intrinsic activity (α). It can be seen that as the affinity of a drug for its receptor decreases (K_A becomes larger), so there is a parallel shift of the curves to the right, and as the intrinsic activity (α) of a drug decreases, the height and slope of the curves decrease. Log concentration-response curves of drugs are therefore characterized by the maximum height, the slope of the curve and its position on the log concentration axis. Drugs can therefore be classified by means of differences in the quantitative parameters of affinity, intrinsic activity or efficacy.

The affinity of an agonist for a specific receptor in linear relationships is given by the pD_2 value which is defined as the negative log of the dissociation constant K_A or the negative log of that molar concentration of the agonist that produces 50% of its own maximum effect (EC50)

$$\text{i.e.} \quad pD_2 = - \log EC50 \quad \dots\dots\dots 8$$

In the derivation of these equations which provide a mathematical model for drug-receptor interactions, the simplest although not the most probable case was taken

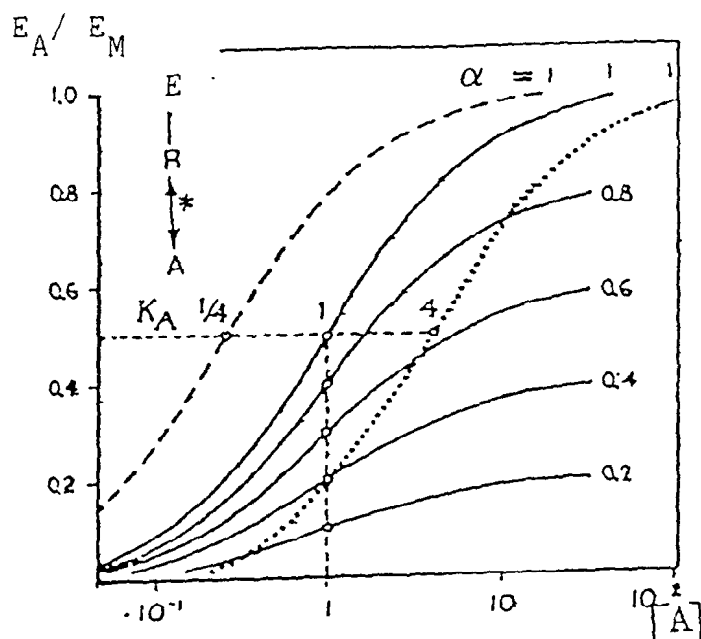


Fig. 4. Theoretical log concentration-response curves for compounds with varying values for affinity ($1/K_A$) and intrinsic activity (α). (After Ariëns et al (1964a)).

i.e. that the effect of a drug is proportional to the quantity of drug-receptor complex formed, and that a linear relationship exists between the stimulus evoked and the effect produced. In practice, however, a direct proportionality between stimulus and effect does not always exist. A non-linear relationship between stimulus and effect occurs when a "receptor reserve" and/or "threshold value" exists in a receptor-effector system.

A full agonist may elicit a maximal effect before all the specific receptors in a receptor-effector system are occupied. The receptors which are not occupied by

the agonist are then known as the receptor reserve (Ariëns, Simonis and Van Rossum, 1964c). Many organs possess receptor reserves and this is possibly a biological savings mechanism e.g. the bronchial muscle can have a receptor reserve of as high as 90% (Venter, 1982). The presence of a receptor reserve reduces the K_A value of an agonist i.e. the affinity of the agonist for the receptor would appear higher than it actually is. The EC50 can therefore not be used to determine the K_A and PD_2 values when a receptor reserve exists.

A threshold value is defined as the size of the stimulus an agonist must elicit before an effect can be observed (Ariëns et al. 1964c). This could be as a result of a certain inertia that exists in the organ. The stimulus is, by definition, directly proportional to the number of occupied receptors and this should therefore relate to a specific concentration of the agonist (threshold concentration) (Venter, 1982). The K_A value obtained in the presence of a threshold value does not correspond with the EC50 value. It appears higher than it should be i.e. the agonist would appear to possess a lower affinity for the receptor. The EC50 value can therefore not be used to calculate K_A and PD_2 when a threshold value exists.

2.3.1.1 Classification of drug-receptor interactions

2.3.1.1.1 Competitive interactions on specific receptors

In this type of interaction, two or more drugs compete for occupation of the same specific receptors in a receptor-effector system. There are three basic types of competitive interaction which are differentiated from one another by the value of the intrinsic activity of the competing drug (B).

2.3.1.1.1.1 Competitive synergism

This interaction occurs when both drug A and drug B possess similar intrinsic activities (e.g. $\alpha = 1$). This interaction is represented by the theoretical log concentration - response curves in figure 5. The presence of drug B increases the effect of each concentration of drug A and the effect of A and B together is not greater than the sum of their individual effects.

2.3.1.1.1.2 Competitive dualism

If drug B is a partial agonist (e.g. $\alpha = 0,5$) and drug A is a full agonist, then at low concentrations of drug A, drug B acts as a competitive synergist (see figure

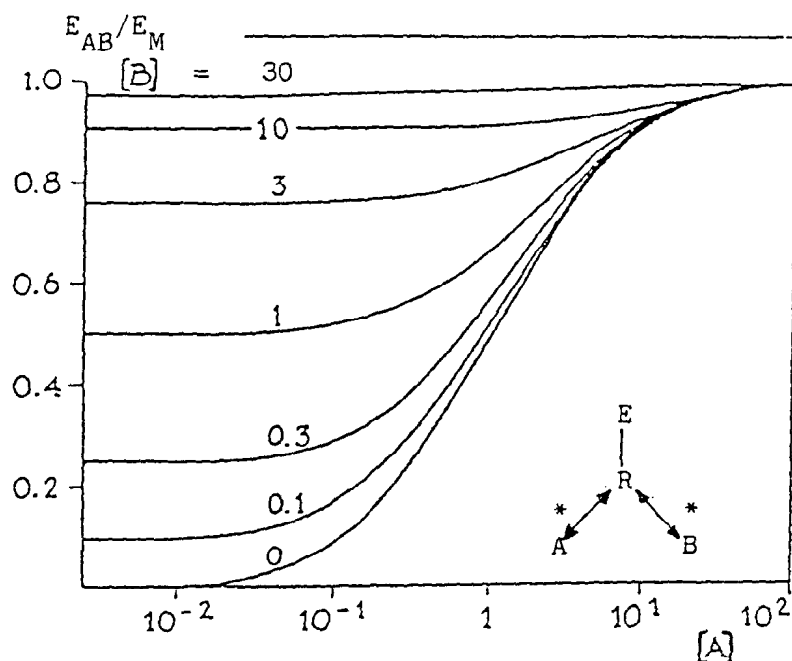


Fig. 5. Competitive synergism (after Van den Brink (1977) and Venter (1982)).

6). At high concentrations of A, however, B acts as a competitive antagonist (i.e. if A had occupied the receptors on its own, the effect would have been greater).

2.3.1.1.1.3 Competitive antagonism

In this case, drug A is an agonist and drug B is a competitive antagonist (i.e. B possesses affinity for the receptor but $\alpha = 0$). This interaction is illustrated in figure 7 where it can be seen that the shape and height of the agonist curve is not influenced by the presence of drug B, but there is a parallel

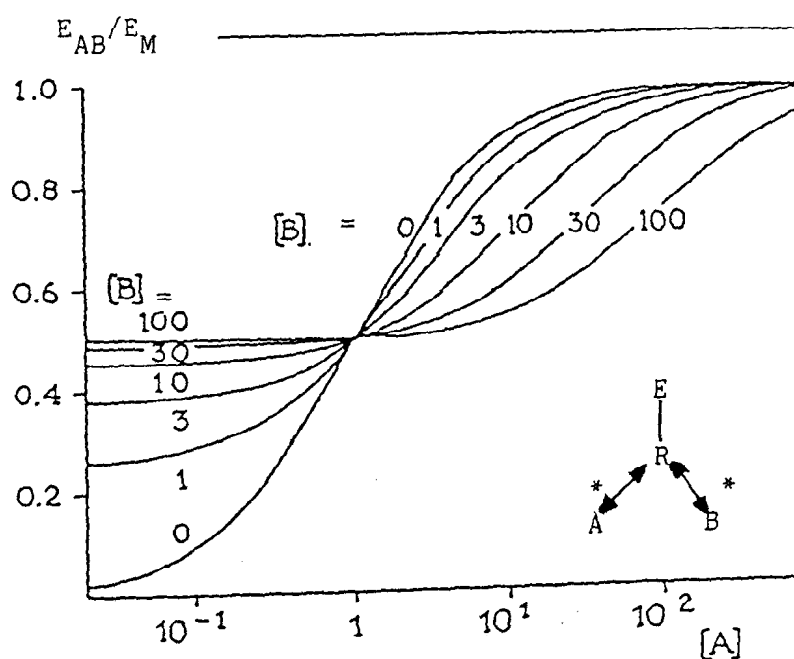


Fig. 6. Competitive dualism (after Van den Brink (1977) and Venter (1982)).

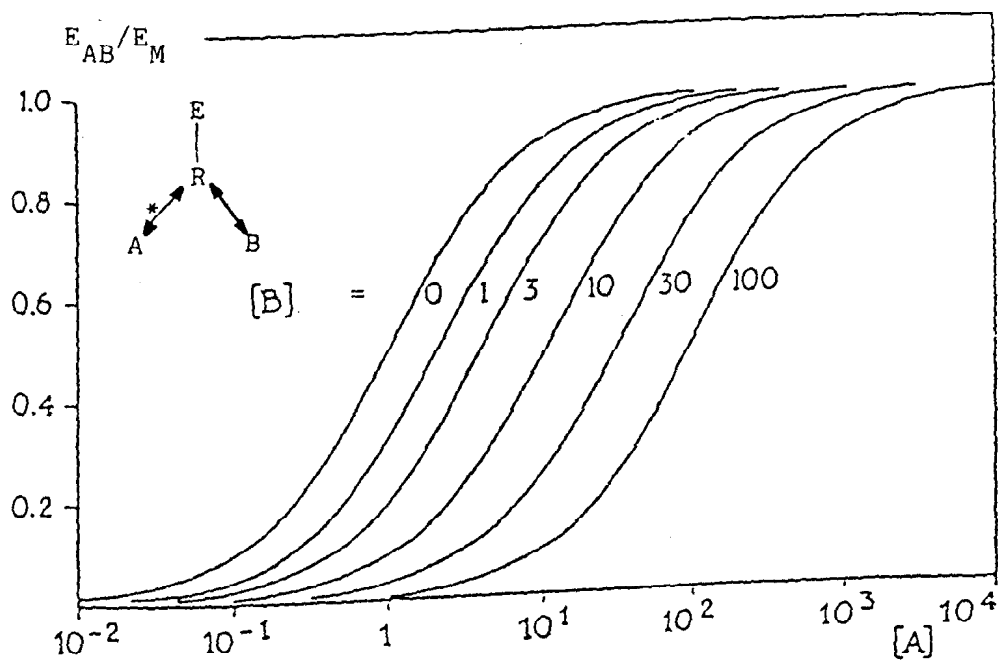


Fig. 7. Competitive antagonism (after Van den Brink (1977) and Venter (1982)).

shift of the curve to the right. The magnitude of the shift depends only on the concentration and affinity of the antagonist (Van den Brink, 1977). As the concentration of the agonist A is increased, so the competitive antagonist B is displaced from the receptors and maximal effect is again achieved. The presence of a receptor reserve has no effect on the curves obtained. This is a reversible interaction.

The affinity of the competitive antagonist for the specific receptor is given by the pA_2 value. The pA_2 is defined as the negative log of the dissociation constant of the competitive antagonist-receptor complex (K_B) or, alternatively, as the negative log of that molar concentration of a competitive antagonist in the presence of which the concentration of the agonist must be doubled in order to obtain an effect which is equal to the effect obtained in the absence of the competitive antagonist.

2.3.1.1.2 Non-competitive interactions

Here drugs A and B interact with two different kinds of receptors - specific (R) and non-specific (R') receptors. Non-specific receptors are involved in this type of interaction. A non-specific receptor can be defined as a receptor which is occupied by "non-

specific" drugs that can only show a pharmacological effect in the presence of an agonist (Venter, 1982) i.e. they are able to modify the effects of an agonist. Non-specific drugs are agonists on their own non-specific receptors. In non-competitive interactions the receptors R and R' are independent of one another regarding their interactions with drugs A and B, but the receptor-effector systems are interrelated beyond the level of the receptors (Van den Brink, 1977). There are two types of non-competitive interactions, namely metactoid interactions and metaaffinoid interactions.

2.3.1.1.2.1 Metactoid interactions

Two different receptor types are involved here as illustrated in figure 8. R represents the specific receptor and R' the non-specific receptor. These receptors function independently of one another w.r.t. their interactions with the agonist, A, and B, the non-specific drug but the interaction of B with R' leads to a change in the stimulus-effect chain of A. It therefore appears as though the intrinsic activity of A has changed.

In the case of metactoid antagonism (figure 9), there is an apparent decrease in or even loss of the

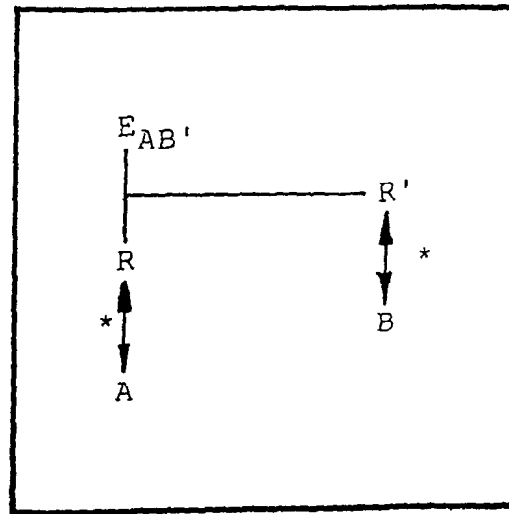


Fig. 8. Model of the mechanism of metactoid interaction (after Venter (1982)).

intrinsic activity of A and the agonist curve is depressed. There is no shift in the curves. This is an irreversible antagonism.

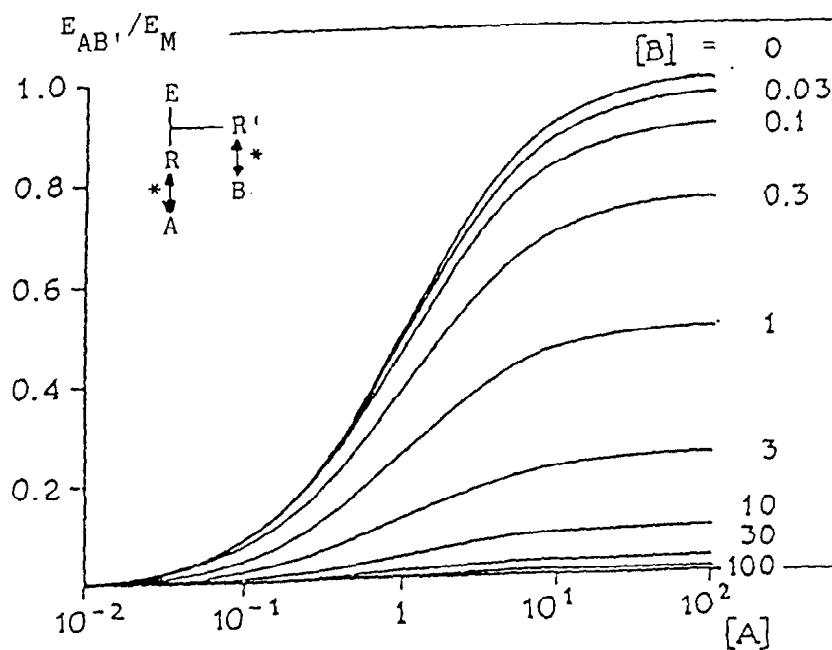


Fig. 9. Metactoid antagonism (after Van den Brink (1977) and Venter (1982)).

If the stimulus-effect relation in the agonistic system is nonlinear however, and the agonistic system possesses a receptor reserve, then the shape of the curves will not follow the normal pattern as just described. As the concentration of B increases, the curve will not at first be depressed, but it will become less steep and as long as the receptor reserve lasts, the original maximal effect will be retained. Once the receptor reserve has been depleted however, depression of the curve will occur.

In the case of metactoid sensitization, the interaction of B with R' appears to increase the intrinsic activity of A. The maximum effect observed remains the same, but the slope of the agonist curve increases i.e. a receptor reserve is created (see figure 10). The affinity of a metactoid drug for its non-specific receptor is expressed by the PD_2' value.

2.3.1.1.2.2 Metaffinoid interactions

A metaffinoid drug possesses both affinity and intrinsic activity for the metaffinoid receptor R' but has no pharmacological effect on its own (Venter, 1982). Receptor occupation of R' by a metaffinoid drug B causes the conformation of the specific receptor R to change and the affinity of an agonist A for R is therefore changed. In the generalized metaffinoid

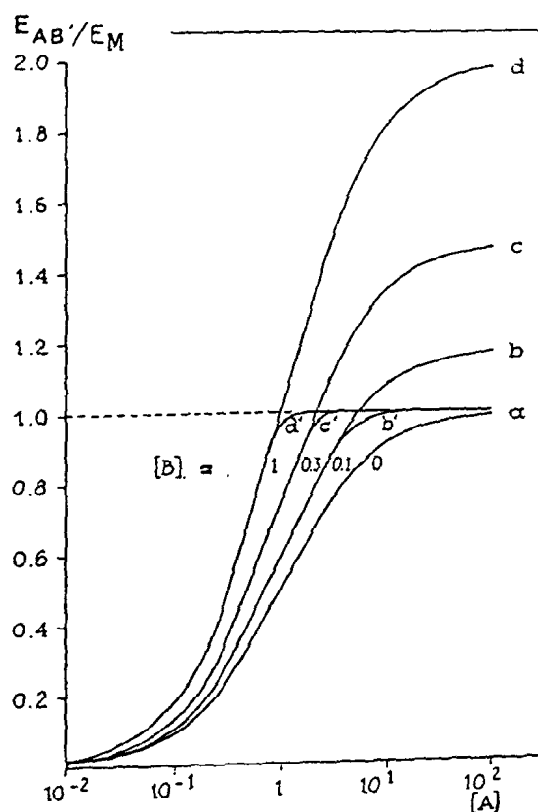


Fig. 10. Metactoid sensitization (after Van den Brink (1977) and Venter (1982)).

model of Van den Brink (1977) the following assumptions are made:

- i. Both the agonistic and metaaffinoid receptors can occur in two conformational states (changed and unchanged).
- ii. Interaction of B and a certain fraction of metaaffinoid receptors leads to a conformational change of the same fraction of the agonistic receptors and interaction of A and a certain fraction of the agonistic receptors leads to a conformational change of the same fraction of the metaaffinoid receptors. No assumptions are made regarding the ratio of the numbers of both kinds of receptors and it is not supposed that the

agonistic and metaaffinoid receptors occur in interdependant couples.

In the model of metaaffinoid antagonism described by Ariëns, Simonis and Van Rossum (1964b), the R'B complex changes the conformation of R in an allosteric manner such that A no longer fits R well. The affinity of A for R is therefore reduced and this is evidenced by a parallel shift of the dose-response curves to the right (figure 11). This is a reversible antagonism. Competitive antagonism can therefore be seen to be a special case of metaaffinoid antagonism where the agonist and competitive antagonist interact with the same receptors (Van den Brink, 1977).

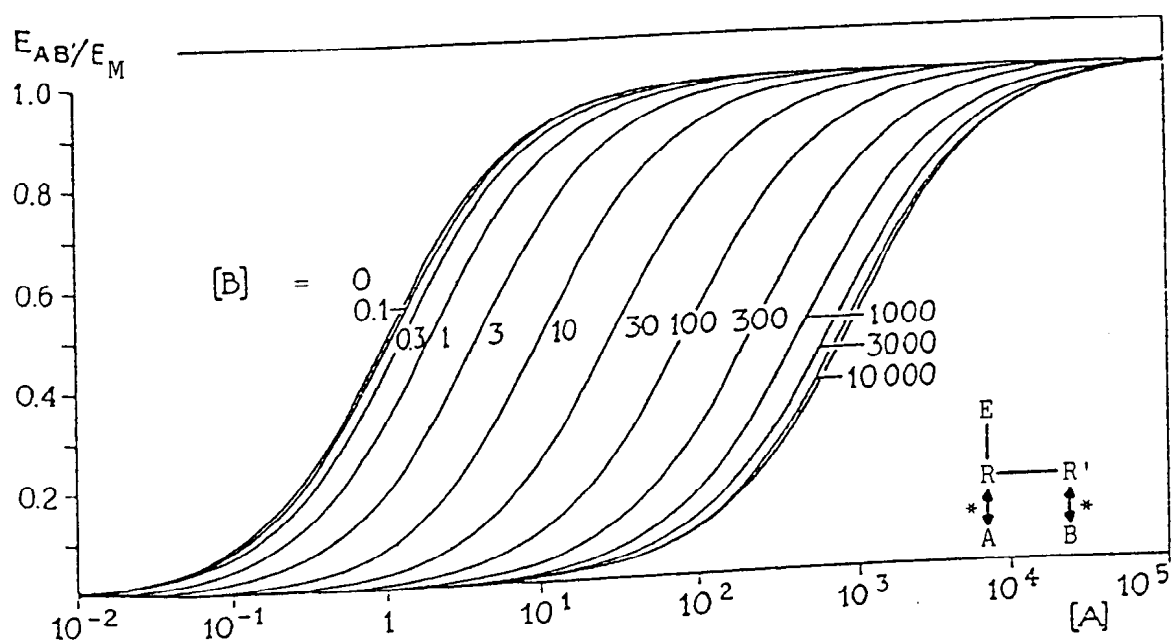


Fig. 11. Metaaffinoid antagonism (after Van den Brink (1977)).

The alternative model for metaaffinoid interaction proposed by Offermeier and Brandt (Brandt, 1973) differs from the model of Ariëns et al. (1964b) in that it does not assume that the numbers of agonistic and metaaffinoid receptors are equal, and it does assume that the affinity between B and R' is not influenced by the interaction between A and R. The effect produced by the agonist A in the presence of the metaaffinoid drug B is equal to the sum of the effects produced by A on the conformationally changed (R_C) and on the conformationally unchanged (R_U) agonistic receptors. The theoretical log concentration - response curves obtained with this model may therefore differ from those based on the model of Ariëns et al. (1964b) as illustrated in figure 12. As the concentration of B increases, the conformation of R_C might change sufficiently to reduce the affinity of A for R_C to such an extent that A no longer has affinity for the changed receptors and therefore can exert no effect via these receptors i.e. as the concentration of B increases, the intrinsic activity of A for the changed receptors would decrease. The first part of the log concentration - response curves could therefore be mistaken for metactoid antagonism (Van den Brink, 1977).

A metaaffinoid drug can also exert a sensitizing effect on an agonist. The R'B complex can bring about a

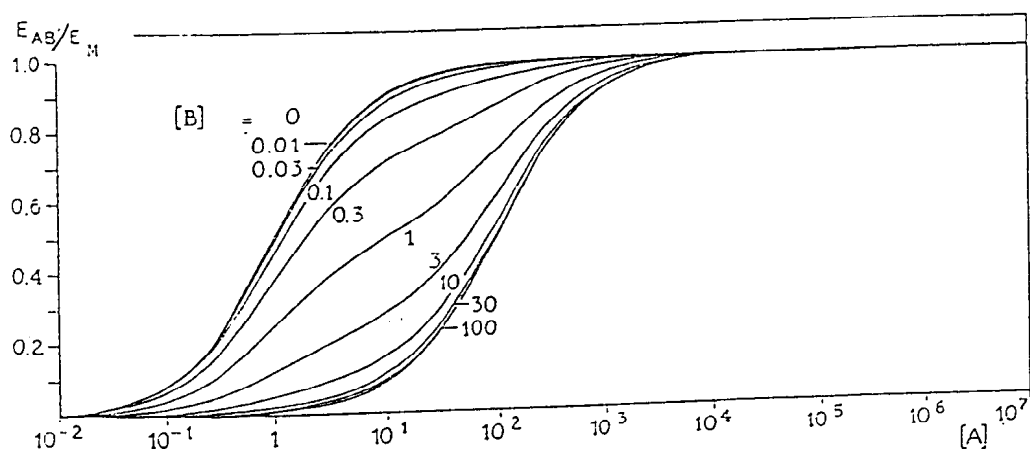


Fig. 12. Metaffinoid antagonism after the model of Offermeier and Brandt (Van den Brink, 1977).

change in the conformation of the specific receptor R which results in an increase in the affinity of A for R. The agonist curves will then show a parallel shift to the left.

2.3.1.1.3 Functional interactions

These interactions occur between two agonists, A and B, which interact with two independent receptor systems R_I and R_{II} which are situated in the same organ (see fig.13). The resultant net effect is therefore seen in the same effector system (Ariëns et al., 1964b; Van den Brink, 1977; Venter, 1982). The total stimulus-effect chain can be divided into three parts, which all

may or may not have a reserve. According to Van den Brink (1977), " a relationship between a causative and a resultant factor has a reserve when the latter can reach its maximal value at a submaximal value of the former".

I. A and B induce subeffects P_{IA} and P_{IIB} as a result of the stimuli S_I and S_{II} respectively. These subeffects cannot be observed individually and precede the functional interaction.

II. Subeffect P_{IAIIB} is then formed as a result of subeffects P_{IA} and P_{IIB} and this forms part of the functional interaction.

III. The final effect E_{IAIIB} is formed as a result of the subeffect P_{IAIIB} (Van den Brink, 1977).

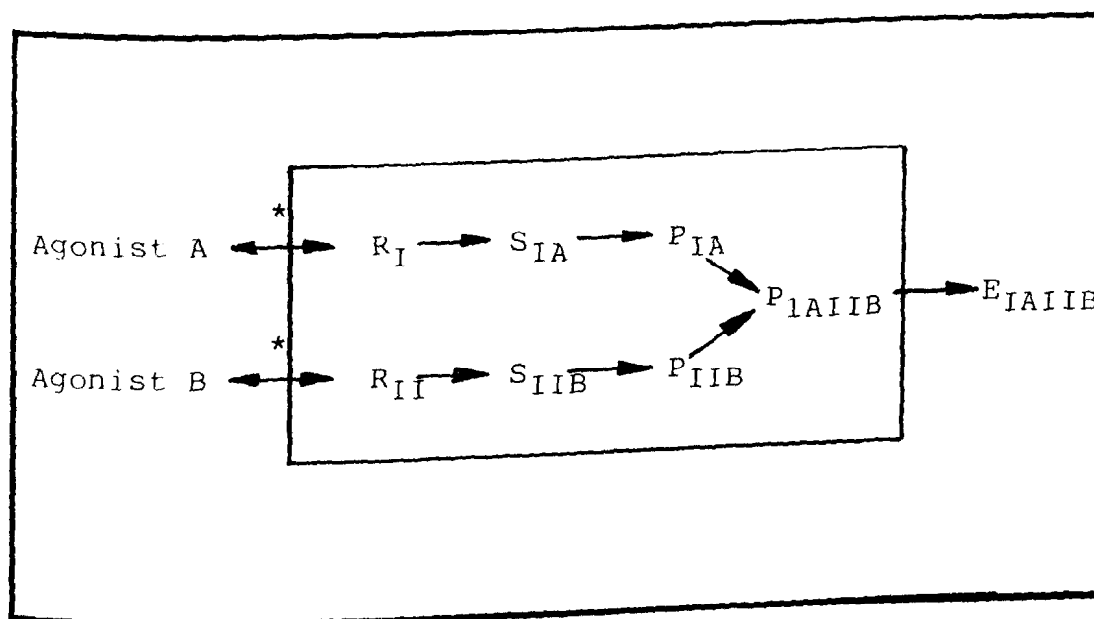


Fig. 13. Schematic model for functional interactions (after Venter (1982)).

If the induced subeffects work together, the interaction is known as functional synergism and if they oppose one another it is known as functional antagonism. A functional antagonist can therefore be defined as an agonist which is able to produce an opposing effect to another agonist on the same effector (Venter, 1982). Due to the fact that the agonist and functional antagonist act on separate receptor-effector systems, the inhibitory activity of the antagonist is independent of the concentration of the agonist and the antagonism is therefore insurmountable (Venter, 1982).

Figure 14 illustrates the theoretical curves for agonist A in the presence of various concentrations of functional antagonist B (Van den Brink 1977). In figures 14a and 14b the intrinsic activity of B is 0,2 and 0,6 respectively and the maximal value of subeffect P_{IIB} is smaller than the agonist system's reserve and there is a shift of the curves to the right before they become stationary. In figure 14c the intrinsic activity of B is 0,8 and larger than the reserve but not large enough to totally inhibit the formation of P_{IAIIB} and the curves are shifted to the right and subsequently depressed before they become stationary. As illustrated in figure 14d, when the intrinsic activity of B is equal to 1, P_{IIB} is large enough to prevent the formation of P_{IAIIB} and the

curves are shifted and then depressed until they lie below the ordinate base-line. In this situation, not even maximal concentrations of A will result in an effect in the presence of high enough concentrations of the functional antagonist B. The shifting of the curves in this model is therefore different from the true shifting of the curves as observed in competitive and metaaffinoid antagonism because all that is happening is that the subeffect curve is moved up and down and the effect curve therefore shows a pattern of shifting (Van den Brink, 1977).

2.3.1.1.4 Physiological interactions

In a physiological interaction, two agonists, A and B, activate two completely independent receptor-effector systems ($R_I \longrightarrow S_{IA} \longrightarrow E_{IA}$ and $R_{II} \longrightarrow S_{IIB} \longrightarrow E_{IIB}$ respectively) which are situated in different effector organs (see fig. 15). Each agonist produces a measurable effect on its own effector and the combination of these two effects results in a final communal effect, E_{IAIIB} . If E_{IA} and E_{IIB} are complimentary, the interaction is physiological synergism and if the effects are opposing, the interaction is physiological antagonism (Venter, 1982). Functional interactions can therefore be seen to be a special type of physiological interaction where the receptors are located in the same effector organ.

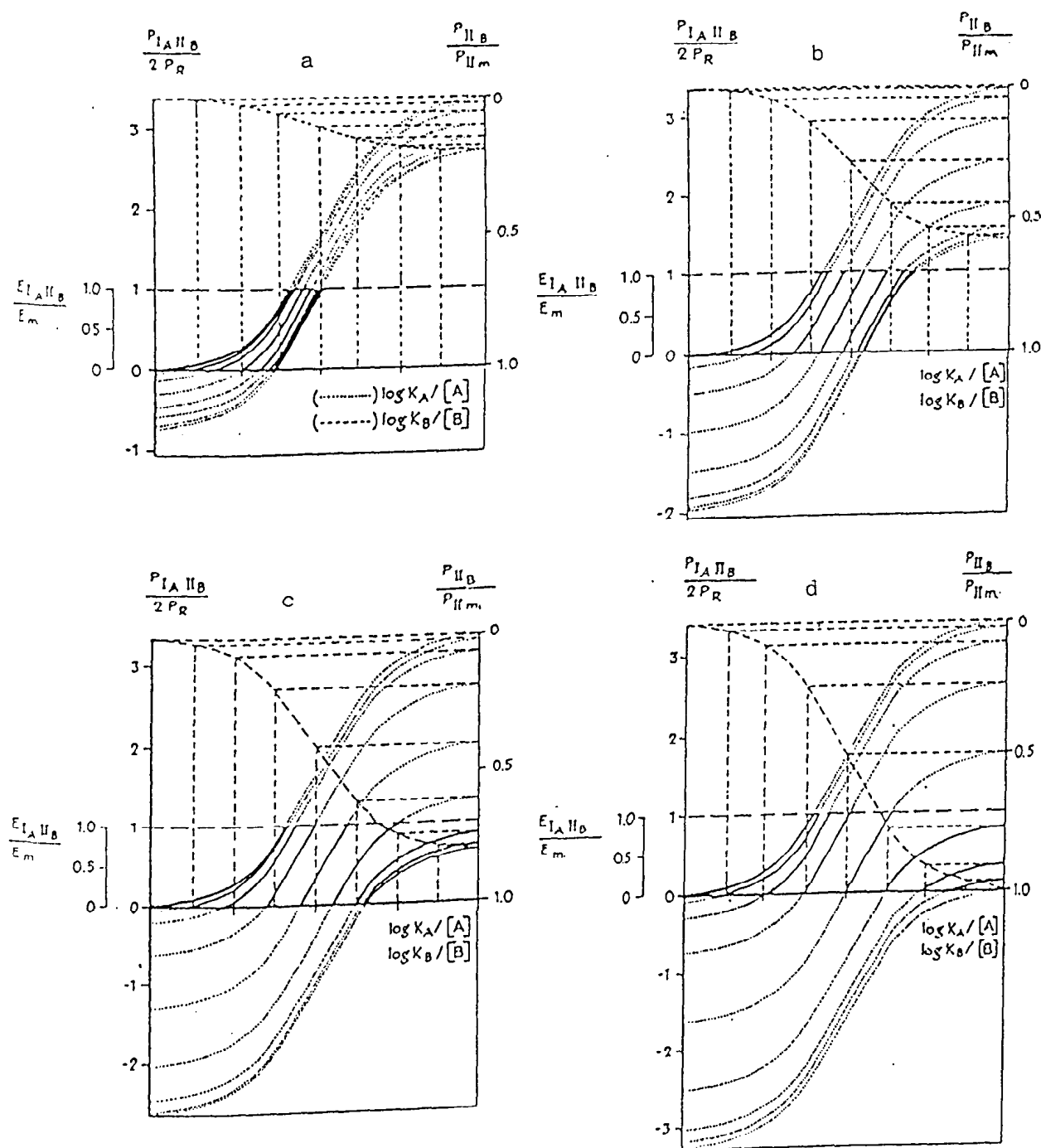


Fig. 14 a-d. Theoretical log concentration-effect curves illustrating the effects of functional antagonists with various potencies. The dotted line represents the concentration-subeffect curve and the continuous line represents the concentration-effect curve (after Van den Brink (1977)).

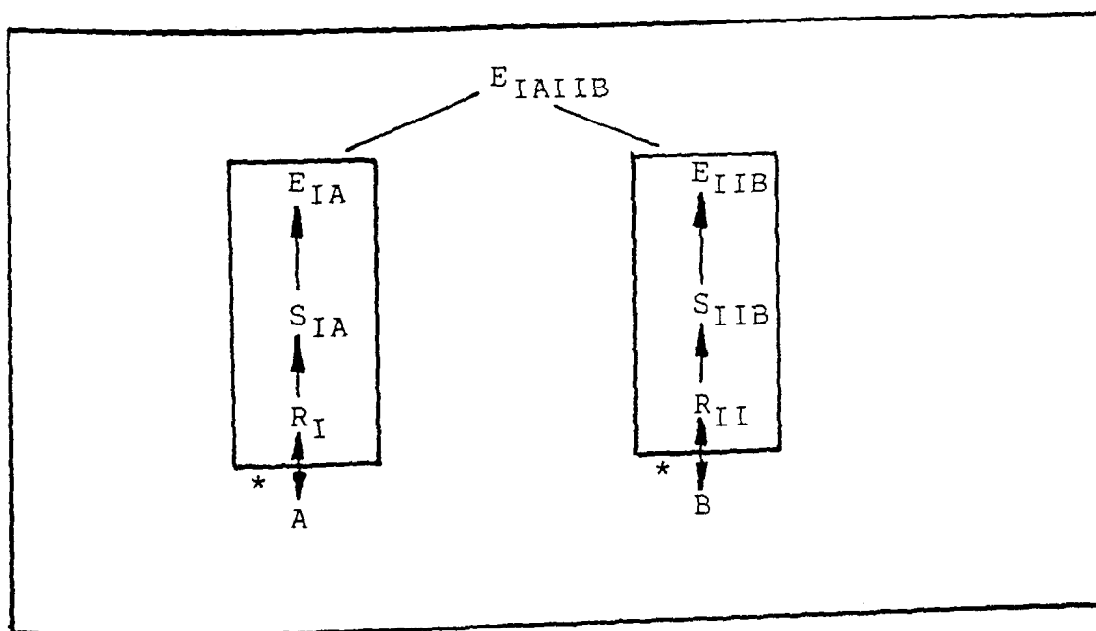


Fig. 15. Schematic model for physiological interactions (after Venter (1982)).

2.3.1.1.5 Dualistic interactions

Many drugs have affinity for more than one type of receptor and are therefore able to interact with more than one receptor simultaneously. These drugs are known as dualists and one can differentiate between dualistic antagonism and dualistic agonism (Venter, 1982).

2.3.1.1.5.1 Dualistic antagonism

Many competitive antagonists exist which also possess affinity for non-specific (metactoid) receptors. This interaction is also known as "dualism in antagonism" (illustrated schematically in figure 16).

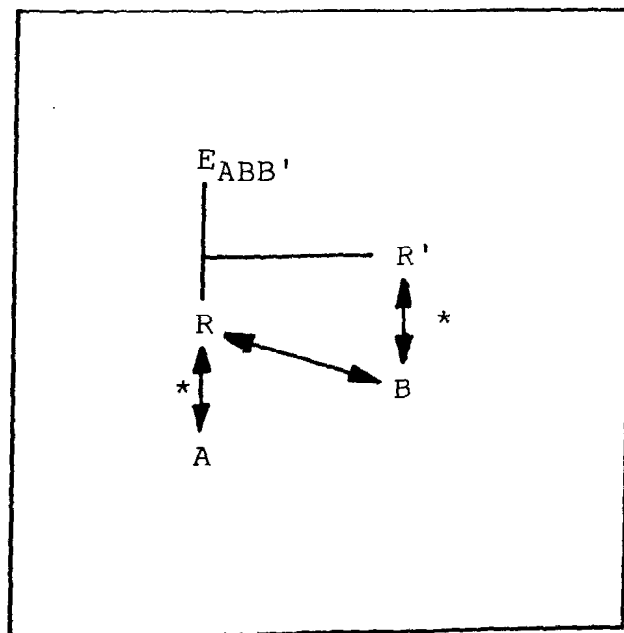


Fig. 16. Schematic model for dualism in antagonism (after Venter (1982)).

If the affinity of the dualistic antagonist, B, for the specific receptor, R, is higher than its affinity for the metactoid receptor, R', then there is first a parallel shift of the agonist curve to the right followed by suppression (see fig.17). Therefore, at high concentrations of B, maximal effect is no longer obtained by the agonist, A, and this becomes an insurmountable antagonism. If the affinity of B for both the specific and non-specific receptors does not differ much, then there is no parallel shift of the agonist curve, just a shift to the right with suppression (Venter, 1982).

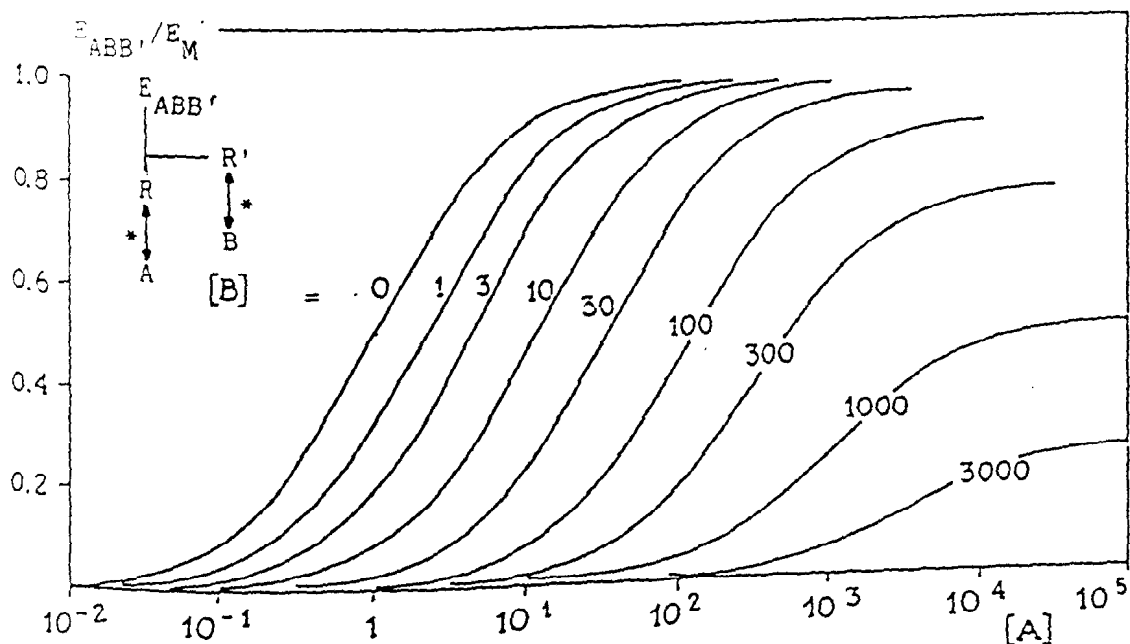


Fig. 17. Dualistic antagonism (after Van den Brink (1977)).

2.3.1.1.5.2 Dualistic agonism

There are two types of dualistic agonism, namely, auto-inhibition and antagonistic agonism.

2.3.1.1.5.2.1 Auto-inhibition

This interaction is well-described by Venter (1982) and is illustrated in figure 18 where it can be seen that the agonist (A) possesses both affinity and intrinsic activity for both the specific receptor, R and the non-specific (metactoid) receptor, R'. At high concentrations of A, it acts as a metactoid antagonist

against its own agonistic effect. A "bell-shaped" concentration - effect curve is obtained and the height of the curve is dependant on both the affinity and

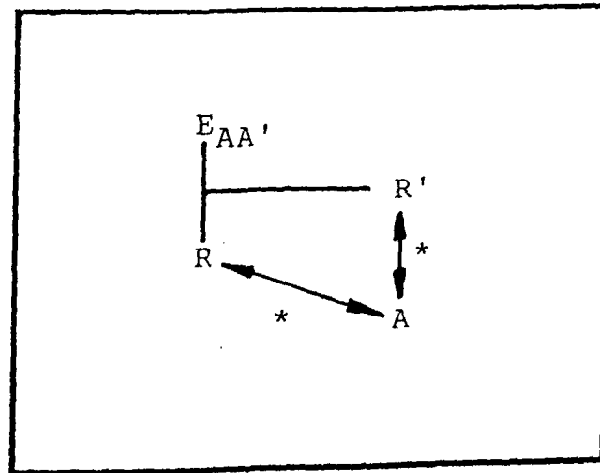


Fig. 18. Schematic model for auto-inhibition (after Venter (1982)).

intrinsic activity of A for the specific and non-specific receptors. The bell-shaped curve is actually comprised of two sigmoid curves which are mirror images and which often overlap.

If the difference between K_A and $K_{A'}$ is small, the curves are shallow and maximum effect is not obtained (see curves 3 to 7 in fig.19.) If $K_{A'}$ is much larger than K_A , then the affinity on the non-specific receptors, R' , will be much smaller than the affinity on the specific receptors, R . In this situation the

sigmoid curves will lie so far apart that there will be no overlapping and a plateau will be formed (see curve 1 in fig.19). A full agonistic effect is therefore obtained first and maximum effect is achieved before the effect of the metactoid antagonism is seen (descending leg). When K_A is much larger than $K_{A'}$, then all the non-specific receptors are occupied before occupation of the specific receptors even starts. The effect of A on the specific receptors can therefore induce no effect and no concentration - effect curve is obtained.

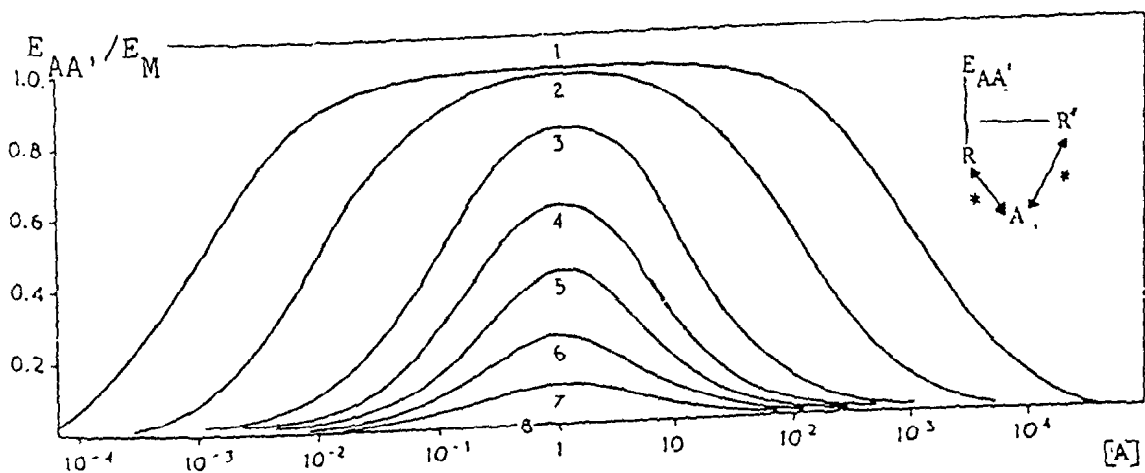


Fig. 19. Auto-inhibition (after Venter (1982)).

Venter (1982) also describes the case where a drug exhibiting auto-inhibition is combined with a competitive antagonist, B, which competes for the same specific receptors, R. There is a parallel shift of the ascending leg of the bell-shaped curve to the right and there is a suppression of the height of the curve (see fig. 20).

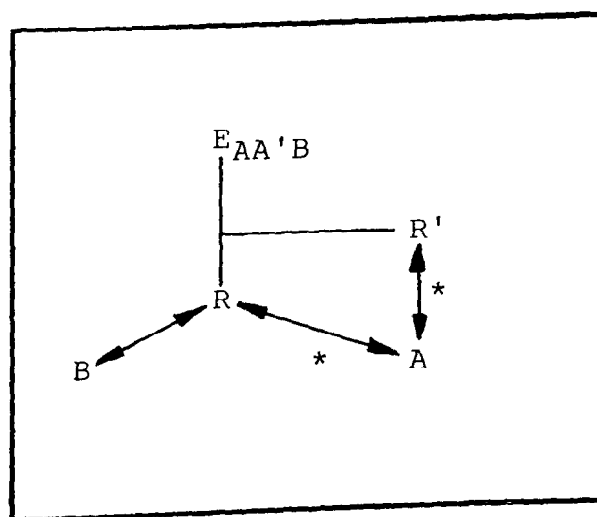


Fig. 20. Auto-inhibition with competitive antagonism (after Venter (1982)).

2.3.1.1.5.2.2 Antagonistic agonism

According to Venter (1982), this interesting phenomenon was first described by Furchgott (1955) and the term "antagonistic agonism" was coined by Szabadi (1975). Functionally opposite receptors, $R_{(+)}$ and $R_{(-)}$, occur

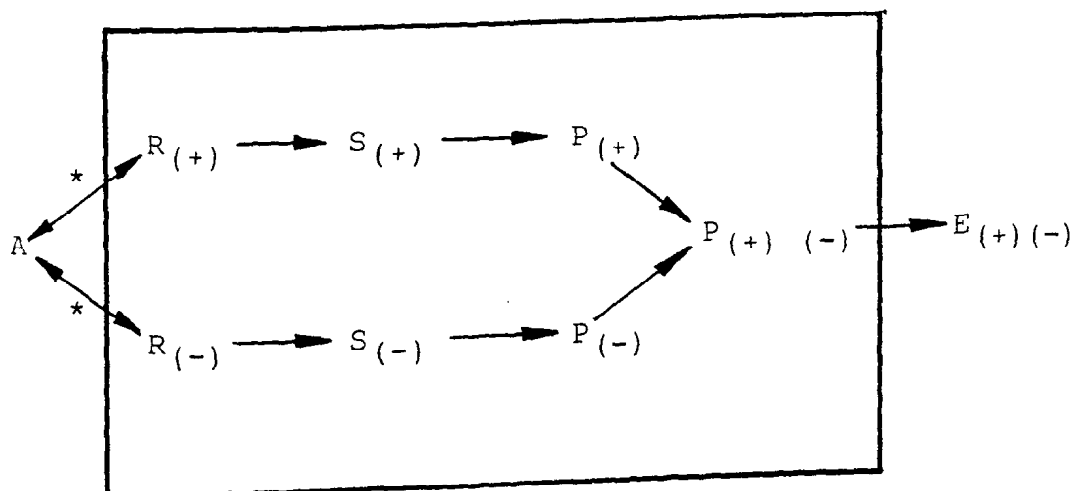


Fig. 21. Model for antagonistic agonism (after Venter (1982)).

on the same organ or smooth muscle and the agonist, A, interacts with them simultaneously as illustrated in fig. 21. Five different cases of antagonistic agonism can be distinguished and they are illustrated as concentration - effect curves in fig. 22.

Case I: $\alpha_{(+)} = \alpha_{(-)}$; $K_{A(+)} = K_{A(-)}$. The two concentration - effect curves are mirror images of one another. The functional antagonism is complete and no effect is observed.

Case II: $\alpha_{(+)} = \alpha_{(-)}$; $K_{A(+)} < K_{A(-)}$. The concentration - effect curve is a monophasic bell - shape. As the concentration of A increases, the effect will increase to a maximum and then decrease to zero. The effect of the stimulation of $R_{(+)}$

overrules the effect of the stimulation of $R_{(-)}$ at all concentrations of A.

Case III: $\alpha_{(+)} > \alpha_{(-)} ; K_{A(+)} = K_{A(-)}$. The concentration - effect curve is a monophasic saturation curve. The full maximal effect the system is capable of is never attained.

Case IV: $\alpha_{(+)} > \alpha_{(-)} ; K_{A(+)} < K_{A(-)}$. The concentration - effect curve increases to a peak and then declines to a plateau.

Case V: $\alpha_{(+)} > \alpha_{(-)} ; K_{A(+)} > K_{A(-)}$. This is a biphasic curve, consisting of a negative and a positive phase. When the affinity of A for $R_{(-)}$ is greater than its affinity for $R_{(+)}$, then it is going to react with the inhibitory receptor first and the curve proceeds in a negative direction. At a certain concentration, A will start to react with $R_{(+)}$ and then the curve changes to a positive direction. The receptor population can therefore be described as either "masked" or "dominant", depending on the direction of the response.

According to Szabadi (1975), a competitive antagonist which could act at either or both receptors, could change the relationship between the concentration - effect curves. A blockade of masked receptors could cause potentiation and a blockade of dominant receptors could cause a reversal of the effect. If both

receptors were blocked by the antagonist, then either potentiation or antagonism could be observed.

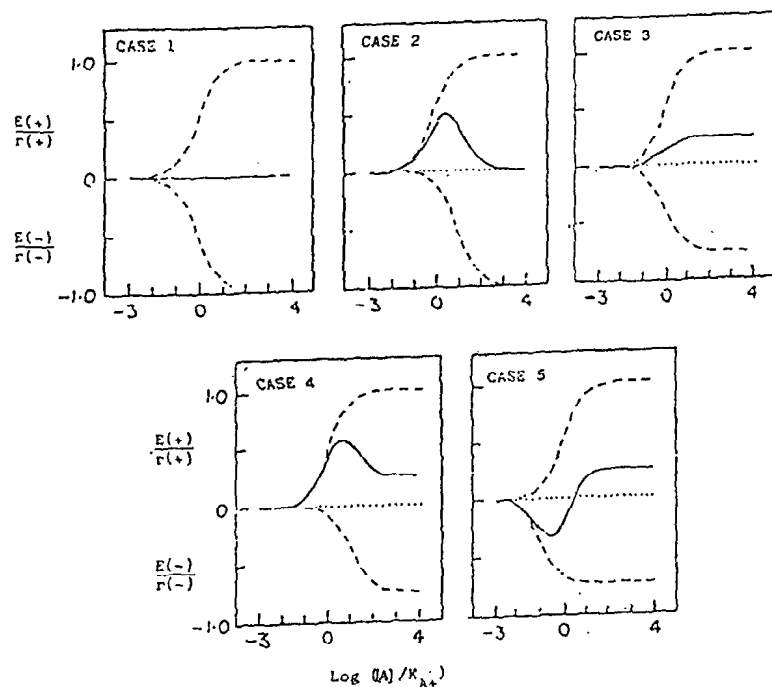


Fig. 22 Antagonistic agonism (after Szabadi (1977)).

2.3.2 Receptor classification by measurement of agonist affinity and relative efficacy

These are quantitative methods derived from the classical receptor theory and are aimed at classifying receptor types in isolated tissues. They rely on null procedures which are assumed to cancel out the effects of stimulus-response coupling i.e. the tissue dependent factors in the classic equation describing agonism (Kenakin, 1984)

$$\text{Response} = f(S) = f\left(\frac{E[R_t]}{K_A + [A]}\right) \dots\dots\dots 9$$

where the tissue related factors are: f , the function relating stimulus and response and $[R_t]$, the concentration of the total receptor population and the receptor related factors are: K_A and E .

Furchgott (1966) developed a method to quantify the relative efficacy of agonists based on the partial alkylation of receptors. Concentration-response curves are obtained before and after irreversible inactivation of a portion of the receptors and equiactive doses are compared. He derived the following equation:

$$\frac{1}{[A]} = \frac{1}{q[A']} + \frac{(1-q)}{qK_A} \dots\dots\dots 10$$

where $[A]$ and $[A']$ are equiactive concentrations of full agonist before and after alkylation of the receptors and q is the fraction of receptors remaining after alkylation. A double reciprocal plot of $1/[A]$ against $1/[A']$ is made and K_A is calculated as follows:

$$K_A = \frac{\text{slope} - 1}{\text{intercept}} \dots\dots\dots 11$$

If relative potency is measured in the same tissue, tissue related factors such as receptor number and the efficiency of stimulus-effect coupling cancel out and relative potency depends only on the receptor parameters of affinity and intrinsic efficacy. Potency ratios are calculated according to the following equation:

$$\frac{[A_1]}{[A_2]} = \frac{E_2 K_{A1}}{E_1 K_{A2}} \dots\dots\dots 12$$

where A_1 and A_2 are high efficacy full agonists in a given tissue (Kenakin, 1989). Differences in potency ratio indicate a difference in receptor type.

2.4 New trends in Molecular Pharmacology

The classical receptor theory has been modified somewhat during the last decade. Early concepts represented the receptor in a fairly static mode where receptor occupancy and the initiation of response were regarded to be independant events. Recent biochemical studies of receptors in membrane fragments and whole cells indicate that receptors can exist in different affinity states and that these different states may in turn depend on non-receptor membrane protein constituents (Kenakin, 1989). Membrane bound regulatory transducer proteins seem to play a vital

role in the translation of the type and strength of messages that agonists are able to transmit to cells (Kenakin and Morgan, 1989). They are known as G proteins due to the fact that they bind guanine nucleotides. Four classes of G proteins have been described - G_s , G_i , G_o and G_t proteins. G_s proteins stimulate adenylyl cyclase and G_i proteins inhibit adenylyl cyclase. Each G protein consists of an alpha, beta and gamma subunit (in order of decreasing mass) (Graziano and Gilman, 1987). The regulation of transmembrane signalling by G proteins is explained in figure 23.

Signal transduction due to agonist-receptor activation seems to depend on the type and concentration of transducer proteins available. Experimental evidence now exists to show that agonists can elicit multiple cellular signals. This could be due to multiple receptor subtypes, or due to divergent biochemical cascades within the cell as a result of a single agonist-receptor interaction, or due to a receptor that can interact with multiple membrane bound G proteins i.e. receptor "promiscuity" (Kenakin et al., 1989).

The quantitative methods used to classify drugs in the past, eg. affinity, intrinsic activity and relative efficacy were based on the receptor occupation theory

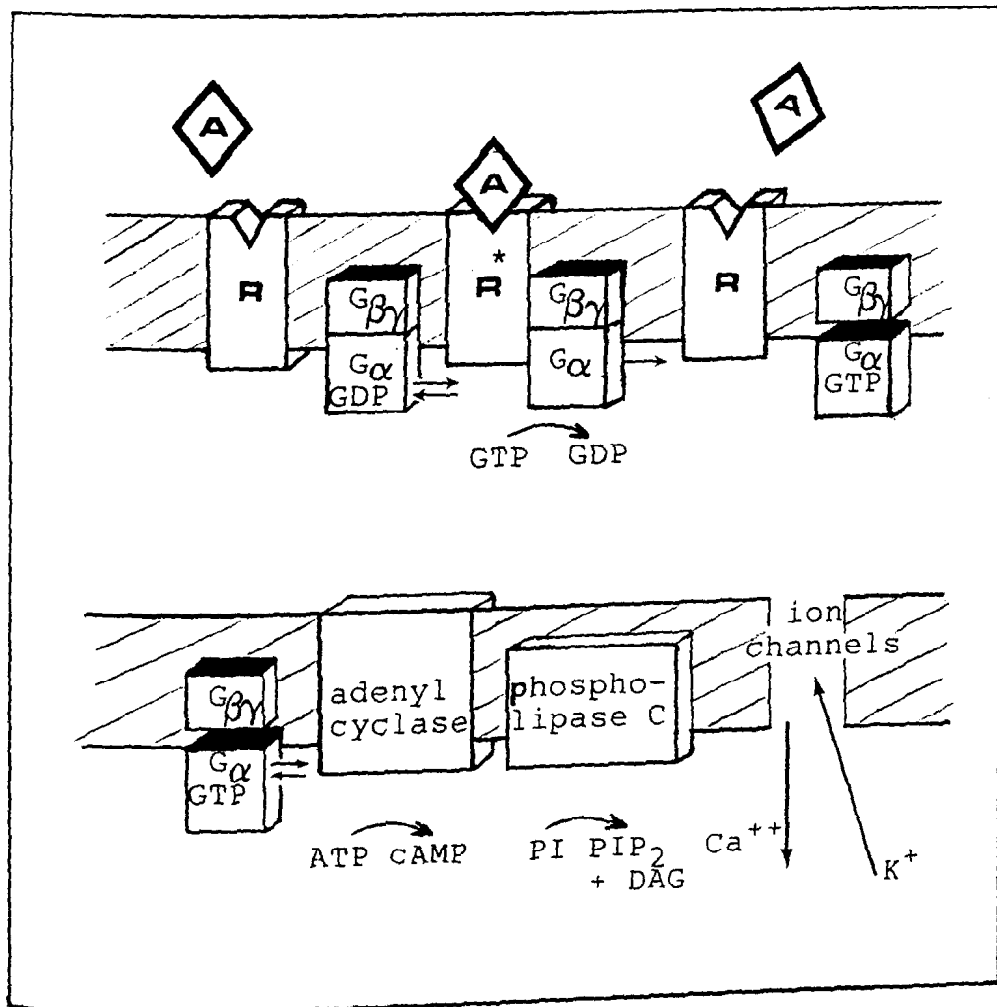


Fig. 23. G protein regulation of transmembrane signalling. The agonist-receptor complex catalyses the dissociation of GDP from the alpha-subunit of the G protein. Subsequent binding of GTP is then followed by the dissociation of the G protein alpha-subunit from the beta-gamma-subunit. Once the G protein subunits are dissociated, they modulate the activity of a given effector (after Graziano et al. (1987)).

where it was assumed that the magnitude of the stimulus was proportional to the fraction of receptors occupied by the agonist i.e. the agonist specific step in the stimulus-response chain was related only to the drug receptor. However with the biochemical evidence now on

hand that the receptor forms a complex with the agonist, is qualitatively altered by the interaction and may or may not subsequently combine with one or more G proteins to bring about a pharmacological effect, the concept of drug action has changed dramatically and the classic quantitative techniques of classifying drugs will have to be reviewed (Kenakin, 1988; Kenakin, 1989; Kenakin et al. , 1989; Mackay, 1990 and Kenakin, Morgan and Lutz, 1990).

Classical receptor theory regards the agonist recognition area of receptors facing the extracellular compartment as being critical to the classification of receptors. However, according to Kenakin (1988) it is now recognized that there must be at least one other recognition area, situated intracellularly, in order for the receptor to pass information on to the stimulus-response components of the cell. The possibility therefore exists that different cytosolic recognition areas for transducer proteins could exist or be created on receptors which possess identical extracellular recognition areas for agonists (see fig. 24). This could lead to conflicting data in experiments with agonists and antagonists because simple competitive antagonists would only interact with the extracellular recognition area and not be influenced by the cytosolic transducer binding area, whereas agonists could be involved with both areas (Kenakin, 1989). Receptor

classification based on drug affinity would indicate that these receptors were pharmacologically similar while studies of relative efficacy and potency ratios would show that the receptors could be different (Kenakin, 1988).

Figure 24 represents a model of the effects of transducer proteins on drug receptor interactions in isolated organ systems according to Kenakin et al. (1989). Agonist A interacts with a transmembrane protein, R, which has at least two recognition areas, namely an extracellular area for the binding of drugs and also an intracellular area for the binding of G proteins. R is a promiscuous receptor and can interact with two separate stimulus transduction proteins (G_1 and G_2) which are both membrane bound and which each control separate cellular functions. Ternary complexes ARG_1 and ARG_2 are formed which initiate the production of intermediate stimuli (S_1 and S_2 respectively). These intermediate stimuli combine at some stage to yield a summation stimulus S_3 . The final cellular effect or response (E) is then postulated to be a function of S_3 .

The tools of receptor classification, intrinsic efficacy and relative potency ratios, may now have to be modified to fit the new model. If intrinsic

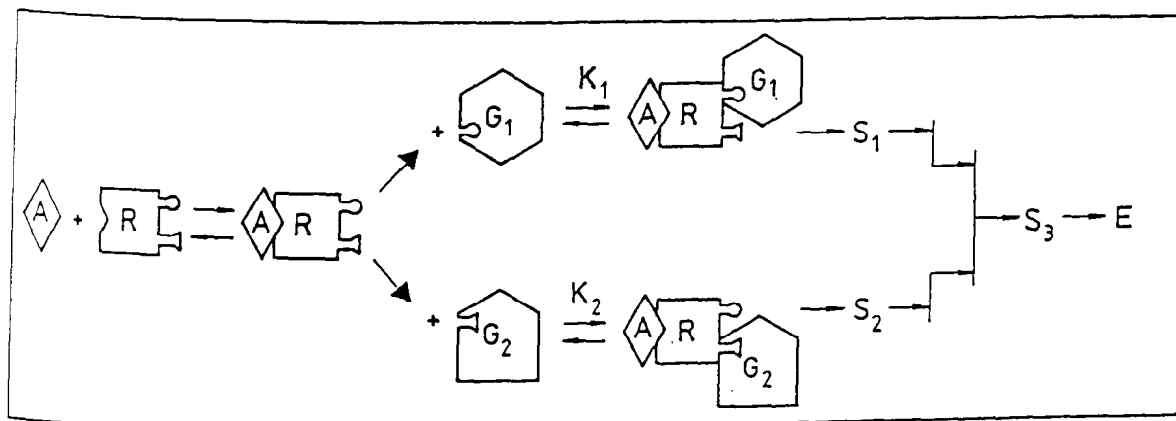


Fig. 24. Model of the theoretical effects of transducer receptor coupling proteins on drug-receptor interactions.

efficacy is related to the conformational change in the receptor that determines transducer binding, then relative efficacy should depend on the nature and concentration of the G proteins available in the membrane (Kenakin, 1989). Previously, if two agonists were found to have different potency ratios in two different tissues, it was assumed the tissues contained different receptors. However, the new model now suggests that the same receptors in different tissues can produce different values for relative potency due, not to receptor difference, but to different transducing proteins with which they interact

(Kenakin et al., 1989).

The measurement of agonist affinity by the method of Furchgott (1966) is based on the premise that the only effect of partial alkylation of receptors is a decrease in receptor number. The presence of transducer proteins in the tissue, however, changes the situation and alkylation may change the ratio of receptor to transducer coupling protein (Kenakin, 1989). Equation 10 changes accordingly:

$$\frac{1}{[A]} = \frac{1}{[A'](1-q)} + \left(\frac{q}{K_A(1-q)} \right) \left(1 + \frac{[G_t]}{K_G} \right) \dots\dots 13$$

where K_G is the dissociation constant of the ternary complex and $[G_t]$ is the total concentration of coupling protein. The apparant K_A derived from a double reciprocal plot would therefore be an underestimation of the true K_A , i.e. equation 11 changes as follows:

$$K_{App} = \frac{K_A}{1 + \frac{[G_t]}{K_G}} \dots\dots\dots 14$$

This implies that in systems where affinity for the coupling protein is very high (as it would be for high efficacy full agonists), there is a considerable error in the estimate of the true K_A . This could result in

artificially high estimates of agonist affinity. Drug receptor classification by means of agonist affinity calculations would depend on tissue factors and would therefore be unreliable (Kenakin, 1989). The whole concept of drug action has therefore been altered in the light of the evidence of the existence of transducer proteins and the possibility that they form ternary complexes with the agonist-receptor complex to bring about a cellular response.

CHAPTER 3

Literature Review - Smooth Muscle Contraction
Uterus and Ileum

3.1 The biochemistry of smooth muscle contraction in the uterus and ileum.

The uterus and ileum are examples of "unitary" smooth muscle. They behave like a single unit and are usually spontaneously active (Burnstock, 1970). Smooth muscle cells communicate with one another by means of gap junctions. The gap junctions are important for synchronized uterine muscle contraction during labour and it has been found that there is an increase in the number of gap junctions towards the end of pregnancy (Carsten and Miller, 1987). The actin and myosin filaments in smooth muscle are not organised into fibers and fibrils as in skeletal muscle, but are arranged in random bundles throughout the muscle cells. This allows smooth muscle to contract in any direction, unlike skeletal muscle where the direction of contraction is always aligned with the axis of the muscle fibres. Smooth muscle is also able to shorten more than skeletal muscle (Huszar, 1986).

Skeletal muscle contraction is triggered by a stimulus at the cell membrane which initiates an action potential. In smooth muscle, however, contraction can be triggered either electrically or hormonally. According to Carsten et al., (1987), hormonal control of smooth muscle contraction may take place as follows:

Hormonal or agonist receptor binding at the cell membrane activates the enzyme phospholipase C and initiates phosphoinositide hydrolysis in the cell membrane. G-proteins are possibly involved in the modification of this reaction. Phosphoinositol 4,5 biphosphate (PIP_2) is hydrolysed to inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 then acts as a second messenger and diffuses to the cell interior and activates the release of calcium from the sarcoplasmic reticulum. DAG activates protein kinase C, which phosphorylates various proteins. DAG is then converted to phosphatidic acid and/or arachidonic acid (the precursor for prostaglandin synthesis). Prostaglandins release calcium from the sarcoplasmic reticulum and inhibit calcium uptake. Oxytocin and other uterotonic agents stimulate the production of $\text{PGF}_{2\alpha}$ and PGE_2 in pregnant myometrium (Hensby, Seed and Williams, 1986) and this could be as a result of their activation of phosphoinositide hydrolysis.

The calcium released from the sarcoplasmic reticulum (SR) forms a complex with the protein calmodulin. This complex binds to and activates myosin light-chain kinase (MLCK), the enzyme which is the key regulator of contractility in smooth muscle. MLCK activation causes the phosphorylation of myosin which then interacts with actin to bring about smooth muscle

contraction. The activity of MLCK is modulated by a cyclic adenosine monophosphate (cAMP)-dependent protein kinase which catalyzes the phosphorylation of MLCK. Phosphorylated MLCK has much less affinity for the calcium-calmodulin complex and is thus inhibited and the dephosphorylation of myosin and smooth muscle relaxation follow.

Shrey, Cornford, Read and Steer (1988) confirm this proposed mechanism in studies of the effects of oxytocin on phosphoinositide hydrolysis in human gestational myometrium. Activation of muscarinic receptors is believed to lead to at least three well-defined G protein-dependent cellular responses, namely, inhibition of adenylyl cyclase, activation of phospholipase C and the opening of a certain class of potassium channels (Mutschler, Feifel, Moser, Tacke, Wess and Lambrecht, 1990). Muscarinic receptor stimulation by carbachol was found to enhance phosphoinositide breakdown in guinea-pig ileum (Sekar and Roufogalis, 1984) and in guinea-pig myometrium (Marc, Leiber and Harbon, 1986). Uterine contractions mediated by alpha-1-adrenergic receptors also appear to be linked to activation of the phosphatidylinositol pathway (Kirk-Riemer and Roberts, 1986).

3.2 The pharmacology of smooth muscle contraction in the uterus and ileum

3.2.1 Ileum

The receptors responsible for the peristaltic reflex are found in the muscle layers of the small intestine. These distension-sensitive receptors are stimulated by passive distension and can also be activated by 5-hydroxytryptamine (5-HT) and acetylcholine (ACh) (Daniel, 1968). The longitudinal muscle layer contracts first, shortening the intestine and this increase in radial distension activates the sensory receptors of the circular layer of muscle. The circular muscle then contracts in a wave-like motion to empty the contents of the gut. The longitudinal muscle relaxes abruptly during the circular muscle contraction (Kottegoda, 1970). According to Costa and Furness (1976), the peristaltic reflex consists of an ascending excitatory reflex (which might involve neurons releasing ACh and 5-HT) and a descending inhibitory reflex.

Activation of the following receptor types is believed to be responsible for a spasmogenic effect on the intestine:

Muscarinic cholinergic receptors:- The M-3 receptors

are believed to be located on smooth muscle cells in the gastrointestinal tract (Mutschler et al., 1990). Smooth muscle contraction caused by the stimulation of these receptors by ACh can be antagonized by atropine.

Histamine receptors:- H-1 receptors are located on smooth muscle and nerves and stimulation of these receptors by histamine causes contraction of the intestinal smooth muscle. These effects can be antagonized by mepyramine (Black, Duncan, Durant, Ganellin and Parsons, 1972).

Serotonergic 5-HT-2 receptors:- They are located on ganglion cells located in the intestinal wall and in smooth muscle. Stimulation of 5-HT-2 receptors by 5-HT contracts the intestine, increases tone and facilitates peristalsis (Burkhalter and Frick, 1989). 5-HT is believed to be involved in the control of the peristaltic reflex. The effects of 5-HT on the ileum can be blocked by methysergide (Costa et al., 1976).

The isolated rat ileum was used in this study to investigate the possible effects of *Clivia* on intestinal motility. The rat ileum is not an ideal model for the study of the effects of histamine (Van Rossum, 1963) and 5-HT (Born, 1970), and therefore only the interactions of *Clivia* with the muscarinic cholinergic system of the ileum was examined.

3.2.2 Uterus

The response of the uterus to hormones and drugs is controlled by the hormones oestrogen and progesterone. Progesterone is the predominant hormone during pregnancy and is essential for the maintenance of pregnancy. In the rat, there is increased binding of progesterone to specific protein at term and its effective concentration decreases. This increases the [oestrogen]/[progesterone] ratio in the uterus which then becomes oestrogen-dominated. The increase in this ratio is believed to be integral to the initiation of labour. In the human, however, there seems to be no reduction in progesterone concentration prior to parturition (Sakamoto, Maclusky and Naftolin, 1986). Under the influence of oestrogen there is increased synthesis and release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and an increase in the numbers of myometrial oxytocic and alpha-adrenergic receptors (Kirk Riemer et al., 1986). There is also an increase in sarcolemmal resting potential and gap formation (Huszar and Roberts, 1982). The exact mechanisms and molecular events which initiate labour are as yet unknown.

Oxytocin, $PGF_{2\alpha}$ and PGE_2 are the physiological hormones that cause uterine contraction during spontaneous labour. Oxytocin is the most potent of

all the known uterotonic agents and oxytocin receptors are located in the myometrial cell membrane and in the endometrium (Fuchs 1986). Oestrogen increases the number of oxytocin receptors and progesterone inhibits this increase (Carsten et al., 1987). PGE₂ and PGF_{2α} receptors do not increase in number during pregnancy and labour as do the oxytocin receptors, but an increased synthesis of prostaglandins is believed to be an important factor in the initiation of labour. Prostaglandin receptors are found in the cell membrane and SR of the myometrium (Carsten et al., 1987). Oxytocin seems to be more important in the initial stages of labour whereas PGF_{2α} production is essential for the progress of labour and cervical dilatation (Fuchs, 1986). Prostaglandins enhance the production of myometrial gap junctions and PGF_{2α} stimulates the influx of calcium into myometrial cells and the release of calcium from the SR (Caritis, Darby and Chan, 1988). Indomethacin and other prostaglandin synthesis inhibitors have been used to treat premature labour. Oestradiol has been shown to increase cyclooxygenase activity in rat uterus, thereby escalating prostaglandin synthesis (Carsten et al., 1987).

Alpha- and beta-adrenergic receptors are also found in the uterus. Stimulation of alpha-1 receptors results in uterine contraction and beta-2 receptor stimulation

causes the uterus to relax (Hoffman, Lavin, Lefkowitz and Ruffolo, 1981; Olssen, Ahlquist, Gustafsson and Perrson, 1984). The pregnant uterus is beta-2 receptor dominated. Beta-adrenergic response is increased when plasma progesterone levels are high (Digges, 1982). There is an increase in alpha receptor responsiveness in late pregnancy and early post partum. Pretreatment of non-pregnant rats with oestrogens has a similar effect on the uterus. It has been proposed that alpha-1 receptors predominate in the circular smooth muscle layer of the myometrium and beta-2 receptors predominate in the longitudinal smooth muscle layer during late pregnancy (Digges, 1982). The uterus contracts in response to noradrenaline when propranolol is used to block the beta-2 receptor response (Paton, 1968).

Serotonergic 5-HT-2 receptors and cholinergic M-3 receptors are present in the uterus and stimulation of these receptors also results in uterine contraction (Born, 1970; Mutschler, 1990). Oestrogen treatment increases the sensitivity of the uterus to the effects of 5HT and muscarinic receptor stimulation. Methysergide is a classical non-competitive antagonist of 5-HT on the uterus (Ichida, Hayashi and Terao, 1983) and atropine is the classic muscarinic antagonist (Shew, Yee and Pang, 1984). The uterus also contains

histamine receptors and in the rat, H-2 receptor stimulation results in uterine relaxation (Bertaccini, Molina, Vitali and Zappia, 1979)

The oestrogen-pretreated isolated rat uterus was used in this study to examine the effects of Clivia on the uterus. Figure 25 summarizes the pharmacological and biochemical regulation of smooth muscle contraction.

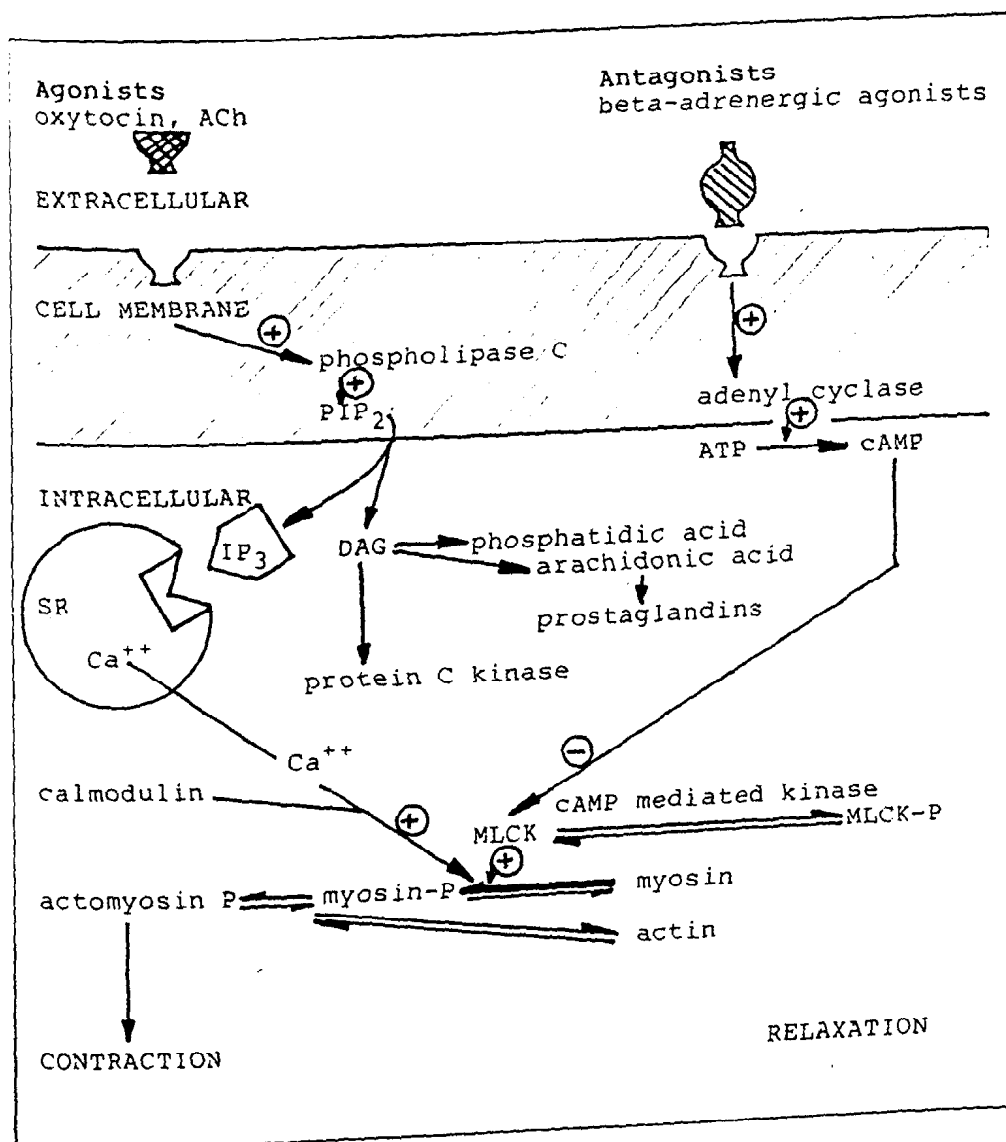


Fig. 25. A model for the pharmacological and biochemical regulation of smooth muscle contraction.

3.3 The pharmacological approach to the experimental protocol

The objective of this study was to examine the effects of Clivia on isolated rat uterus and ileum. Studies in smooth muscle preparations usually distinguish between three types of pharmacological activity i.e. agonistic, antagonistic or potentiating (Blattner, Classen, Dehnert and Doring, 1980). Bearing the general pharmacology of the ileum and uterus in mind, the experimental procedure was planned as follows:

i. Investigation of the direct effects of Clivia on the ileum and the uterus

The first step in this study was to be the investigation of the direct effects of Clivia on smooth muscle, using the cumulative response to acetylcholine as a standard reference in both organs. This would indicate whether or not the plant possessed agonistic activity on the uterus and ileum.

ii. Investigation of the interaction of Clivia with the muscarinic receptors in the ileum and uterus

The investigation of the effects of pretreatment with Clivia extract on the response of the ileum and uterus to ACh was to be the next phase of the study. As both the uterus and ileum contain muscarinic receptors, these results would indicate whether the extract potentiated or antagonized the cholinergic response to

muscarinic receptor activation. The interaction of this system with the classic muscarinic antagonist, atropine, would also be investigated. The possible indirect action of Clivia by stimulation of prostaglandin synthesis was to be tested by including a series of experiments where the interaction of indomethacin with the system was examined.

iii. Investigation of the interaction of Clivia with the oxytocic receptors in the uterus

The effects of pretreatment with the extract on the uterine response to oxytocin was to be tested in order to ascertain whether Clivia potentiated or antagonized the effects of oxytocin. As oxytocin is believed to be critically involved in the initiation of spontaneous labour, these experiments could help predict the possible outcome of the ethnic use of the plant to induce labour. The system was also to be tested with the inclusion of pretreatment with either atropine or indomethacin in order to investigate possible interactions of the muscarinic cholinergic system or prostaglandins with the uterine response to Clivia and oxytocin.

iv. Investigation of the interaction of Clivia with 5-HT receptors in the uterus

The cumulative response of the uterus to 5-HT after pretreatment with the extract was compared to the normal uterine response to 5-HT. The ergot alkaloids,

well-known uterotonic agents of plant origin, interact with 5-HT receptors and this series of experiments could therefore possibly relate the effects of **Clivia** on the uterus to the effects of the ergot alkaloids.

v. **Investigation of the interaction of Clivia with adrenergic receptors in the uterus**

The normal uterine response to cumulative addition of noradrenaline in the presence of propranolol was compared with the uterine response to noradrenaline after pretreatment with propranolol and extract. The activation of alpha-1 receptors causes contraction of the uterus and therefore the effects of **Clivia** on the adrenergic system was also examined.

CHAPTER 4

Methodology

4.1 Preparation of the Clivia miniata extract

Clivia miniata plants were purchased from nurseries in the Johannesburg area and were established in a garden in Sandton. The plants were authenticated at the Herbarium of the Botanical Research Institute in Pretoria as *Clivia miniata* Regel.

Clivia miniata is prepared as inembe by making an aqueous infusion or "tea" of the leaves (Gerstner 1941, Bryant 1966). It was decided to investigate the properties of the ethnic preparation (i.e. a crude aqueous extract) itself in an attempt to directly confirm the traditional medicinal use of the plant. Extraction and identification of the active principles could then form the basis of a subsequent study. A similar rationale was followed by Akubue, Mittal and Aguwa (1983), Carlini, Contar, Silva-Filho, Da Silveira-Filho, Frochtengarten and Bueno (1986), Feng, Haynes, Magnus, Plimmer, and Sherratt (1962) and Gran (1973).

Fresh *Clivia* leaves were harvested during the period March 1987 to August 1989. The leaves were washed, wiped dry, cut into small pieces and weighed. The *Clivia* was boiled in distilled water for 4 hours to extract water soluble principles. The aqueous

filtrate was then divided into aliquots of 200ml, frozen and freeze dried. The extract yield was 1% w/w relative to the wet starting material.

4.2 Apparatus

The biological system used in this study was the 50ml double-walled "Magnus" organ bath which provided standardised, optimal in-vitro conditions for the isolated organs. The organ bath is illustrated in figure 26 . A thermostatically controlled water bath continuously pumped water of the correct temperature through the outer cavity of the water bath. The water bath also contained a reservoir of Tyrode physiological solution heated to the correct temperature and used to fill and rinse the inner cavity of the organ bath containing the isolated organ. The organ bath was rinsed by overflow so that the organ remained totally immersed in the physiological solution throughout the experiment.

The organ bath was fitted with a loose stainless steel organ holder (see fig.26). The bottom of the organ holder was fitted with a stainless steel clip to which one end of the preparation was first attached. A second clip, attached to the other end of the isolated organ, connected the organ to the lever-transducer

system by means of a thread. The organ holder was gently lowered into the bath filled with physiological solution at the correct temperature. Care was taken not to overstretch the preparation and the organ holder was firmly attached to the organ bath wall. The stem of the organ holder was hollow and open at both ends. It was connected to the aerating gas supply at the upper end and the gas was allowed to aerate the physiological solution inside the bath by gently bubbling through the lower end (see fig. 26).

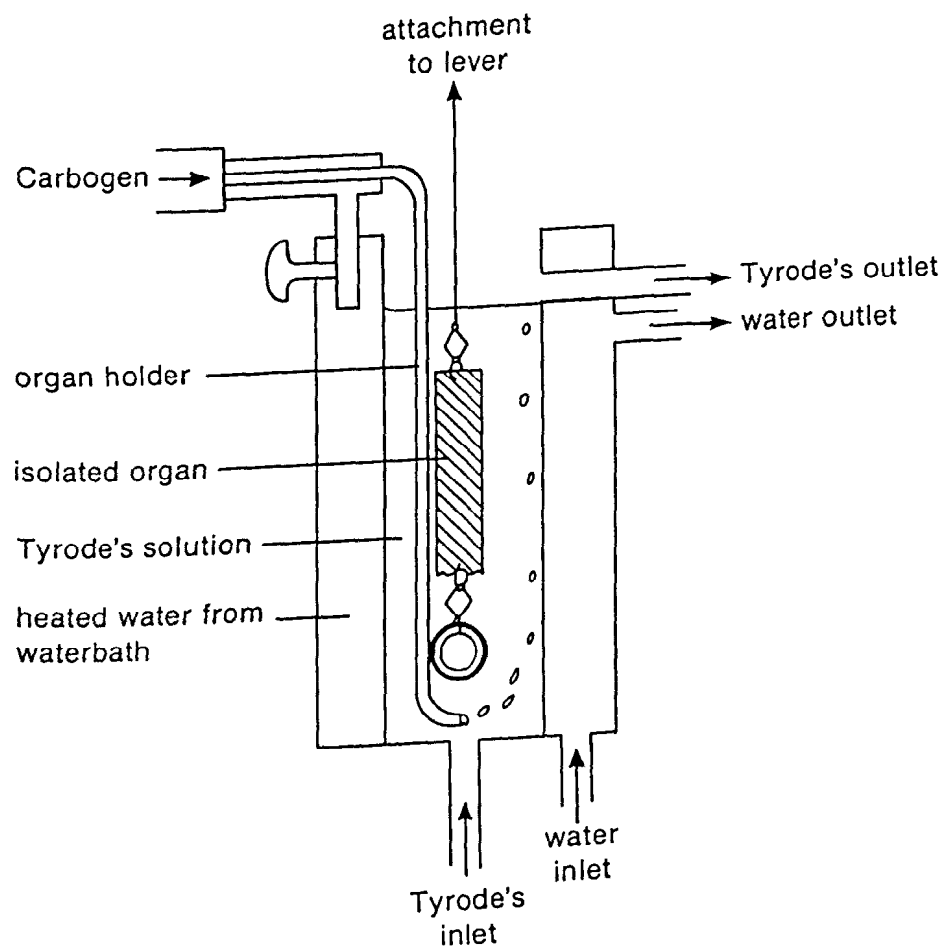


Fig. 26. Isolated organ bath (50ml) fitted with organ holder.

The measuring system consisted of a lever connected to the isolated organ at one end and a metal rod at the other end. The metal rod or "core" rested inside a solenoid which was connected to a Metrohm Labograph Potentiometric Recorder (model E428).

The physiological solution used throughout the study was Tyrode's solution. This is the recommended solution for the study of the isolated ileum (Blattner et al., 1980). Various other "physiological" solutions have been recommended for the isolated uterus preparation e.g. De Jalon's, calcium deficient Tyrode's, Sund's solution and Kochman's solution (Blattner et al., 1980) in order to reduce the interference of spontaneous contractile activity. These solutions however, do not contain normal concentrations of electrolytes. It was therefore decided to use Tyrode's solution as recommended for the model of the spontaneously contracting uterus for the study of oxytocic agents (Blattner et al., 1980) and to restrict the spontaneous motility by reducing the bath temperature as recommended by Kumegai, Ebashi and Takeda (1952) and Blattner et al., (1980). The Tyrode's solution was freshly made up before every experiment and had the following composition (mmol/l): NaCl 137; KCl 2.68; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 1.80; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 1.05; NaHCO_3 11.90; $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ 0.36; dextrose monohydrate 5.05.

4.3 Animals

The rat was chosen as an experimental animal for this study. Rats are inexpensive, easily obtainable and the rat uterus has been used as a standard preparation for the assay of oxytocin (British Pharmacopoeia, 1958), 5-HT and ergometrine (Pennefather, 1961). The rat ileum is a good preparation for the study of parasympathetic agents although it is unsuitable for the study of histaminic agents (Van Rossum, 1963). According to Bertaccini et al. (1979) the rat uterus contains only Histamine-2 receptors and is relaxed by histaminic agents. The study of histaminic substances in combination with *Clivia* was therefore excluded from the study. The effects of *Clivia* on both the uterus and the ileum could therefore be studied in a female rat, thus reducing the number of experimental animals needed for the project.

Virgin female Sprague-Dawley rats weighing 150-200g were used. Animal Ethics Clearance was obtained from the Animal Ethics Screening Committee of the University of the Witwatersrand (protocol nos. 86/4, 88/57/1 and 90/102/1). The rats were supplied and housed by the Central Animal Unit, Medical School, University of the Witwatersrand.

The rats were brought into oestrus by pretreatment with stilboestrol. Stilboestrol was diluted in arachis oil and 0,1mg/Kg was administered intraperitoneally 24 h before each experiment (Department of Pharmacology Manual, University of Edinburgh, 1970). This ensured that all rats used in the study were in the same stage of their sexual cycle. According to Marshall (1962) and Csapo (1962), uterine muscle from an immature animal in anoestrus exhibits very weak and uncoordinated contractions. Pretreatment with oestrogen causes the myometrium to contract spontaneously and rhythmically and become pharmacologically reactive. Soloff, Alexandrova and Fernstrom (1979) found that oestrogenisation of the rat increased the concentration of oxytocin receptors in the uterus and this correlated well with the fact that levels of plasma estradiol-17-beta were maximal on the day of parturition. Oestrogenisation of the rat can therefore benefit the experiment further by inducing a state of oestrogen dominance similar to that occurring at parturition.

The rats were starved overnight and killed by inhalation of CO₂. Food was withdrawn 24 hours before dissection to ensure that the gut was empty and less prone to spontaneous motor activity after dissection (Blattner et al., 1980). Vaginal smears were taken

to confirm that the rats were in oestrus at the time of the experiment. Shorr's stain (Drury and Wallington, 1980) was used and the degree of cornification determined according to the method of Mahoney (1973).

4.4 Isolated organ preparations

The abdomen of the rat was opened and the uterine horns were exposed. A section approximately 2cm in length was dissected from the central portion of each horn and immediately transferred to a dish containing Tyrode's solution. The tissue was trimmed of fat and cut open longitudinally at the mesenteric border so that the preparation finally formed a sheet of muscle approximately 1cm in length. Each uterine strip was mounted in the organ bath containing 50 ml Tyrode's solution and aerated with a mixture of 95% O₂ and 5% CO₂, giving a pH of 7.4. The bath temperature was maintained at 26°C which inhibited spontaneous contractility in most cases (Kumagai et al., 1952).

A piece of ileum about 10cm in length was dissected out about 10cm away from the caecum and immediately placed in a bowl containing fresh Tyrode solution. It was trimmed of fat and carefully washed out. Two sections about 1.5cm in length were cut off and each piece of ileum was mounted in a 50 ml organ bath containing

Tyrode's solution and aerated with the same gaseous mixture as the uterine strips. Care was taken to ensure that the preparation was attached by diagonally opposite sides of its open ends. The bath temperature was maintained at 37°C.

An equilibration period of at least 30 minutes was allowed and during this period the bath was rinsed by upward displacement at least 3 times. Isotonic contractions against a load of 1g were recorded electronically. The organ was allowed to rest for at least 30 minutes between drug challenges and the bath was rinsed at least 3 times during this rest period. Cumulative concentration-response curves were constructed according to Van Rossum (1963). At least two observations per concentration per rat were recorded and each experiment was repeated with at least 3 different rats. Control as well as experimental treatments were always given to each preparation. The results were expressed as the mean $\pm$ S.E.M.

4.5 Reference Drugs

The following drugs were used in the experiments in final organ bath concentrations as indicated:
Oxytocin (Syntocinon, Sandoz) - $[6,3 \times 10^{-8}$ to $6,3 \times 10^{-2}$ IU/ml]; acetylcholine chloride (BDH) - $[3 \times 10^{-9}$

to 1×10^{-2} M]; atropine sulphate (BDH) - [1×10^{-9} to 1×10^{-7} M]; serotonin creatininesulphate (Aldrich) - [5×10^{-10} to $1,5 \times 10^{-3}$ M]; methysergide (Deseril, Sandoz) - [$2,8 \times 10^{-9}$ M]; noradrenaline bitartrate (Levophed, Winthrop) - [4×10^{-9} to $1,3 \times 10^{-4}$ M]; propranolol (Inderal, ICI) - [$1,35 \times 10^{-5}$ M]; indomethacin (Sigma) - [2×10^{-6} to 5×10^{-6} M] and stilboestrol dipropionate (Maybaker) - [0,1 mg/Kg]. All solutions of agonists and antagonists were made up in Tyrode's solution immediately before the experiment.

CHAPTER 5

Results

5.1 Direct activity of Clivia miniata extract on rat uterus and ileum

5.1.1 Ileum

The results of cumulative additions in ascending doses of the herbal extract on the ileum are illustrated as a cumulative concentration-response curve in Fig. 27. A graded dose-response was obtained. These results indicated that a concentration of 2×10^{-3} g/ml of Clivia extract would be suitable as the pretreatment dose in subsequent experiments on the ileum.

5.1.2 Uterus

The results of cumulative additions in ascending doses of Clivia on the non-spontaneously contracting uterus are illustrated as a cumulative concentration-response curve in fig.28. The results were similar to those obtained with the ileum preparation - a graded dose-response was obtained. A concentration of 2×10^{-3} g/ml of the Clivia extract was selected from these results as a suitable pretreatment dose in all subsequent experiments with the uterus.

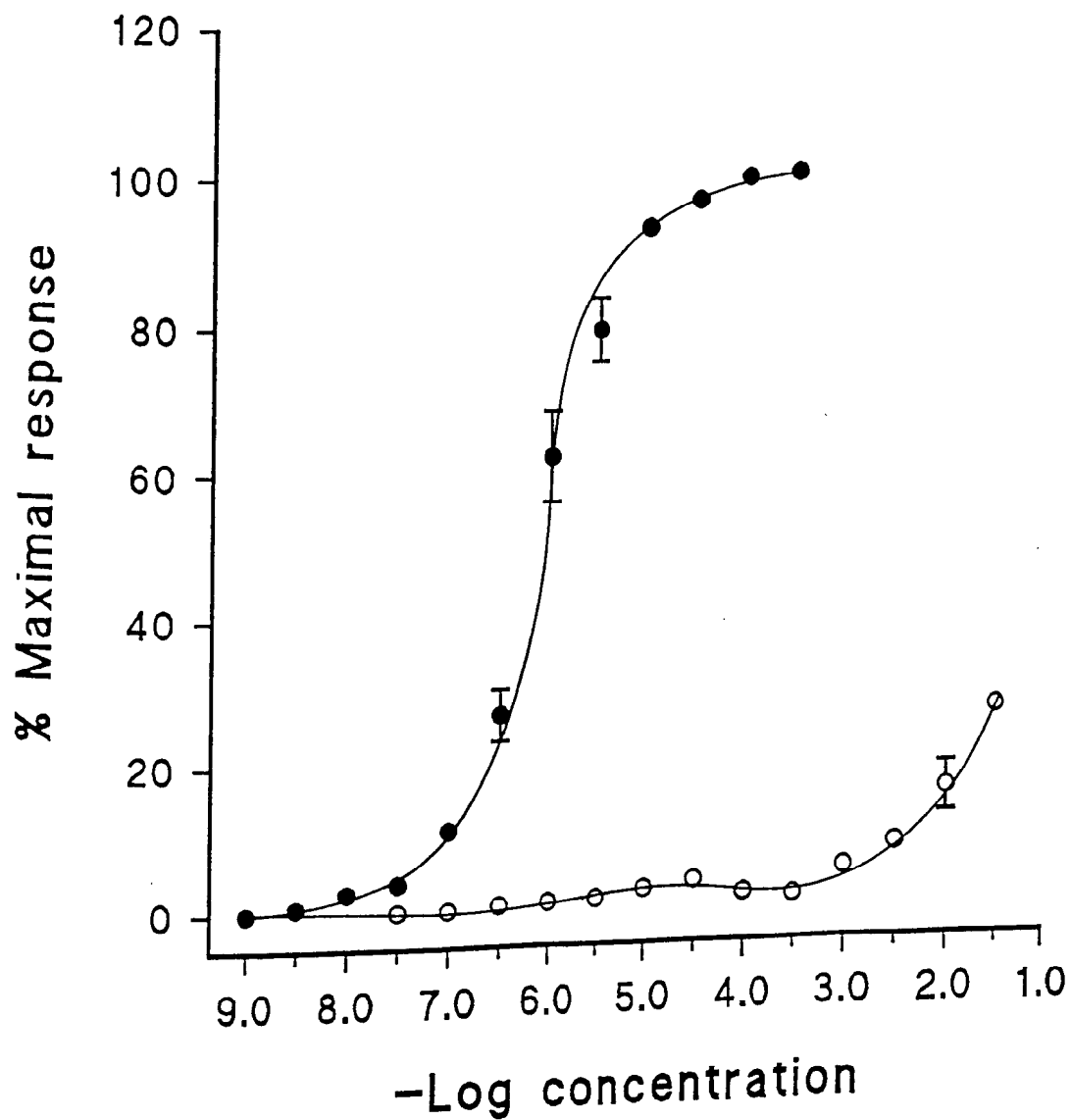


Fig. 27. Mean cumulative concentration-response curves for ACh () and Clivia extract () on the isolated rat ileum. Each point represents the mean of mean experimental values obtained with 5 rats and the vertical bars represent the S.E.M. Bath concentrations are expressed as g/ml for Clivia extract and M for ACh.

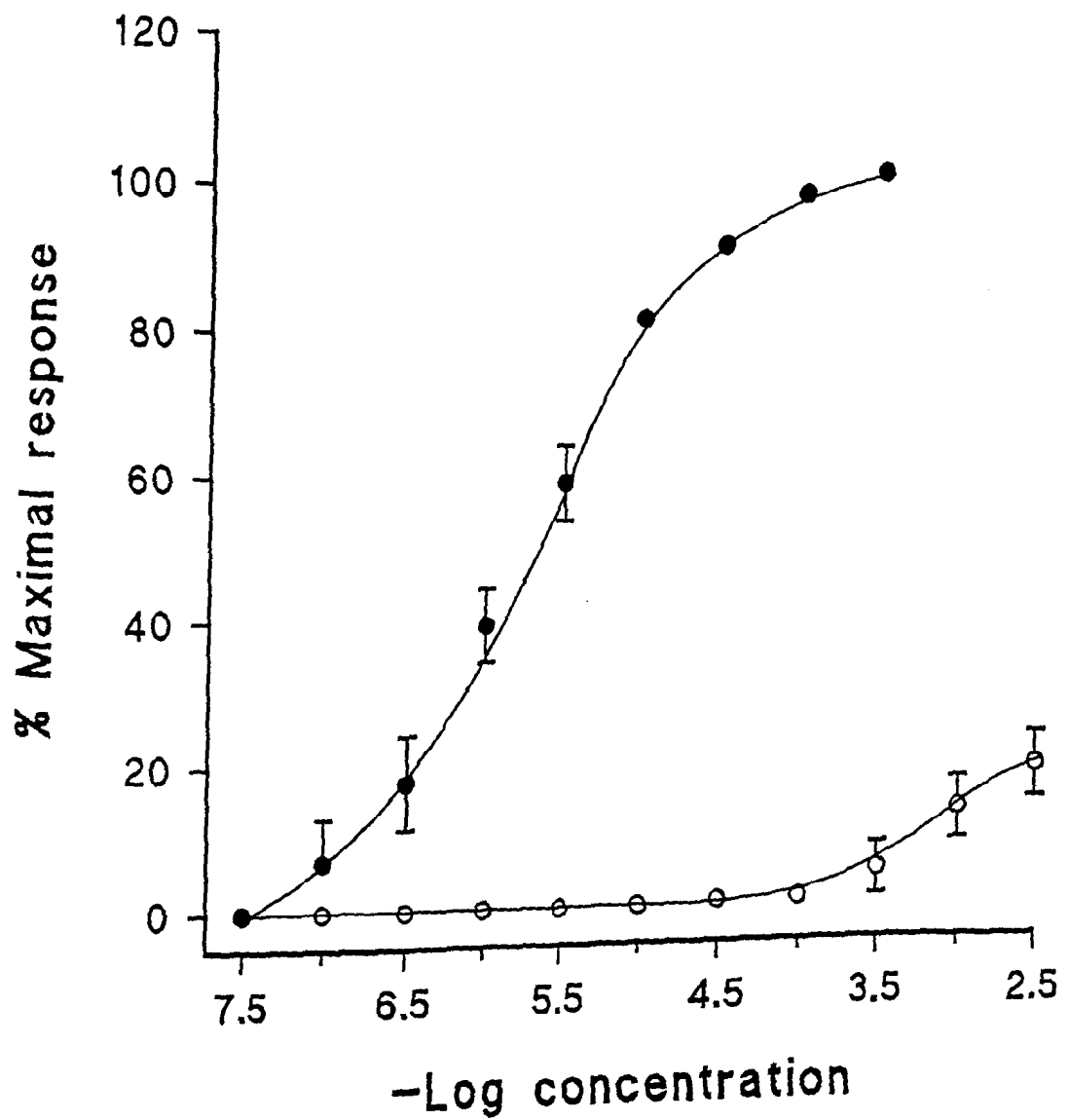


Fig. 28. Mean cumulative concentration-response curves for ACh (●) and Clivia extract (○) on the isolated rat uterus. Each point represents the mean of mean experimental values obtained with 5 rats and the vertical bars represent the S.E.M. Bath concentrations are expressed as g/ml for Clivia extract and M for ACh.

5.2 Effects of pretreatment with Clivia extract alone or in combination with atropine or indomethacin on the response of the isolated organs to acetylcholine

5.2.1 Ileum

5.2.1.1 Effects of pretreatment with the extract on the response of the ileum to ACh

Concentration-response curves obtained by cumulative additions of ACh to the ileum were compared to those obtained when the ileum was pretreated with Clivia extract (2×10^{-3} g/ml) for 5 min before cumulative additions of ACh (Fig. 29). The Clivia augmented the effects of subthreshold and threshold concentrations of ACh on the ileum but it inhibited the maximal response of the ileum to ACh.

5.2.1.2 Effects of pretreatment with atropine and the extract on the response of the ileum to ACh

Cumulative concentration-response curves obtained with ACh on the ileum were also compared to those where the ileum was pretreated with atropine (1×10^{-7} M or 3×10^{-8} M) for 5 minutes before pretreatment with Clivia,

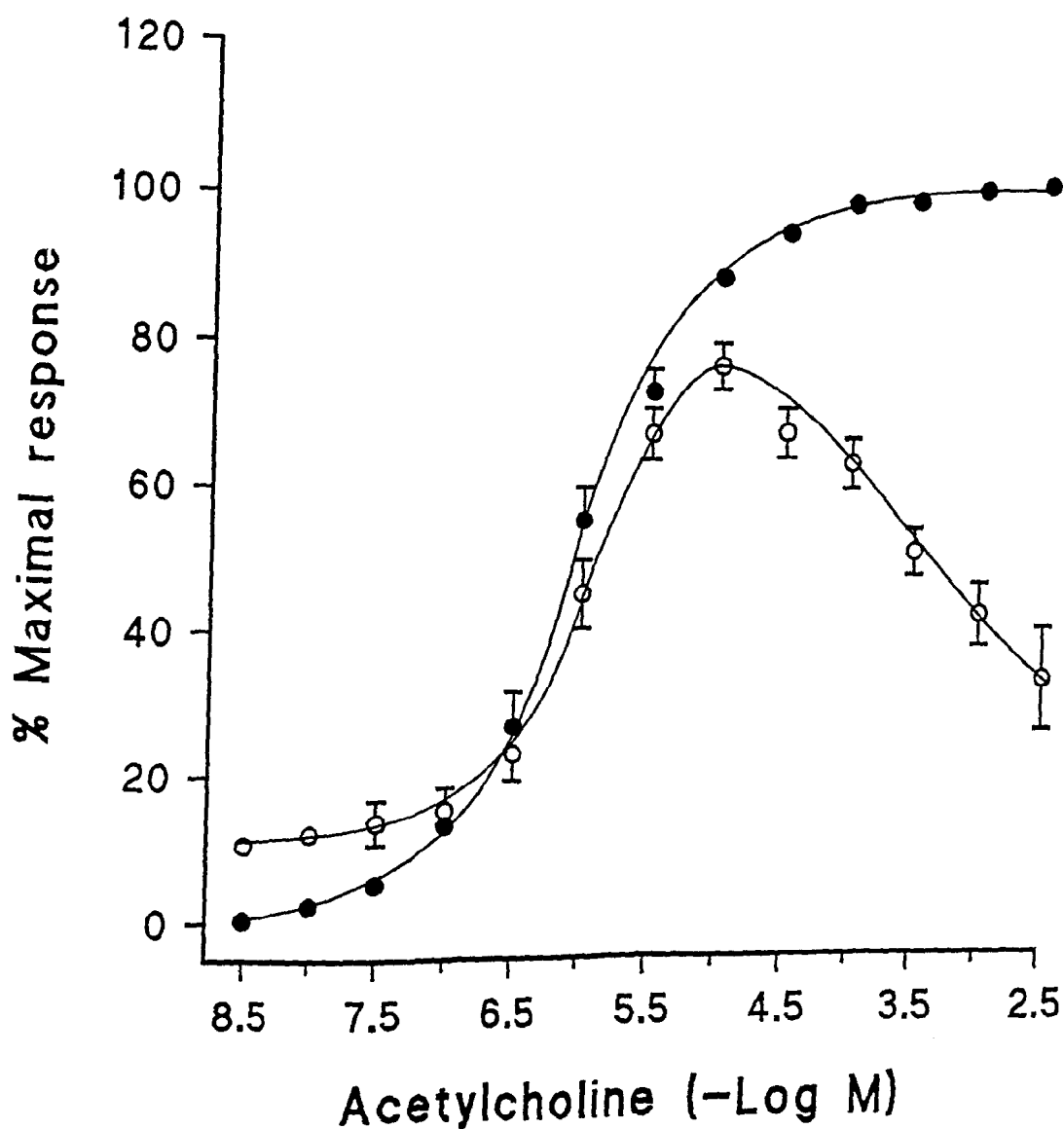


Fig. 29. Mean cumulative concentration-response curves for ACh on the isolated rat ileum in the absence (●) or in the presence (○) of *Clivia* extract (2×10^{-3} g/ml). Each point represents the mean of mean experimental values obtained with 18 to 19 rats and the vertical bars represent the S.E.M.

followed by cumulative administration of ACh (fig. 30). Atropine inhibited the effects of *Clivia* on the

response of the ileum to ACh and shifted the curves to the right.

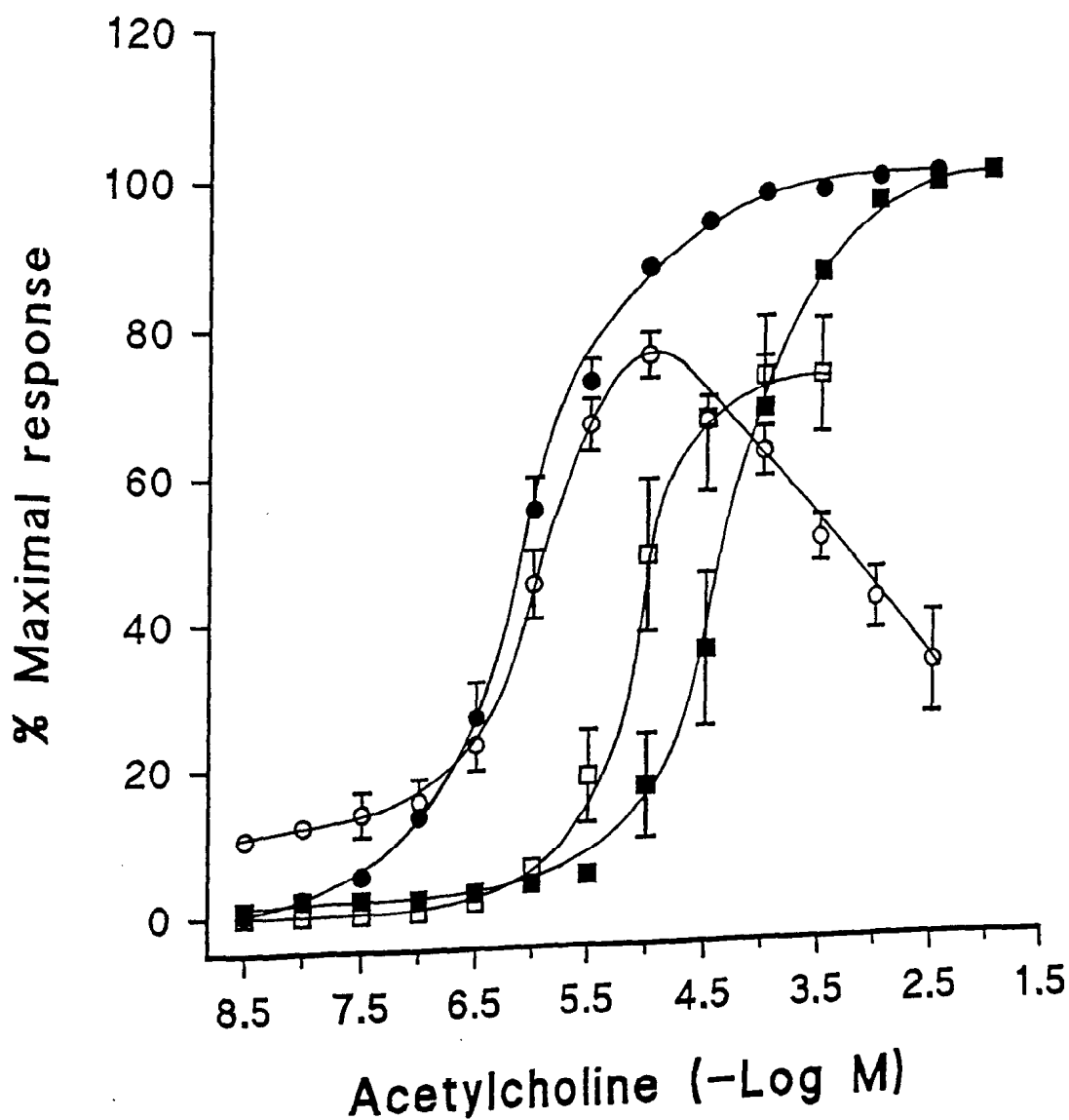


Fig. 30. Mean cumulative concentration-response curves for ACh on the isolated rat ileum in the absence (●) or in the presence (○) of *C. miniata* extract (2×10^{-3} g/ml) or in the presence of atropine (1×10^{-7} M) and extract (■) or atropine (3×10^{-8} M) and extract (□). Each point represents the mean of mean experimental values obtained with 5 to 19 rats and the vertical bars represent the S.E.M.

5.2.1.3 Effects of pretreatment with indomethacin and the Clivia extract on the response of the ileum to ACh

Cumulative concentration-response curves obtained with ACh on the ileum were compared to those obtained where the organ was pretreated with indomethacin ($5 \times 10^{-6} \text{M}$ for 5 min) before pretreatment with the extract ($2 \times 10^{-3} \text{ g/ml}$ for 5 min) followed by cumulative dosing with ACh. Indomethacin did not alter the effects of Clivia on the ileal response to ACh (fig. 31). Pretreatment with indomethacin alone did not alter the response of the ileum to ACh.

5.2.2 Uterus

5.2.2.1 Effects of pretreatment with Clivia on the response of the uterus to ACh

Cumulative concentration-response curves obtained with ACh on the non-spontaneously contracting uterus were compared to those where the organ was pretreated with the extract ($2 \times 10^{-3} \text{ g/ml}$) for 5 min before the cumulative addition of ACh (Fig. 32). The Clivia significantly enhanced the effects of subthreshold and threshold concentrations of ACh on the uterus. The pretreatment with Clivia clearly did not affect the maximal response to ACh.

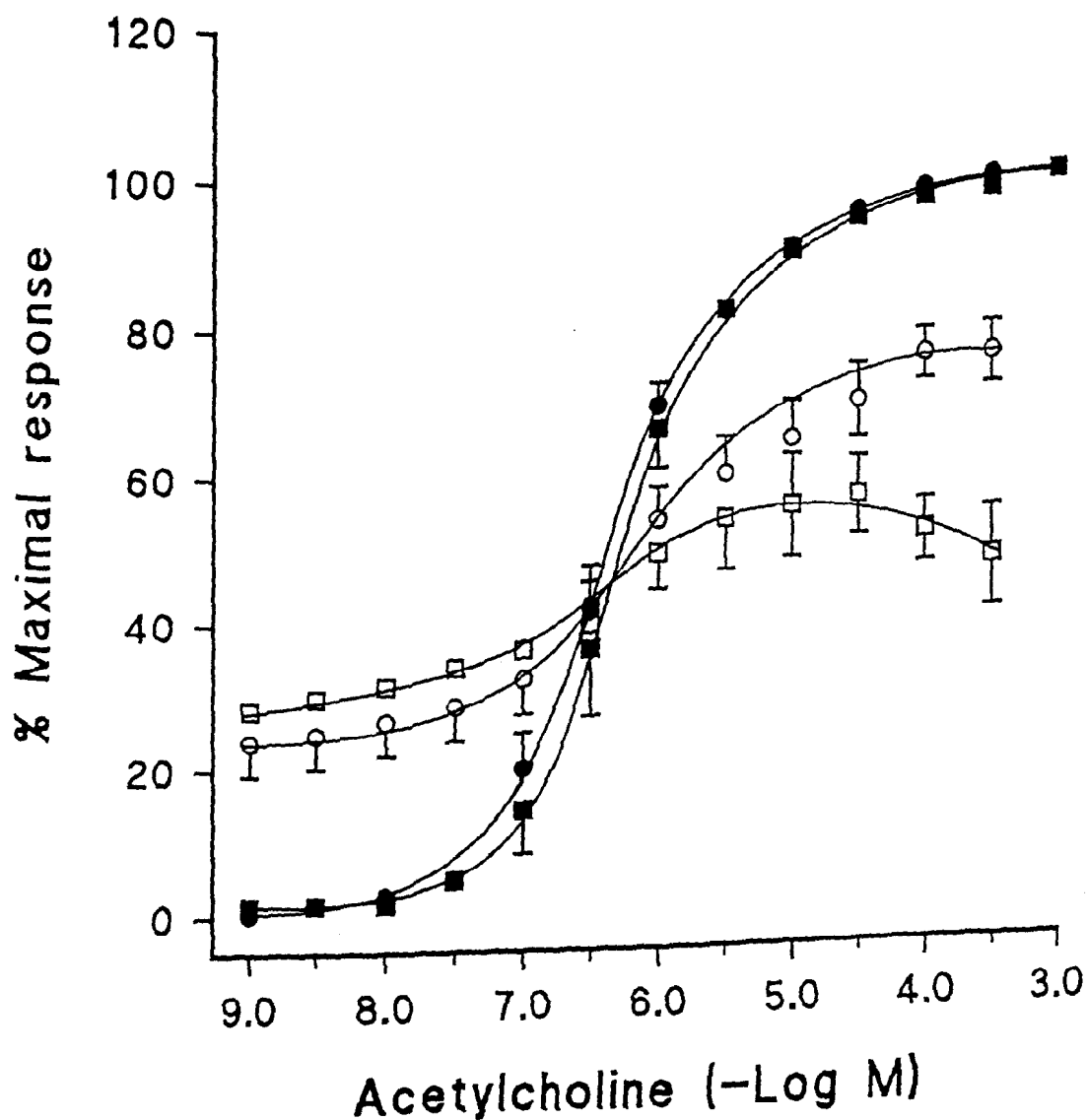


Fig. 31. Mean cumulative concentration-response curves for ACh on the isolated rat ileum in the absence (●) or in the presence of *C. miniata* extract (2×10^{-3} g/ml) (○) or in the presence of indomethacin (5×10^{-6} M) (■) or in the presence of indomethacin and *Clivia* (□). Each point represents the mean of mean experimental values obtained with 3 to 7 rats and the vertical bars represent the S.E.M.

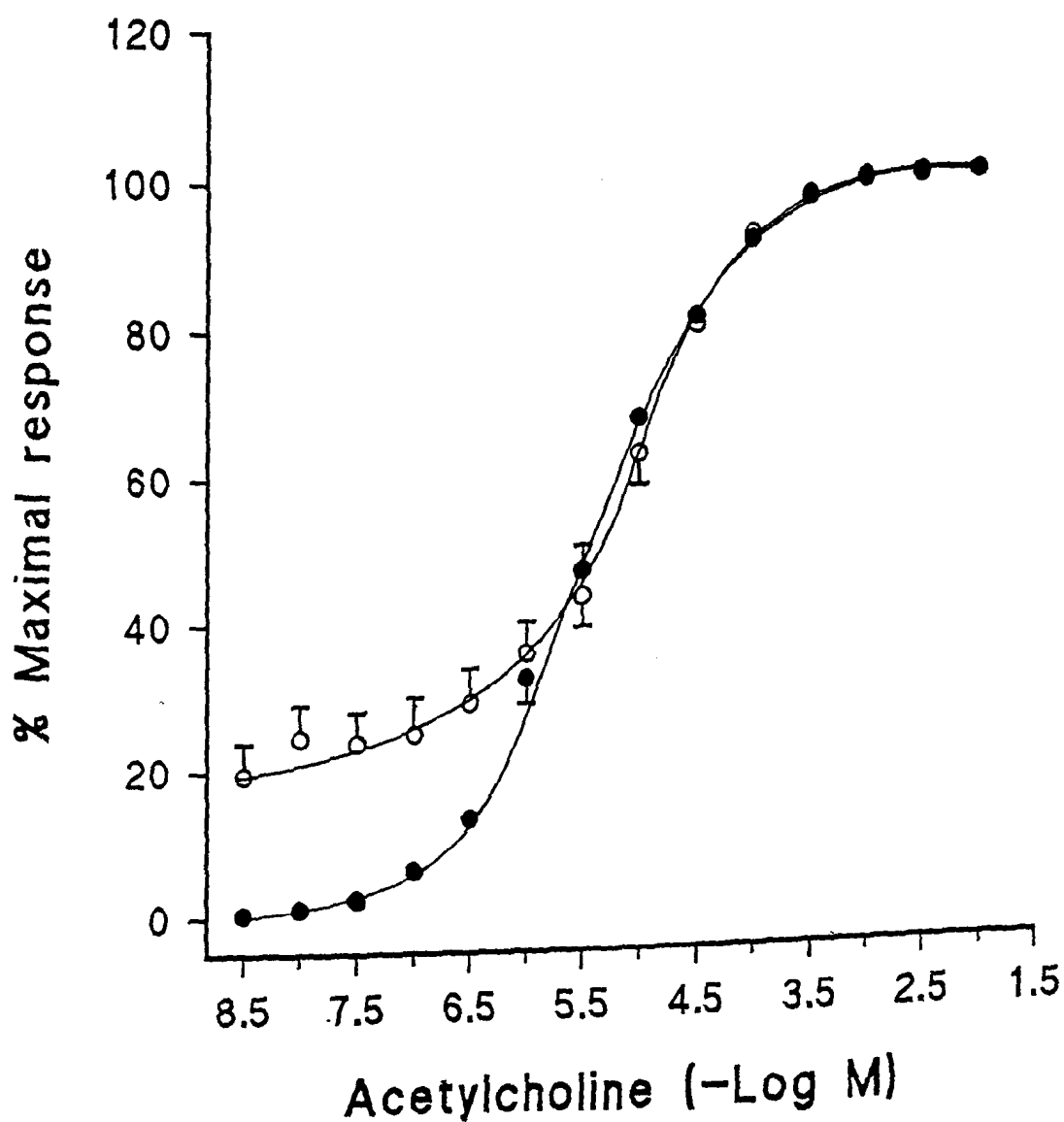


Fig. 32 . Mean cumulative concentration-response curves for ACh on the isolated rat uterus in the absence (●) and in the presence (○) of Clivia extract (2×10^{-3} g/ml). Each point represents the mean of mean experimental values obtained with 14 to 18 rats and the vertical bars represent the S.E.M.

5.2.2.2 Effects of pretreatment with atropine and Clivia on the response of the uterus to ACh

Pretreatment of the isolated uterus with atropine ($1 \times 10^{-8}\text{M}$ or $1 \times 10^{-9}\text{M}$) for 5 min before pretreatment with the extract followed by cumulative administration of ACh inhibited the initial effects of the Clivia on the response of the uterus to ACh. The curves were shifted to the right (fig. 33) and pretreatment with atropine ($1 \times 10^{-8}\text{M}$) and Clivia inhibited the maximal response of the uterus to ACh.

5.2.2.3 Effects of pretreatment with indomethacin and extract on the response of the uterus to ACh

Cumulative concentration-response curves obtained with ACh on the uterus were compared to those obtained where the organ was pretreated with indomethacin ($5 \times 10^{-6}\text{M}$ for 5 min) before pretreatment with the extract ($2 \times 10^{-3}\text{ g/ml}$ for 5 min) followed by cumulative dosing with ACh. Indomethacin inhibited the synergistic effect of the extract on the uterine response to ACh (fig. 34). Pretreatment with indomethacin on its own caused a slight shift of the foot of the curve to the right.

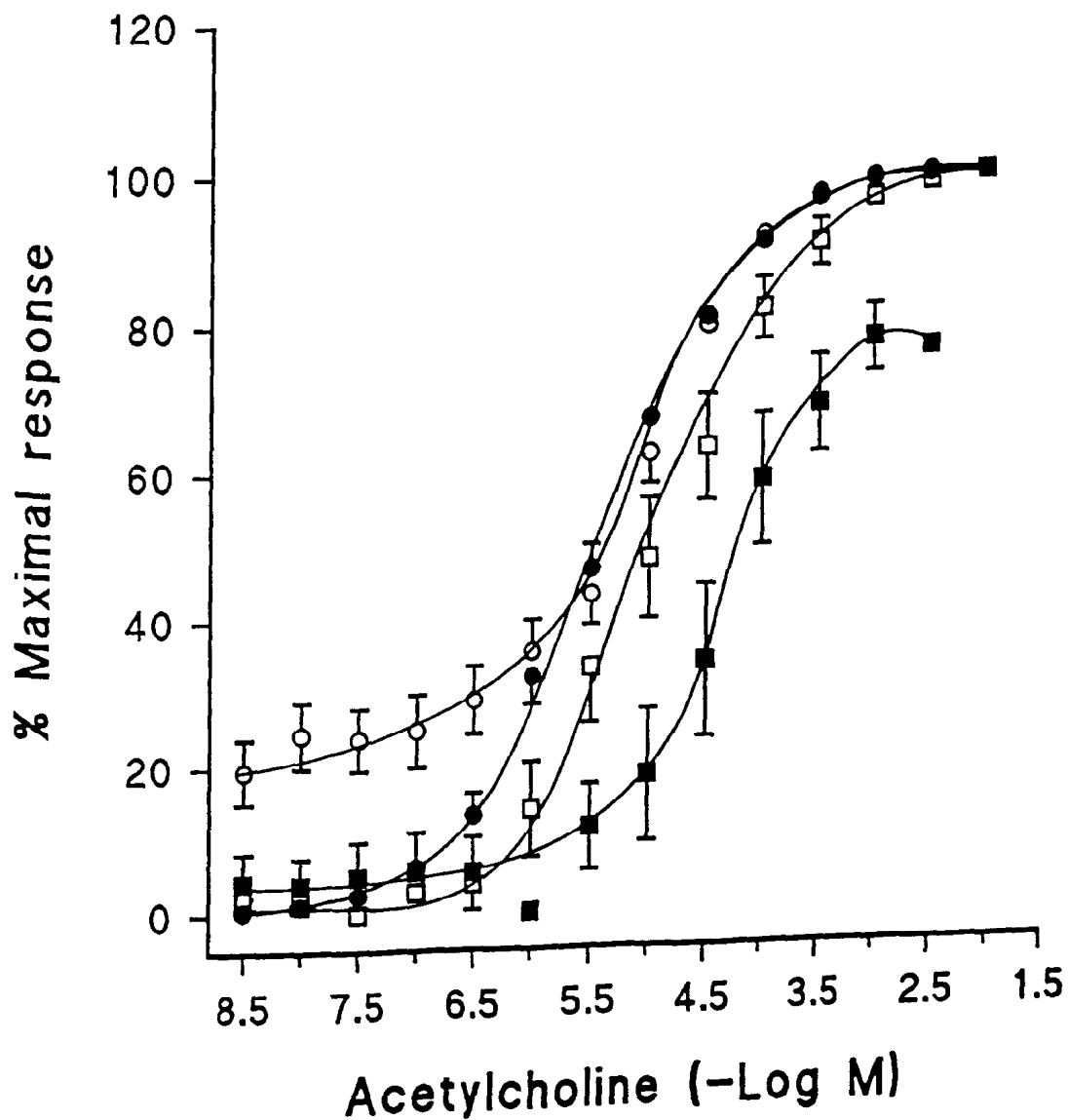


Fig. 33. Mean cumulative concentration-response curves for ACh on the isolated rat uterus in the absence (●) or in the presence (○) of *C. miniata* extract (2×10^{-3} g/ml), or in the presence of atropine (1×10^{-8} M) and extract (■) or atropine (1×10^{-9} M) and extract (□). Each point represents the mean of mean experimental values obtained with 6-18 rats and the vertical bars represent the S.E.M.

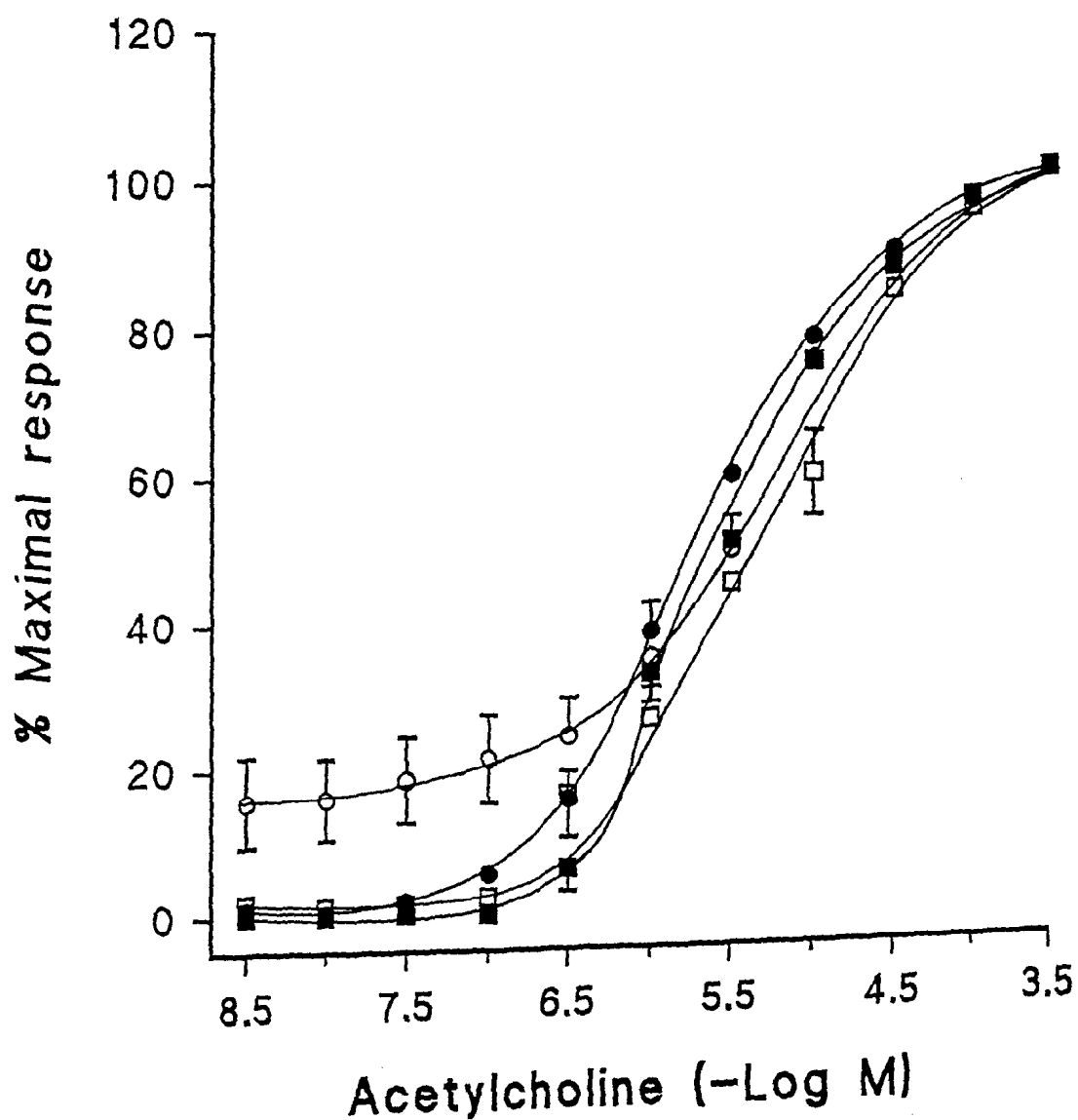


Fig. 34. Mean cumulative concentration-response curves for ACh on the isolated rat uterus in the absence (●) or in the presence of *C. miniata* extract (2×10^{-3} g/ml) (○) or in the presence of indomethacin (5×10^{-6} M) (■) or in the presence of indomethacin and extract (□). Each point represents the mean of mean experimental values obtained with 5-6 rats and the vertical bars represent the S.E.M.

5.3 Effects of pretreatment with Clivia alone or in combination with atropine or indomethacin on the uterine response to oxytocin

5.3.1 Effects of pretreatment with the extract on the response of the spontaneously and non-spontaneously contracting uterus to oxytocin

Cumulative concentration-response curves obtained with oxytocin on the spontaneously contracting and non-contracting uterus were compared to those where the uterus was pretreated with the extract (2×10^{-3} g/ml) for 5 min before cumulative additions of oxytocin.

Pretreatment with Clivia extract increased the frequency of contractions in the spontaneously contracting uterus at both threshold and subthreshold concentrations of oxytocin (Fig. 35). Clivia extract also significantly potentiated the response of the non-spontaneously contracting uterus to subthreshold and threshold concentrations of oxytocin (Fig. 36). The maximal response of the uterus to oxytocin was retained. Clivia therefore seemed to augment the initial uterine response to oxytocin by increasing both the tonicity and frequency of contractions.

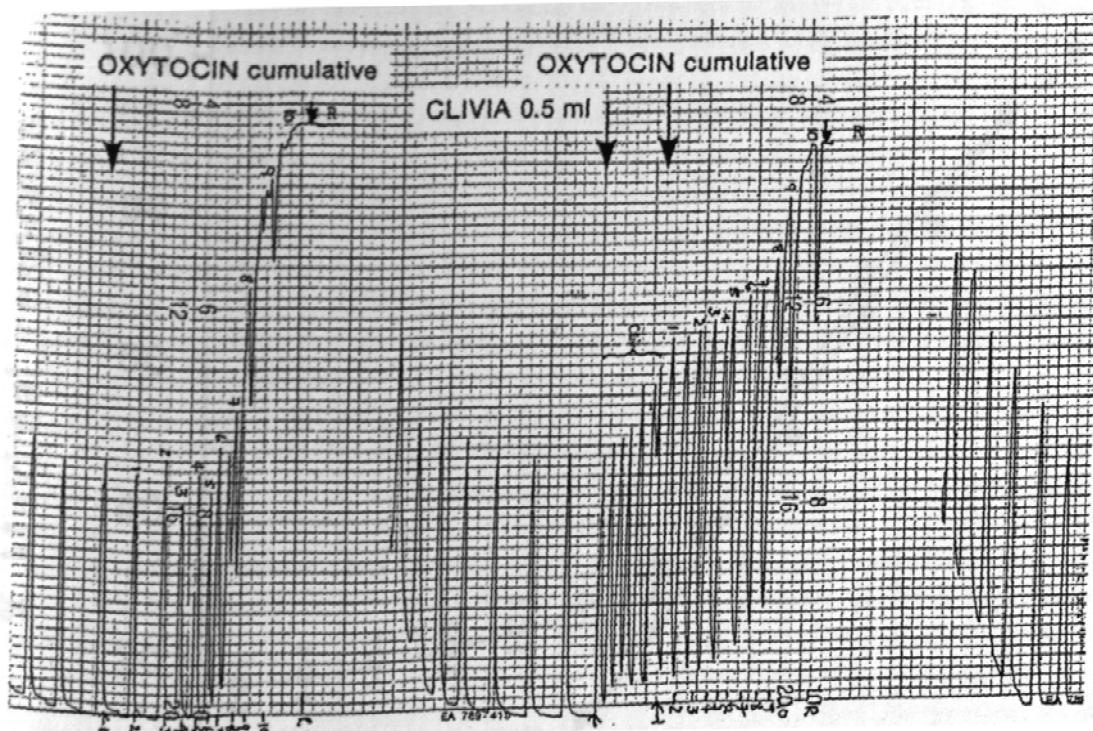


Fig. 35. Effects of Clivia extract on the response of the spontaneously contracting isolated rat uterus to cumulative dosage of oxytocin.

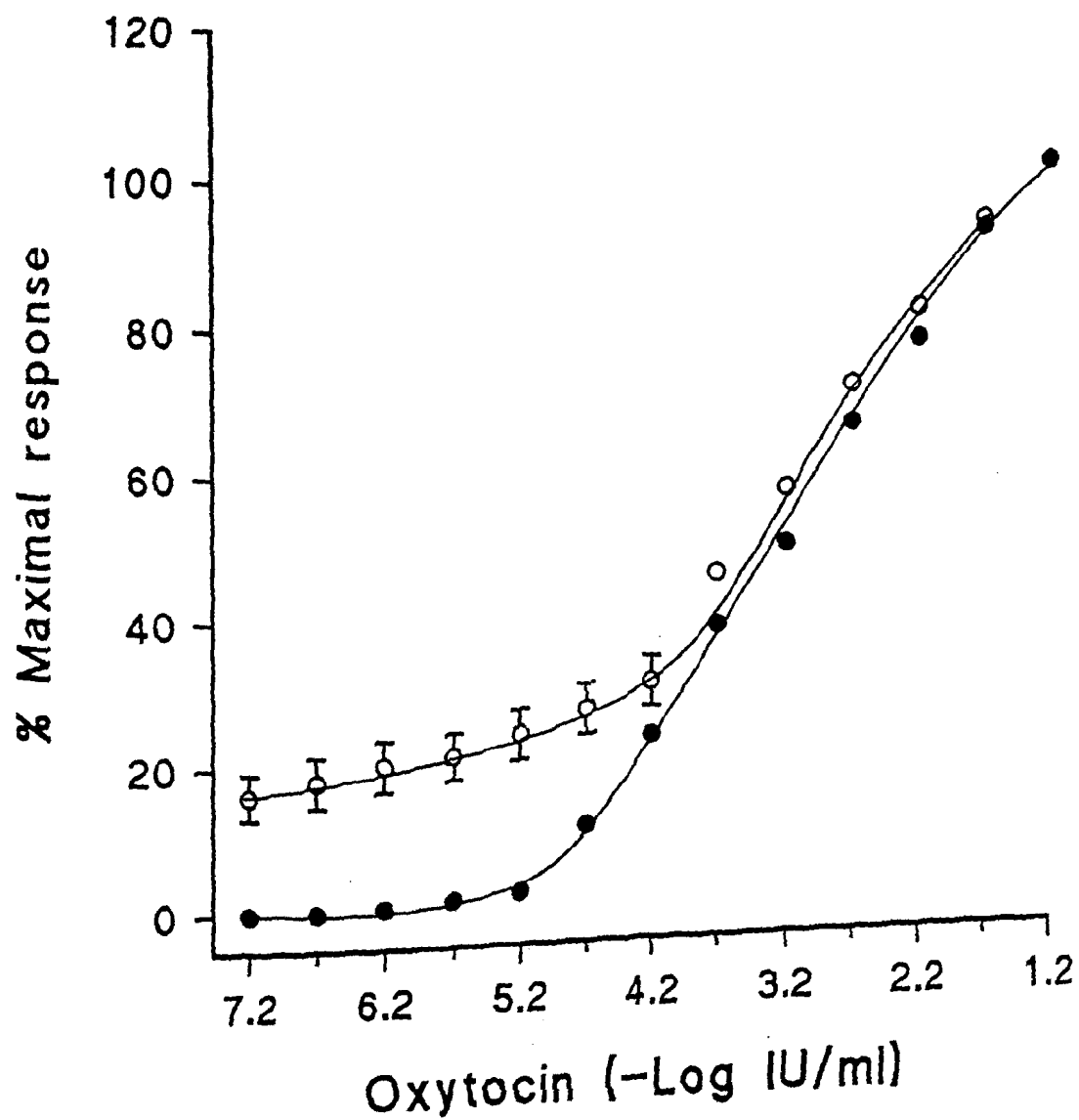


Fig. 36. Mean cumulative concentration-response curves for oxytocin on the isolated rat uterus (no spontaneous contractions) in the absence (●) or in the presence (○) of *Clivia* extract (2×10^{-3} g/ml). Each point represents the mean of mean experimental values obtained with 23 rats and the vertical bars represent the S.E.M.

5.3.2 Effects of pretreatment with atropine and Clivia on the uterine response to oxytocin

The curves obtained by cumulative dosing of the uterus with oxytocin were compared to those where the uterus was pretreated with atropine (3×10^{-9} M or 1×10^{-8} M) for 5 minutes before pretreatment with the extract followed by cumulative administration of oxytocin. Atropine (3×10^{-9} M) seemed to augment the initial effects of Clivia on the uterine response to oxytocin, whereas atropine (1×10^{-8} M) reduced the effects of Clivia (fig. 37). Pretreatment with atropine alone had no effect on the response of the uterus to oxytocin.

5.3.3 Effects of pretreatment with indomethacin and the extract on the response of the uterus to oxytocin

Cumulative concentration-response curves obtained with oxytocin on the uterus were compared to those where the uterus was pretreated with indomethacin (2×10^{-6} M or 5×10^{-6} M) for five minutes before pretreatment with Clivia followed by cumulative administration of oxytocin (fig. 38). Neither concentration of indomethacin reduced the potentiating effect of Clivia on the uterine response to oxytocin. Pretreatment with indomethacin (5×10^{-6} M) alone shifted the foot

of the curve slightly to the right.

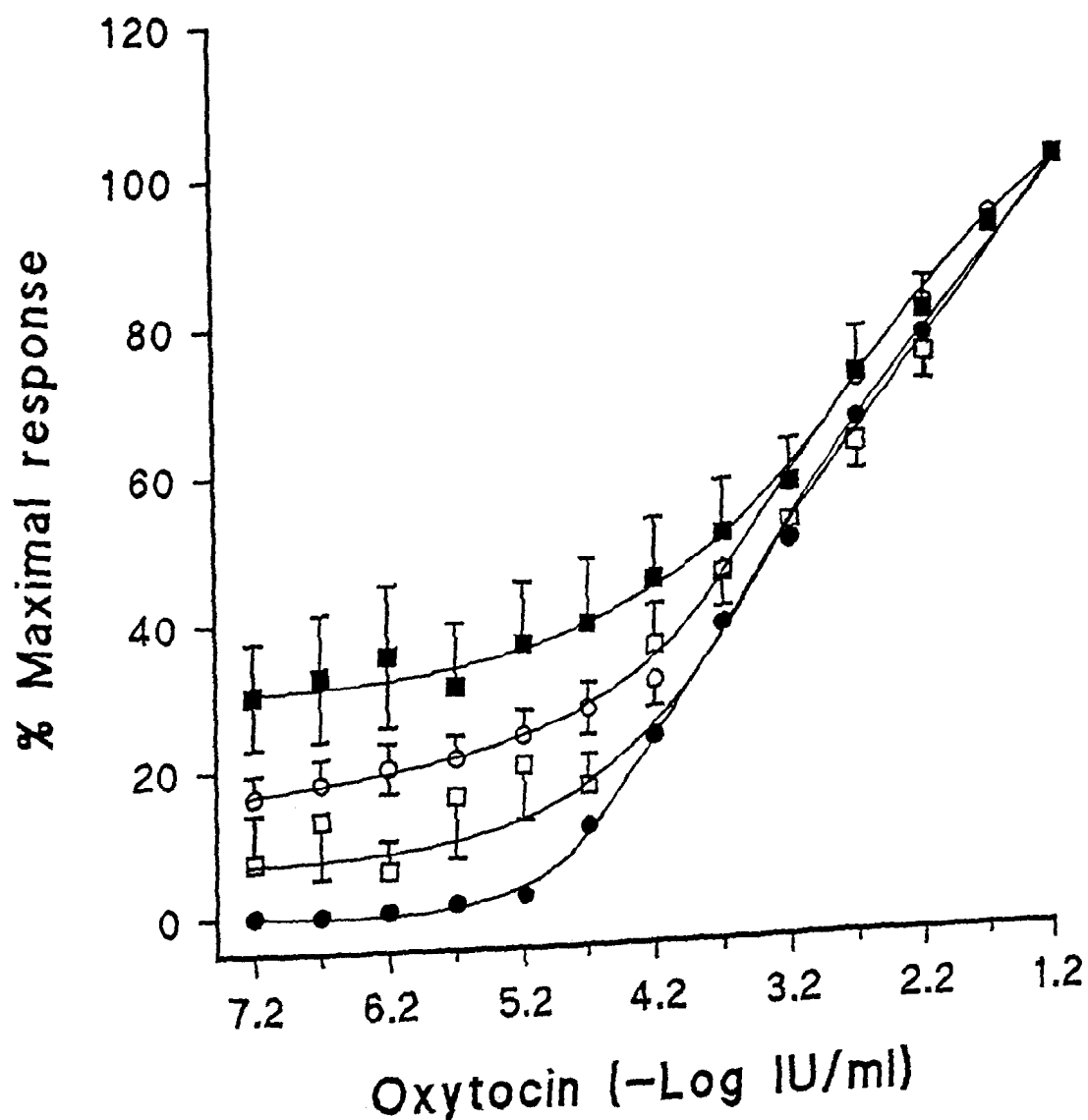


Fig. 37. Mean cumulative concentration-response curves for oxytocin on the isolated rat uterus in the absence (●) or (○) in the presence *C. miniata* extract (2×10^{-3} g/ml) or in the presence of atropine (3×10^{-9} M) and extract (■) or in the presence of atropine (1×10^{-8} M) and extract (□). Each point represents the mean of mean experimental values obtained with 5-23 rats and the vertical bars represent the S.E.M.

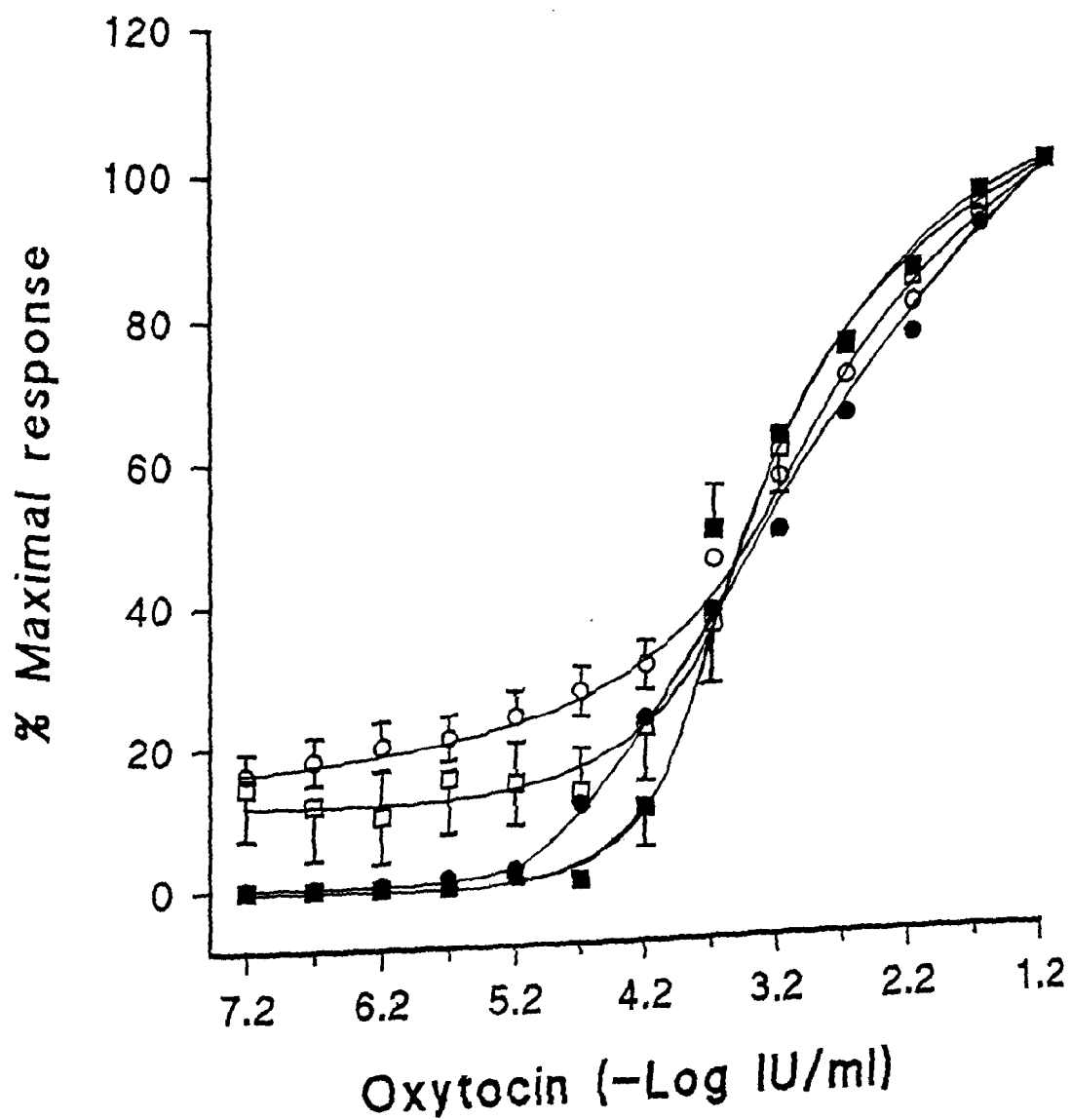


Fig. 38. Mean cumulative concentration-response curves for oxytocin on the isolated rat uterus in the absence (●) or (○) in the presence of *Clivia* extract (2×10^{-3} g/ml) or in the presence of indomethacin (5×10^{-6} M) and extract (□) or in the presence of indomethacin (5×10^{-6} M) (■). Each point represents the mean of mean experimental values obtained with 10 to 23 rats and the vertical bars represent the S.E.M.

5.4 Effects of pretreatment with Clivia on the uterine response to serotonin (5-HT).

Concentration-response curves obtained by cumulative dosing of 5-HT on the isolated rat uterus were compared to those where the uterus was pretreated with Clivia (2×10^{-3} g/ml or 2×10^{-4} g/ml for 5 min) or methysergide ($2,8 \times 10^{-9}$ M for 5 min) before cumulative addition of 5-HT. Pretreatment with the extract caused a parallel shift of the curves to the right whereas pretreatment with methysergide depressed the maximal response of the uterus to 5-HT (fig. 39). The initial synergistic effect of the extract obtained when combined with ACh or oxytocin was absent.

5.5 Effects of pretreatment with propranolol and Clivia on the response of the uterus to noradrenaline

Cumulative concentration-response curves obtained with noradrenaline on the rat uterus pretreated with propranolol ($1,35 \times 10^{-5}$ M) were compared to those where the uterus was pretreated with propranolol and the extract (2×10^{-3} g/ml) before cumulative addition of noradrenaline. The extract did not appear to interact significantly with alpha-adrenergic stimulation of uterine contractility or interfere with the beta-blocking effect of propranolol.

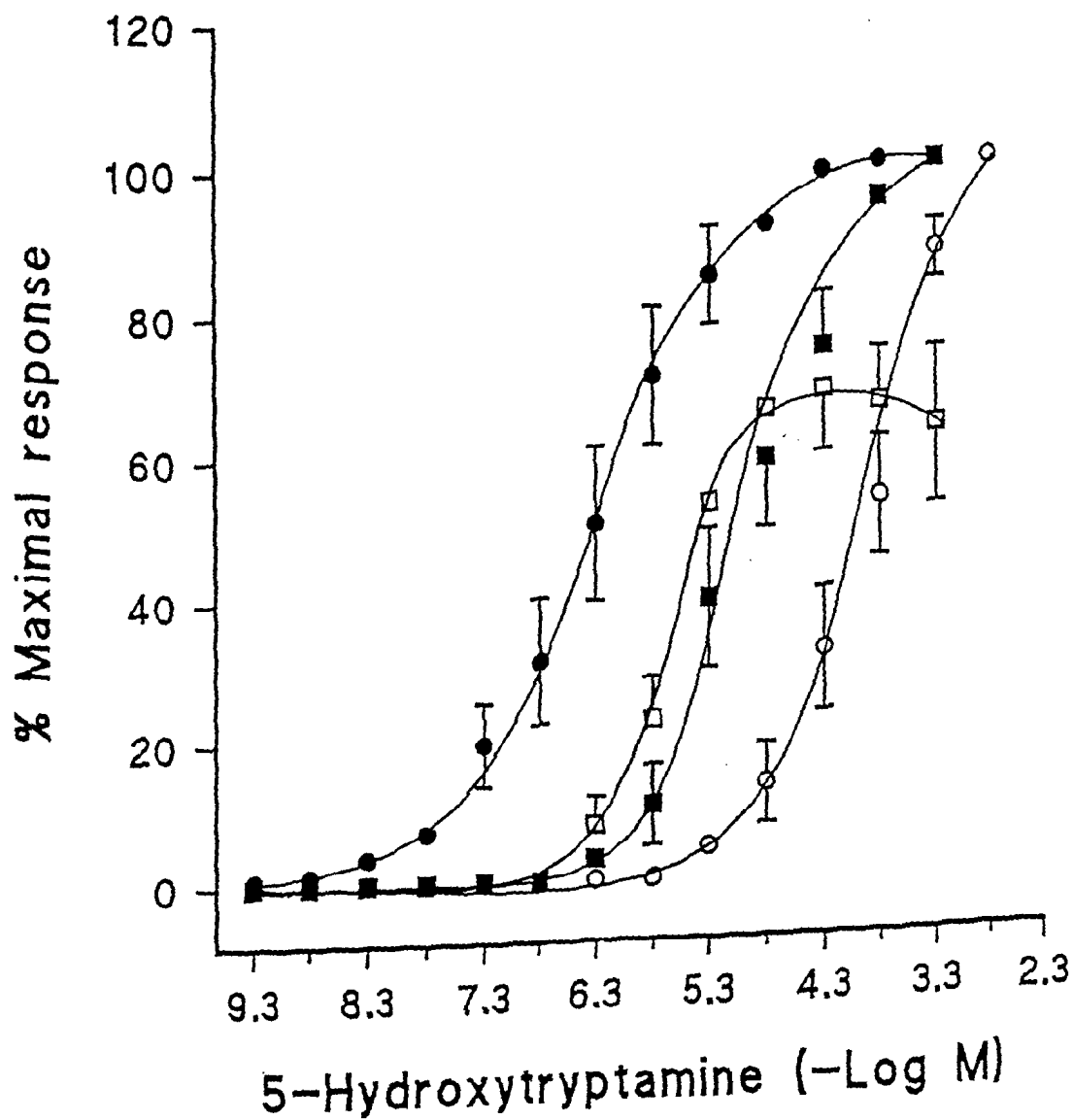


Fig. 39. Mean cumulative concentration-response curves for 5-HT on the isolated rat uterus in the absence (●) or in the presence of *C. miniata* extract (2×10^{-3} g/ml (○) or 2×10^{-4} g/ml (■) or methysergide (2.8×10^{-9} M) (□). Each point represents the mean of mean experimental values obtained with 3-7 rats and the vertical bars represent the S.E.M.

CHAPTER 6

Discussion

In this study the pharmacological effects of an aqueous extract of *Clivia miniata* leaves on isolated rat uterus and ileum have been examined in order to gain insight into its use by Black South African women, as an oxytocic agent.

To date five alkaloids have been isolated and identified in *Clivia* (Ali et al., 1983). One of the major constituents, lycorine, was found to possess antiviral properties (Ieven et al., 1982) and an aqueous extract of *Clivia* was antimicrobial (Ieven et al., 1979). No other pharmacological effects of *Clivia* have been described in the literature.

This study was performed using a crude aqueous extract which is the ethnic method of preparing the traditional medicine. The most concentrated solution that could be obtained from the lyophilized extract without precipitation occurring was 0,2 g/ml. The highest concentration of the extract that could be obtained by one dosage without compromising the prescribed ratio of final volume of test substance to volume of organ bath (1:20 according to Van Rossum (1963)) was 2×10^{-3} g/ml. This concentration corresponded roughly to the submaximal dosage described by Blattner et al. (1980) and was thus used as the pretreatment dosage in all subsequent experiments.

The extract exhibited direct smooth muscle stimulant activity on both the ileum and uterus (figs. 27 and 28 respectively).

The isolation of the pharmacologically active principles in the *Clivia* extract did not form part of the current study. The actual chemical composition and concentration of the active principle/s in the extract that produced maximal effects in the isolated organs could therefore not be determined using the crude extract and this fact was kept in mind when the results were evaluated.

Pharmacological effects of *Clivia* on the cholinergic system of the isolated rat ileum

A concentration of 2×10^{-3} g/ml of the aqueous extract of *Clivia* augmented the effects of subthreshold and threshold concentrations of ACh on the ileum. At higher concentrations of ACh, however, the extract inhibited the maximal response of the ileum to ACh in a manner that appeared not to involve competition with specific receptors (fig. 29).

Pretreatment of the ileum with atropine, the classic competitive antagonist of muscarinic receptors, blocked the initial synergistic effect of the extract (fig.

30). This indicated that the active principle/s in the **Clivia** extract possess/es activity on muscarinic receptors. At low concentrations of atropine, the maximal response to ACh remained depressed. At higher concentrations of atropine however, the depressant effect of **Clivia** on the maximal response of the ileum to ACh was also blocked and there was a parallel shift of the curves to the right.

The curves in figure 29 would be expected to be those of competitive synergism if one of the active components of the **Clivia** extract was simply a full agonist but this was not the case (compare fig. 5 and fig. 29). The curves in fig. 29 do not resemble those of competitive dualism either (see fig. 6) because, in the presence of the extract there was no parallel shift of the curves to the right at higher concentrations of ACh, but a depression of the curve. The extract appears not to act as a competitive antagonist on cholinergic receptors (fig. 29).

These results indicate the possibility of multiple receptor interactions. The fact that the extract may contain one or more active principles acting as agonists and/or antagonists could explain these results. A functional interaction of the **Clivia** extract on the ileum smooth muscle is therefore possible.

Clivia does not appear to promote prostaglandin synthesis in the gut as indomethacin did not inhibit the effects of the extract on the ileal response to ACh (fig. 31).

Pharmacological effects of Clivia on the cholinergic receptors in the isolated rat uterus

When the uterus was pretreated with 2×10^{-3} g/ml of the extract and then dosed cumulatively with ACh, the response to subthreshold and threshold doses of ACh was increased, in a similar manner to the effects of the extract on the ileum. **Clivia**, however, did not affect the maximal response of the uterus to ACh (fig. 32). From the appearance of the concentration-response curves in fig. 32, **Clivia** seems to affect the response of the uterus to ACh in a synergistic manner. Ariëns et al. (1964b) describe similar sets of curves as being either those of competitive synergism or functional synergism. Competitive synergism implies that **Clivia** has the same intrinsic activity as ACh, which could not be determined because of the chemical complexity of the extract. If **Clivia** contained only a single active component and was acting as a partial agonist on cholinergic receptors, the classic curves of competitive dualism (fig. 6) would have been expected, which was not the case (fig. 32).

Atropine inhibited the synergistic effects of the extract on the response of the uterus to ACh and shifted the curves to the right (fig. 33). At nanomolar concentrations of atropine, Clivia and ACh could overcome the atropine antagonism, but at higher concentrations of atropine (1×10^{-8} M), the antagonism was insurmountable. This indicates that the Clivia extract does not interact with the muscarinic receptors in the uterus alone but seems to act on more than one type of receptor. If the extract contained more than one active principle, they could each interact on a different receptor system in a functional type of interaction.

Pretreatment with indomethacin before pretreatment with Clivia followed by cumulative administration of ACh blocked the initial synergistic effect of the extract on the uterine response to ACh. This indicates that Clivia could be acting indirectly on the uterus by promoting prostaglandin synthesis.

Effects of Clivia on the oxytocic receptors in the isolated rat uterus

The concentration-response curves obtained when the effects of Clivia on the uterine response to oxytocin were examined were very similar to those obtained with

Atropine inhibited the synergistic effects of the extract on the response of the uterus to ACh and shifted the curves to the right (fig. 33). At nanomolar concentrations of atropine, *Clivia* and ACh could overcome the atropine antagonism, but at higher concentrations of atropine (1×10^{-8} M), the antagonism was insurmountable. This indicates that the *Clivia* extract does not interact with the muscarinic receptors in the uterus alone but seems to act on more than one type of receptor. If the extract contained more than one active principle, they could each interact on a different receptor system in a functional type of interaction.

Pretreatment with indomethacin before pretreatment with *Clivia* followed by cumulative administration of ACh blocked the initial synergistic effect of the extract on the uterine response to ACh. This indicates that *Clivia* could be acting indirectly on the uterus by promoting prostaglandin synthesis.

Effects of Clivia on the oxytocic receptors in the isolated rat uterus

The concentration-response curves obtained when the effects of *Clivia* on the uterine response to oxytocin were examined were very similar to those obtained with

Clivia and ACh (compare figs 32 and 36). Pretreatment with atropine alone had no effect on the concentration-response curves to oxytocin. However, when the uterus was pretreated with atropine and extract before the cumulative administration of oxytocin, there was no significant change in the initial augmentation by Clivia of the uterine response to oxytocin. In fact, the lower concentration of atropine ($3 \times 10^{-9}M$), seemed to increase the positive effects of the extract. There was no shift of the curves to the right (compare figs. 33 and 37). These results seem to indicate that the augmentation of the uterine response to oxytocin by Clivia is not entirely related to its activity on uterine muscarinic receptors and that this contribution is, in fact, minor. The interaction here seems to be one of functional synergism where Clivia is acting directly on multiple receptors or indirectly by promoting the release of other oxytocic agents such as the prostaglandins.

Pretreatment with indomethacin in order to test the effect of Clivia on prostaglandin synthesis in the presence of oxytocin (fig. 38) resulted in a negligible reduction in the effects of the extract as opposed to the effects of an indomethacin block on the response of the uterus to the extract and ACh (fig. 34). This indicates that although Clivia might promote prostaglandin synthesis in the uterus, this effect is

minor in its overall augmentation of the uterine response to oxytocin.

Effects of Clivia on serotonergic receptors in the uterus

Clivia was found to act as a competitive antagonist on 5-HT receptors in the uterus (fig. 39) causing a parallel shift of the curves to the right. This is unlike the non-competitive antagonism of methysergide (fig. 39) and similar to the competitive antagonism of ergometrine described by Ichida et al. (1983). Ergometrine is a well-known uterotonic agent which potentiates the effects of oxytocin, acting by an unknown mechanism. Ergometrine also causes increased synthesis of prostaglandins in the uterus (Hensby et al., 1985) as Clivia appears to do.

The synergistic effect of the extract as observed when it was combined with ACh or oxytocin was notably absent when administered with 5-HT. Although the ergot derivative methysergide is only a weak oxytocic (Douglas, 1985), a slight synergistic effect would also have been anticipated when the uterus was pretreated with methysergide before cumulative addition of 5-HT, but this did not occur. This correlates with results obtained by Gran (1973). Ichida et al (1983) did not

report an initial synergistic effect when they treated the isolated rat uterus with ergometrine and 5-HT. The absence of a synergistic effect could possibly be due to the desensitizing effect of prior administration of 5-HT as a control in these experiments as observed in the ileum by Huidboro-Toro and Foree (1980).

Although *Clivia* has a prominent effect on the serotonergic system, the contribution of the serotonergic system to the uterine response to oxytocin is negligible according to Ichida et al. (1983). It would have been interesting to calculate the pA_2 value of *Clivia* on 5-HT receptors, but as this study was performed on a crude extract of unknown composition, this was not possible.

The profound competitive antagonism of 5-HT receptors by *Clivia* could be of interest as 5-HT receptors are currently being related to the pathophysiology of migraine and antagonism of 5-HT receptors has beneficial effects in the treatment of migraine. Peatfield (1988) states: "studies of the clinical effectiveness of agents with clearly defined interactions with 5-HT receptors are likely to be a particularly profitable avenue in our understanding of migraine".

The fungus, ergot, contains alkaloids which are such potent uterine stimulants that they cannot be used safely to induce or facilitate labour (Rall and Schleifer, 1985). The uterine contractions they induce can tend to compress rather than expel the uterine contents (Bowman and Rand, 1980). The intensity and duration of their effects are so great that the foetus may be injured and rupture of the uterus may also occur (Petrie and Danilo, 1984). Ergometrine, the water soluble uterotonic principle of ergot, is therefore used clinically only in the third phase of labour where the sustained uterine contractions it causes helps to prevent post partum haemorrhage (Rall et al., 1985). The results of this study indicate that the pharmacological activity of *Clivia* on the uterus is similar to that of ergometrine. *Clivia* could contain active principles which resemble those contained in ergot with similar potential toxicity. Further investigation and isolation of the active principles contained in *Clivia* leaves should be undertaken in order to predict the potential toxicity of the ethnic use of this plant to induce or augment labour.

CHAPTER 7

Conclusion

In conclusion therefore, it seems that the ethnic use of an infusion of *Clivia miniata* leaves as an oxytocic may be based on a direct stimulant effect on the tonicity and frequency of uterine contractions and its ability to augment the effects of threshold concentrations of oxytocin. The extract appears to interact with more than one receptor system in both the ileum and the uterus, one of which appears to be the muscarinic cholinergic receptor. A functional model is proposed for the pharmacological activity of *Clivia*. The inhibition by indomethacin of *Clivia*'s enhancement of the initial contractile response of the uterus to ACh seems to indicate that the extract may also act indirectly by stimulating prostaglandin synthesis. *Clivia* acts as a competitive antagonist on serotonergic receptors in the uterus in a similar manner to the uterotonic agent, ergometrine.

Although the observations made in this study cannot be directly transposed to a human clinical condition, they are significant. They indicate a similarity between the pharmacological effects of *Clivia* and ergometrine on the uterus and therefore the possibility exists that large volumes of *Clivia* infusion used to induce or augment labour could cause toxic uterine hypertonia.

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