TABLE 14. PLASMA PROLACTIN RESPONSES TO EXERCISE

	Basal	Immediately post-exercise	5 minutes post-exercise	20 minutes post-exercise
Normal subjects (n=12)	7.0(1.5)	10.7(4.1)⁄	11.7(5.0)⁄	10.4(4.1)⁄
Asthmatic patients without premedication (n=10)	6.6(1.5)	7.8(2.2)	8.3(3.0)	7.7(3.4)
Asthmatic patients with promedication (n=10)	6.1(1.8)	7.9(2.5)	8.0(2.9)	7.0(2.2)

Values are mean(SD) ng/ml

⁄ Value significantly different from basal (p = <0.05)

lower immediately after the completion of exercise in the asthmatic patients with premedication than in the normal subjects ($p = <0.006$). When the plasma ACTH responses were expressed as ratios (value:basal) in order to correct for the relatively large inter-group differences between the basal values, the ratio was lower immediately after exercise in the asthmatic patients with premedication than in the normal subjects. This difference approached statistical significance ($p = <0.05$). The plasma ACTH responses are shown in Table 15.

The plasma cortisol level varied significantly in association with exercise in the normal subjects ($p = <0.002$), and in the asthmatic patients under both study conditions (with premedication $p = <0.001$; without premedication $p = <0.05$). The plasma cortisol level fell significantly from the basal value both immediately and 5 minutes after exercise in the asthmatic patients with premedication ($p = <0.05$). The plasma cortisol level was lower 20 minutes after exercise in the asthmatic patients with premedication than in the normal subjects. This difference approached significance ($p = <0.05$). When the plasma cortisol responses were expressed as ratios (value:basal) in order to correct for the relatively large inter-group differences in the basal values, the plasma cortisol response was smaller at all times after exercise in the asthmatic patients with premedication than in the normal subjects (immediately after exercise $p = <0.02$; 5 minutes after exercise $p = <0.006$; 10 minutes after exercise $p = <0.02$). In the asthmatics without premedication the cortisol ratio w s lower than in the normal

TABLE 15. PLASMA ACTH RESPONSES TO EXERCISE

	Basal	Immediately post-exercise	5 minutes post-exercise	20 minutes post-exercise
Normal subjects (n = 12)	124.3(88.7)	208.2(204.8)**	129.6(88.9)	94.7(71.3)
Asthmatic patients without premedication (n = 10)	80.0(15	116.5(32.6)+	108.5(48.5)	102.4(38.3)
Asthmatic patients with premedication (n = 10)	84.8(24.4)	85.0(24.5)**	145.9(123.7)	83.9(25.8)

Values are mean(SD) pg/ml

+ Value significantly different from basal (p = <0.05)

** Normal subjects vs asthmatic patients with premedication significantly different (p = <0.01)

subjects 5 minutes after exercise. This difference approached significance ($p = <0.05$). The plasma cortisol responses are shown in Tables 16 and 17.

Pairwise comparison of the two sets of data from the asthmatic patients showed that the plasma growth hormone responses were somewhat greater when they had significant bronchospasm in association with exercie, having received no premedication. This was only significant immediately after exercise ($p = 0.01$). There was no difference in any other variable at any time.

9.3.4 Discussion

The present study provided no significant information regarding possible abnormalities of the alpha-adrenergic responses in asthma. It is possible that the greater plasma growth hormone response in the patients without premedication may have been related to the stress associated with exercise-induced bronchospasm.

An interesting finding in the present study was that of an apparently diminished plasma cortisol response to exercise in the asthmatic patients when they had received premedication which was effective in preventing the occurrence of significant bronchospasm. In addition, although the differences were not statistically significant, the plasma cortisol responses of the asthmatic patients without premedication also appeared to be somewhat smaller than those of the normal subjects.

Plasma cortisol levels usually exhibit a significant

TABLE 16. PLASMA CORTISOL RESPONSES TO EXERCISE

	Basal	Immediately post-exercise	5 minutes post-exercise	20 minutes post-exercise
Normal subjects (n = 12)	80.1(16.9)	79.7(10.5)	96.7(24.7)	119.6(31.2)≠**
Asthmatic patients without premedication (n = 10)	95.9(29.0)	82.5(29.0)	86.0(29.3)	106.5(33.6)
Asthmatic patients with premedication (n = 10)	98.8(24.8)	79.0(20.2)≠	76.7(21.3)≠	90.2(26.1)**

Values are mean(SD) ng/ml

≠ Value significantly different from basal ($p = <0.05$)

** Normal subjects vs asthmatic patients with premedication ($p = <0.05$)

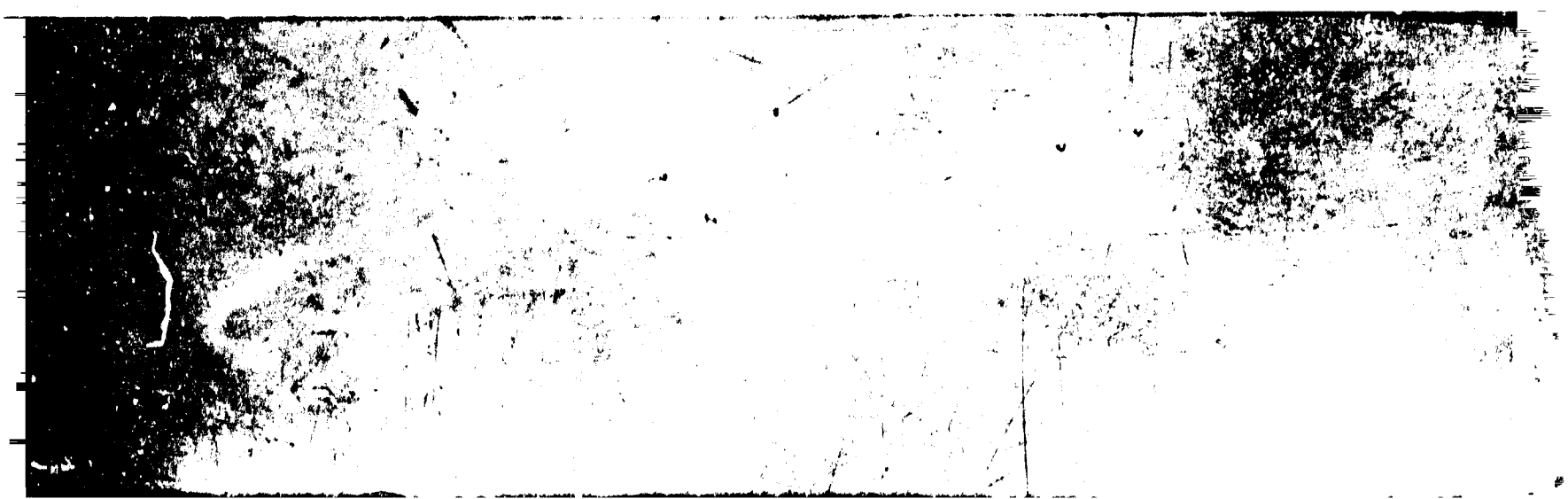


TABLE 17. PLASMA CORTISOL RESPONSES TO EXERCISE
(Expressed as ratios value:basal)

	Immediately post-exercise	5 minutes post-exercise	20 minutes post-exercise
Normal subjects (n = 12)	1.04(0.26)*	1.32(0.71)**	1.62(0.75)*
Asthmatic patients without premedication (n = 10)	0.86(0.15)	0.90(0.20)***	1.1 (0.38)
Asthmatic patients with premedication (n = 10)	0.81(0.11)	0.78(0.12)	0.94(0.29)

Values are mean(SD)

Normal subjects vs asthmatic patients with premedication
* p = <0.02
** p = <0.006

*** Normal subjects vs asthmatic patients without premedication p = <0.05

response to exercise, with peak levels often occurring in the post-exercise period (Brandenberger et al. 1984). The response appears to be enhanced by opioid (Grossman et al. 1984) and beta-adrenergic receptor blockers (Jezova et al. Adrenopituitary hormone response to exercise combined with propranolol infusion in man. Endocrinologia experimentalis 17, 91-7, 1983; cited in Galbo, 1986).

The present findings clearly require analysis in the light of the use by the asthmatic patients of corticosteroid medications. As only one of them had ever been treated with systemic corticosteroids - for a short period some years prior to the study - it is only the possible effect of beclomethasone dipropionate on adrenal cortical responses that is of direct relevance. Four of the 10 asthmatic patients studied had been using beclomethasone dipropionate aerosol (maximum dose 400 /ug/day) until 24 hours prior to the study. The data regarding the effect of beclomethasone dipropionate on hypothalamic-pituitary-adrenal function will be reviewed in detail in Chapter 11. It is sufficient to note here that while it has been reported that children using relatively large doses (300-800/ug/day) may have diminished responses to insulin-induced hypoglycaemia (Vaz et al. 1982) and metyrapone (Wyatt et al. 1978), there is no convincing evidence that hypothalamic-pituitary-adrenal suppression occurs in adults using doses of up to 400/ug/day. There is also ample documentation of adequate functional reserve, as assessed by the responses to both ACTH and metyrapone in both adults and children (Godfrey and König, 1974; British Thoracic

and Tuberculosis Association, 1975; Francis, 1976; Klein et al. 1977; Yernault et al. 1977; Sherman et al. 1982; Goldstein and König, 1983; Smith and Hodson, 1983; Williams et al. 1984), even in patients using 1000-2000 ug/day (Costello and Clark, 1974; Vlasses et al. 1981; Ebden et al. 1986). These data, together with the fact that the minority of the patients were actually using beclomethasone dipropionate, militate against the present finding being the result of this therapy.

A number of factors which may possibly affect the plasma cortisol response to exercise, could not have been responsible for the disparity in the magnitudes of the responses in the present study. These include the relationship of the exercise to a meal (Brandenberger et al. 1982), and chronic training, which has been shown to diminish the adrenal cortical response to exercise in animals (Tharp and Buuck, 1974) as well as in asthmatic children (Leisti et al. 1979).

Previous studies of the plasma cortisol response to exercise in asthmatic patients have yielded markedly conflicting results. It has been reported that plasma corticosteroid levels fall significantly in association with exercise in asthmatic patients and, to a lesser extent, normal subjects, after premedication with disodium cromoglycate while remaining unchanged in the absence of premedication (Holmes et al. 1972). It has been suggested that this may be related to increased tissue uptake of glucocorticoid hormones mediated in some way by the substance (Holmes et al. 1972). Plasma glucocorticoid levels have also been reported to be unchanged in association with exercise regardless

of the occurrence of bronchospasm (Sly et al. 1973), and to increase more in asthmatic patients than in normal subjects despite the use of systemic corticosteroid therapy, possibly in relation to the stress of bronchospasm (Jaffe et al. 1973). A possible source of error with these studies may, however, have been the relatively inaccurate methods of plasma corticosteroid determination used.

It has been observed that asthmatic children have a negligible cortisol response to the stress of severely symptomatic asthma (Kumar et al. 1971). It would be tempting to attribute the diminished response observed in the asthmatic patients in the present study to a depression in adrenal cortical, or possibly even hypothalamic-pituitary responsiveness associated, in some way, with the disease. However, the glucocorticoid response to exercise is extremely complex, being critically dependent on the work load (Tharp, 1975; Farrell et al. 1983; Galbo, 1986) and possibly on such factors as the accumulation of lactic acid (Farrell et al. 1983) and the clearance of glucocorticoid hormones from the circulation (Galbo, 1986). The latter does not appear to differ in asthmatic patients and normal subjects (Weller et al. 1968; Boye et al. 1974). Furthermore, the intensity of the exercise, at least as assessed by the cardiovascular responses, appeared to be similar in the different groups of subjects in the present study. Nonetheless, it is felt that further studies of the different components of the hormonal response to exercise in asthma, and confirmation of the present findings are necessary before the latter can be

considered in relation to the pathogenesis of the disease.

9.4 A STUDY OF NOCTURNAL GROWTH HORMONE SECRETION

The secretion of growth hormone appears to occur predominantly during the night, with major secretory bursts occurring approximately 70 minutes after the onset of sleep (Takahashi et al. 1968). Although the mechanism of sleep-induced growth hormone secretion has not been clearly defined, it is unaffected by either phentolamine or propranolol, and does not, therefore, appear to be directly mediated by adrenergic mechanisms (Lucke and Glick, 1971). However, as part of the investigation into nocturnal hormonal and autonomic variables (Chapter 11), the nocturnal secretion of growth hormone was studied.

9.4.1 Subjects

Thirteen asthmatic patients and 11 normal subjects were studied. Each of the asthmatic patients was being treated with a sympathomimetic bronchodilator aerosol, and 10 were, in addition, using beclomethasone dipropionate aerosol (200-500/ug/day). Five had been treated with systemic corticosteroids - 4 for only short periods - at least 2 years prior to the study. All bronchodilator therapy was discontinued at least a week prior to the study. Beclomethasone dipropionate was discontinued on the morning of the study.

9.4.2 Methods

The subjects were studied individually in the recumbent position in a quiet room to which they were admitted at approximately noon. A 19-gauge or larger cannula was immediately inserted into an arm vein and kept patent with a saline infusion. Each subject received a standardised light meal at 18h00, and water was the only oral fluid permitted. Blood was sampled through the cannula at 16h00, 16h30, midnight and 08h00.

The blood was placed into iced tubes and immediately centrifuged and the separated plasma stored at -20°C until assayed.

Biochemical Analysis:

See Appendix "A".

Statistical Methods:

Pairwise comparisons were done using the two-tailed Mann-Whitney U test.

The basal level was estimated by mixing equal aliquots of the 16h00 and 16h30 specimens, and obtaining an integrated basal value.

9.4.3 Results

Demographic and pulmonary function data are shown in Table 25 (Chapter 11). The asthmatics had a significantly lower forced expiratory volume in one second (FEV_1) and a significantly greater airway resistance and fall in peak expiratory flow rate (PEFR) on exercise than the normal subjects.

Plasma growth hormone levels are shown in Table 18. Although basal levels in the 2 groups were similar, the asthmatic patients had lower plasma growth hormone levels at all other times during the study. These differences at no time, however, attained statistical significance. When the results were expressed as ratios (value:basal), there was still no difference between the asthmatic patients and the normal subjects.

9.4.4 Discussion

Although the differences were not statistically significant, the present study suggests the possibility that asthmatic patients may have lower nocturnal plasma growth hormone levels than normal subjects. Asthmatic children using beclomethasone dipropionate in relatively large doses (300-1300/ug/day) have recently been shown to have low nocturnal plasma cortisol levels, the finding being attributed to hypothalamic-pituitary-adrenal suppression by the agent (Law et al. 1986). It has been reported that children using 200-400/ug/day, and adults using 400-500/ug/day show no change in the secretion of growth hormone (Wilken-Jensen, 1975). However, the possibility that beclomethasone dipropionate may have a depressant effect on the nocturnal secretion of growth hormone requires further systematic investigation.

9.5 CONCLUSIONS

No significant difference could be detected between the asthmatic patients and the control subjects in any of the plasma

TABLE 18. NOCTURNAL PLASMA GROWTH HORMONE LEVELS

	Basal	Midnight	04h00	0800
Normal Subjects (n=11)	6.6(6.7)	9.1(9.6)	7.3(9.0)	8.0(7.9)
Asthmatic Patients (n=13)	7.1(6.5)	7.5(8.1)	1.8(0.9)	3.7(3.3)

Values are mean(SD) ng/ml

growth hormone responses studied. The suggestion of the presence among the asthmatic patients of a subgroup of "hyperresponders" (Chapter 9.2) was not subsequently confirmed, and both "hyperresponders" and "hyporesponders" were found among both the asthmatic patients and the control subjects in each of the different studies. The occurrence of alpha-adrenergic overactivity in asthma could not, therefore, be proved.

Growth hormone is secreted in a pulsatile manner and individual responses to stimuli are, as a result, extremely difficult to evaluate (Rose, 1985). The choice of the growth hormone responses as a means of assessing the alpha-adrenergic system was not, therefore, a good one. However, as has been previously explained, the present investigations were undertaken because it was not possible to study the haemodynamic and metabolic responses to a potent intravenously administered alpha-1 agonist such as phenylephrine.

CHAPTER 10

A STUDY OF BETA-ADRENERGIC RESPONSES

10.1 THE THEORY OF BETA-ADRENERGIC SUBSENSITIVITY IN ASTHMA

Zaid and Beall demonstrated in 1966 that the intravenous administration of propranolol to asthmatic patients resulted in a diminution in airway calibre. There was, in addition, a marked increase in the subsequent bronchoconstrictor response to methacholine, suggesting the induction of a significantly greater degree of bronchial hyperreactivity than had previously been present. The bronchoconstrictor effect of beta-adrenergic blockade in asthmatic patients was confirmed by others (MacDonald et al. 1967; Grieco and Pierson, 1971).

Subsequently, Szentivanyi (1968) presented his "beta-adrenergic theory of the atopic abnormality in bronchial asthma" in which he suggested that beta-adrenergic subsensitivity was the principal underlying pathogenetic mechanism in asthma. His theory was developed from the observations made in two experimental animal models:

(i) Guinea-pigs with hypothalamic "imbalance":

This model was based on the concept that the posterior hypothalamus mediates sympathetic responses and the anterior hypothalamus parasympathetic responses. It was noted that electrical stimulation of one of these areas, or ablation of the other, profoundly altered the anaphylactic reactivity of the animals to both immunological and pharmacological stimuli.

(ii) Mice and rats inoculated with B. pertussis:

It was demonstrated that inoculation with B. pertussis induced a state of increased sensitivity to histamine, serotonin,

bradykinin and acetylcholine, as well as to cold and various respiratory irritants, and also stimulated marked peripheral blood eosinophilia. These changes were associated, moreover, with a reduction in the sensitivities of the animals to various catecholamine-stimulated reactions (Szentivanyi, 1968).

10.1.1 A Review of the Experimental Data Linking Beta-Adrenergic Subsensitivity to the Pathogenesis of Bronchial Asthma

The data from numerous studies provided evidence in support of the existence of a state of beta-adrenergic subsensitivity in asthmatic patients, some suggesting, moreover, that it correlated in degree with the severity of the disease. The hyperglycaemic response to catecholamines was shown to be inversely proportional to the degree of severity of the disease in asthmatic children (Inoue, 1967), as well as being smaller in asthmatic patients than normal subjects (Lockey et al. 1967; Middleton and Finke, 1968). The responses in the levels of lactate and pyruvate were also diminished in asthmatic patients (Middleton and Finke, 1968). In a group of patients with asthma of a varying degree of severity, the magnitude of the rise in the levels of blood glucose and lactate, and of the fall in the peripheral eosinophil count induced by intravenous epinephrine appeared to be directly related to the dose of inhaled acetylcholine required to produce significant bronchoconstriction (Makino et al. 1970). It was also demonstrated that epinephrine produced a significant increase in the urinary levels of 3,5 cyclic AMP in normal subjects but not asthmatic patients, while glucagon produced a comparable increase

in the urinary levels of the nucleotide in both groups (Bernstein et al. 1972).

An in vitro study of peripheral blood leukocytes showed that the adenyl cyclase response to isoproterenol was significantly diminished in cells from asthmatic compared with non-asthmatic individuals (Logsdon et al. 1972). Moreover, in two sets of monozygotic twins, each with one asthmatic member, the asthmatic patients had lower basal and isoproterenol-stimulated leukocyte adenyl cyclase activity than their twins (Falliers et al. 1971).

A significant shortcoming of most of these earlier studies was their failure to take into account the effect of sympathomimetic therapy on the adrenergic responses studied (see Chapter 6.1), and data were soon obtained suggesting that the reported manifestations of beta-adrenergic subsensitivity were related to the use in the asthmatic patients of adrenergic bronchodilators, rather than to any primary abnormality of the beta-receptor mechanism. The lymphocyte 3,5 cyclic AMP responses to isoproterenol were shown to be significantly diminished only in those asthmatic patients being treated with sympathomimetic therapy and to become normal following the withdrawal of sympathomimetic bronchodilators. Significant depression of the responses of leukocytes from normal obstetric patients were evident, moreover, following only 48 hours of therapy with the adrenergic-agonist, isoxsuprine (Conolly and Greenacre, 1976).

Studies of beta-receptor numbers showed that therapy with adrenergic agonists produced a diminution in the number of beta-adrenergic receptors in the leukocytes of both normal and

asthmatic subjects (Galant et al. 1978; Scarpace et al. 1982), which was reversible after the discontinuation of the agent (Galant et al. 1978). There was, moreover, no difference between asthmatic patients not receiving sympathomimetic therapy and normal subjects in the density of beta-adrenergic receptors, the receptor affinity for isoproterenol, the adenyl cyclase responses or the net generation of 3,5 cyclic AMP (Galant et al. 1980a; Galant et al. 1980b).

The depressant effect of sympathomimetic therapy on the adrenergic responses of normal subjects was confirmed by the finding that the haemodynamic responses as well as those in the plasma levels of glucose, lactate and free fatty acids to intravenous epinephrine, were significantly depressed by the prior administration of ephedrine for one week (Nelson, 1973; Nelson et al. 1975). Similarly, the bronchodilator effect of beta-adrenergic agonists appeared to be rapidly attenuated by chronic therapy (Nelson et al. 1977).

Despite these contradictory data, evidence, providing some support for Szentivanyi's theory has continued to accumulate. Thus, while the state of beta-adrenergic subsensitivity induced by the administration of beta-2 agonists appears to last for 7-10 days after they have been discontinued (Holgate et al. 1977; Galant et al. 1978), a number of studies have documented the presence of attenuated beta-adrenergic responses in asthmatic patients, despite the cessation of all sympathomimetic therapy, for periods often considerably in excess of this. Asthmatic patients who have not used any adrenergic therapy for at least

one month have significantly smaller heart rate responses (Larsson, 1977) to beta-adrenergic stimulation than normal subjects, and require larger doses to elicit given changes in pulse pressure (Shelhamer et al. 1980b; Davis et al. 1986). Asthmatic patients have diminished stress-induced increments in the excretion of urinary catecholamines despite the discontinuation of all bronchodilator therapy for 3 weeks (Ramsey, 1977), and patients with exercise-induced bronchospasm have diminished diastolic blood pressure responses to isoproterenol than either normal subjects or those without significant exercise-induced bronchospasm, despite being off all bronchodilator therapy for a week (Martinsson et al. 1985).

Similar results have been documented in a number of in vitro cell studies. The leukocytes from asthmatic patients have significantly depressed 3,5 cyclic AMP responses to various adrenergic agonists compared with those of normal subjects (Parker and Smith, 1973; Shelhamer et al. 1980b), and similarly depressed reponses cannot be reproduced in normal subjects by the administration of an adrenergic agonist (Parker and Smith, 1973). In a study of the inhibitory effect of isoproterenol on the zymosan-stimulated release of lysosomal enzymes from polymorphonuclear leukocytes, this reaction appears to be markedly diminished in the cells from asthmatic patients, including those off all bronchodilator therapy for at 2 weeks (Busse, 1977).

Studies of beta-receptor numbers suggest that these are diminished in the mononuclear cells from asthmatic patients

compared with those from normal subjects, this being equally apparent in those patients receiving no adrenergic bronchodilators (Kariman and Lefkowitz, 1977; Sano et al. 1983). By contrast, the cells from patients with "inactive asthma" show no diminution in the number of receptors (Kariman and Lefkowitz, 1977). It has been suggested that the degree of diminution in the number of beta-receptors in lymphocytes from asthmatic patients correlates with the severity of airway obstruction (Brooks et al. 1979). The finding of a diminished number of beta-receptors in the leukocytes of asthmatic patients has not been a consistent finding, however (Davis et al. 1986).

The presence of auto-antibodies to beta-2 adrenergic receptors has been recently documented in a small number of patients with asthma (Fraser et al. 1981), and it has been suggested, moreover, that approximately 5% of a large sample of sera from asthmatic patients contain blocking auto-antibodies to smooth muscle beta-2 receptors (Blecher et al. 1984).

In the light of all this conflicting evidence, the evaluation of Szentivanyi's theory is extremely difficult. However, despite the large body of evidence which has been presented in support of the existence of a state of beta-adrenergic subsensitivity as a primary phenomenon in asthma, there are a number of problems regarding the acceptance of this as the sole pathogenetic mechanism underlying bronchial hyperreactivity.

(i) Beta-adrenergic subsensitivity does not appear to be specific for bronchial asthma:

In vivo and in vitro manifestations of beta-adrenergic subsensitivity have been demonstrated in atopic non-asthmatic individuals (Fireman et al. 1970; Busse and Lee, 1976; Reed et al. 1976; Szentivanyi et al. 1980; Shelhamer et al. 1980b; Davis et al. 1986), as well as in patients with irreversible airway obstruction (Lemanske et al. 1980) and cystic fibrosis and healthy heterozygote carriers of the cystic fibrosis gene (Davis et al. 1978; Davis and Kaliner, 1983; Davis, 1984). These findings do not appear to be related to sympathomimetic therapy. Similarly, autoantibodies to beta-2 receptors have been found to be present in atopic non-asthmatic individuals, patients with cystic fibrosis and completely normal subjects (Fraser et al. 1981).

(ii) Manifestations of different autonomic abnormalities appear to co-exist in individuals with asthma as well as other diseases:

Manifestations of both alpha-adrenergic and cholinergic hypersensitivity appear to co-exist with those of beta-adrenergic subsensitivity in patients with asthma, non-asthmatic atopic individuals, patients with cystic fibrosis and healthy cystic fibrosis heterozygotes (Kaliner et al. 1982; Davis and Kaliner, 1983; Davis, 1984). This suggests that while manifestations of beta-adrenergic subsensitivity undoubtedly occur in asthma, they may be part of a broader spectrum of unexplained autonomic abnormalities in patients with this disease, as well as others.

(iii) Beta-adrenergic blockade alone appears to have little effect on the airways of normal individuals:

Propranolol may induce a degree of increased sensitivity to the inhalation of cholinergic agonists in some normal subjects (Zaid and Beall, 1966; Orehek et al. 1975) and in non-asthmatic individuals with allergic rhinitis (Townley et al. 1976). In addition, beta-adrenergic blockade increases the bronchoconstrictor response to intravenous histamine (Ploy-Song-Sang et al. 1978) and inhaled cigarette smoke (Zuskin et al. 1974) in normal individuals. Beta-adrenergic blockade alone, however, has, at most, a small effect on the calibre of normal airways in the absence of another bronchoconstrictor stimulus (MacDonald et al. 1967; Richardson and Sterling, 1969; Tattersfield et al. 1973; Ploy-Song-Sang et al. 1978), and a recent study has suggested that even its effect on the airway reactivity of normal subjects is negligible (Kiyingi et al. 1985). These findings suggest that beta-adrenergic subsensitivity is unlikely to be the sole pathogenetic mechanism underlying the bronchial hyperreactivity associated with asthma.

10.1.2 Possible Mechanisms of Beta-Adrenergic Subsensitivity

Although the existence and importance of beta-adrenergic subsensitivity remain subjects of controversy, a number of causes for such a state may be postulated:

(i) Infection:

In the original presentation of his theory, Szentivanyi (1968) discussed the induction by the inoculation of B. pertussis

into rodents, of a state resembling atopy, which was associated with beta-adrenergic subsensitivity. A number of studies have since suggested that such a state may be associated with infection due to various micro-organisms. The subsensitivity in the neutrophils from asthmatic patients of the isoproterenol-mediated inhibition of zymosan-stimulated lysosomal enzyme release appears to be exaggerated during upper respiratory infections, and incubation of the cells with rhinovirus results in a similarly depressed response in a degree proportional to the viral dose (Busse, 1977; Busse et al. 1980). Moreover, the protective effect of a beta-adrenergic agonist on the in vitro responses of tracheal and bronchial smooth muscle from guinea pigs is significantly smaller in animals which have been inoculated with parainfluenza 3 virus (Buckner et al. 1981).

Various bacteria also appear to affect beta-adrenergic receptor function. The vaccination of H. influenzae into guinea pigs induces:- (a) Increased sensitivity to ovalbumin-stimulated release of mediators from pulmonary tissue, as well as a significant attenuation of the inhibitory effect on this reaction of isoproterenol (Schreurs et al. 1980b). (b) A diminution of the in vitro relaxation of tracheal muscle in response to isoproterenol, associated with a simultaneous increase in the contractile response to carbachol (Schreurs et al. 1980a; Norris and Eyre, 1981). (c) A loss of beta-receptors from pulmonary tissue and tracheal muscle proportional to the diminution in the relaxant effect of isoproterenol (Schreurs and Nijkamp, 1982). A similar effect on the number of beta-receptors in guinea-pig lung

has been observed with S. pneumoniae, B. pertussis and E. coli (Schreurs et al. 1983).

(ii) Adrenal insufficiency:

(a) Abnormalities of catecholamine secretion: A number of studies have suggested that whereas asthmatic patients have normal levels of plasma catecholamines while in a stable condition (Barnes et al. 1982c), they may have an abnormality of catecholamine secretion in various clinical and experimental situations. The response in plasma free fatty acid levels and urinary catecholamines to mental stress (Mathe and Knapp, 1969), as well as the responses in the levels of plasma catecholamines and 3,5 cyclic AMP to exercise and hyperventilation, appear to be significantly diminished in asthmatic patients compared with normal individuals (Barnes et al. 1981a; Hartley et al. 1981; Warren et al. 1982; Ind et al. 1983). There appears, however, to be no difference in the plasma catecholamine responses to insulin (Ind et al. 1983). In other studies, however, there has been no difference between asthmatic patients and normal subjects in the plasma catecholamine responses to both exercise and the maintenance of the upright position (Larsson et al. 1982), and it has even been suggested that asthmatic patients may have markedly exaggerated catecholamine responses to exercise (Griffiths et al. 1972).

In contrast to the rise in plasma catecholamine levels - particularly that of epinephrine - which has been shown to occur in non-asthmatic patients requiring intensive care management (Wortsman et al. 1984), plasma epinephrine levels do not appear

to increase in association with acute severe asthma requiring emergency hospital treatment (Ind et al. 1985b). Plasma norepinephrine concentrations, on the other hand, do rise markedly, being significantly higher in patients requiring admission (Ind et al. 1985b). It has been noted that neither methacholine- nor allergen-provoked bronchospasm elicit a significant catecholamine response in asthmatic patients (Sands et al. 1985; Larsson et al. 1985), and a recent study has demonstrated that non-atopic middle-aged patients with "chronic partially reversible airways obstruction" have diminished nocturnal urinary catecholamine excretion (Postma et al. 1985).

While these contradictory findings cannot be explained with certainty, they appear, at any rate, to indicate an abnormality of catecholamine release, rather than of beta-adrenergic receptor mechanisms. The magnitude of the airway responses to inhaled epinephrine in asthmatic subjects is significantly greater at 04h00 than at 16h00, this being related to the lower pretreatment value at the former time (Barnes et al. 1982b). Similarly, infusions of epinephrine designed to produce plasma levels approximating those induced by postural changes combined with volume depletion completely reverse methacholine-induced bronchospasm in both normal and asthmatic subjects, the increase in airway conductance being considerably greater in the latter (Sands et al. 1985). These findings suggest that while pronounced fluctuations of airway calibre in asthmatic patients may be related to variations in plasma catecholamine levels, the beta-receptor responses are, themselves, intact.

(b) Adrenocortical insufficiency: It has been suggested that glucocorticoid deficiency may be associated with a state of adrenergic "resistance" (Ramey and Goldstein, 1957). The effect of corticosteroids on the regulation of both the number and function of beta-receptors has been extensively dealt with in Chapter 6.9. The evidence related to a possible diminution in the secretion of glucocorticoid hormones in association with asthma will be dealt with in Chapter

(iii) Autoimmunity:

The finding of antibodies to beta-adrenergic receptors in patients with asthma (Fraser et al. 1981; Blecher et al. 1984) suggests the possibility that the disease may, like so many others, ultimately be found to have an autoimmune component.

(iv) Reciprocal Adrenergic Receptor Interconversion:

It was reported by Kunos and Szentivanyi in 1968 that the myocardial adrenergic receptors of an isolated frog heart preparation exhibited the properties of alpha-receptors when the ambient temperature was low, but those of beta-receptors when the perfusion fluid was warmed. The concept has subsequently been developed that adrenergic receptors may exist in a state of reciprocal interconversion (Kunos et al. 1985), and it has been suggested that an exaggerated interconversion of beta- to alpha-adrenergic receptors may be the underlying abnormality in asthma - as well as in the atopic state in general (Kunos et al. 1985; Szentivanyi, 1985) (see Chapter 10.2.4).

10.2 A STUDY OF THE METABOLIC AND CARDIOVASCULAR RESPONSES TO SALBUTAMOL

In 1979 a small pilot study was performed by Kallenbach et al. in which the cardiovascular and metabolic responses of extremely mild asthmatic patients each having a forced expiratory volume in one second greater than 90% of the predicted value, and atopic non-asthmatic subjects to 100/ug intravenous salbutamol were compared. The dose of salbutamol was chosen on the basis of an earlier study showing that 100/ug caused near maximal metabolic stimulation with minimal side effects (Goldberg et al. 1975).

No difference could be demonstrated between the asthmatic patients and the non-asthmatic atopic individuals in the responses in heart rate, blood pressure and the plasma levels of insulin, glucose, free fatty acids, potassium and lactate to beta-2 adrenergic stimulation. While these findings suggested that beta-adrenergic subsensitivity was unlikely to be the sole cause of bronchial hyperreactivity in asthma, the study design clearly did not enable exclusion of the possibility that it was related to the atopic state. In the present investigation the following modifications were made to the design of the pilot study:

(i) The beta-2 adrenergic responses of larger numbers of asthmatic patients and normal subjects were compared.

(ii) Patients with asthma of a greater degree of severity were included.

(iii) Two doses of salbutamol were used (25 and 100 /ug) with the object of investigating the possibility that subsensitivity of the beta-adrenergic responses in asthma might be more readily discernible with lower doses of agonist.

10.2.1 Subjects

Eleven asthmatic patients and 11 control subjects were studied. Each of the patients had been using a sympathomimetic bronchodilator aerosol regularly. Eight, in addition, were long-term users of beclomethasone dipropionate aerosol (maximum dose 400/ug/day) and/or disodium cromoglycate. Four of the asthmatic patients had received short courses of systemic corticosteroids at least two years prior to the study. All of the asthmatic patients were in a stable condition at the time of the study. All forms of bronchodilator therapy were discontinued at least 10 days prior to the study. Beclomethasone dipropionate was discontinued 24 hours prior to the study.

10.2.2 Methods

The subjects were studied in the recumbent position in a quiet room following an overnight fast. A 19-gauge or larger cannula was inserted into an arm vein ard kept patent with a slow infusion of normal saline. Not less than 30 minutes later, blood was sampled through the cannula. This was repeated after a further 15 minutes. Twenty-five /ug salbutamol was subsequently injected intravenously over one minute and blood sampled 2, 5, 15 and 30 minutes after the completion of the injection. A further

100/ug salbutamol was then injected over one minute and blood sampled 2, 5, 15 and 30 minutes after the completion of the second injection. Readings of heart rate and blood pressure were taken prior to each blood sample.

Aliquots of the blood sampled at each time were used for the determination of the plasma levels of free fatty acids, insulin, glucose and potassium. Plasma specimens were immediately separated and analysed for glucose and potassium. For free fatty acid and insulin determinations, blood was placed into iced tubes and immediately centrifuged and the separated plasma subsequently stored at -20°C until assayed.

Biochemical Methods:

See Appendix "A".

Statistical Analysis:

Pairwise comparisons were done for each variable using the two-tailed Mann-Whitney U test (Siegel, 1956). The significance of the variation in each variable following salbutamol was assessed within each group using Friedman's 2-way analysis of variance (Siegel, 1956). If the variation in a particular variable was significant, pairwise comparison of the value at each time with the basal value was done within each group using the 95% extreme range limits of mean ranks (Steel, 1961).

The basal value for each variable was the arithmetic mean of the two values obtained prior to the first injection of salbutamol.

10.2.3 Results

Demographic and pulmonary function data are shown in Table 19. The forced expiratory volume in one second (FEV_1) was significantly lower, and the airway resistance and fall in peak expiratory flow rate (PEFR) on exercise significantly greater in the asthmatic patients than in the normal subjects.

The heart rate varied significantly in both groups of subjects following salbutamol ($p = <0.0001$), as did pulse pressure ($p = <0.01$ in normal subjects; $p = <0.02$ in asthmatic patients). However, while both the systolic and diastolic blood pressures of the normal subjects varied significantly ($p = <0.05$), there was no significant variation in either the systolic or the diastolic blood pressure in the asthmatic patients. There was no significant difference between the groups in any of these variables at any time in the study. The cardiovascular responses to salbutamol are shown in Tables 20a and 20b.

The plasma insulin level varied significantly in both the asthmatic patients and the normal subjects following salbutamol ($p = <0.0001$). The plasma insulin levels of the asthmatic patients were significantly higher than those of the normal subjects 15 and 30 minutes following 25/ug salbutamol, and at all times following the 100/ug dose. When the results were expressed as ratios (value:basal), the maximum response - 2 minutes following 100/ug salbutamol - was significantly greater in the asthmatic patients than in the normal subjects ($p = <0.03$). The plasma insulin responses to salbutamol are shown in Table 21.

TABLE 19. DEMOGRAPHIC AND PULMONARY FUNCTION DATA

	Age (yrs)	Sex	Mass (kg)	FEV_1 (% predicted)	Airway Resistance ($cmH_2O/l/sec$)	Fall in PEFR on exercise (%)
Normal subjects (n=11)	21.2(3.2)	5F,6M	61.5(9.6)	107.2(11.2)*	1.5(0.5)**	2.0(2.7)**
Asthmatic patients (n=11)	21.1(4.7)	4F,7M	63.0(7.9)	83.6(21.4)	3.8(2.3)	29.6(.0.7)

Values are mean(SD)
Normal subjects vs asthmatic patients significantly different
* p = <0.005
** p = <0.0002

TABLE 20a. CARDIOVASCULAR RESPONSES TO SALBUTAMOL

		Basal	25/ug	2 min	5 min	15 min	30 min	100/ug	2 min	5 min	15 min	30 min
Normal subjects (n=11)	Systolic Blood pressure	121 (11)		126 (13)	122 (13)	120 (11)	123 (10)		130 (17)	129≠ (8)	125 (10)	128 (12)
	Diastolic Blood pressure	69 (10)		66 (10)	65 (10)	66 (9)	69 (8)		63≠ (8)	65 (8)	68 (8)	8 (9)
Asthmatic patients (n=11)	Systolic Blood pressure	121 (12)		122 (8)	124 (9)	124 (12)	125 (10)		133 (18)	129 (13)	127 (10)	126 (10)
	Diastolic Blood pressure	68 (8)		67 (6)	65 (7)	68 (8)	71 (6)		66 (13)	67 (6)	67 (6)	69 (7)

Values are mean(SD) mmHg

≠ Value significantly different from basal (p = <0.05)

TABLE 20b. CARDIOVASCULAR RESPONSES TO SALBUTAMOL

		Basal	25/ug	2 min	5 min	15 min	30 min	100/ug	2 min	5 min	15 min	30 min
Normal subjects (n=11)	Pulse Pressure (mmHg)	51 (11)		60 (16)	57 (14)	55 (11)	54 (8)		67↗ (12)	64↗ (12)	57 (10)	60 (13)
	Heart rate (beats/min)	63 (12)		74 (15)	68 (12)	66 (12)	65 (11)		99↗ (25)	89↗ (18)	80↗ (17)	81↗ (15)
Asthmatic patients (n=11)	Pulse Pressure (mmHg)	53 (11)		55 (9)	59 (7)	56 (12)	54 (8)		66↗ (18)	62 (12)	61 (8)	57 (10)
	Heart rate (beats/min)	65 (10)		74↗ (12)	72↗ (9)	69 (10)	70 (9)		99↗ (17)	85↗ (11)	80↗ (10)	79↗ (10)

Values are mean(SD)

↗ Value significantly different from basal (p = <0.05)

TABLE 21. PLASMA INSULIN RESPONSES TO SALBUTAMOL

	Basal	25/ug	2 min	5 min	15 min	30 min	100/ug	2 min	5 min	15 min	30 min
					*	**		****	***	*	**
Normal subjects (n=11)	6.7 (3.0)		13.9≠ (4.1)	10.1≠ (2.2)	8.0 (2.9)	7.9 (5.3)		20.6≠ (5.4)	16.4≠ (3.7)	13.9≠ (5.7)	10.1≠ (3.4)
Asthmatic patients (n=11)	7.2 (1.6)		22.1≠ (12.6)	14.3≠ (6.0)	10.8 (3.1)	10.9 (3.7)		34.1≠ (8.6)	26.8≠ (9.6)	18.9≠ (5.6)	13.2≠ (2.4)

Values are mean(SD) /uUnits/ml

≠ Value significantly different from basal (p= <0.05)

Normal subjects vs asthmatic patients significantly different

* p = <0.05
** p = <0.02
*** p = <0.005
**** p <0.0005

The level of the plasma free fatty acids varied significantly in both groups of subjects following salbutamol ($p = <0.01$). However, no significant change from the basal value could be detected in the asthmatic patients at any time using the 95% extreme range limits of mean ranks. The plasma level of free fatty acids tended to be lower in the asthmatic patients than in the normal subjects and the difference was statistically significant 5 minutes after 25/ug salbutamol ($p = <0.05$). However, when the results were expressed as ratios (value:basal) in order to correct for the relatively large difference between the groups in the basal value, there was no significant difference between the groups of subjects at any time. The responses to salbutamol in the levels of plasma free fatty acids are shown in Table 22.

The plasma glucose and potassium levels varied significantly in both groups of subjects following salbutamol ($p = <0.005$). There was no significant difference, however, between the groups in either of these variables at any time. The plasma glucose and potassium responses to salbutamol are shown in Tables 23 and 24 respectively.

10.2.4 Discussion

The secretion of insulin has been shown to be stimulated by the non-selective beta-adrenergic agonist, isoproterenol (Porte, 1967; Imura et al. 1971). A similar effect occurs in both dogs and humans in association with the administration of the selective beta-2 agonist, salbutamol (Loubatieres et al. 1971;

TABLE 22. PLASMA FREE FATTY ACID RESPONSES TO SALBUTAMOL

	Basal	25/ug	2 min	5 min	15 min	30 min	100/ug	2 min	5 min	15 min	30 min
Normal subjects (n=11)	1304 (293)		1325 (282)	1564≠* (358)	1609 (373)	1309 (349)		1339 (425)	1565≠ (263)	1785≠ (455)	1483 (318)
Asthmatic patients (n=11)	1228 (188)		1261 (169)	1298 (209)	1341 (266)	1207 (220)		1315 (320)	1383 (238)	1505 (450)	1280 (359)

Values are mean(SD) /uEq/l

≠ Value significantly different from basal ($p < 0.05$)

* Normal subjects vs asthmatic patients significantly different ($p < 0.05$)

TABLE 23. PLASMA GLUCOSE RESPONSES TO SALBUTAMOL

	Basal	25/ug	2 min	5 min	15 min	30 min	100/ug	2 min	5 min	15 min	30 min
Normal subjects (n=11)	4.6 (0.7)		4.6 (0.4)	4.7 (0.5)	4.7 (0.4)	4.7 (0.3)		4.8 (0.4)	5.0≠ (0.5)	5.1 (0.7)	5.1 (0.5)
Asthmatic patients (n=11)	4.5 (0.4)		4.7 (0.3)	4.9≠ (0.4)	4.8 (0.3)	4.6 (0.4)		4.9≠ (0.3)	5.1≠ (0.4)	5.2≠ (0.4)	5.2≠ (0.3)

Values are mean(SD) mmol/l

≠ Value significantly different from basal ($p < 0.05$)

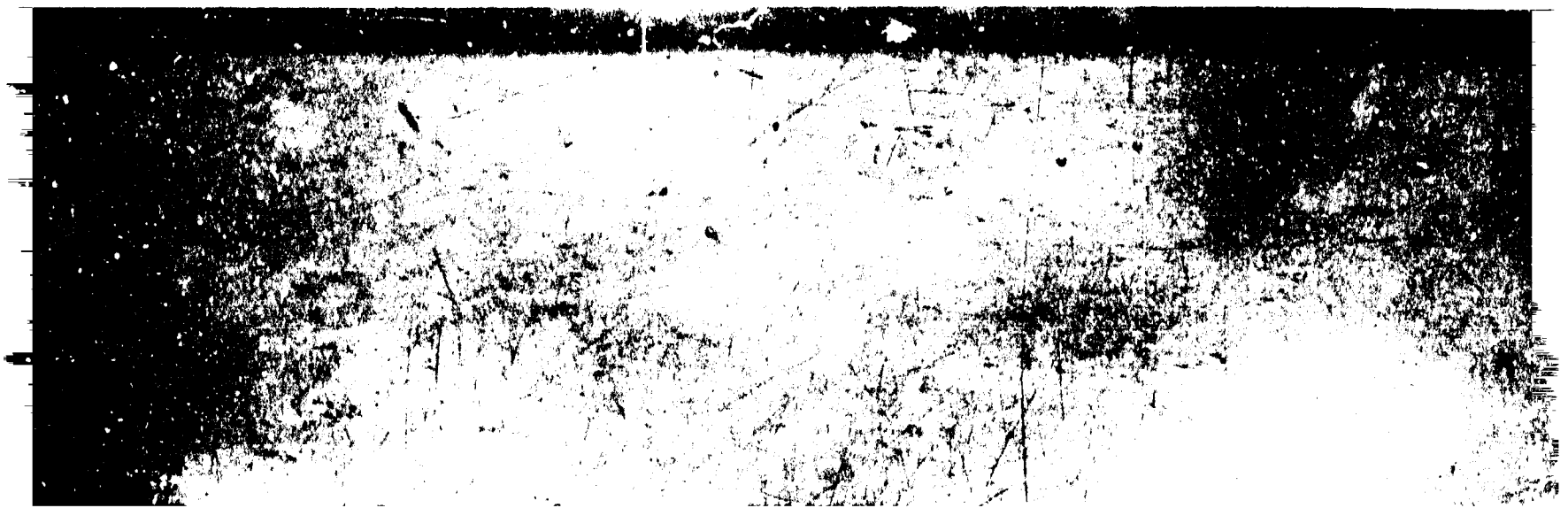


TABLE 24. PLASMA POTASSIUM RESPONSES TO SALBUTAMOL

	Basal	25/ug	2 min	5 min	15 min	30 min	100/ug	2 min	5 min	15 min	30 min
Normal patients (n=11)	3.9 (0.2)		3.8 (0.3)	3.9 (0.4)	3.8 (0.2)	3.8 (0.3)		3.7≠ (0.3)	3.6≠ (0.3)	3.6≠ (0.3)	3.5≠ (0.2)
Asthmatic patients (n=11)	3.9 (0.3)		3.9 (0.2)	3.8 (0.3)	3.9 (0.5)	3.7 (0.2)		3.8 (0.5)	3.5≠ (0.3)	3.4≠ (0.2)	3.5≠ (0.3)

Values are mean(SD) meq/l

≠ Value significantly different from basal (p = <0.05)

Goldberg et al, 1975). This response appears to be attenuated in humans by the beta-1 antagonist, practolol, suggesting its partial dependence on the stimulation of beta-1 adrenergic receptors (Goldberg et al. 1975).

There are few reported studies in which the insulin response to beta-adrenergic stimulation has been examined in asthma. A significant increase in plasma insulin levels has been shown to occur following the administration of intravenous salbutamol to asthmatic patients (Tickner et al. 1977; Neville et al. 1977; Nogrady et al. 1977). In addition, it has been reported that the plasma insulin response to epinephrine in asthmatic patients shows no "gross abnormality" (Makino et al. 1970). None of these studies have specifically compared the response of asthmatic patients to that of normal subjects. The results of a study of the plasma insulin response to beta-2 adrenergic stimulation, using the agent trimetoquinol, suggest that it is diminished in asthmatic compared with normal individuals (Kurosawa et al. 1980). Sympathomimetic medications were not discontinued for an adequate period prior to the study, however. In another study, no difference could be detected between asthmatic and non-asthmatic subjects in the insulin response to salbutamol (Kallenbach et al. 1979). As has been discussed in Chapter 10.2, the asthmatic patients in this study had extremely mild disease and the non-asthmatic subjects were, in fact, atopic, not normal, individuals.

There is ample documentation of the occurrence of a blunted hyperglycaemic response to beta-adrenergic stimulation in

asthmatic patients compared with normal subjects (Inoue, 1967; Lockey et al. 1967; Middleton and Finke, 1968; Fireman et al. 1970; Morris et al. 1978; Mue et al. 1979), which has, in the absence of measurements of insulin levels, been attributed to beta-adrenergic subsensitivity. Another major problem in the interpretation of the data from these studies is, once again, the fact that sympathomimetic bronchodilator therapy was not discontinued for an adequate period prior to any of them.

In the present study, plasma glucose levels remained relatively constant in both groups of subjects despite the significant increments in the plasma insulin levels. This may possibly be attributable to the independent hyperglycaemic effect of salbutamol (Goldberg et al. 1975) which appears to be related to beta-adrenergically mediated glycogenolysis (Arnold et al. 1968; Brody and McNeill, 1970; Hornbrook, 1970). Although the anti-lipolytic effect of the higher insulin levels appeared to be manifested in the asthmatic patients by the occurrence in them of a smaller salbutamol-stimulated rise in the plasma free fatty acid levels, the blood glucose levels of the two groups of subjects were similar throughout. While no definite explanation can be given for this, it is possible that it may be related to the well-known phenomenon whereby the inhibition of lipolysis is more sensitive to insulin than the stimulation of peripheral glucose uptake (Rabinowitz and Zierler, 1965; Porte and Halter, 1981).

The finding of a manifestation of beta-adrenergic hyper-responsiveness as manifested by the increased insulin response in

asthmatic patients is both surprising and difficult to explain, being at variance with the existing experimental data and with the theory of beta-adrenergic subsensitivity. It is important to note, however, that there may be a considerable lack of homogeneity in beta-receptor numbers as well as in the intensity of beta-adrenergically mediated responses occurring simultaneously in different tissues. It has been suggested that, while sympathomimetic bronchodilator therapy may induce beta-2 receptor desensitisation in both skeletal muscle and the heart, a similar effect does not occur in the airway (Svedmyr et al. 1976). More recently it has been demonstrated that the administration of terbutaline produces a significant reduction in the number of beta-receptors in lymphocytic, but not pulmonary tissue (Hasegaw and Townley, 1983). Similarly, it appears that the administration of oral terbutaline for 4-5 weeks to both normal and asthmatic individuals results in no significant reduction in the bronchodilator responsiveness to the agent, despite a marked reduction in the number of lymphocyte beta-receptors (Tashkin et al. 1982). There appears to be a considerable divergence between the time-course of the bronchodilator response to orally administered terbutaline and of its effects on both plasma 3,5 cyclic AMP levels and leukocyte 3,5 cyclic AMP responses in both normal subjects and asthmatic patients (Morris et al. 1983). It has also been clearly shown that while asthmatic patients have abnormal haemodynamic responses to isoproterenol, which bear a relationship to the degree of bronchial hyperreactivity, they have no abnormality in

either the number of leukocyte beta-receptors or their 3,5 cyclic AMP responses (Davis et al. 1986).

It is of interest to consider the increased insulin response in the light of the concept that adrenergic receptors exist in a state of reciprocal interconversion (Kunos et al. 1985; Szentivanyi, 1985). A conversion of the glycogenolytic response of rat hepatocytes from a reaction mediated by alpha-1 receptors to one mediated by beta-receptors occurs in association with a number of conditions, including hypothyroidism and glucocorticoid deficiency (Malbon et al. 1978; Chan et al. 1979; Preiksaitis et al. 1982; Nog . 1983; Kunos et al. 1985). It has recently been suggested tha. may be associated with an actual diminution in the number epatic alpha-receptors (Preiksaitis et al. 1982; Noguch'). Conversely, pulmonary homogenates from guinea-pigs w. ovalbumin-induced asthma contain a greater number of alpha-receptors and fewer beta-receptors than those of control animals (Barnes et al. 1980c). Alpha-receptor predominance has been documented in lymphocytes from patients with atopic skin disease compared with those from both normal subjects and patients with non-atopic skin diseases (Szentivanyi et al. 1980). A similar increase in the . r of alpha-receptors has been found in lymphocytes and surgical specimens of pulmonary tissue obtained from patients with asthma compared with those from patients without airway disease or with irreversible airway obstruction (Szentivanyi et al. 1979). In the light of these results it has been suggested that exaggerated interconversion of beta- to alpha-1 adrenergic receptors may be the underlying

defect in asthma - as well as in the atopic state in general (Kunos et al. 1985; Szentivanyi, 1985).

The data regarding the enzyme phospholipase A2 in relation to beta-adrenergic receptors have been discussed in Chapter 6.9.3, and it is possible to postulate a theoretical relationship of the process of exaggerated receptor interconversion to this enzyme. The conversion from alpha- to beta-receptor mediated glycogenolysis occurring in rat hepatocytes which is associated with corresponding changes in receptor numbers (Nakamura et al. 1984) can be blocked by lipomodulin - the protein which inhibits phospholipase A2 (Kunos et al. 1984). Furthermore, if it is postulated that phospholipase A2 is involved in the pathogenesis of asthma, it would have to be capable of mediating changes in the number of beta-receptors in the opposite direction in pulmonary tissue, and the enzyme has, in fact, recently been shown to be present in greater amounts in the lungs of guinea-pigs with ovalbumin-induced asthma than in those from control animals, in association with a diminished number of beta-receptors (Taki et al. 1986). In hypothyroidism there is an apparent lack of homogeneity of the number of beta-receptors in different organs, a diminution in their number occurring in the myocardium (Stiles et al. 1984) associated with an increased number in hepatic tissue (Preiksatis et al. 1978; Malbon et al. 1982; Noguchi, 1983). It is, at least theoretically possible, therefore, that the exaggerated insulin response to salbutamol observed in the asthmatic patients in the present study could be related to an increase in the number of pancreatic beta-receptors

in association with a diminished number in the airway - conceivably a phospholipase A2-mediated effect.

Almost certainly, however, the most important conclusion that can be drawn from the present results is that the premise on which most of the studies quoted in this dissertation is based, namely that autonomic events in the lungs are mirrored by those occurring at other, often remote sites, may be false. It is of considerable interest, moreover, that the number of lymphocyte beta-receptors, as well as their adenyl cyclase activity, has been shown to diminish in asthmatic patients following house dust mite antigen-induced bronchospasm, suggesting the interesting possibility that a beta-receptor abnormality may be the result rather than the cause of asthma (Meurs et al. 1982). It is clear from the data which have been reviewed, as well as from the results of the present study, that attempts to relate extrapulmonary autonomic abnormalities to the pathogenesis of bronchial asthma are fraught with difficulty.

CHAPTER 11

A STUDY OF THE CIRCADIAN VARIATIONS IN AUTONOMIC AND HORMONAL VARIABLES IN RELATION TO CIRCADIAN CHANGES IN AIRWAY CALIBRE

11.1 INTRODUCTION

An exacerbation of symptoms in the early morning hours, often referred to as "morning dipping", occurs commonly in patients with asthma, and is associated with a deterioration in pulmonary function, which is characteristic of acute bronchospasm (Hetzel et al. 1977). A circadian rhythm in airway calibre appears to be present in man with the bathyphase or trough occurring in the early morning hours (Kerr, 1973; Gaultier et al. 1977; Hetzel and Clark, 1980), and the amplitude of variation being considerably greater in asthmatic patients than in normal individuals (Hetzel and Clark, 1980). It is probable that "morning dipping" is associated with this exaggerated circadian variation in pulmonary function, the precise cause of which, however, remains undefined. The numerous mechanisms to which it has been attributed include circadian variations in the secretion of various hormones in the release of inflammatory mediators such as histamine, and in parasympathetic neural output, each of which could have a more marked effect on the sensitive airways of asthmatic patients (Barnes, 1985; Muers, 1984; Douglas, 1985).

The therapeutic effect of both epinephrine and cortisol in asthma lends credence to the postulate that "morning dipping" is related to the nocturnal troughs in the plasma levels of these hormones. A temporal association has been shown, moreover, to exist between the nadirs in the levels of plasma epinephrine (Barnes et al. 1980b) and urinary catecholamines (Soutar et al. 1977), plasma cortisol (Barnes et al. 1980a) and urinary

corticosteroids (Reinberg et al. 1963) and early morning bronchospasm in asthmatic patients. A definite cause-effect relationship between fluctuations in these variables and changes in airway function has not, however, been easy to establish and neither the infusion of cortisol (Soutar et al. 1975) nor the achievement of therapeutic levels of salbutamol by the oral administration of a sustained release preparation (Fairfax et al. 1980), has been completely effective in abolishing "morning dipping" in asthmatic patients.

Asthmatic reactions induced by both antigens (Nagy et al. 1982; Durham et al. 1984) and exercise (Lee et al. 1983) have been shown to be associated with increased neutrophil chemotactic activity in the serum, probably originating from mast cells. A similar phenomenon could conceivably be occurring in relation to "morning dipping" as a result of increased mediator release precipitated by the nocturnal nadirs in the plasma levels of both catecholamine and corticosteroid hormones. Although beta-adrenergic blockade is not associated with an increase in the plasma level of histamine (Ind et al. 1985a), or an increase in the serum neutrophil chemotactic activity (Ind et al. 1984), no data regarding the latter variable have been reported in relation to "morning dipping".

A circadian variation in heart rate has been reported to occur in normal subjects with the nadir occurring in the early morning hours, probably related to a degree of sympathetic withdrawal and a shift to dominant vagal tone (Clarke et al. 1976). The possibility that "morning dipping" may be closely

related to vagal tone is suggested by the finding that it can be attenuated to a greater extent by ipratropium bromide than by salbutamol (Cox et al. 1984).

It has been suggested (Barnes, 1985) that "morning dipping" may be related to the coincidence of a number of these factors during the early morning hours as a result of biological rhythms originating in the hypothalamus. The object of this study was to assess the relative importance of these variables, each of which has been implicated in producing early morning airway narrowing. This was done by investigating the circadian changes occurring in them in relation to fluctuations in airway calibre in a group of asthmatic patients with "morning dipping" of a widely varying degree of severity, as well as in normal subjects.

11.2 SUBJECTS

Thirteen asthmatic patients and 11 control subjects were studied. The asthmatic patients were divided into two subgroups: (i) "dippers" (ii) "non-dippers", on the basis of whether or not they demonstrated significant "morning dipping" during the study. Significant "dipping" was defined as a fall of 20% or greater in the PEFR at 04h00 compared with the basal value the previous afternoon. This was based on the data obtained from a large study suggesting that peak expiratory flow rate (PEFR) may exhibit a circadian variation of almost 20% (mean amplitude of variation 8.3%; standard deviation 5.2%) in normal subjects (Hetzel and Clark, 1980).

Each of the asthmatic patients was using a beta-adrenergic agonist bronchodilator aerosol regularly. This was discontinued one week prior to the study. No patient had received systemic corticosteroid therapy for at least 2 years prior to the study. Ten patients were using a beclomethasone dipropionate aerosol which was discontinued on the morning of the study. Further details of corticosteroid therapy in the 2 subgroups of asthmatic patients are given in the "Results" section.

11.3 METHODS

The subjects were studied individually in the recumbent position in a quiet room, to which they were admitted at approximately noon on the study day. A 19-gauge or larger cannula was immediately inserted into an arm vein and kept patent with a saline infusion. At approximately 14h00 the subject was attached to a 24-hour ambulatory electrocardiograph (ECG) recorder. A standardised light meal was given at 18h00. Water was the only oral fluid permitted. Blood was sampled through the indwelling cannula at 16h00, 16h30, midnight, 04h00 and 08h00. Aliquots of the blood taken at each time were stored for determination of the plasma levels of cortisol, epinephrine, norepinephrine, adrenocorticotropic hormone (ACTH) and for studies of the serum neutrophil chemotactic activity. However, as the number of investigations was limited by cost, measurements of plasma catecholamines and serum neutrophil chemotactic activity were omitted at midnight, and measurements of plasma ACTH levels only

done at midnight and 08h00.

For plasma specimens, aliquots of blood were placed into iced tubes, immediately centrifuged, and the separated plasma stored at -20°C until assayed.

For serum specimens, blood was allowed to clot at room temperature for 20 minutes then centrifuged, and the separated serum stored at -20°C until analysed.

Measurements of PEFR were made subsequent to the sampling of blood at 16h00, 16h30, 04h00 and 08h00, using a Wright's peak flow meter. The best of three readings was recorded at each time.

Biochemical Analysis:

See Appendix "A".

Neutrophil chemctactic activity:

This was assessed by: (i) a polymorphonuclear leukocyte migration assay; (ii) a study of polymorphonuclear leukocyte membrane-associated oxidative metabolism. For methods see Appendix "B".

Electrocardiographic Analysis:

ECG strips of 20 beats each, taken 5, 10 and 15 minutes prior to each sampling of blood were analysed. From these 60 beats, the maximum, minimum and mean heart rates, and the magnitude of respiratory sinus arrhythmia - maximum minus minimum heart rate - were calculated for each time in the study.

Statistical Analysis:

For each variable, 3 comparisons were made:

(i) The asthmatic "dippers" were compared with the asthmatic "non-dippers".

(ii) The asthmatic "dippers" were compared with the normal subjects.

(iii) The asthmatic "non-dippers" were compared with the normal subjects.

Pairwise comparisons of the data from the different groups were done using the two-tailed Mann-Whitney U test (Siegel, 1956) conducted at a simultaneous 5% Bonferroni level. Assessment of the significance of the variation in each variable during the study was done within each group using Friedman's two-way analysis of variance (Siegel, 1956). Correlations between variables were done using Spearman's rank correlation test (Siegel, 1956).

For basal levels of PEFR and heart rates, the arithmetic means of the values obtained at 16h00 and 16h30 were used. For basal hormone levels, equal aliquots of the 16h00 and 16h30 specimens were mixed, and integrated basal levels obtained. The basal values in the tests for neutrophil chemotactic activity were the arithmetic mean of the 16h00 and 16h30 values.

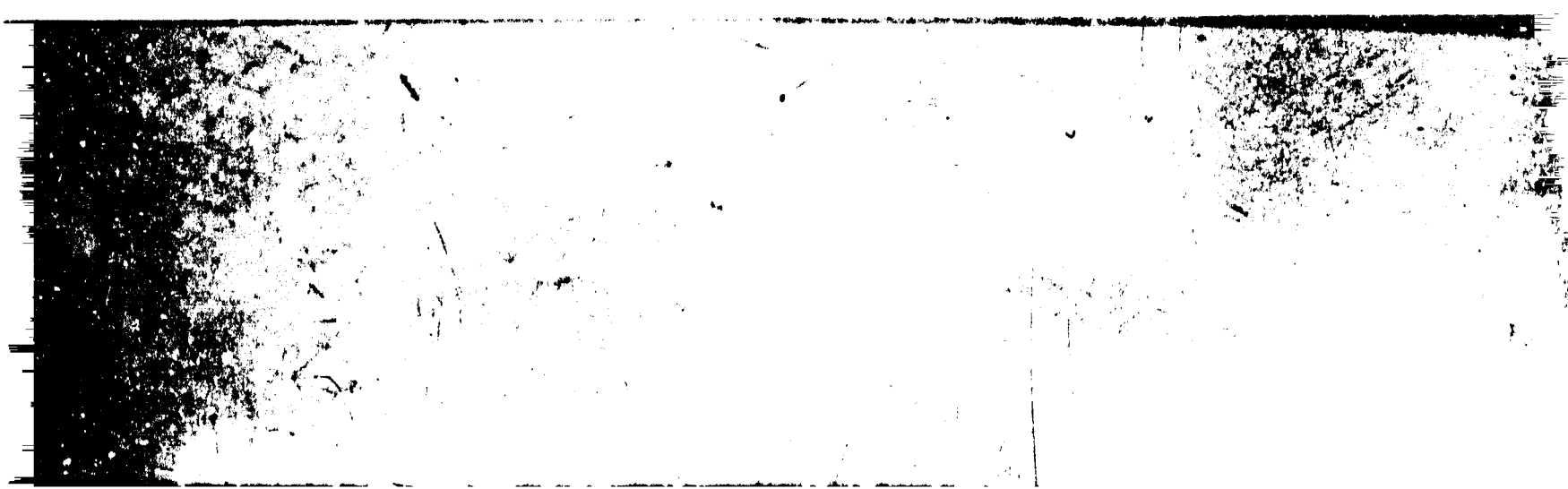
11.4 RESULTS

The demographic and pulmonary function data of the asthmatic patients and the normal subjects are shown in Table 25. The

TABLE 25. DEMOGRAPHIC AND PULMONARY FUNCTION DATA

	Age (Yrs)	Sex	FEV_1 (% predicted)	Airway Resistance ($cmH_2O/l/sec$)	Fall in PEFR on exercise (%)
Normal subjects (n = 11)	21.2(3.2)	6M,5F	107.2(11.2)*	1.5(0.5)**	1.7(2.8)**
Asthmatic patients (n = 13)	20.8(4.3)	7M,6F	78.6(21.9)	4.9(3.9)	31.9(11.1)
"Dippers" (n = 6)	20.3(2.9)	3M,3F	67.0(22.5)	6.9(4.9)***	32.8(14.3)
"Non-dippers" (n = 7)	21.1(5.5)	4M,3F	88.6(16.9)	3.2(1.9)	31.1(8.7)

* Normal subjects vs asthmatic patients significantly different ($p = <0.002$)
** Normal subjects vs asthmatic patients significantly different ($p = <0.0002$)
*** "Dippers" vs "non-dippers" significantly different ($p = <0.02$)



forced expiratory volume in one second (FEV_1) was significantly lower and the airway resistance and fall in PEFR on exercise significantly greater in the asthmatic patients than in the normal subjects. On the basis of the fall in PEFR at 04h00 compared with the basal value the previous afternoon, the asthmatic patients were subdivided into 6 "dippers" and 7 "non-dippers". The demographic and pulmonary function data of these two subgroups of asthmatic patients are shown in Table 25. Although the FEV_1 was lower in the "dippers" than in the "non-dippers", the difference was not statistically significant. The airway resistance was significantly higher in the "dippers" than in the "non-dippers". There was no difference between the "dippers" and the "non-dippers" in the fall in PEFR on exercise.

The results of measurements of PEFR in the different groups during the study are shown in Table 26. The mean fall in the PEFR at 04h00 compared with the basal value was $28 \pm 14\%$ in the "dippers" and $10 \pm 4\%$ in the "non-dippers" ($p = <0.0001$). The PEFR exhibited a significant degree of variation in both the "dippers" and the "non-dippers" during the study ($p = <0.01$), but did not vary significantly in the normal subjects ($p = >0.1$).

Hormones: The plasma cortisol level exhibited a significant degree of variation in each group of subjects during the study ("dippers" and "non-dippers" $p = <0.0001$; normal subjects $p = <0.001$). The midnight plasma cortisol levels were significantly lower in the asthmatic "dippers" than in either the asthmatic "non-dippers" ($p = <0.005$) or the normal subjects ($p = <0.001$).

TABLE 26. PEAK EXPIRATORY FLOW RATES

	Basal	04h00	08h00
Normal subjects (n = 11)	490(73)	477(69)	490(72)
"Dippers" (n = 6)	323(94)	238(102)	284(111)
"Non-Dippers" (n = 7)	443(107)	402(106)	416(112)

Values are mean(SD) litres/min

(Percent fall in PEFR at 04h00 compared with basal value - "dippers" = 28±14%; "non-dippers" = 10±4% -- p = <0.0001)

There was no difference, however, between the asthmatic "non-dippers" and the normal subjects in the midnight plasma cortisol levels ($p = >0.38$). The asthmatic "dippers" exhibited the greatest degree of fluctuation in plasma cortisol levels during the study. When the results were expressed as ratios (basal:value), the basal:midnight ratio was higher in the "dippers" than in the "non-dippers" ($p = <0.025$) or the normal control subjects ($p = <0.001$). There was no difference, however, between the asthmatic "non-dippers" and the normal subjects in the value for the basal:midnight ratio ($p = >0.16$). The results of the measurements of plasma cortisol levels are shown in Table 27.

The results of the measurements of plasma ACTH levels are shown in Table 28. The mean values in each group of subjects were consistent with the normal diurnal levels in the laboratory where the assays were performed (Department of Medicine, University of the Witwatersrand). There was no significant difference between any of the groups at either time.

The plasma norepinephrine level exhibited a significant degree of variation in the normal subjects during the study ($p = <0.02$). There was no significant variation in the plasma norepinephrine levels, however, in either the asthmatic "dippers" or the "non-dippers". Although plasma epinephrine levels appeared to exhibit minor degrees of variation, this was not statistically significant in any group of subjects. There were no significant differences between the groups of subjects in either the epinephrine or norepinephrine levels at any time. When the

TABLE 27. PLASMA CORTISOL LEVELS

	Basal	Midnight	04h00	08h00	Ratio Basal:Midnight
[illegible] (n=11)	74.8(26.1)	50.5(32.3)*	97.3(40.8)	151.8(32.5)	1.8(0.7)*
"dippers" (n=8)	57.3(16.9)	13.8(3.7)	63.8(29.2)	119.2(35.8)	4.3(1.7)
"non-dippers" (n=7)	87.1(50.3)	50.4(48.5)**	81.3(44.1)	173.7(48.6)	2.5(1.1)***

Values are mean(SD) ng/ml
(Conversion to nmol/l x 2.76)

* Normal subjects vs "dippers" significantly different (p = <0.001)
** "Non-dippers" vs "dippers" significantly different (p = <0.005)
*** "Non-dippers" vs "dippers" (p = <0.025)

TABLE 28. PLASMA ACTH LEVELS

	Midnight	08h00
Normal subjects (n = 11)	76.9(24.6)	100.8(40.9)
"Dippers" (n = 6)	71.3(8.8)	110.0(24.6)
"Non-Dippers" (n = 7)	98.0(34.6)	113.6(21.7)

Values are mean(SD) pg/ml

Conversion to pmol/l x 0.22

results were expressed as ratios in order to correct for inter-group differences in the basal values, there was still no difference between the groups at any time. The results of the measurements of plasma catecholamine levels are shown in Tables 29 and 30.

Serum neutrophil chemotactic activity: Serum neutrophil activity, as assessed by the two tests performed, did not exhibit a significant degree of variation in any group of subjects during the study. There were no significant differences between the groups of subjects at any time. The results of the studies of serum neutrophil chemotactic activity are shown in Tables 31 and 32.

Electrocardiographic Analysis:

There was no significant variation in any of the heart rates or in the magnitude of respiratory sinus arrhythmia in either the normal subjects or the asthmatic "non-dippers" during the study. The asthmatic "dippers" on the other hand, exhibited a significant degree of variation in each of the heart rates, as well as in the magnitude of respiratory sinus arrhythmia (maximum heart rate $p = <0.005$; minimum heart rate $p = <0.05$; mean heart rate $p = <0.05$; magnitude of respiratory sinus arrhythmia $p = <0.005$). The maximum heart rate tended to diminish to a greater degree in the asthmatic "dippers" than in either of the other groups of subjects at midnight and 04h00, with a corresponding diminution in the magnitude of respiratory sinus arrhythmia at

TABLE 29. PLASMA EPINEPHRINE LEVELS

	Basal	04h00	08h00
Normal subjects (n=11)	37.1(17.4)	27.1(12.6)	39.1(17.4)
"Dippers" (n=6)	23.5(12.1)	19.2(1.5)	30.2(7.5)
"Non-dippers" (n=7)	33.9(18.4)	25.6(11.6)	29.4(11.0)

Values are mean(SD) pg/ml

Conversion to nmol/l x 0.0055

TABLE 30. PLASMA NOREPINEPHRINE LEVELS

	Basal	04h00	08h00
Normal subjects (n=11)	224(104)	154(43)	136(65)
"Dippers" (n=6)	137(35)	106(59)	243(230)
"Non-Dippers" (n=7)	218(157)	145(51)	138(58)

Values are mean(SD) pg/ml
Conversion to nmol/l x 0.0059

TABLE 31. POLYMORPHONUCLEAR LEUKOCYTE MIGRATION ASSAY

	Basal	04h00	08h00
Normal subjects (n=11)	53(28)	53(29)	53(24)
"Dippers" (n=6)	54(25)	63(25)	58(23)
"Non-dippers" (n=7)	57(30)	58(19)	60(25)

Values are mean(SD) polymorphonuclear leukocytes/high powered field

TABLE 32. POLYMORPHONUCLEAR LEUKOCYTE MEMBRANE-ASSOCIATED OXIDATIVE METABOLISM

	Basal	04h00	0h800
Normal subjects (n=11)	6160(4115)	5890(3977)	5856(4028)
"Dippers" (n=6)	6211(3718)	6132(3879)	5725(4011)
"Non-Dippers" (n=7)	4980(1322)	5119(1665)	4395(1290)

Values are mean(SD) fmoles I^{12}

these times. There were no significant differences, however, between the different groups of subjects in any of the ECG variables at any time during the study.

Re-analysis of the ECG data using a parametric statistical method (analysis of variance) similarly showed no significant difference between the groups in any variable at any time during the study.

The ECG data obtained from the different groups of subjects are shown in Tables 33 to 36.

Correlations:

When the data from the two subgroups of asthmatic patients ("dippers" plus "non-dippers" n = 13) were combined, there was a strong inverse correlation between the midnight plasma cortisol levels and the magnitudes of the fall in PEFR from the basal value to that at 04h00 ($r = -0.79$, $p = <0.0002$). There was no correlation between the 04h00 plasma cortisol levels and the fall in PEFR ($r = -0.15$, $p = >0.5$). Similarly, no correlation could be demonstrated between either the 04h00 plasma epinephrine or norepinephrine levels and the magnitude of the fall in PEFR ($r = -0.49$, $p = >0.09$ and $r = -0.23$, $p = >0.4$ respectively). There was no correlation between any of the ECG variables and the magnitude in the fall in PEFR (minimum heart rate $r = -0.22$, $p = >0.4$; maximum heart rate $r = -0.33$, $p = >0.2$; mean heart rate $r = -0.28$, $p = >0.3$; magnitude of respiratory sinus arrhythmia $r = -0.31$, $p = >0.2$).

TABLE 33. MAXIMUM HEART RATES

	Basal	Midnight	04h00	08h00
Normal subjects (n = 11)	72(9)	67(10)	70(12)	76(13)
"Dippers" (n = 6)	72(9)	64(11)	64(11)	80(11)
"Non-Dippers" (n = 7)	79(9)	74(5)	75(4)	82(9)

Values are mean(SD) beats/min

TABLE 34. MINIMUM HEART RATES

	Basal	Midnight	04h00	08h00
Normal subjects (n = 11)	54(8)	51(9)	50(8)	57(11)
"Dippers" (n = 6)	52(7)	50(10)	48(7)	56(10)
"Non-Dippers" (n = 7)	56(12)	55(8)	55(7)	59(12)

Values are mean(SD) beats/min

TABLE 35. MEAN HEART RATES

	Basal	Midnight	04h00	08h00
Normal subjects (n = 11)	62(9)	59(8)	59(10)	65(11)
"Dippers" (n = 6)	60(9)	56(11)	56(11)	66(11)
"Non-Dippers" (n = 7)	67(11)	65(6)	65(5)	72(9)

Values are mean(SD) beats/min

TABLE 36. MAGNITUDE OF RESPIRATORY SINUS ARRHYTHMIA

	Basal	Midnight	04h00	08h00
Normal subjects (n = 11)	18(6)	16(4)	21(6)	19(9)
"Dippers" (n = 6)	21(6)	14(5)	17(6)	26(6)
"Non-Dippers" (n = 7)	23(7)	18(5)	20(6)	22(9)

Values are mean(SD) beats/min

Details of corticosteroid therapy in the asthmatic patients:

None of the asthmatic patients had been treated with systemic corticosteroids for at least 2 years prior to the study. Two asthmatic "dippers" had received short courses of systemic corticosteroids during childhood and 2 asthmatic "non-dippers" had received short courses 2 and 3 years prior to the study respectively. A further asthmatic "non-dipper" had received systemic corticosteroid therapy for 2 years 14 years prior to the study.

Regular beclomethasone dipropionate aerosol therapy had been used for at least 2 years prior to the study by 3 of the asthmatic "dippers" in doses ranging from 200-400/ug/day and 2 of the asthmatic "non-dippers" (dose in each 300/ug/day). One asthmatic "dipper" had used the compound regularly for less than a month (maximum dose 500/ug/day) and 4 "non-dippers" had used it irregularly for less than a month prior to the study. Beclomethasone therapy was discontinued on the morning of the study.

11.5 DISCUSSION

The most important finding in the present study was that of the occurrence of extremely low midnight plasma cortisol levels in the asthmatic patients with "morning dipping". The results are at variance with those of Postma et al. (1985), who found no abnormality in the nocturnal plasma cortisol levels, but lower than normal nocturnal urinary catecholamine levels in a group of patients with airway obstruction and significant fluctuations in

airway calibre. The patients in their study, however, were middle-aged non-atopic individuals with "chronic partially reversible airway obstruction", a significant number of whom were smokers, and it is possible that they represented a different population of patients.

The finding of low midnight plasma cortisol levels in the "dippers" clearly requires careful analysis in the light of their use of corticosteroid medications. As only two of the "dippers" had received short courses of systemic corticosteroids some years prior to the study, it is primarily the effect of inhaled beclomethasone dipropionate on hypothalamic-pituitary-adrenal function that is of direct relevance in the interpretation of the present results.

The greater proportion of any drug given by oral inhalation is deposited in the mouth and throat and subsequently swallowed. Although beclomethasone dipropionate is actively absorbed from the gastrointestinal tract (Martin et al. 1974), it has been claimed that it is relatively lacking in systemic corticosteroid effects, possibly due to its rapid conversion in the liver to inactive metabolites (Harris, 1975). It is also possible that beclomethasone may have a considerably less profound depressant effect on the anterior pituitary gland than other glucocorticoids (Mahmoud et al. 1984).

An extremely important study in relation to the present findings has recently been reported suggesting that children using relatively large doses of beclomethasone dipropionate (300-1300/ug/day) may have nocturnal suppression of cortisol secretion

(Law et al. 1986). Clinical data, with special reference to "dipping", were not given, however, neither were plasma ACTH levels reported. The possibility cannot, therefore, be excluded that the findings of these authors reflect the occurrence of a similar phenomenon to that seen in the present study, and that this is directly related in some way to the disease itself, rather than being a side effect of beclomethasone therapy.

While previous studies have suggested the occurrence in children of some depression of plasma cortisol levels by beclomethasone dipropionate used in doses ranging from 300-800 /ug/day (Wyatt et al. 1978; Vaz et al. 1982; Pijls and Driessen, 1984), most studies in children using the compound in similar doses have provided no support for the occurrence of hypothalamic-pituitary-adrenal suppression (Godfrey and König, 1974; Francis, 1976; Kerrebijn, 1976; Klein et al. 1977; Goldstein and König, 1983). Although many of these earlier studies did not specifically focus on nocturnal or midnight cortisol levels, a recent investigation of hypothalamic-pituitary-adrenal function by means of saliva cortisol concentrations in children using 400/ug/day of beclomethasone dipropionate showed no evidence of suppression, nor any difference between users and non-users of the compound in midnight saliva cortisol levels (Williams et al. 1984). It is interesting, moreover, that in a study which showed minor differences between non-users and long-term users of beclomethasone dipropionate in the early morning plasma cortisol levels, there was no difference between the groups in the

midnight values (Pijls and Driessen, 1984). Studies in adults using beclomethasone dipropionate have demonstrated the occurrence of hypothalamic-pituitary-adrenal suppression with 1600-2000/ug/day (Choo-Kang et al. 1972; Vlasses et al. 1981; Sherman et al. 1982; Smith and Hodson, 1983), but not with doses similar to those used by the patients in the present study (British Thoracic and Tuberculosis Association, 1975; Sherman et al. 1982; Smith and Hodson, 1983). The compound appears, moreover, to have no effect on nocturnal cortisol levels (Sherman et al. 1982).

The asthmatic "dippers" in the present study were adolescents or adults, only two-thirds of whom were using beclomethasone dipropionate (200-500/ug/day, mean 300/ug/day). There is no convincing evidence of hypothalamic-pituitary-adrenal suppression occurring in adults in association with doses of this magnitude. In addition, there was no significant difference between the midnight plasma cortisol levels of the asthmatic "non-dippers" and those of the normal subjects, despite the use in a number of the former group of beclomethasone dipropionate. Furthermore, a strong inverse correlation appeared to exist between the midnight plasma cortisol levels and the magnitude of the fall in PEFR in the asthmatic patients ("dippers" plus "non-dippers").

Another important piece of evidence mitigating against a pituitary-depressant effect of beclomethasone in the "dippers" is the finding in them of normal plasma ACTH levels. ACTH is secreted in a pulsatile fashion with a delay between the

occurrence of a pulse and the subsequent cortisol response (Daughaday, 1985). It is difficult, therefore, to clearly interpret the relationship between the levels of these two hormones when obtained from a single blood sample. In addition, the responses to insulin and metyrapone are considerably more sensitive tests of hypothalamic-pituitary-adrenal function. The finding, however, of plasma ACTH levels in the normal range makes it somewhat less likely that pituitary function was suppressed by beclomethasone in the "dippers". It must be emphasised, however, that as more of the "dippers" than the "non-dippers" had been using regular beclomethasone, the possibility cannot completely be excluded that the low midnight plasma cortisol levels seen in the former subgroup were related to this therapy.

In contrast to the results of Barnes et al. (1980b), no statistically significant diurnal variation in the plasma epinephrine levels was demonstrable in any group of subjects during the study. Although the asthmatic "dippers" appeared to exhibit the greatest degree of variation in this variable, it appears unlikely that the small changes which occurred were closely related to the large fluctuations in simultaneously recorded expiratory flow rates which were observed in this group of subjects. It is noteworthy that in a recent study (Bolli et al. 1984), the plasma epinephrine levels of normal human subjects were shown to commence their rise from nocturnal nadirs before 04h00. The number of estimations of plasma catecholamine levels which could be performed in the present study was limited by cost, and the measurement performed at 04h00 may have missed the

early morning nadir, resulting in an underestimation of the degree of variation in the plasma epinephrine level. A further shortcoming of the p. esent study was the inability, for logistic reasons, to have the subjects spend a night becoming accustomed to the room where they slept. It is possible that more pronounced nadirs in the plasma epinephrine levels may have occurred had the subjects been studied dur'ng a more relaxed second night in the room.

Of the different groups of subjects only the "dippers" exhibited significant variations in each of the ECG variables analysed, during the study, the most marked being that in maximum heart rate which, in turn, appeared to result in corresponding changes in the magnitude of respiratory sinus arrhythmia. Heart rate is at any time the net result of both sympathetic and parasympathetic factors. It is clearly difficult, therefore, to attribute the diminution in maximum heart .ate which occurred at midnight and 04h00 in this group of subjects to an increase in parasympathetic neural output. Furthermore, vagal activity has been shown to be directly related to the magnitude of respiratory sinus arrhythmia (Katona et al. 1970; Katona and Jih, 1975) which actually diminished at these times in association with decreased maximum heart rate. There was no difference between the different groups of subjects in any of the ECG variables analysed, nor any correlation with the magnitude of the fall in PEFR in the combined subgroups of asthmatic patients. It is difficult, however, to completely exclude the possibility that a nocturnal increase in parasympathetic neural output was related to the

early morning diminution in airway calibre which occurred in the "dippers". Other workers have demonstrated the occurrence of an increased magnitude of respiratory sinus arrhythmia in older non-atopic patients with "chronic partially reversible airway obstruction" although the differences between the patients and the control subjects were relatively small (Postma et al. 1985). It is possible that heart rate variations would, likewise, have been more marked in all the groups of subjects during a more relaxed second night in the study-room.

It has been suggested that the nocturnal nadirs in plasma cortisol and epinephrine levels may not only have a direct effect on the airway smooth muscle of asthmatic patients, but may also predispose to the release of mediators from mast cells (Barnes, 1985). The latter is inhibited by beta-adrenergic agonists (Butchers et al. 1980; Peters et al. 1982; Hughes et al. 1983) and is inversely related to the intracellular level of 3,5 cyclic AMP (Austen, 1974), and therefore, to the state of beta-receptor "activation". In the same way that low plasma cortisol levels could diminish the "sensitivity" of airway beta-receptors, they could simultaneously affect those of mast cells in a similar manner. There was, however, no significant variation in the serum neutrophil chemotactic activity in any group of subjects, nor any difference between the groups at any time in the study.

The possible mechanisms and time-course of the glucocorticoid effect on beta-adrenergic receptor function have been extensively reviewed (Chapter 6.9). It has been suggested that glucocorticoid deficiency may result in a state of beta-

receptor "resistance" (Ramey and Goldstein, 1957). The experimental evidence reviewed in Chapter 6 is compatible with the theoretical concept that an exaggerated midnight trough in plasma cortisol levels could precipitate a deterioration in pulmonary function within a few hours by the induction of a state of beta-adrenergic subsensitivity.

If "morning dipping" is related to excessively low midnight plasma cortisol levels, it should be preventable by corticosteroid therapy. It has been shown, however, that infusions of corticosteroids do not abolish "morning dipping" (Soutar et al. 1975). However, the fluorometric method used in this study to determine the adequacy of the corticosteroid doses is subject to spurious elevations related to various medications (Bondy, 1985) and it is noteworthy, in addition, that the values obtained by this method in patients with adrenal insufficiency, were considerably higher than the midnight cortisol levels in the "dippers" in the present study (Mattingly, 1962). In addition, the fact that the cortisol infusion did not abolish the diurnal variation in the plasma cortisol level in some of the patients suggests the possibility that the doses may have been too low. Clearly, a similar study should be undertaken, using the more sensitive radioimmunoassay method of plasma glucocorticoid measurement.

The possibility that patients with asthma may have abnormal plasma corticosteroid levels has long been the subject of a controversy. A number of early studies suggested that asthmatic patients have abnormal adrenal cortical function (Eriksson-Lihr,

1951; Rose et al. 1955; Vaccarezza, 1961). These were, however, based largely on the analysis of urinary corticosteroids which is a poor method of assessing adrenal cortical function. Later investigators suggested that the adrenal cortical function of asthmatic patients was, in fact, normal (Blumenthal et al. 1966; Collins et al. 1975). However, it has been claimed that asthmatic patients who have been off all corticosteroid therapy for at least 6 months, as well as those never treated with it, have lower plasma cortisol levels than normal subjects (Weller et al. 1968; Morris et al. 1978), and in addition, asthmatic children do not appear to exhibit a significant plasma cortisol response to the stress of severely symptomatic asthma (Kumar et al. 1971). A problem with many of these earlier investigations may also have been the relatively insensitive methods of glucocorticoid assay used.

A more recent study using a sensitive radioimmunoassay for plasma cortisol determinations, has reported that the morning plasma cortisol levels, as well as the responses to metyrapone and ACTH in asthmatic patients do not differ from those of normal subjects (Morrish et al. 1979). The subject has been further complicated by recent reports that the fall in FEV_1 associated with late, but not immediate, asthmatic reactions is accompanied by a significant fall in plasma cortisol levels (Nakazawa et al. 1985; Nakazawa et al. 1986). This unexplained but interesting finding, as well as the results of the present study, indicate the need for further investigation into the relationship between the plasma corticosteroid levels and bronchospasm occurring under

various clinical and experimental conditions. In addition, it is, clearly, extremely important that the effect of beclomethasone dipropionate therapy on hypothalamic-pituitary-adrenal function should be elucidated.

REFERENCES

Aarons RD, Molinoff PB. Changes in the density of beta adrenergic receptors in rat lymphocytes, heart and lung after chronic treatment with propranolol. J Pharmacol Exp Ther 1982; 221: 439-443.

Aarons RD, Nies AS, Gal J, Hegstrand LR. Elevation of β-adrenergic receptor density in human lymphocytes after propranolol administration. J Clin Invest 1980; 65: 949-957.

Abraham GE, Manlimos FS, Garza R. Radioimmunoassay of steroids. In: Handbook of Radioimmunoassay. Abraham GE ed. Marcel Dekker Inc, New York, Basel: 1977; 591-656.

Adelstein RS, Conti MA, Hathaway DR. Phosphorylation of smooth muscle myosin light chain kinase by the catalytic subunit of adenosine 3':5'-monophosphate-dependent protein kinase. J Biol Chem 1978; 253: 8347-8350.

Adelstein RS, Hathaway DR. Role of calcium and cyclic adenosine 3':5' monophosphate in regulating smooth muscle contraction. Mechanisms of excitation-contraction coupling in smooth muscle. Am J Cardiol 1979; 44: 783-787.

Adolphson RL, Abern SB, Townley RG. Human and guinea pig respiratory smooth muscle - Demonstration of alpha-adrenergic receptors. J Allergy 1971; 47: 110-111.

Afifi AA, Azen SP. Regression and correlation analysis. In: Statistical Analysis: A Computer Orientated Approach. Academic Press, New York, London: 1979; 123-196.

Ahlquist RP. A study of the adrenotropic receptors. Am J Physiol 1948; 153: 586-600.

Ahlquist RP. The adrenergic receptor. J Pharm Sci 1966; 55: 359-367.

Al-Bazzaz FJ, Kelsey JG, Kaage WD. Substance P stimulation of chloride secretion by canine tracheal mucosa. Am Rev Respir Dis 1985; 131: 86-89.

Altiere RJ, Diamond L. Comparison of vasoactive intestinal peptide and isoproterenol relaxant effects in isolated cat airways. J Appl Physiol 1984; 56: 986-992.

Altounyan REC. Variation of drug action on airway obstruction in man. Thorax 1964; 19: 406-415.

American Thoracic Society Statement: Chronic bronchitis, asthma and pulmonary emphysema. Am Rev Respir Dis 1962; 85: 762-768.

Andersson RG, Kovesi G, Ericsson E. Beta-adrenoceptor stimulation and cyclic AMP levels in bovine tracheal muscle of old and young animals. Acta Pharmacol Toxicol (Kbh) 1978; 43: 323-327.

Arnold A, McAuliff JP, Colella DF, O'Connor WV, Brown TG. The β-2 receptor mediated glycogenolytic responses to catecholamines in the dog. Arch Int Pharmacodyn Ther 1968; 176: 451-457.

Assimacopoulos-Jeannet FD, Blackmore PF, Exton JH. Studies on α-adrenergic activation of hepatic glucose output. Studies on role of calcium in α-adrenergic activation of phosphorylase. J Biol Chem 1977; 252: 2662-2669.

Austen KF. Reaction mechanisms in the release of mediators of immediate hypersensitivity from human lung tissue. Fed Proc 1974; 33: 2256-2262.

Axelrod J, Weinshilboum R. Catecholamines. N Engl J Med 1972; 287: 237-243.

Babcock DF, Chen J-LJ, Yip BP, Lardy HA. Evidence for mitochondrial localization of the hormone-responsive pool of Ca^{2+} in isolated hepatocytes. J Biol Chem 1979; 254: 8117-8120.

Baker AP, Hillegass LM, Holden DA, Smith WJ. Effect of kallidin, substance P, and other basic polypeptides on the production of respiratory macromolecules. Am Rev Respir Dis 1977; 115: 811-817.

Baker SP, Potter LT. Effect of propranolol on β-adrenoceptors in rat hearts. Br J Pharmacol 1980; 68: 8-10.

Bandouvakis J, Cartier A, Roberts R, Ryan G, Hargreave FE. The effect of ipratropium and fenoterol on methacholine- and histamine-induced bronchoconstriction. Br J Dis Chest 1981; 75: 295-305.

Barnes P, Brown M, Dollery C. Letter. N Engl J Med 1980a; 303: 1301.

Barnes P, FitzGerald G, Brown M, Dollery C. Nocturnal asthma and changes in circulating epinephrine, histamine and cortisol. N Engl J Med 1980b; 303: 263-267.

Barnes PJ. Asthma as an axon reflex. Lancet 1986; i: 242-245.

Barnes PJ. Beta-adrenoceptors in lung tissue. In: Beta-adrenoceptors in Asthma. Morley J ed. Academic Press. London, Orlando, San Diego, New York. 1984; 67-90.

Barnes PJ. Circadian variation in airway function. Am J Med 1985; 79 (Suppl 6A) 5-9.

Barnes PJ. Pathogenesis of asthma: A review. J R Soc Med 1983; 76: 580-586.

Barnes PJ, Basbaum CB, Nadel JA. Autoradiographic localization of autonomic receptors in airway smooth muscle. Marked differences between large and small airways. Am Rev Respir Dis 1983a; 127: 758-762.

Barnes PJ, Basbaum CB, Nadel JA, Roberts JM. Localization of adrenoreceptors in mammalian lung by light microscopic autoradiography. Nature 1982a; 299: 444-447.

Barnes PJ, Basbaum CB, Nadel JA, Roberts JM. Pulmonary α-adrenoceptors: autoradiographic localization using [^{3}H]Prazosin. Eur J Pharmacol 1983b; 88: 57-62.

Barnes PJ, Brown MJ, Dollery CT, Fuller RW, Heavey DJ, Ind PW. Histamine is released from skin by substance P but does not act as the final vasodilator in the axon reflex. Br J Pharmacol 1986a; 88: 741-745.

Barnes PJ, Brown MJ, Silverman M, Dollery CT. Circulating catecholamines in exercise and hyperventilation induced asthma. Thorax 1981a; 36: 435-440.

Barnes PJ, Conradson T-BG, Dixon CMS, Fuller RW. A comparison of the cutaneous actions of substance P, neurokinin A and calcitonin gene-related peptide in man. J Physiol (Lond) 1986b; 374: 48P.

Barnes PJ, Cuss FM, Palmer JB. The effect of airway epithelium on smooth muscle contractility in bovine trachea. Br J Pharmacol 1985; 86: 685-691.

Barnes PJ, Dixon, CMS. The effect of inhaled vasoactive intestinal peptide on bronchial reactivity to histamine in humans. Am Rev Respir Dis 1984; 130: 162-166.

Barnes PJ, Dollery CT, MacDermot J. Increased pulmonary α-adrenergic and reduced β-adrenergic receptors in experimental asthma. Nature 1980c; 285: 569-571.

Barnes PJ, Fitzgerald GA, Dollery CT. Circadian variation in adrenergic responses in asthmatic subjects. Clin Sci 1982b; 62: 349-354.

Barnes PJ, Ind PW, Brown MJ. Plasma histamine and catecholamines in stable asthmatic subjects. Clin Sci 1982c; 62: 661-665.

Barnes PJ, Ind PW, Dollery CT. Inhaled prazosin in asthma. Thorax 1981b; 36: 378-381.

Barnes PJ, Karliner JS, Dollery CT. Human lung adrenoreceptors studied by radioligand binding. Clin Sci 1980d; 58: 457-461.

Barnes PJ, Nadel JA, Roberts JM, Basbaum CB. Muscarinic receptors in lung and trachea: autoradiographic localization using [^{3}H]quinuclidinyl benzilate. Eur J Pharmacol 1983c; 86: 103-106.

Barnes PJ, Nadel JA, Skoogh B-E, Roberts JM. Characterization of beta adrenoceptor subtypes in canine airway smooth muscle by radioligand binding and physiological responses. J Pharmacol Exp Ther 1983d; 225: 456-461.

Barnes PJ, Skoogh B-E, Brown JK, Nadel JA. Activation of α-adrenergic response in tracheal smooth muscle: a postreceptor mechanism. J Appl Physiol 1983e; 54: 1469-1476.

Barnes PJ, Skoogh B-E, Nadel JA, Roberts JM. Postsynaptic alpha2-adrenoceptors predominate over alpha1-adrenoceptors in canine tracheal smooth muscle and mediate neuronal and hormonal alpha-adrenergic contraction. Mol Pharmacol 1983f; 23: 510-515.

Barnes PJ, Wilson NM, Vickers H. Prazosin, an alpha1-adrenoceptor antagonist, partially inhibits exercise-induced asthma. J Allergy Clin Immunol 1981c; 68: 411-415.

Becker EL, Showell HJ, Henson PM, Hsu LS. The ability of chemotactic factors to induce lysozomal enzyme release. I. The characteristics of the release, the importance of surfaces and the relation of enzyme release to chemotactic responsiveness. J Immunol 1974; 112: 2047-2054.

Beil M, de Kock MA. Role of alpha-adrenergic receptors in exercise-induced bronchoconstriction. Respiration 1978; 35: 78-86.

Bell RM. Protein kinase C activation by diacylglycerol second messengers. Cell 1986; 45: 631-632.

Bennett T, Farquhar IK, Hosking DJ, Hampton JR. Assessment of methods for estimating autonomic nervous control of the heart in patients with diabetes mellitus. Diabetes 1978; 27: 1167-1174.

Berkin KE, Inglis GC, Ball SG, Thomson NC. Effect of low dose adrenaline and noradrenaline infusions on airway calibre in asthmatic patients. Clin Sci 1986; 70: 347-352.

Bernstein RA, Linarelli L, Facktor MA, Friday GA, Drash AL, Fireman P. Decreased urinary adenosine 3',5' monophosphate (cyclic AMP) in asthmatics. J Lab Clin Med 1972; 80: 772-779.

Berridge MJ. Inositol triphosphate and diacylglycerol as second messengers. Biochem J 1984; 220: 345-360.

Berridge MJ. Rapid accumulation of inositol triphosphate reveals that agonists hydrolyse polyphosphoinositides instead of phosphatidylinositol. Biochem J 1983; 212: 849-858.

Berson SA, Yalow RS. Radioimmunoassay of ACTH in plasma. J Clin Invest 1968; 47: 2725-2751.

Berthelsen S, Pettinger WA. A functional basis for classification of α-adrenergic receptors. Life Sci 1977; 21: 595-606.

Besse JC, Bass AD. Potentiation by hyd[illegible]sone of responses to catecholamines in vascular smooth [illegible] J Pharmacol Exp Ther 1966; 154: 224-238.

Bianco S, Griffin JP, Kamburoff PL, Prime FJ. Prevention of exercise-induced asthma by Indoramin. Br Med J 1974; 4: 18-20.

Billah MM, Michell RH. Phosphatidylinositol metabolism in rat hepatocytes stimulated by glycogenolytic hormones. Effects of angiotensin, vasopressin, adrenaline, ionophore A23187 and calcium-ion deprivation. Biochem J 1979; 182: 661-668.

Black JL, Temple DM, Anderson SD. Long-term trial of an alpha adrenoceptor blocking drug (Indoramin) in asthma. A preliminary report. Scand J Respir Dis 1978; 59: 307-312.

Blackard WG, Heidingsfelder SA. Adrenergic receptor control mechanism for growth hormone secretion. J Clin Invest 1968; 47: 1407-1414.

Blackmore PF, Brumley FT, Marks JL, Exton JH. Studies on α-adrenergic activation of hepatic glucose output. Relationship between α-adrenergic stimulation of calcium efflux and activation of phosphorylase in isolated rat liver parenchymal cells. J Biol Chem 1978; 253: 4851-4858.

Blackmore PF, Exton JH. α1-Adrenergic stimulation of Ca^{2+} mobilization without phosphorylase activation in hepatocytes from phosphorylase b kinase-deficient gsd/gsd rats. Biochem J 1981; 198: 379-383.

Blackwell GJ, Carnuccio R, Di Rosa M, Flower RJ, Parente L, Persico P. Macrocortin: a polypeptide causing the anti-phospholipase effect of glucocorticoids. Nature 1980; 287: 147-149.

Blackwell GJ, Flower RJ, Nijkamp FP, Vane JR. Phospholipase A2 ativity of guinea-pig isolated perfused lungs: Stimulation and inhibition by anti-inflammatory steroids. Br J Pharmacol 1978; 62: 79-89.

Blecher M, Lewis S, Hicks JM, Josephs S. Beta-blocking autoantibodies in pediatric bronchial asthma. J Allergy Clin Immunol 1984; 74: 246-251.