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The autonomic nervous system and bronchial asthma

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Experimental evidence obtained from studies of cultured cells suggests that specific catecholamine-induced receptor "downregulation" occurs in the later stages of a multi-step process (Su et al. 1980). Thus, following the incubation of astrocytoma cells with isoproterenol, a rapid attenuation in the catecholamine-stimulated 3,5 cyclic AMP accumulation occurs ($t^1/2$=3 minutes), associated with a marked reduction in the ability of isoproterenol to bind to the beta-receptors with high affinity. Exposure of the cells to the agonist for approximately 45 minutes appears to produce no change in beta-receptor numbers despite a decline in 3,5 cyclic AMP responsiveness to about half of the control level. Prolongation of the exposure beyond 2 hours, however, produces a r pid and progressive loss of receptors. While cells exposed to isoproterenol for less than 60 minutes recover their responsiveness rapidly upon its removal ($t^1/2$=7 minutes), those exposed for 24 hours appear to retain little potential for recovery, and the degree, moreover, to which the cells recover their responsiveness appears to be related to the extent of receptor loss.

It has also been demonstrated that astrocytoma cells incubated for only 15 minutes with isoproterenol can be resolved into a normal population and one that can only bind to agonists with a low affinity, and it has been suggested that this acute catecholamine-induced desensitisation may be related to a functional "uncoupling" of the receptors from adenyl cyclase (Harden et al. 1980). The occurrence of both beta-receptor "downregulation" and functional "uncoupling" have been

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demonstrated in rat lung membranes in association with desensitisation induced by the in vivo administration of the selective beta-2 agonist metaproterenol (Scarpace and Abrass, 1982). Similar results have been obtained in human neutrophils following incubation with isoproterenol (Davies and Lefkowitz, 1983).

The "downregulation" of receptors in association with catecholamine-induced desensitisation may result from their removal from the cell membrane into the interior of the cell (Chuang and Costa, 1979). These relocated receptors can subsequently be recovered from intracellularly sequestered vesicles in frog erythrocytes (Stadel et al. 1983b), and appear to be functionally intact, retaining their response to beta-adrenergic stimulation after fusion with Xenopus laevis erythrocytes which characteristically lack beta-receptors, but possess adenyl cyclase which is responsive to prostaglandin E_1 (Strulovici et al. 1983). It appears, therefore, that this form of desensitisation may be related to physical separation from the other components of the adenyl cyclase system rather than to any functional alteration in the receptors themselves (Stadel et al. 1983; Strulovici et al. 1983). While these internalised receptors retain the ability to recycle to the cell surface and reassociate with the other components of the adenyl cyclase system following the removal of the agonist, prolonged exposure may, on occasion, result in degradation, possibly by lysosomal proteases (Harden, 1983).

In avian erythrocytes desensitisation to catecholamines

appears to involve mainly the functional "uncoupling" of beta-receptors, as manifested by their inability to combine with agonists with high affinity (Stadel et al. 1981). The reaction appears to be dependent on 3,5 cyclic AMP and the beta-receptors appear to be phosphorylated during this process, as assessed by increased labelling with P^{32} (Stadel et al. 1983a). It has been suggested that this reaction is mediated by a 3,5 cyclic AMP-dependent protein kinase (Lefkowitz et al. 1984).

It must be emphasised that the biochemical mechanisms underlying these different processes remain largely undefined. Detailed in vivo correlates of these mechanisms are, moreover, for the most part, lacking.

6.2 ANTAGONIST-INDUCED RECEPTOR SENSITISATION

There is little doubt that a "withdrawal" syndrome occurs in patients following the abrupt cessation of therapy with beta-adrenergic antagonists, and is characterised by arrhythmias and ischaemic events (Shand and Wood, 1978). Normal subjects to whom propranolol has been administered for 2 days and then abruptly discontinued, subsequently display increased inotropic and chronotropic responses to isoproterenol suggesting a possible increase in beta-receptor numbers (Boudoulas et al. 1977).

This hypothesis has been supported by the results of a number of studies. The density of myocardial beta-receptors has been shown to double in rats following 2 weeks of propranolol administration (Glaubiger and Lefkowitz, 1977). A similar effect

has been observed in lymphocytes obtained from humans treated with propranolol for 8 days and is associated with hypersensitivity of beta-adrenergically mediated haemodynamic responses (Aarons et al. 1980). In addition, propranolol has been shown to induce an increase in the density of beta-receptors in the lymphocytes, lungs and hearts of rats (Aarons and Molinoff, 1982), although these results could not be confirmed in a rat heart model by other workers (Baker and Potter, 1980; Stiles et al. 1984). The mechanisms responsible for the "propranolol withdrawal" syndrome have not yet, therefore, been defined with complete certainty.

6.3 THE EFFECT OF DENERVATION

The chemical or surgical denervation of adrenergically innervated tissue may result in increased catecholamine responses. A marked increase in the number of beta-receptors in rat heart has been demonstrated following denervation produced by the systemic administration of 6-hydroxydopamine (Yamada et al. 1980; Kajiyama et al. 1982). This appears to be due to an increase in the number of beta-1 receptors only (Kajiyama et al. 1982). Similarly, the depletion of the catecholamine stores in rats by chronic guanethidine administration results in an increase in the number of cardiac beta-receptors associated with an augmentation in the 3,5 cyclic AMP response to isoproterenol (Glaubiger et al. 1978).

6.4 THE EFFECT OF AGE

A number of studies have suggested that catecholamine-mediated cardiovascular responses may diminish with age (Vestal et al. 1979; Lakatta, 1980). The 3,5 cyclic AMP content of bovine tracheal smooth muscle is considerably higher in young than in old animals, and the 3,5 cyclic AMP response of the tissue to isoproterenol appears, likewise, to be age-dependent (Andersson et al. 1978). The data from another study, however, have suggested that the basal intracellular 3,5 cyclic AMP levels in the leukocytes from normal humans show an increase with advancing age despite a diminished response to the beta-2 agonist, terbutaline (Pauwels and van der Straeten. 1980).

A study of rat adipocyte beta-receptors has suggested that their number declines markedly with age, with a parallel diminution in adenyl cyclase responsiveness, while the binding affinity of the receptors remains unaffected (Giudicelli and Pecquery, 1978). Studies using the maturation of rat reticulocytes to erythrocytes as a model of advancing cellular age have also demonstrated that this process is associated with diminished beta-receptor numbers paralleled by an attenuation in adenyl cyclase responsiveness. In addition, the ability of receptor occupancy by agonists to promote combination with the guanine nucleotide regulatory protein is lost in the mature erythrocytes (Limbird et al. 1980b).

Although diminished adenyl cyclase responsiveness associated with advancing age has been demonstrated in a wide variety of

tissues, it has not been possible to correlate this consistently with a decrease in the number of beta-receptors (Stiles et al. 1984), and the mechanisms underlying this phenomenon may vary in different tissues. It has recently been suggested that the altered beta-adrenergic sensitivity in lymphocytes from aged human subjects is related to an alteration in the receptor affinity for agonists (Feldman et al. 1984).

6.5 THE EFFECT OF THE CIRCADIAN RHYTHM

Pulmonary function exhibits a significant circadian variation in normal human subjects with a diminution of airway calibre occurring in the early morning hours (Kerr, 1973; Gaultier et al. 1977; Hetzel a. Clark, 1980). The fact that this can be abolished by beta-adrenergic stimulation or by cholinergic antagonists suggests its possible relation to a circadian autonomic rhythm (Gaultier et al. 1977).

A circadian rhythm has also been demonstrated in the basal 3,5 cyclic AMP levels of normal human leukocytes, which are significantly lower at 16h00 and midnight than at 08h00 and noon (Pauwels and van der Straeten, 1980), and a significant circadian variation also occurs in the number of beta-receptors in normal human lymphocytes (Titinchi et al. 1984). Radioligand studies have demonstrated the existence of a marked circadian fluctuation in the numbers of both alpha- and beta-adrenergic and muscarinic receptors in the brains of rats, which can be abolished by ablation of the suprachiasmatic nuclei (Kafka et al. 1983).

6.6 THE EFFECT OF CARDIOVASCULAR DISEASE

Various diseases of the cardiovascular system, including ischaemia, hypertension, cardiac hypertrophy and cardiac failure, have been shown to be associated with alterations in beta-receptor number and possibly function, mainly affecting the heart (Lefkowitz et al. 1984; Stiles et al. 1984). These will not, however, be dealt with in this review.

6.7 THE EFFECT OF THYROID HORMONES

There is a large body of data addressing the subject of the effect of thyroid hormones on the number and function of beta-adrenergic receptors in different tissues. The general principle that has emerged is that hyperthyroidism is associated in most tissues with an increase, and hypothyroidism with a decrease, in the number of receptors (Stiles et al. 1984).

Thyroxine has been shown to produce a rapid increase in the number of beta-receptors in rat heart reaching a maximum after 2 hours. This is followed by a period of slower receptor "upregulation", reaching a maximum after about 15 hours. Whereas the latter slow phase can be completely blocked by cycloheximide, an inhibitor of protein synthesis, the earlier rapid phase appears to be unaffected by it (Kempson et al. 1978). It has been suggested that thyroxine stimulates both the insertion into the cell membrane of a plasma pool of preformed receptors and the synthesis of a new receptor population (Kempson et al. 1978). The

treatment of rats with thyroxine appears to induce not only an increase in receptor numbers, but also an increase in the affinity with which they bind to isoproterenol (Stiles and Lefkowitz, 1981). It has also been demonstrated that hyperthyroidism is associated with an increase and hypothyroidism with a decrease in the affinity of isoproterenol for the beta-receptors in rat adipocyte membranes, the modulatory effect of guanine nucleotides on receptor affinity being, moreover, lost in both states (Malbon, 1980).

The effect of thyroid hormones on pulmonary and airway beta-receptors remains uncertain. Triiodothyronine appears to have no effect on the density of either lymphocyte or lung receptors in rats (Scarpace and Abrass, 1981). Thyroid hormones may, on the other hand, have a significant effect on the maturing lung, as demonstrated by the marked diminution in the number of beta-receptors in propylthiouracil-treated neonatal rats, but not those treated with propylthiouracil combined with thyroxine (Whitsett et al. 1980).

6.8 THE EFFECT OF SEX HORMONES

The gonadal hormones may be involved in the dynamic regulation of adrenergic receptor density in various tissues. The heart rate response to isoproterenol is depressed by chronic oestrogen administration in ovariectomised female rats (Fregly and Thrasher, 1977). Moreover, progesterone, testosterone and 17-beta-oestradiol each have a significant potentiating effect on

the isoproterenol-induced relaxation of porcine bronchus (Foster et al. 1983). It has been demonstrated that intact female rats have reduced beta-adrenergic responses in cerebral cortical slices compared with male animals, and that this is associated with a diminished density of beta-receptors (Wagner and Davies, 1980). These differences may be reversed by ovariectomy and reoccur following the treatment of ovariectomised female rats with oestrogens (Wagner et al. 1979). In addition, the density of ovarian beta-receptors in rats varies considerably during the oestrus cycle, being higher during pre-oestrus than oestrus (Jordan, 1981). Testosterone appears to increase the number of renal beta-receptors in female mice (Petrovic et al. 1981).

6.9 THE EFFECT OF GLUCOCORTICOID HORMONES

The existence of an interaction between glucocorticoid hormones and adrenergic responses has been recognised for some time. Hydrocortisone has been shown to potentiate the catecholamine responses of mammalian vascular smooth muscle, in vivo and in vitro (Besse and Bass, 1966), and reversibly potentiates the cardiac stimulatory effects of isoproterenol - but not norepinephrine - on feline cardiac muscle (Kaumann, 1972). In addition, glucocorticoids increase the response of both animal and human airway smooth muscle to various catecholamines, the effect being most pronounced with isoproterenol (Townley et al. 1970; Pun et al. 1973; Geddes et al. 1974; Foster et al. 1983). Hydrocortisone stimulates the accumulation of 3,5 cyclic

AMP in the leukocytes of normal human subjects (Logsdon et al. 1972; Parker et al. 1973; Marone et al. 1980), as well as potentiating the 3,5 cyclic AMP response to catecholamines (Parker et al. 1973; Marone et al. 1980).

A number of studies have also demonstrated the attenuation by corticosteroids of catecholamine-induced desensitisation of the 3,5 cylic AMP response in various tissues, including rat lung (Scarpace and Abrass, 1982), human neutrophils (Davies and Lefkowitz, 1983), human lymphocytes (Hui et al. 1982) and pig skin (Iizuka and Ohkawara, 1983). It appears, in addition, that tachyphylaxis of inhibition by isoproterenol of methacholine-induced bronchospasm in dogs is significantly reversed by methylprednisone (Stephan et al. 1980). Likewise, in normal human subjects with attenuation of the bronchodilator responses to both inhaled and intravenous salbutamol, induced by regular inhalations of salbutamol, sensitivity to the beta-2 agonist is rapidly restored by hydrocortisone (Holgate et al. 1977).

The precise mechanism of the enhancement of catecholamine action by glucocorticoids remains uncertain, and a number of different hypotheses have been proposed in an attempt to explain it. It appears, however, that the effect is most likely to be related to one or more of the mechanisms dealt with below.

6.9.1. "Upregulation" of Receptors

A considerable amount of evidence supports the hypothesis that the facilitation by corticosteroids of the action of

catecholamines occurs as the result of an increase in the number of beta-receptors in cell membranes. Hydrocortisone induces a 100% increase in the density of beta-receptors in cultured human lung cells within 24 hours, the effect probably being related to increased synthesis and incorporation of the receptors into the cell membranes (Fraser and Venter, 1980). A number of studies in intact animals have confirmed this finding. Nine days of therapy with hydrocortisone has been shown to increase the density of beta-receptors in lung membranes from rats by 70% (Mano et al. 1979) and betamethasone to produce a 26% increase after only 24 hours (Salonen and Mattila, 1984). Furthermore, the administration of betamethasone to rabbits at 25 days of pregnancy stimulates a 75% increase in the concentration of pulmonary beta-receptors in the foetuses, compared with the foetuses of control animals (Cheng et al. 1980). It has also been shown in rats that receptor "downregulation" induced in lung tissue by the in vivo administration of metaproterenol can be prevented by simultaneous therapy with methylprednisolone (Scarpace and Abrass, 1982). A similar effect of glucocorticoids on beta-receptor numbers has been observed in a number of other cell types, including human neutrophils (Davies and Lefkowitz, 1980) and human astrocytoma cells (Foster and Harden, 1980), but not rat mast cells (Tolone et al. 1979). As opposed to the results obtained with human granulocytes, however, the incubation of human lymphocytes with cortisone acetate produces an initial acute fall in beta-receptor numbers after 4 hours, with a subsequent rise above the control values after 24 hours (Davies

and Lefkowitz, 1980).

6.9.2 Effects on Receptor Affinity for Agonists

The data from a number of studies have suggested that glucocorticoids increase the affinity with which beta-adrenergic receptors bind to agonists. The administration of cortisone to normal human subjects has been shown to increase the proportion of high affinity beta-receptors in neutrophils after 4 hours, and similar results have been obtained by the in vitro exposure of human neutrophils to glucocorticoids (Davies and Lefkowitz, 1981). It has also been demonstrated that catecholamine-induced desensitisation of the adenyl cyclase response in human neutrophils can be attenuated by incubation with hydrocortisone, and that this appears to be related to the prevention of functional "uncoupling", rather than to the reversal of receptor "downregulation" which may, in fact, persist (Davies and Lefkowitz, 1983).

6.9.3 Glucocorticoids and Phospholipase A2

A possible mechanism by which glucocorticoids may affect beta-receptor homeostasis involves the enzyme phospholipase A2. Various molecules, including concanavalin A and catecholamines, stimulate a series of methylation reactions, resulting in the conversion of the membrane phorpholipid, phosphatidyl-ethanolamine, to phosphatidylcholine. The latter compound is subsequently hydrolysed by phospholipase A2 with the generation of lysophosphatidylcholine and arachidonic acid (Hirata et al.

1979; Hirata and Axelrod, 1980). Arachidonic acid is the precursor of a number of mediators, and the lysolecithin product of this phospholipase A2-mediated reaction has been shown to have an inhibitory effect on cellular adenyl cyclase activity (Shier et al. 1976).

Isolated perfused guinea-pig lungs have a low background level of phospholipase A2 activity which can be increased by a number of stimuli, including trauma, antigen challenge and histamine. Both the basal and stimulated phospholipase A2 activity can be inhibited by dexamethasone and by the non-steroidal anti-inflammatory substance mepacrin (Blackwell et al. 1978). This glucocorticoid effect is associated with binding of the dexamethasone molecule to a cytosolic receptor site, and can be abolished or reduced by inhibitors of RNA or protein synthesis such as actinomycin D, puromycin and cycloheximide (Flower and Blackwell, 1979). Similarly, the incubation of rabbit peritoneal neutrophils with various glucocorticoids produces a decrease in the level of cellular phospholipase A2 activity, as measured by the release of 14C-labelled arachidonic acid from membrane phospholipids. This reaction can also be attenuated by actinomycin D and cycloheximide, and appears to be associated with the induction of a protein with the ability to inhibit phospholipase A2 (Hirata et al. 1980; Blackwell et al. 1980). This has been named "lipomodulin" (Hirata, 1981).

That phospholipase A2 may be associated with catecholamine-induced beta-receptor desensitisation is suggested by the demonstration that non-steroidal inhibitors of the enzyme block

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isoproterenol-induced desensitisation of 3,5 cyclic AMP production as well as the "downregulation" of beta-adrenergic receptors in cultured human astrocytoma cells, whereas activators produce a decreased 3,5 cyclic AMP response to isoproterenol (Mallorga et al. 1980). It has recently been demonstrated that the in vitro treatment of guinea-pig lung with phospholipase A2 results in a considerable decrease in both beta-receptor numbers and isoproterenol-stimulated adenyl cyclase activity (Taki et al. 1986). Furthermore, the "upregulation" of beta-adrenergic receptors by glucocorticoid hormones in cultured human lung adenocarcinoma cells can be totally inhibited by incubation with a monoclonal antibody to lipomodulin (Kuncs et al. 1985). It is possible, therefore, that glucocorticoid hormones may attenuate or reverse catecholamine-induced beta-receptor desensitisation by their inhibitory effect on phospholipase A2.

6.9.4 Effects Distal to the Receptor

A number of studies have suggested that the facilitatory action of glucocorticoids on catecholamine responses may be mediated by a mechanism distal to the site of agonist-receptor interaction (Exton et al. 1972; Tolone et al. 1979). It has been demonstrated that hepatic extracts from hydrocortisone-treated rats have diminished incorporation of p^{32}, as compared with control animals, into a protein which may be the regulatory subunit of type II 3,5 cyclic AMP-dependent protein kinase, suggesting an increased in vivo state of phosphorylation of the enzyme (Liu et al. 1981). The phosphorylated form of the

regulatory subunit of the enzyme has a reduced affinity for the catalytic subunit as compared with the non-phosphorylated form (Rangel-Aldao and Rosen, 1976). It is possible that glucocorticoid hormones may promote a state of phosphorylation of the regulatory subunit of protein kinase II, resulting in reduced affinity for the catalytic subunit, and, therefore, in diminished regeneration of the inactive form of the enzyme.

SECTION III

A DESCRIPTION OF THE SUBJECTS AND THEIR SELECTION

CHAPTER 7

SUBJECTS AND PULMONARY FUNCTION TESTS

In those studies, the role of the autonomic nervous system in the pathogenesis of bronchial asthma was investigated by comparing the autonomically-mediated responses of asthmatic patients and non-asthmatic control subjects to various physiological and pharmacological stimuli.

7.1 INCLUSION CRITERIA FOR ASTHMATIC PATIENTS

The asthmatic patients were recruited either from the Asthma Outpatients Clinic of the Johannesburg Hospital or from among the hospital staff and their friends and families. Each was a life-long non-smoker with a history of atopy. Each patient gave a definite history of bronchial asthma, as defined by the American Thoracic Society (1962), namely: a history of transitory or prolonged episodes of dyspnoea, cough and wheezing. Each asthmatic patient was otherwise healthy. No patient older than 30 years was included in any study.

Except where otherwise stated (Chapter 8.5), the diagnosis was confirmed in each patient by the finding of at least one of the following: (i) Airway obstruction with significant reversibility; (ii) Bronchial hyperreactivity.

(i) Airway obstruction with significant reversibility was defined as:

(a) a forced expiratory volume in 1 second (FEV_1) which was less than 70% of the predicted value and less than 75% of the forced vital capacity; AND

(b) an increase in the FEV_1 of 20% or more following the

inhalation of a sympathomimetic bronchodilator.

(ii) Bronchial hyperreactivity was defined as:

A fall of not less than 20% in the peak expiratory flow rate (PEFR) on exercise.

7.2 INCLUSION CRITERIA FOR NON-ASTHMATIC CONTROL SUBJECTS

The non-asthmatic control subjects were healthy individuals recruited from among the hospital staff and their friends and families. No subject older than 30 years was included in any study. Each subject was a life-long non-smoker who complied with each of the following requirements.

(i) The subject had a history negative for bronchial asthma as defined by the American Thoracic Society (1962).

(ii) Except where otherwise stated (Chapter 8.4 and Chapter 9.2), the subject had no history of atopy.

(iii) The subject had completely normal pulmonary function on objective testing.

(iv) The subject had no evidence of bronchial hyperreactivity as assessed by the response of the PEFR to exercise.

7.3 PULMONARY FUNCTION STUDIES

The indices of pulmonary function studied in each subject included lung volumes (total lung capacity, vital capacity, fincluded lung volumes (total lung capacity, vital capacity, expiratory manoeuvres (forced vital capacity, FEV_1, and a maximum

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7.3 PULMONARY FUNCTION STUDIES

The indices of pulmonary function studied in each subject included lung volumes (total lung capacity, vital capacity, functional residual capacity and residual volume); and forced expiratory manoeuvres (forced vital capacity, FEV_1, and a maximum

expiratory flow volume curve). Measurements were made using the Pulmolab 5300 (Cardiopulmonary Instruments, Houston, Texas, USA). In addition, airway resistance was measured in each subject using a total body plethysmograph (Pneumotest-Bodytest; Jaeger Instruments, Wurzburg, Federal Republic of Germany).

The results of the measurements of lung volumes were expressed as percentages of the predicted normal values based on the data of Goldman and Becklake (1959). The results of the measurements of the forced vital capacity and of maximal expiratory flow rates were expressed as percentages of the predicted normal values based on the data of Crapo et al. (1981) and Knudson et al. (1976).

In each of the asthmatic subjects the pulmonary function studies were performed during a stable phase of the disease and following the withholding of all bronchodilator therapy for at least 12 hours.

7.4 BRONCHOPROVOCATION EXERCISE TESTING

Except in the study described in Chapter 9.3, the response in the PEFR to exercise was assessed by means of a standardised exercise test. Each subject performed 8 minutes of treadmill running during which the electrocardiograph was continuously monitored and the rate and incline of the treadmill adjusted so that the heart rate attained the predicted maximum value based on the data in the review of Fortuin and Weiss (1977). The PEFR was measured at 2 mirute intervals during, and for 15 minutes after,

completion of the exercise, using a Wright's peak flow meter. Three readings of PEFR were taken at each time, the highest being used as the representative value. The percentage fall from the PEFR prior to exercise was calculated from the lowest representative value obtained during the test.

7.5 THE CONSTITUTION OF THE SUBJECT GROUPS IN THE DIFFERENT STUDIES

The asthmatic patients and the normal subjects were recruited from four separate groups of subjects. A few individuals were included in more than one group.

Those who participated in the studies described in Chapter 8 were recruited from one group. All of the subjects who took part in the study described in Chapter 8.6 were also included in the study described in Chapter 8.2.

The subjects who participated in the study described in Chapter 9.2 were recruited from a second group.

The subjects who participated in the study described in Chapter 9.3 were recruited from a third group.

The subjects who participated in the studies described in Chapters 10 and 11 were recruited from a fourth group. The asthmatic and normal populations in the studies described in these chapters were identically constituted, with the exception of 2 asthmatic patients who did not take part in that described in Chapter 10. In addition, the study described in Chapter 9.4 was performed simultaneously with that described in Chapter 11.

7.6 INFORMED CONSENT

The subjects, or in the case of minors, a parent or guardian each gave informed consent prior to participation in any investigation. The individual investigations were each approved by The Committee for Research on Human Subjects of the University of the Witwatersrand.

SECTION IV

STUDIES OF AUTONOMIC FUNCTION IN ASTHMATIC PATIENTS AND NON-ASTHMATIC CONTROL SUBJECTS

CHAPTER 8

STUDIES OF PARASYMPATHETIC RESPONSES

8.1 A REVIEW OF THE EXPERIMENTAL DATA LINKING PARASYMPATHETIC MECHANISMS TO THE PATHOGENESIS OF BRONCHIAL ASTHMA

Cholinergic agonists stimulate the contraction of smooth muscle in various sites, including the respiratory tract. In 1917 Eppinger and Hess suggested that the parasympathetic nervous system was involved in bronchial hyperreactivity in their description of an association betweer a state of "vagotonia" and bronchial asthma. Since this time a considerable amount of experimental evidence has been accumulated in support of the existence of a parasympathetic component in the bronchoconstriction which occurs under a variety of clinical and experimental circumstances. An extension of this observation is the concept that parasympathetic overactivity may be an important underlying pathogenetic mechanism in bronchial asthma.

8.1.1 Evidence of Parasympathetic Involvement in Bronchoconstriction

The evidence implicating the parasympathetic nervous system in the mediation of bronchoconstriction is based on:

(i) The bronchoconstrictor effect of cholinergic agonists:

In 1947 Curry reported that each of 27 asthmatic subjects to whom the cholinergic agonist acetyl-beta-methyl-choline (methacholine) had been administered by various routes, exhibited a significant diminution in vital capacity. The response was less consistent and less pronounced in normal subjects and atopic individuals without asthma. It has since been demonstrated that

the inhalation of methacholine provokes a significant fall in peak expiratory flow rate (PEFR) in the majority of asthmatic patients (Laitinen, 1974). In addition, the inhalation of the cholinesterase inhibitor, edrophonium, has been shown to produce a significant diminution in airway calibre in both asthmatic and non-asthmatic individuals (Quigley et al. 1985). Pharmacological bronchoprovocation challenge testing using, among other agents, methacholine, has become a widely used procedure and the response is sufficiently consistent to make it of value in the assessment of patients in whom bronchial asthma is suspected, but has not been proved (Pratter and Irwin, 1984).

(ii) The bronchodilator effect of cholinergic antagonists:

In 1959, Herxheimer reported that the smoking of cigarettes to which atropine sulphate had been added resulted in a significant increase in the vital capacities of asthmatic patients. It has since been demonstrated that inhaled atropine methonitrate potentiates the bronchodilator effect of inhaled isoproterenol in patients with asthma (Chamberlain et al. 1962), and itself significantly improves airway function in asthmatic children in a dose-dependent manner, with maximal responses roughly equivalent to those following the inhalation of 1mg isoproterenol (Cavanaugh and Cooper, 1976). Studies in asthmatic patients have also demonstrated that ipratropium bromide, a synthetic atropine derivative, is an effective bronchodilator when administered by inhalation, with a potency similar to (Storms et al. 1975) or approaching that of isoproterenol (Gross, 1975). More recently, early morning asthma or "morning dipping"

has been shown to be attenuated by ipratropium bromide to a greater degree than by salbutamol (Cox et al. 1984), and another atropine derivative, oxitropium bromide, has also been shown to be effective in preventing early morning asthma in some patients (Coe and Barnes, 1985). Ipratropium bromide also appears to be of value in the management of patients with acute severe asthma (Ward et al. 1981).

(iii) The efficacy of cholinergic antagonists in blocking bronchoconstriction provoked by various stimuli:

In 1967, Simonsson et al. demonstrated that the provocation of airway obtruction in patients with bronchial asthma and chronic bronchitis, as well as in normal subjects, by various stimuli, could be prevented or attenuated by the prior administration of atropine sulphate. The protective effect of anticholinergic compounds against bronchial hyperreactivity and the bronchoconstriction provoked by a number of diverse stimuli has subsequently been demonstrated in a large number of studies. These stimuli include:

(a) Ozone:

Bronchial hyperreactivity induced by the exposure of normal subjects and non-asthmatic atopic individuals to ozone results in an increase in the bronchoconstrictor response to histamine which can be blocked by pretreatment with atropine (Golden et al. 1978; Holtzman et al. 1979)

(b) Upper Respiratory Infections:

It has been demonstrated that the bronchoconstrictor responses to the inhalation of histamine and citric acid in

normal subjects with bronchial hyperreactivity associated with upper respiratory infections can be blocked by atropine (Empey et al. 1976). Similar results have been obtained following the induction of bronchial hyperreactivity by the inoculation of live attenuated influenza virus into normal subjects.

(c) Histamine and Methacholine:

The bronchoconstrictor response to methacholine in both asthmatic and non-asthmatic individuals is effectively blocked by cholinergic antagonists (Casterline et al. 1976; Woenne et al. 1978; Holtzman et al. 1980; Bandouvakis et al. 1981; Gross and Skorodin, 1984;). However, although anticholinergic agents may reduce the bronchial responsiveness to histamine in some asthmatic patients (Boulet et al. 1984), most studies suggest that they have, at best, a partial effect (Gross and Skorodin, 1984).

(d) Sulphur Dioxide:

The inhalation of sulphur dioxide has been shown to result in decreased airway conductance in healthy subjects, the effect being prevented by the subcutaneous injection of atropine (Nadel et al. 1965). It also appears that this bronchoconstrictor response occurs at a lower threshold concentration in asthmatic patients than in either atopic non-asthmatic or normal subjects, and can be prevented by pretreatment with aerosolized atropine (Sheppard et al. 1980).

(e) Prostaglandins:

The bronchoconstrictor effect of prostaglandin F_2-alpha in both dogs and human subjects can be blocked by atropine (Patel,

1975; Wasserman, 1975).

(f) Psychological Factors:

It has been demonstrated that the inhalation of normal saline in the guise of its being allergen can produce an increase in airway resistance in a significant proportion of asthmatic patients, and that intravenous atropine sulphate is effective in preventing this response (McFadden et al. 1969).

(g) Exercise and Hyperventilation:

The induction of bronchospasm by exercise in asthmatic children may be blocked by the prior administration of atropine (Tinkelman et al. 1976; Chen et al. 1981). In addition, ipratropium bromide appears to protect some subjects from the diminution in airway calibre provoked by hyperventilation (Wilson et al. 1982).

(h) Antigen:

Bronchoconstriction induced in asthmatic patients by the inhalation of extracts of housedust and pollen may be significantly reversed by intravenous atropine, and completely prevented by premedication with it (Yu et al. 1972). Similarly, ipratropium bromide may be effective in preventing allergen-induced bronchospasm (Kersten, 1975).

(i) Beta-adrenergic blockade:

The effect of anticholinergic agents used in combination with beta-adrenergic blockers is of particular interest. Zaid and Beall (1966) showed that beta-blockade in patients with mild asthma was associated with some diminution in airway calibre and a marked increase in the sensitivity to subcutaneous

methacholine. It has been shown that the bronchoconstrictor response to beta-blockade in asthmatic patients can be markedly attenuated by pretreatment with atropine, and that likewise, the small changes in airway resistance induced by beta-blockade in normal subjects are completely abolished by it (MacDonald et al. 1967; Grieco and Pierson, 1971). Especially in view of the negligible effect of propranolol on the calibre of normal airways (MacDonald et al. 1967; Richardson and Sterling, 1969; Tattersfield et al. 1973; Ploy-Song-Sang et al. 1978), it has been suggested that unopposed parasympathetic neural activity is the main cause of the bronchial hyperreactivity induced by beta-adrenergic blockade in asthmatic patients.

It must be noted that while most investigators have agreed that anticholinergic agents are effective in blocking the bronchoconstrictor response to methacholine (Casterline et al. 1976; Woenne et al. 1978; Bandouvakis et al. 1981; Gross and Skorodin, 1984), it has been suggested that they are considerably less effective in preventing the responses associated with histamine (Altounyan, 1964 Casterline et al. 1976; Woenne et al. 1978; Bandouvakis et al. 1981; Gross and Skorodin, 1984), antigen (Altounyan, 1964; Fish et al. 1977) and exercise (Fisher et al. 1970; Chan-Yeung et al. 1971; Deal et al. 1978). These groups of workers have accordingly concluded that the parasympathetic nervous system is not an important factor in the bronchoconstriction provoked by these stimuli. It is possible, however, that the efficacy of anti-cholinergic agents in protecting against a particular bronchoconstrictor stimulus may

be related not only to the stimulus itself, but also, critically, to the dose and route of administration chosen (Hartley and Davies, 1980; Sheppard D et al. 1982; Sheppard D et al. 1983).

8.1.2 Possible Sites of Parasympathetic Overactivity

A number of sites in the parasympathetic pathway could theoretically be involved in an abnormal increase in neural activity, resulting in bronchial hyperreactivity.

(i) The Afferent Sensory Pathway:

The inhalation by anaesthetised rabbits of a number of substances known to provoke bronchoconstriction, including aerosolised histamine and citric acid, can provoke an increase in the electrical activity of vagal sensory fibres (Sellick and Widdicombe, 1971), and it is also known that histamine stimulates the electrical discharge of "rapidly adapting" sensory receptors in the airways of mechanically ventilated dogs (Vidruk et al. 1977). The inhalation of histamine by normal subjects with upper respiratory infections produces a significantly greater degree of bronchoconstriction than in control subjects, or following recovery (Empey et al. 1976) and tne integrity of the airway epithelium appears to have a profound effect on the contractile response to histamine, as well as on the maximal effects of various smooth muscle relaxants (Goldie et al. 1986). These results suggest that damage to the airway epithelium may result in the exposure and therefore the sensitisation of the underlying sensory receptors.

Similarly, a number of studies have demonstrated that ozone

can induce a state of bronchial hyperreactivity, possibly by causing epithelial damage. The exposure of dogs to ozone increases the effect of inhaled histamine on airway calibre and this may be abolished either by the administration of atropine or by cooling blockade of the vagus nerve (Lee et al. 1977). In addition, exposure to ozone results in an exaggeration of the rapid shallow breathing pattern that occurs following the inhalation of histamine (Lee et al. 1979), and this can be abolished by vagal blockade. These results suggest that ozone may induce epithelial damage and inflammation, with sensitisation of vagal sensory receptors.

The studies describing the induction of bronchial hyperreactivity by the inhalation of ozone in normal subjects and non-asthmatic atopic individuals have already been cited (Golden et al. 1978; Holtzman et al. 1979). It is noteworthy that ozone may also increase the bronchoconstrictor response to methacholine, suggesting a possible effect of the substance on the efferent side of the reflex arc (Holtzman et al. 1979). The magnitude of the airway response to the inhalation of acetylcholine in dogs following ozone exposure appears to correlate closely with the number of neutrophils seen in the epithelium, suggesting that the induction of bronchial hyperreactivity may be critically dependent on the development of an acute inflammatory response (Holtzman et al. 1983). Similarly, the inflammatory mediator, prostaglandin D2, has been shown to potentiate the bronchoconstrictor responses to methacholine in asthmatic patients (Fuller et al. 1986).

(ii) The Central Pathways:

There is little doubt that the central connections of the parasympathetic reflex pathways may have a profound effect on airway calibre. This is suggested by the study cited above (McFadden et al. 1969) showing that the inhalation of saline in the guise of its being an allergen, can produce an increase in airway resistance, which can be blocked by anticholinergic premedication. Similarly, the inhalation of an inert diluent flavoured in the same way as a previously inhaled methacholine solution may reduce airway calibre in patients with asthma (Spector et al. 1976), and aerosolised saline, presented as a bronchoconstrictor, has been shown to have a significantly greater effect on airway resistance than when presented as a neutral substance (Horton et al. 1978). It has been shown in hypnotised asthmatic subjects that suggestions linked to fear, anger or an attack of asthma, can provoke increased airway resistance (Smith et al. 1970). The importance of the central pathways in the control of airway calibre has been confirmed by the finding that the central synapses of the parasympathetic reflex pathway are depressed by general anaesthesia, with simultaneous inhibition of reflex bronchoconstriction (Holtzman et al. 1982).

(iii) The Efferent Motor Pathway:

The application of serotonin to the airways of ventilated dogs produces bronchoconstriction which can be inhibited by vagal cooling blockade, and also markedly increases the bronchoconstrictor effect of electrical vagal stimulation in

doses which have little effect on baseline airway calibre (Hahn et al. 1978). In addition, the bronchoconstrictor response produced by electrical stimulation of the cut-ends of the cervical vagi in dogs is greatly increased by serotonin, while the response to acetylcholine remains virtually unchanged, thus excluding a direct effect on muscarinic receptors (Sheller et al. 1982). These studies suggest that serotonin may produce bronchial hyperreactivity by what appears to be a specific facilitatory effect on vagal efferent pathways.

8.1.3 Evidence of Extrapulmonary Parasympathetic Overactivity in Asthma

Although it is reasonable to postulate that an increase in parasympathetic activity in any of a number of sites in the neural pathway could lead to bronchial hyperreactivity, there is at present little direct evidence of a generalised increase in parasympathetic neural activity in asthmatic compared with non-asthmatic individuals.

It has been demonstrated that atopic subjects have increased eccrine sweat gland responses to intradermal methacholine. The responses of asthmatic patients do not, however, appear to differ from those of non-asthmatic atopic subjects (Kaliner, 1976). The few non-allergic asthmatic patients tested appear to resemble allergic patients more closely than normal control subjects in their responses (Kaliner, 1976). Likewise, a study of the pupillary responses to carbachol in similar groups of subjects has shown that there is approximately the same degree of

responsiveness in allergic subjects with and without asthma, this being, once again, significantly greater than that found in normal individuals. Patients with "intrinsic asthma" appear to have an even greater degree of hyperresponsiveness (Smith et al. 1980). These findings suggest the possible existence of a generalised cholinergic abnormality in relation to the atopic state, but not specifically to bronchial asthma.

8.2 A STUDY OF THE REFLEX CONTROL OF HEART RATE

As the rate of discharge of the sino-atrial node is modulated by the vagus nerve, it was reasoned that any abnormality in the parasympathetic control of airway calibre might be reflected by a parallel change in the control of heart rate. In this study, reflex heart rate control in asthmatic patients and normal control subjects was compared in an attempt to investigate the possible relationship between bronchial hyperreactivity and parasympathetic neural overactivity.

8.2.1 Subjects

Fifteen asthmatic patients and 15 normal subjects were studied. The asthmatic patients were all using a sympathomimetic bronchodilator. Each, in addition, was using disodium cromoglycate and/or beclomethasone dipropionate aerosol (maximum dose 400 ug/day). Only one had ever required systemic corticosteroids, for a short period some months prior to the study. All bronchodilators were discontinued for at least a week

prior to the study. The asthmatic patients were all in a stable condition at the time of the study. No subject was a trained athlete.

8.2.2 Methods

Each subject was studied early in the morning following an overnight fast.

Care was taken to achieve familiarity with the various manoeuvres by the performance of numerous practice tests. Each test was performed twice, and the mean of the results calculated. Following each manoeuvre, the heart rate was allowed to return to the resting level before proceeding with the next.

The variations in heart rate induced by deep breathing (respiratory sinus arrhythmia), the Valsalva manoeuvre and standing up from the recumbent position were studied.

(i) Variations in heart rate induced by deep breathing (respiratory sinus arrhythmia):

With the subject semi-recumbent and breathing deeply at a rate of 6 breaths per minute (Wheeler and Watkins, 1973; Ewing, 1978) the electrocardiograph (ECG) was recorded for 60 seconds. The duration of each respiratory cycle was exactly 10 seconds with equal time being allowed for inspiration and expiration. The seconds were counted aloud by a technologist, who ensured that the breathing was continuous. Forced breaths and maximum expiratory manoeuvres were specifically avoided because of the possibility of provoking bronchospasm in the asthmatic patients.

From each ECG strip, the longest and shortest R-R intervals

were selected and measured, and the results converted from milliseconds to beats per minute. The magnitude of respiratory sinus arrhythmia was expressed as the difference between the maximum and minimum heart rate in beats per minute (Ewing et al. 1981). The mean heart rate during the deep breathing manoeuvre was estimated from the average of the maximum and minimum heart rates.

The tidal volumes of 8 asthmatic patients and 8 control subjects, breathing at a rate of 6 breaths per minute for 2 minutes in the semi-recumbent position were measured and compared. In order to correct for height and weight differences, the average tidal volume of the 12 breaths from each subject was expressed as a percentage of both the observed and predicted vital capacities.

(ii) Variation in heart rate induced by the Valsalva manoeuvre:

While the ECG was being recorded, and with the subject in the semi-recumbent position, an expiratory pressure of 40mmHg was maintained for 10 seconds through a mouthpiece attached to a sphygmomanometer and then released. Care was taken to ensure that the pressure changes occurred abruptly at the onset and termination of the strain period (Levin, 1966). The difference between the shortest R-R interval during, and the longest R-R interval after the strain period was expressed in beats per minute (Levin, 1966).

(iii) Variation in heart rate induced by standing up from the recumbent position:

While the ECG was being recorded, the subject rose rapidly

from the recumbent to the erect position. The difference between the shortest R-R interval at, or around, the 15th beat, and the longest R-R interval at, or around, the 30th beat after standing was expressed in beats per minute (Ewing, 1978).

Statistical Analysis:

Pairwise comparisons were done using the unpaired t-test.

8.2.3 Results

Demographic and pulmonary function data are shown in Table 1. The forced expiratory volume in one second (FEV_1) was significantly lower, and the airway resistance and fall in the peak expiratory flow rate (PEFR) on exercise significantly greater in the asthmatic patients than in the normal subjects.

The magnitude of respiratory sinus arrhythmia and the heart rate responses to the Valsalva manoeuvre, and to standing, are shown in Table 2. The magnitude of respiratory sinus arrhythmia was significantly greater in the asthmatic patients than in the normal subjects ($p = <0.001$). There was no significant difference between the asthmatic patients and the normal subjects in the heart rate responses to the other two manoeuvres.

There was no significant difference between the average tidal volume of 8 asthmatic patients (49.6 $\pm$ 14.1% observed vital capacity; 44.8 $\pm$ 12% predicted vital capacity) and 8 control subjects (60.9 $\pm$ 18.2% observed vital capacity; 60.3 $\pm$ 19.4% predicted vital capacity), breathing at a rate of 6 breaths per minute in the semi-recumbent position.

TABLE 1. 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=15)	21.5(3.6)	7F,8M	94.5(11.2)*	1.7(0.3)*	2.2(2.8)*
Asthmatic patients (n=15)	21.9(4.8)	7F,8M	64.5(15.0)	6.7(4.1)	37.7(19.1)≠

Values are mean(SD)

* Normal subjects vs asthmatic patients significantly different ($p = <0.0005$)

≠ n = 12

TABLE 2. REFLEX HEART RATE RESPONSES

	Deep breathing (Respiratory sinus arrhythmia)	Valsalva manoeuvre	Standing from the recumbent position
Normal subjects (n=15)	32.1(6.1)*	29.3(13.6)	25.8(5.7)
Asthmatic patients (n=15)	39.9(5.1)	32.2(13.1)	21.9(5.8)

Values are mean(SD) beats/min

* Normal subjects vs asthmatic patients significantly different (p = <0.001)

The mean heart rate during the deep breathing procedure was similar in the asthmatic patients and the normal subjects (73.4 ± 9.1; 73.9 ± 9.9 beats per minute, respectively).

No ectopic beats occurred during any of the ECG recordings.

8.2.4 Discussion

The reflex control of heart rate is dependent upon intact autonomic function, and the manoeuvres performed in the present study have been extensively employed in the evaluation of patients with suspected autonomic neuropathy due to various causes (Wheeler and Watkins, 1973; Ewing, 1978; Bennett et al. 1978; Ewing et al. 1981; Smith, 1982). Several physiological mechanisms may contribute to respiratory heart rate variations, including reflexes involving pulmonary and atrial stretch receptors and baroreceptors. There may also be a direct interaction between the respiratory and cardiovascular centres in the brainstem (Guyton, 1981a; Katona and Jih, 1975).

Respiratory sinus arrhythmia is abolished by atropine (Ewing, 1978; Fouad et al. 1984) and either unaffected (Ewing, 1978) or increased by propranolol (Fouad et al. 1984). It has been shown to be closely related to the activity of vagal fibres (Katona et al. 1970; Katona and Jih, 1975). In addition, it appears that vagal efferent activity ceases in dogs during spontaneous but not mechanical inspiration, regardless of arterial pressure (Katona et al. 1970), suggesting that brainstem connections are the primary origin of respiratory variations in heart rate. The results of a clinical study in diabetic patients,

however, have suggested that there is a close relationship between baroreceptor function and respiratory heart rate variations (Bennett et al. 1978).

The magnitude of respiratory sinus arrhythmia has been well evaluated clinically as an index of autonomic function, and appears to have an acceptable degree of intrasubject repeatability (Smith and Smith, 1981; Smith, 1982), even at an interval of 3-8 months (Smith and Smith, 1981). Abnormally diminished respiratory variations in heart rate are generally accepted as providing proof of the presence of autonomic neuropathy. The finding, therefore, of an increased magnitude of respiratory sinus arrhythmia in asthmatic patients compared with normal subjects may be interpreted as evidence of enhanced autonomic neural activity, primarily involving the paraysmpathetic nervous system.

Although vagal pathways appear to be important in the heart rate responses to both the Valsalva manoeuvre and assumption of the upright posture (Leon et al. 1970; Ewing et al. 1981), there was an absence of correlation in the present study, as well as in others, between the magnitude of the changes in heart rate induced by these manoeuvres, and the magnitude of respiratory sinus arrhythmia (Bennett et al. 1978; Wieling et al. 1982). It has been suggested that, while the vagus may be the efferent pathway for all these reflexes, the intensity of the afferent input may vary considerably between them (Bennett et al. 1978; Wieling et al. 1982).

The magnitude of respiratory sinus arrhythmia has been shown

to be related to mean heart rate, rate of respiration and age (Davies and Neilson, 1967; Bennett et al. 1978; Smith and Smith, 1981; Wieling et al. 1982; Eckberg, 1983). None of these variables could have accounted for the observed difference between the asthmatic patients and the normal subjects in the present study. The effect of tidal volume on respiratory heart rate variations is small, a 50% increase in tidal volume, resulting in only a 15% increase in the degree of respiratory sinus arrhythmia (Eckberg, 1983). Although the difference was not statistically significant, the average tidal volume of the 8 asthmatic patients in whom it was studied, tended to be smaller than that of the control subjects. It is most unlikely, therefore, that the increased magnitude of respiratory sinus arrhythmia found in the asthmatic patients was related to a difference in tidal volume.

The only study which has attempted to establish age-related normal values for the magnitude of respiratory sinus arrhythmia in which the protocol followed was similar to that in the present study, was performed by Wieling et al (1982). However, whereas their measurements were also made in the supine position and at a respiratory rate of 6 per min, the heart rate variations were elicited by forced maximal breaths which were specifically avoided in the present study. Although, as has been stated, the effect of tidal volume on respiratory sinus arrhythmia is small (Eckberg, 1983), the relative effect of forced, as opposed to quiet, breathing is uncertain. In addition, as the study of Wieling et al (1982) was designed to establish normal values for

comparison with those from patients with autonomic neuropathy, it is quite possible that the subjects were not carefully screened to exclude individuals with a history of current or remote asthma which may be present in almost 10% of a random population group (Cockcroft et al. 1983). Nonetheless, superimposition of the present data on their regression curve still shows that the majority of the asthmatic patients fall at or near the upper limit of the very wide normal range (Wieling et al. 1982).

The present study demonstrates the existence in a group of asthmatic patients of an increased magnitude of respiratory sinus arrhythmia, suggesting the presence in them of increased parasympathetic neural output, at least to the sino-atrial node. It is of considerable interest that other workers have recently reported the finding of decreased nocturnal heart rates together with an increased magnitude of respiratory sinus arrhythmia in a group of non-atopic middle-aged patients with "chronic partly reversible airway obstruction", suggesting the possibility of the presence in them of parasympathetic overactivity (Postma et al. 1985).

8.3 A STUDY OF THE EFFECT OF RESISTANCE BREATHING ON RESPIRATORY SINUS ARRHYTHMIA IN NORMAL SUBJECTS

The asthmatic patients in the study of reflex heart rate control (Chapter 8.2) all had relatively mild disease, in that each was able, without difficulty, to discontinue all bronchodilator drugs for at least a week, and only one had ever

required systemic corticosteroids. In addition, all of them were in a stable condition at the time of the study. However, large changes in intrathoracic pressure such as may occur in association with severely obstructed breathing may cause fluctuations in cardiac output, and therefore in arterial blood pressure (Buda et al. 1979). These may, in turn, provoke finely modulated compensatory heart rate changes mediated by the separate outputs of intrathoracic and extrathoracic baroreceptors (Fitzgerald et al. 1981).

The possibility was investigated, therefore, that the increased respiratory heart rate variations observed in the asthmatic patients might have been the result of a baroreceptor effect caused by fluctuations in cardiac output, associated with airway obstruction. For this reason, an attempt was made to simulate the pulmonary mechanical abnormalities associated with asthma by the application of a resistive load to the airways of normal subjects. This manoeuvre may also produce a degree of pulmonary hyperinflation, with a rise in the functional residual capacity (Zechman et al. 1957; Edstrom et al. 1976).

8.3.1 Subjects and Methods

Ten normal subjects were studied. The magnitude of respiratory sinus arrhythmia during both the application of a resistance to the airway and unloaded breathing was measured as described in 8.2.1.

The resistance consisted of a metal disc with a 4mm diameter perforation which was inserted into a pneumotachograph. The

magnitude of the resistance was assessed by measuring the pressure drop across it at a flow rate of 500ml/second with a water manometer consisting of two lengths of plastic tubing which were attached to the pneumotachograph on either side of the disc. The resistance was estimated to be approximately 24cmH_2O/l/sec.

Pairwise comparison was done using the paired t-test.

8.3.2 Results

The magnitude of respiratory sinus arrhythmia was 29 $\pm$ 6.4 beats per minute during resistance breathing, and 32.5 $\pm$ 6.7 beats per minute during unloaded breathing. The difference was not statistically significant.

8.3.3 Discussion

There was no change in the magnitude of respiratory sinus arrhythmia in normal subjects during resistance breathing. Although an extrathoracic resistance is not strictly analagous to diffuse intrathoracic airway obstruction, it appears unlikely that the increased respiratory heart rate variations observed in the asthmatic patients were primarily related to increased airway resistance. However, although the application of a resistive load to the airways of normal subjects has been shown to increase the functional residual capacity (Zechman et al. 1957; Edstrom et al. 1976), the effect of the resistance on functional residual capacity could not be measured in the present study for technical reasons, and it was not possible, therefore, to assess whether or not a degree of pulmonary hyperinflation was produced by it. The

possibility can not be completely excluded, therefore, that pulmonary hyperinflation was in some way a contributory factor to the increased magnitude of respiratory sinus arrhythmia observed in the asthmatic patients.

8.4 A STUDY OF THE MAGNITUDE OF RESPIRATORY SINUS ARRHYTHMIA IN NON-ASTHMATIC ATOPIC SUBJECTS

It has been demonstrated that atopic non-asthmatic individuals as well as patients with asthma, demonstrate hyperresponsiveness compared with normal subjects to cholinergically induced pupillary and eccrine sweat gland responses (Kaliner, 1976; Smith et al. 1980; Kaliner et al. 1982). In view of the finding of increased parasympathetic neural activity in asthmatic patients, as manifested by an increased magnitude of respiratory sinus arrhythmia, it was decided to study the heart rate responses to deep breathing in non-asthmatic patients with a history of allergic rhinitis.

8.4.1 Subjects

Fifteen subjects were studied, each of whom had a strong history of allergic rhinitis as well as a family history of atopic disease. None had a history of asthma, as defined by the American Thoracic Society (1962). Each had normal pulmonary function with an insignificant fall in peak expiratory flow rate (PEFR) on exercise, and multiple strongly positive responses when undergoing skin testing with a standard panel of 14 common

allergens (South African Institute for Medical Research, Johannesburg).

8.4.2 Methods

No medications of any kind were taken by any subject for a week prior to the study. The magnitude of respiratory sinus arrhythmia was measured in each subject as described in Chapter 8.2.2.

Statistical Analysis:

The results obtained from the subjects with allergic rhinitis were retrospectively compared with those from the asthmatic patients and the normal individuals (Chapter 8.2.3), using the unpaired t-test and correcting for multiple comparisons by the Bonferroni method.

8.4.3 Results

Demographic and pulmonary function data are shown in Table 3. There were no differences between the subjects with allergic rhinitis and the normal subjects (Table 1, Chapter 8.2.3). The magnitude of respiratory sinus arrhythmia in the subjects with allergic rhinitis is also shown in Table 3. There was no significant difference between the magnitude of respiratory sinus arrhythmia in the subjects with allergic rhinitis, compared with either the asthmatic patients or the normal individuals, the mean value being intermediate between that of these two groups. The difference between asthmatic patients and normal subjects

TABLE 3. DEMOGRAPHIC AND PULMONARY FUNCTION DATA AND MAGNITUDE OF RESPIRATORY SINUS ARRHYTHMIA IN NON-ASTHMATIC ATOPIC SUBJECTS

Age (yrs)	Sex	FEV_1 (% predicted)	Airway Resistance ($cmH_2O/l/sec$)	Fall in PEFR on exercise (%)	Respiratory sinus arrhythmia (beats/min)
20.7(4.8)	6F,9M	103.5(14.4)	1.8(0.5)	2.4(3.0)	36.1(7.9) *

Values are mean(SD)

* Not significantly different from asthmatic patients or normal subjects (Table 2)

remained significant after Bonferroni correction.

8.5 A STATISTICAL ANALYSIS TO INVESTIGATE THE RELATIONSHIP BETWEEN THE MAGNITUDE OF RESPIRATORY SINUS ARRHYTHMIA AND THE DEGREE OF BRONCHIAL HYPERREACTIVITY IN ASTHMATIC PATIENTS

8.5.1 Subjects

The data from all 12 asthmatic patients who had performed exercise tests prior to the study of reflex heart rate control were utilised.* In addition, 3 subjects with a history of atopy and bronchial asthma, as defined by the American Thoracic Society (1962) were studied. Each of these had been excluded from the study of reflex heart rate control, because they did not comply with the requirements for asthmatic patients, in that despite a clear history of asthma, none had a sufficiently severe impairment in pulmonary function on objective testing, or a sufficient degree of demonstrable bronchial hyperreactivity (Chapter 7.1). They were included in the present study, however, to broaden the spectrum of the severity of the disease among the patients.

* Three patients refused to perform exercise tests.

8.5.2 Methods

The measurement of the magnitude of respiratory sinus arrhythmia in the additional 3 subjects was done as described in 8.2.2.

Statistical Analysis:

This was done by means of a stepwise multiple regression procedure (Afifi and Azen, 1979) using the BMDP statistical package (Dixon and Jennrich, 1981). The magnitude of respiratory sinus arrhythmia was entered as the dependent variable and the percent fall in peak expiratory flow rate (PEFR) on exercise, age, baseline PEFR and mean heart rate during the deep breathing manoeuvre as independent variables.

8.5.3 Results

There was a strong correlation between the magnitude of respiratory sinus arrhythmia and the degree of bronchial hyperreactivity as manifested by the percentage fall in PEFR on exercise in the 15 asthmatic subjects ($r = 0,70$; $p = <0.005$) (Figure 1). However, age and mean heart rate were also significantly related to the magnitude of respiratory sinus arrhythmia ($r = 0.57$ and 0.55 respectively; $p = <0.05$). After adjustment for the effects of both of these variables, the partial correlation between the magnitude of respiratory sinus arrhythmia and the percentage fall in PEFR on exercise generated an r value of 0.55 ($p = 0.05$).

8.5.4 Discussion

Despite the relatively low r value of 0.55, these results suggest the existence of a relationship between the magnitude of respiratory sinus arrhythmia and the degree of bronchial hyperreactivity. It must be taken into account that the number of

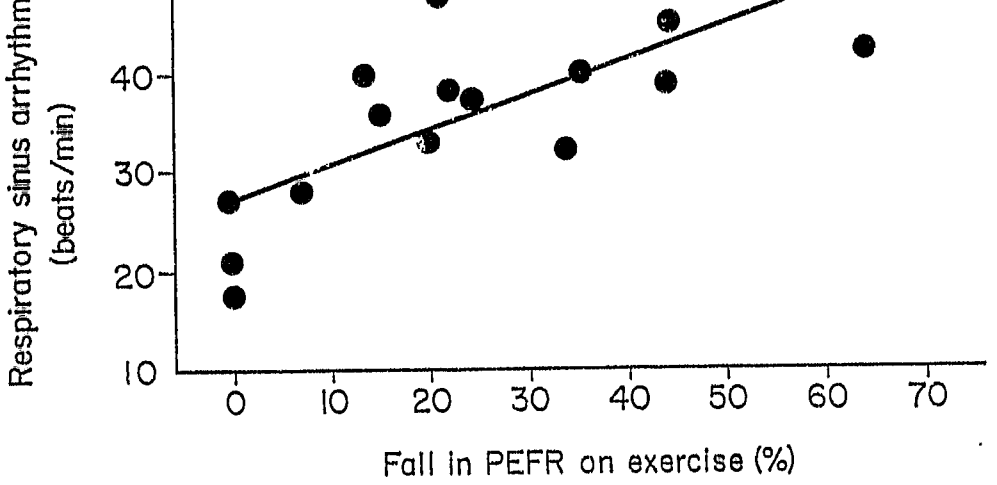


Figure 1. Relationship between the magnitude of respiratory sinus arrhythmia and the degree of bronchial hyperreactivity in 15 asthmatic patients.

subjects studied was relatively small and that after adjustment for the effects of both age and mean heart rate an extremely strong correlation would have been required to generate a greater r value.

8.6 A STUDY OF THE HEART RATE RESPONSES TO ATROPINE

Atropine causes an increase in heart rate by blocking the parasympathetic effect on the sino-atrial pacemaker. Its influence is most noticeable in healthy young adults in whom vagal tone is most marked. In contrast, in infancy and old age even large doses of atropine may fail to significantly increase heart rate (Weiner, 1980). It has also been observed that uraemic patients have a blunted heart rate response to intravenous atropine, presumably as a result of autonomic neuropathy (Lowenthal and Reidenberg, 1972). It is of interest, in addition, that patients with Down's syndrome may respond to atropine with a greater mydriatic response and greater increments in heart rate than normal subjects, suggesting the interesting possibility that an abnormally increased degree of parasympathetic neural output has been abolished (Harris and Goodman, 1968).

It was reasoned, therefore, that an increase in vagal tone in asthmatic patients might be reflected by a greater increase in heart rate in response to the vagolytic effect of atropine.

8.6.1 Subjects

All of the asthmatic patients were using a sympathomimetic bronchodilator aerosol. Each, in addition, was using disodium cromoglycate and/or beclomethasone dipropionate aerosol (maximum dose 400/ug/day), and one had received a short course of systemic corticosteroids some months prior to the study. All bronchodilator therapy was discontinued at least a week prior to the study. All the asthmatic patients were in a stable condition at the time of the study. None of the subjects was a trained athlete.

8.6.2 Methods

Each subject was studied in the recumbent position early in the morning following an overnight fast. Not less than 30 minutes after an intravenous cannula had been inserted into an arm vein, heart rate was recorded electrocardiographically for one minute. This was repeated after 5 minutes. Atropine sulphate (0.6mg) was then injected intravenously over one minute and heart rate measured electrocardiographically for 20 seconds, 1, 2, 3, 4 and 5 minutes after completion of the injection. A further 0.6mg atropine sulphate was then injected intravenously over one minute and heart rate again measured electrocardiographically for 20 seconds, 1, 2, 3, 4 and 5 minutes following completion of the injection.

Statistical Analysis:

Pairwise comparison of the data was done using the unpaired

t-test. The basal heart rate in each subject was the arithmetic mean of the two values obtained prior to the first injection of atropine.

8.6.3 Results

Demographic and pulmonary function data are shown in Table 4. The asthmatic patients were younger than the normal subjects, and 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. The heart rate responses to intravenous atropine are shown in Table 5. There was no significant difference between the asthmatic patients and the normal subjects in the heart rate responses at any time after either dose of atropine.

8.7 CONCLUSIONS

The finding of an increased magnitude of respiratory sinus arrhythmia in the asthmatic patients suggests the presence in them of enhanced parasympathetic output to the sino-atrial node. In addition, the apparent existence of a relationship between the magnitude of respiratory sinus arrhythmia and the degree of bronchial hyperreactivity in the relatively small group of asthmatic patients studied suggests that there may be a parallel enhancement in the parasympathetic neural output to the sino-atrial node and the airways. The complexity of autonomic interactions in the lungs and elsewhere is considerable, however,

TABLE 4. 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=10)	25.7(2.6)*	8F,3M	59.9(6.9)	94.0(16.0)**	1.8(0.4)***	2.8(3.4)**
Asthmatic patients (n=11)	21.4(5.2)	6F,5M	61.3(12.0)	60.3(13.2)	7.2(4.3)	38.9(21.3)

Values are mean(SD)

Normal subjects vs asthmatic patients significantly different
* p = <0.05
** p = <0.0005
*** p = <0.001

TABLE 5. HEART RATE RESPONSES TO ATROPINE

	Basal	0.6mg	1 min	2 min	3 min	4 min	5 min	0.6mg	1 min	2 min	3 min	4 min	5 min
Normal subjects (n=10)	75 (9)		88 (11)	86 (12)	87 (12)	90 (13)	90 (12)		107 (11)	107 (14)	111 (12)	110 (11)	109 (11)
Asthmatic patients (n=11)	74 (13)		84 (14)	88 (13)	87 (15)	87 (15)	87 (14)		105 (16)	107 (16)	108 (17)	107 (17)	108 (17)

Values are mean(SD) beats/min

and one component of the system may be involved in the function of others (Skoogh et al. 1986). In addition, the results obtained do not permit complete exclusion of the possibility that the increased respiratory heart rate variations observed in the asthmatic patients may be, in some way, caused by asthma, rather than being the result of a primary autonomic abnormality. Moreover, the results of the study of the heart rate responses to atropine provide no support for the suggestion of an increase in parasympathetic neural output in asthmatic patients.

CHAPTER 9

STUDIES OF ALPHA-ADRENERGIC RESPONSES

9.1 A REVIEW OF THE EXPERIMENTAL DATA LINKING ALPHA-ADRENERGIC MECHANISMS TO THE PATHOGENESIS OF BRONCHIAL ASTHMA

Alpha-1 adrenergic agonists stimulate the contraction of smooth muscle in various sites, and there is evidence that an alpha-adrenergic component may be associated with the bronchoconstriction which occurs in both asthmatic and non-asthmatic individuals under a variety of conditions. An extension of this observation is the concept that alpha-adrenergic overactivity may be an important underlying pathogenetic mechanism in bronchial asthma.

9.1.1 Evidence of Alpha-Adrenergic Involvement in Bronchoconstriction

The evidence of the involvement of alpha-adrenergic mechanisms in the mediation of bronchoconstriction is based on:-

(i) The bronchoconstrictor effect of alpha-adrenergic agonists:

A number of studies have demonstrated that the administration of alpha-adrenergic agonists diminishes airway calibre in patients with asthma or chronic obstructive airway disease following the induction of beta-adrenergic and cholinergic blockade (Prime et al. 1972; Simonsson et al. 1972; Patel and Kerr, 1973; Snashall et al. 1978). This bronchoconstrictor effect of alpha-agonists has not, however, been a consistent finding (Thomson et al. 1982). Studies in non-asthmatic individuals have also yielded conflicting results (Prime et al. 1972; Snashall et al. 1978).

(ii) The bronchodilator effect of alpha-adrenergic antagonists:

Alpha-adrenergic blockade induced by intravenous thymoxamine has been shown to produce a rapid increase in specific airway conductance in asthmatic patients (Griffin et al. 1972). Similarly, both thymoxamine and phentolamine potentiate the bronchodilator effect of isoproterenol in asthmatic patients (Patel and Kerr, 1975), as well as independently increasing airway calibre (Patel and Kerr, 1975; Campbell, 1982). The bronchodilator effect of alpha-adrenergic antagonists has not, however, been a consistent finding (Palmer et al. 1974). The results of long-term therapy of asthma with the alpha-blocker indoramin have been disappointing (Campbell and Dyson, 1977; Black et al. 1978), and a study with the selective alpha-1 blocker prazosin has demonstrated that it has little acute effect on the pulmonary function of asthmatic patients (Barnes et al. 1981b).

(iii) The efficacy of alpha-adrenergic antagonists in blocking the bronchoconstriction produced by various stimuli:

(a) Histamine: It has been demonstrated that the bronchoconstrictor effect of histamine can be attenuated or abolished in both asthmatic patients and normal subjects by the induction of alpha-adrenergic blockade with various alpha-antagonists (Kerr et al. 1970; Gaddie et al. 1972; Prime et al. 1972; Bleecker et al. 1983). However, neither oral phentolamine (Bleecker et al. 1983; Walden et al. 1984) nor inhaled prazosin (Barnes et al. 1981c;) appear to be effective in this regard.

(b) Exercise: Gross et al. (1974) reported the case of a single

asthmatic patient in whom exercise-induced asthma, which had been refractory to numerous medications, including beta-adrenergic agonists, was successfully treated with phentolamine. It has subsequently been clearly demonstrated that various alpha-adrenergic antagonists administered by different routes abolish or attenuate the bronchoconstrictor responses to exercise or hyperventilation with cold air (Bianco et al. 1974; Patel et al. 1976; Beil and de Kock, 1978; Barnes et al. 1981c; Bleecker et al. 1983; Walden et al. 1984).

9.1.2 Possible Mechanisms of Alpha-Adrenergic Overactivity

Whereas smooth muscle from both humans and dogs exhibits virtually no contractile response to alpha-adrenergic agonists in vitro, in the absence of pretreatment with histamine or potassium chloride (Kneussl and Richardson, 1978; Barnes et al. 1983e), diseased human pulmonary tissue reacts to norepinephrine without pretreatment (Kneussl and Richardson, 1978). This reaction is blocked by phentolamine. Similarly, alpha-adrenergic mechanisms may produce bronchoconstriction in association with fairly marked beta-blockade (Leff et al. 1986). These findings suggest that alpha-adrenergically mediated bronchoconstriction may be a significant pathogenetic mechanism only in association with an existing disease process or bronchoconstrictor stimulus.

Barnes et al. (1980c) have reported the finding of an increased number of alpha-adrenergic receptors, associated with decreased beta-receptors in guinea-pigs with ovalbumin-induced asthma. The concept of reciprocal adrenergic receptor

interconversion (Kunos et al. 1985; Szentivanyi, 1985), and the suggestion that both asthma and the atopic state, in general, may be related to an increase in the number of alpha-receptors and decreased beta-receptors (see Chapter 10.2.4) are clearly of considerable interest in this regard. However, the potentiating effect of histamine on alpha-adrenergically mediated contraction in canine tracheal smooth muscle appears to be associated with no change in the density of either alpha-1 or alpha-2 adrenergic receptors, nor any change in alpha-2 receptor-agonist affinity (Barnes et al. 1983e), suggesting its mediation by a post-receptor mechanism.

9.1.3 Evidence of Extrapulmonary Alpha-Adrenergic Overactivity in Asthma

There is relatively little evidence of the presence of alpha-adrenergic overactivity in patients with bronchial asthma. It has been demonstrated that asthmatics require a smaller dose of phenylephrine to produce a given degree of pupillary constriction (Henderson et al. 1979; Davis and Lieberman, 1982) and cutaneous vasoconstriction (Henderson et al. 1979) than either normal subjects or non-asthmatic atopic individuals. These manifestations of alpha-adrenergic hypersensitivity appear to be associated, in addition, with evidence of both cholinergic hypersensitivity and beta-adrenergic subsensitivity, and also occur in patients with cystic fibrosis (Davis and Kaliner, 1983), healthy heterozygote carriers of the cystic fibrosis gene (Davis, 1984) and patients with allergic rhinitis (Kaliner et al. 1982).

There appears to be no abnormality in the alpha-2 adrenergically mediated inhibition of the reaction in which prostaglandin E_1 stimulates the accumulation of 3,5 cyclic AMP in the platelets of asthmatic patients. There is, likewise, no abnormality in either alpha-2 receptor density or agonist affinity in platelets from asthmatic patients (Davis and Lieberman, 1982; Davis et al. 1985).

9.2 A STUDY OF THE RESPONSES TO THE ALPHA-2 ADRENERGIC AGONIST, CLONIDINE

It was originally intended to study the alpha-adrenergic component of the autonomic nervous system by comparing the metabolic and haemodynamic responses of asthmatic and non-asthmatic individuals to the intravenous administration of the alpha-1 adrenergic agonist, phenylephrine. The protocol for this study was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand, subject to the following conditions:

(i) That the subject should be a member of either the medical or paramedical staff, who could be made fully aware of the potential dangers involved.

(ii) That the study should immediately be discontinued in the event of a serious side effect occurring in any subject.

Unfortunately, although the protocol was completed uneventfully in two subjects, the third suffered a severe

hypertensive response to the intravenous administration of 0.1mg phenylephrine which was associated with marked slowing of the sino-atrial rate and the emergence of a nodal rhythm. It was not, therefore, possible for the investigation to continue, and it was for this reason that oral clonidine was used to study alpha-adrenergic responses.

Clonidine is a selective alpha-2 adrenergic agonist (Berthelsen and Pettinger, 1977; Hoffman and Lefkowitz, 1980). It has a potent anti-hypertensive action, the mechanism of which is complex and controversial, and may be related to the stimulation of either peripheral alpha-2 receptors (Hoffman and Lefkowitz, 1980) or inhibitory central nervous system adrenergic receptors (van Zwieten, 1973; Sciabine et al. 1976) which have characteristics in common with peripheral alpha-2 receptors (Berthelsen and Pettinger, 1977; Miach et al. 1978).

The administration of clonidine produces a rise in the plasma level of growth hormone which is thought to be mediated by the stimulation of central alpha-receptors (Lal et al. 1975; Gilad et al. 1979; Lal et al. 1981). In addition, it causes a rise in the plasma potassium levels by the inhibition of renin secretion, an effect which also appears to be mediated by alpha-adrenergic pathways (Chevillard et al. 1978) and a rise in the level of the plasma glucose. The latter effect may be related to the increase in plasma growth hormone secretion (Humphreys and Reid, 1979), but is more likely to be due to the inhibitory effect of clonidine on the secretion of insulin, which is also an alpha-adrenergically mediated effect (Metz et al. 1978).

9.2.1 Subjects

Nine asthmatic patients and 10 non-asthmatic control subjects were studied. Each of the asthmatic patients had been using a sympathomimetic bronchodilator aerosol, and some, in addition, were being treated with beclomethasone dipropionate aerosol (maximum dose 400 /ug/day), and/or disodium cromoglycate. None had ever been treated with systemic corticosteroids. All bronchodilators were discontinued at least 10 days prior to the study. The asthmatic patients were all in a stable condition at the time of the study.

The non-asthmatic control group consisted of 5 normal individuals and 5 subjects with a history of allergic rhinitis. A comparison of the data obtained from the normal subjects and those with allergic rhinitis revealed no differences. The object of this study was to investigate the relationship of alpha-adrenergic overactivity to bronchial asthma rather than to the atopic state in general, and it was considered reasonable, therefore, to pool the data from these two groups of subjects for comparison with those obtained from the asthmatic patients. Vasoconstrictor nose drops were discontinued in a number of subjects with allergic rhinitis at least 10 days prior to the study.

9.2.2 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 and kept patent with a slow

infusion of normal saline. Blood was sampled through the cannula 10 and 20 minutes later. A 150/ug dose of clonidine hydrochloride was then administered orally and blood subsequently sampled half hourly for 3 hours. Blood pressure readings were taken prior to each blood sample.

Aliquots of the blood sampled at each time were used for measurement of the plasma levels of growth hormone, potassium and glucose. Plasma was immediately separated and analysed for potassium and glucose. The blood for the plasma growth hormone determinations was placed into iced tubes, and immediately centrifuged, and the separated plasma stored at -20°C until assayed.

Biochemical Analysis:

See Appendix "A".

Statistical Analysis:

Pairwise comparison was done for each variable using the 2-tailed Mann-Whitney U test (Siegel, 1956). The significance of the variation in each variable following the administration of clonidine 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 following clonidine with the basal value was done within each group using the 95% extreme range limits of mean ranks (Steel, 1961).

The basal level of each variable was the arithmetic mean of

the two values obtained prior to the administration of clonidine.

9.2.3 Results

Demographic and pulmonary function data are shown in Table 6. The forced expiratory volume in one second (FEV_1) was significantly lower and the airway resistance and percentage fall in peak expiratory flow rate (PEFR) on exercise significantly greater in the asthmatic patients than in the non-asthmatic subjects.

The systolic blood pressure varied significantly in both groups of subjects f ...wing clonidine (p = <0.001). The diastolic blood pressur... varied significantly in both groups (p = <0.05 in non-asthm... subjects; p = <0.01 in asthmatic patients). In the non-a...c subjects, however, no significant change from the basal ... could be detected using the 95% extreme range limits of mean ranks. There was no significant difference between the groups at any time. The responses in arterial blood pressure are shown in Table 7.

The plasma growth hormone level varied significantly in the control subjects (p = <0.001) but not the asthmatic patients following clonidine. Neither of the other meta... variables exhibited a significant degree of variation during the study in either group. There was no significant difference between the groups in any of the metabolic variables at any time. When the results of the plasma growth hormone responses were expressed as ratios (value:basal) to correct for the difference between the groups in the basal values, there was, likewise, no difference

TABLE 6. DEMOGRAPHIC AND PULMONARY FUNCTION DATA

	Age (Yrs)	Sex	Mass (kg)	FEV_1 (% predicted)	Airway Resistance $cmH_2O/l/sec$	Fall in PEFR on exercise (%)
Non-asthmatic subjects (n=10)	24.1(4.4)	6F,4M	58.9(7.3)	101.4(14.2)*	1.9(0.4)**	2.7(2.7)**
Asthmatic patients (n=9)	20.7(4.8)	5F,4M	61.9(15.2)	69.3(21.7)	7.1(4.9)	31.3(17.8)

Values are mean(SD)

Asthmatic vs non-asthmatic subjects significantly different:
*p = <0.005
**p = <0.0005

TABLE 7. BLOOD PRESSURE RESPONSES TO CLONIDINE

		Basal	30 min	60 min	90 min	120 min	150 min	180 min
Non-asthmatic subjects (n=10)	Systolic BP	113 (11)	108 (9)	104/ (13)	97/ (10)	97/ (12)	98/ (12)	99 (10)
	Diastolic BP	73 (8)	71 (8)	66 (7)	67 (8)	68 (10)	66 (8)	65 (10)
Asthmatic subjects (n=9)	Systolic BP	114 (11)	106 (14)	102 (9)	98/ (12)	97/ (8)	97/ (10)	98/ (8)
	Diastolic BP	71 (7)	68 (7)	64 (9)	62/ (13)	61/ (9)	65/ (6)	64/ (7)

Values are mean(SD) mmHg

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

between the asthmatic and non-asthmatic subjects at any time. The metabolic responses to clonidine are shown in Tables 8-10.

Examination of the growth hormone data showed that the asthmatic patients had a higher basal level of plasma growth hormone, as well as higher levels following the administration of clonidine, than the normal subjects although these differences never achieved statistical significance. The higher levels in the asthmatic patients appeared to be mainly due to 3 subjects who demonstrated marked hyperresponsiveness when compared with both the other 6 asthmatic patients and with the control subjects (Figure 2).

The only side effect of clonidine which was observed was drowsiness which affected most of the subjects, none of whom however reported any change in respiratory symptoms. No index of pulmonary function was measured during the study since it was felt that changes in growth hormone levels might be induced by even the minor stress and physical activity involved in the performance of respiratory function tests (Merimee and Rabin, 1973; Martin et al. 1977).

9.2.4 Discussion

Despite the definite blood pressure responses to clonidine observed in the study, the metabolic responses were, in general, minimal. The marked hyperresponsiveness of the plasma growth hormone levels in 3 of the asthmatic patients following clonidine administration was considered to be of possible significance, especially since, allowing for differences in the methods of

TABLE 8. PLASMA GROWTH HORMONE RESPONSES TO CLONIDINE

	Basal	30 min	60 min	90 min	120 min	150 min	180 min
Non-asthmatic subjects (n=10)	1.9(2.1)	1.2(1.1)	1.1(0 9)	3.4(3.7)	3.6(3.0)≠	2.6(2.6)	1.2(.8)
Asthmatic patients (n=9)	4.6(5.9)	4.7(9.9)	3.4(5.8)	7.4(8.9)	7.8(8.0)	5.1(8.6)	4.4(9.3)

Values are mean(SD) ng/ml

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

TABLE 9. PLASMA GLUCOSE RESPONSES TO CLONIDINE

	Basal	30 min	60 min	90 min	120 min	150 min	180 min
Non-asthmatic subjects (n=10)	4.8(0.5)	4.9(0.6)	5.3(0.8)	5.0(1.0)	4.8(0.5)	4.7(0.5)	4.9(0.7)
Asthmatic patients (n = 9)	4.8(0.6)	4.7(0.6)	4.9(0.3)	4.9(0.5)	4.8(0.5)	4.6(0.5)	4.6(0.5)

Values are mean(SD) mmol/l

TABLE 10. PLASMA POTASSIUM RESPONSES TO CLONIDINE

	Basal	30 min	60 min	90 min	120 min	150 min	180 min
Non-asthmatic subjects (n=10)	3.6(0.3)	3.7(0.3)	3.9(0.5)	3.8(0.3)	3.8(0.4)	3.9(0.5)	4.0(0.6)
Asthmatic patients (n=9)	3.5(0.2)	3.7(0.2)	3.7(0.3)	3.7(0.3)	3.7(0.3)	3.8(0.2)	3.7(0.2)

Values are mean(SD) meq/l

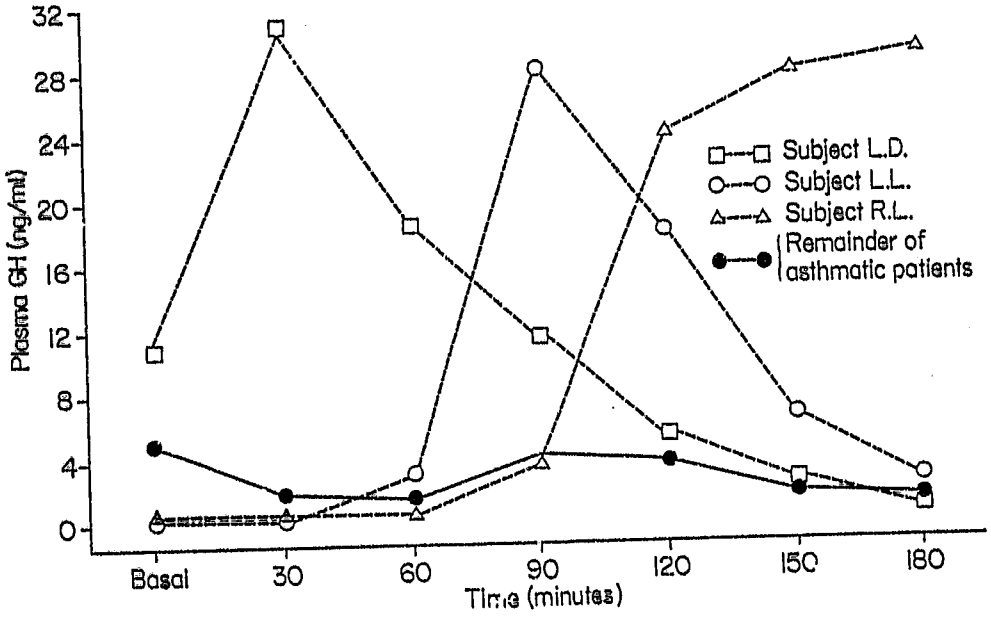


Figure 2. Plasma growth hormone responses to clonidine in 3 asthmatic "hyperresponders" (actual values) and the remainder of the asthmatic patients (mean values).

radioimmunoassay, their responses were also markedly in excess of those of normal subjects to 150 ug intravenous clonidine in other studies (Lal et al. 1975; Lal et al. 1981).

It must be noted that a significant increase in plasma growth hormone levels may occur in simulated tests. This "agnogenic" release of growth hormone which occurs either early - within one hour of needle placement - or late - 3 hours or more after needle placement - in the course of the tests, has been attributed to the stress associated with the procedure (Spitz et al. 1972). While it is conceivable that the high basal level and [illegible]rely early rise in one of these 3 "hyperresponders" was responsible for this phenomenon (Figure 2), the responses of the other 2 [illegible]ok place during the "critical zone" which, it has been suggested, constitutes the optimal time for the true assessment of the response in growth hormone levels to various stimuli (Spitz et al. 1972).

It must be emphasised that frequent determinations of plasma growth hormone levels during the day and night in animals as well as in man show that striking variations may occur in relation to secretory bursts (Finkelstein et al. 1972; Tannenbaum and Martin, 1976). The response of the plasma growth hormone level to various stimuli is, as a result, notoriously difficult to evaluate in individual tests (Rose, 1985).

Despite these difficulties, it was considered possible that the 3 "hyperresponders" could represent a subgroup of asthmatic patients with exaggerated alpha-adrenergic responses. For this

reason it was decided that the patterns of growth hormone secretion in asthmatic patients should be studied further.

9.3 A STUDY OF THE METABOLIC RESPONSES TO EXERCISE

The performance of even such minor physical activity as rising from bed in the morning may stimulate an appreciable rise in plasma growth hormone levels (Martin et al. 1977), and exercise is a potent stimulus to its secretion (Martin et al. 1977; Galbo et al. 1981; Joffe et al. 1985; Galbo, 1986). The physiological control of growth hormone secretion is dually regulated by two peptide hormones, a growth-hormone-releasing-hormone and the inhibitory hormone somatostatin, both of which are synthesised in, and released from, peptide neurones in the medial basal hypothalamus. These neurones are, in turn, under the control of a complex monaminergic system (Martin et al. 1977). A number of the stimuli which increase the secretion of growth hormone, including hypoglycaemia and exercise are, at least partly, alpha-adrenergically mediated and the responses may be blocked by intravenous phentolamine (Blackard and Heidingsfelder, 1968; Hansen, 1971).

In this study the effect of exercise on plasma growth hormone responses was compared in asthmatic patients and normal subjects. In addition, the responses in the plasma levels of prolactin, adrenocorticotropic hormone (ACTH) and cortisol were studied. The plasma levels of each of these hormones also exhibit a vigorous response to exercise (Galbo et al. 1981; Joffe et al.

1985; Galbo, 1986). The response in the plasma prolactin levels appears, as well, to be, at least partly, alpha-adrenergically mediated, and is attenuated by alpha-blockade (Galbo, 1986).

9.3.1. Subjects

Ten asthmatic patients and 12 normal control subjects were studied. All of the subjects were males. Four asthmatic patients were using beclomethasone dipropionate aerosol (maximum dose 400 ug/day; duration of use greater than one year in 3, less than 2 months in one), and one had received a short course of systemic corticosteroids 2 years prior to the study. All bronchodilators were discontinued at least 7 days, and all other medications at least 24 hours, prior to each study. The asthmatic patients were all in a stable condition at the time of the study.

It was anticipated that the asthmatic patients might develop bronchospasm during or after exercise, and that this might itself be a stimulus for increased hormone secretion, both in relation to the work of breathing and by a stress-related effect (Martin et al. 1977; Rose, 1985). In order to investigate this possibility, the study was performed twice in each asthmatic patient; with and without the administration of anti-asthmatic medications prior to exercise. The two studies in the asthmatic patients were performed on separate days, in random order, at least a week apart. The premedication in each patient consisted of the inhalation of salbutamol aerosol (200 ug) and disodium cromoglycate aerosol (2mg) 10 minutes prior to exercise.

The normal subjects were studied once only. As it has been

suggested that disodium cromoglycate may depress the plasma glucocorticoid response associated with exercise (Holmes et al. 1972), each normal subject received the same premedication as the asthmatic patients.

No subject in either group was a trained athlete.

9.3.2 Methods

The exercise bronchoprovocation test was performed in each subject simultaneously with the study. Each study was performed following an overnight fast. After arrival at the exercise laboratory, each subject was placed in the recumbent position in a quiet room. A 19-gauge or larger cannula was immediately inserted into an arm vein and kept patent with a slow infusion of normal saline. Not less than 30 minutes later blood was sampled through the indwelling cannula and this was repeated 15 minutes later. The subjects then ran for 10 minutes on a treadmill of variable speed and incline, during which heart rate was continuously monitored. The incline of the treadmill was progressively increased so that by the end of 7 minutes each subject had attained estimated maximum heart rate based on the data in the review of Fortuin and Weiss (1977). This rate was then maintained for a further 3 minutes, following which the subjects were once again placed in the recumbent position.

Blood was sampled immediately and 5 and 20 minutes following the completion of the exercise. Heart rate and blood pressure were measured prior to each sampling of blood. Blood for hormone determinations was placed into iced tubes and immediately

centrifuged, and the separated plasma stored at -20°C until assayed.

Biochemical Analysis:

See Appendix "A".

Statistical Analysis:

For each variable, normal subjects were compared with asthmatic patients under both study conditions, namely with and without anti-asthmatic premedication. The two sets of data from the asthmatic patients were also compared. The overall significance of any differences between the data from the normal subjects and the 2 sets of data from the asthmatic patients was assessed using the Kruskal-Wallis one-way analysis of variance (Siegel, 1956). The 2-tailed Mann-Whitney U test was used for the pairwise comparison of data from the asthmatic patients with those of the control subjects (Siegel, 1956). The two sets of data from the asthmatic patients were compared using the Wilcoxon rank sign test (Siegel, 1956). All pairwise comparisons were conducted at a simultaneous 5% Bonferroni level.

The significance of the variation in each variable in association with exercise was assessed within each group using Friedman's two-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 exercise.

9.3.3 Results

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

The study with premedication was performed first in 7 of the asthmatic patients, and that without premedication first in the other 3. The fall in PEFR on exercise was markedly attenuated in the asthmatic subjects by premedication with salbutamol and disodium cromoglycate (27.9 + 14.4% fall in PEFR without premedication; 8.3 $\pm$ 9.0% with premedication).

There were significant variations in heart rate and both systolic and diastolic blood pressure in the asthmatic patients, under both study conditions, and the normal subjects in association with exercise ($p = <0.001$). There was no difference between the asthmatic patients under either study condition and the normal subjects in any of these variables at any time. The haemodynamic responses to exercise are shown in Table 12.

The plasma growth hormone level varied significantly in association with exercise in the normal subjects ($p = <0.0005$) and in the asthmatic patients under both study conditions ($p = <0.01$ with premedication; $p = <0.02$ without premedication). There was no significant difference in the plasma growth hormone levels

TABLE 11. AGE AND PULMONARY FUNCTION DATA

	Age (Yrs)	FEV_1 (% predicted)	Airway Resistance ($cmH_2O/l/sec$)	Fall in PEFR on exercise (%)
Normal subjects (n=12)	22.4(2.2)	103.2(9.2)*	1.6(0.5)*	1.5(3.1)*
Asthmatic patients (n=10)	21.0(3.1)	62.7(6.8)	4.0(1.5)	27.9(14.4)

Values are mean(SD)

* Normal subjects vs asthmatic patients significantly different ($p<0.0005$)

TABLE 12. CARDIOVASCULAR RESPONSES TO EXERCISE

		Basal	Immediately post-exercise	5 minutes post-exercise	20 minutes post-exercise
		Heart Rate (beats/min)			
Normal subjects	(n = 12)	56(5)	188(7)	99(7)	87(8)
Asthmatic patients without premedication	(n = 10)	57(5)	189(7)	103(7)	96(12)
Asthmatic patients with premedication	(n = 10)	58(5)	187(5)	101(7)	90(10)
		Systolic BP (mmHg)			
Normal subjects		119(8)	203(15)	147(12)	128(8)
Asthmatic patients without premedication		119(8)	197(14)	141(12)	125(9)
Asthmatic patients with premedication		120(7)	194(15)	147(12)	124(9)
		Diastolic BP (mmHg)			
Normal subjects		68(8)	41(15)	63(5)	72(7)
Asthmatic patients without premedication		69(7)	36(13)	63(9)	73(8)
Asthmatic patients with premedication		68(15)	34(13)	62(5)	71(8)

Values are mean(SD)

between the asthmatic patients under either study condition, and the normal subjects at any time. In order to correct for the large differences between the groups in the basal values, the plasma growth hormone responses were expressed as ratios (value:basal). There was, likewise, no significant difference between the asthmatic patients under either study condition, and the normal subjects at any time. The plasma growth hormone responses are shown in Table 13.

The plasma prolactin level varied significantly in the normal subjects in association with exercise ($p = <0.001$). In the asthmatic patients the plasma prolactin level varied significantly only in the study with premedication ($p = <0.05$), although no difference from the basal level could be detected using the 95% extreme range limits of mean ranks. There was no significant difference between the asthmatic patients under either study condition, and the normal subjects at any time. Similarly, when the plasma prolactin responses were expressed as ratios (value:basal), there was no significant difference between the asthmatic patients under either study condition, and the normal subjects at any time. The plasma prolactin responses are shown in Table 14.

The plasma ACTH levels varied significantly in association with exercise only in the normal subjects, and in the asthmatic patients in the study without premedication ($p = <0.02$). In the normal subjects, however, no significant change from the basal value could be detected at any time using the 95% extreme range limits of mean ranks. The plasma ACTH levels were significantly

TABLE 13. PLASMA GROWTH HORMONE RESPONSES TO EXERCISE

	Basal	Immediately post-exercise	5 mins post-exercise	20 mins post-exercise
Normal subjects (n=12)	0.3(0.2)	8.7(14.8)	10.9(14.3)ϕ	12.3(14.2)ϕ
Asthmatic patients without premedication (n=10)	0.1(0.1)	14.9(17.4)*	15.7(15.8)ϕ	17.2(13.0)ϕ
Asthmatic patients with premedication (n=10)	2.1(4.7)	5.6(12.1)	7.2(10.3)ϕ	9.3(9.1)ϕ

Values are mean(SD) ng/ml

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

* Ratio value:basal significantly greater in asthmatic patients without premedication than with premedication ($p = 0.01$)

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