THE AUTONOMIC MECHANISMS IN EXTRINSIC BRONCHIAL ASTHMA

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SUMMARY

Although the bronchial hyper-reactivity to specific and non-specific stimuli in asthma has been well recognised for many years, the factors involved in its pathogenesis are still unresolved. Salter[7] in 1859 first suggested that airways hyper-reactivity in asthma resulted from hypersensitivity of pulmonary nervous system. More recently, Szentivayni[45] (1968) has postulated that the atopic state and bronchial hyper-reactivity in asthma is due to a functional imbalance between the alpha and beta adrenergic receptors and results from diminished beta receptor function. The theme of work presented in this thesis is to examine the autonomic mechanisms in patients with extrinsic asthma and in particular the role of alpha adrenergic activity in the control of bronchomotor tone both in normal subjects and asthmatic patients.

In sixteen patients with extrinsic bronchial asthma alpha stimulation in presence of beta blockade caused a significant bronchoconstriction both in the central and peripheral airways whereas in ten normal subjects alpha stimulation or beta blockade had no effect on the bronchial calibre. The bronchoconstriction caused by pharmacological stimulation of alpha receptors could be inhibited by pretreatment of these patients with alpha receptor blocking drugs, phenoxybenzamine and thymoxamine. The results of this investigation confirm the presence of alpha receptors in human airways and that the stimulation of these receptors can cause bronchoconstriction in asthmatic patients. Further, it is shown that thymoxamine can effectively inhibit histamine, allergen

and exercise induced bronchoconstriction whereas it had no effect in methacholine and Prostaglandin F_2 alpha induced bronchoconstriction. It is suggested that inhibitory effect of thymoxamine in histamine, allergen and exercise provoked asthma is mediated by increase in the intracellular levels of cyclic AMP which prevents the effect of some of the pharmacological mediators released in the type I reaction on the bronchial smooth muscle. On the other hand, methacholine and Prostaglandin F_2 alpha are now known to cause bronchial smooth muscle contraction through stimulation of cholinergic receptors which are unaffected by alpha receptor blocking drugs. Further, it is shown that thymoxamine and phentolamine when administered with isoprenaline cause significantly greater bronchodilatation compared to bronchodilatation achieved with isoprenaline alone. This observation may have therapeutic significance in the management of patients with chronic labile airways obstruction and especially in those patients in whom beta agonists alone fail to produce significant bronchodilatation.

In Part II of this thesis biochemical studies of leucocyte adenyl cyclase and lymphocyte guanyl cyclase activities in normal and asthmatic patients are described. It is now well recognised that the membrane bound enzyme, adenyl cyclase-cyclic AMP system, mediates beta adrenergic responses and that guanyl cyclase-cyclic GMP system is activated by cholinergic and possibly alpha receptor stimulation. The presence of both these enzyme activities on the peripheral leucocytes has paved the way for more fundamental and basic research to study autonomic

mechanisms in asthma. Conflicting results had been reported previously on the leucocyte adenyl cyclase response to isoprenaline in patients with asthma. Logsdon et al[107] observed diminished leucocyte adenyl cyclase activity in all patients they had examined whereas Gillespie et al[108] failed to show any significant difference in the leucocyte adenyl cyclase activity of normals or asthmatic patients. Parker and Smith[109], in more detailed studies, have reported diminished adenyl cyclase activity in patients with acute asthma whereas in patients in remission the activity of this enzyme was normal. In none of these studies was there any objective assessment of degree of airways obstruction or detailed account of the clinical state of the patients.

In this carefully conducted investigation it is shown that the basal leucocyte adenyl cyclase activity is increased in patients with acute asthma and the response of this enzyme system to isoprenaline is inversely related to the basal levels. These observations indicate that adenyl cyclase activity in acute asthma is maximally stimulated by endogenous factors and further stimulation becomes increasingly difficult. In contrast, the basal adenyl cyclase activity in patients in remission is low and the response to isoprenaline does not differ significantly from normal subjects. The adenyl cyclase stimulation to isoprenaline in asthmatic patients did not relate to the total circulating reagins nor to airways response induced by beta blockade and allergen challenge. These observations together with the failure to produce histamine hyper-reactivity in normal

subjects suggest that diminished beta receptor function is not the cause of atopic state or bronchial hyper-reactivity in asthma as postulated by Szentivayni[45]. In presence of beta receptor function in asthmatic patients the minor alpha receptor stimulating properties of adrenaline may become dominant causing bronchoconstriction through stimulation of alpha receptors in the airways. This phenomenon may explain the adrenaline fastness and adrenaline reversal commonly observed in status asthmaticus.

The possibility of enhanced cholinergic mechanisms in asthmatic patients has also been postulated. In contrast to my expectations, the lymphocyte guanyl cyclase response to cholinergic and alpha stimulation was depressed in the asthmatic group as a whole and no distinction in this activity could be made on the basis of clinical state of the disease as was possible in the adenyl cyclase study. It appears that guanyl cyclase-cyclic GMP abnormality in asthma may be a primary defect which in turn modifies adenyl cyclase activity. Alpha and cholinergic stimulation both cause bronchoconstriction in Man, and this response is grossly exaggerated in asthmatic patients. In guanyl cyclase studies, alpha and cholinergic stimulation have been shown to cause a significant increase in cyclic GMP formation lymphocytes of normal subjects. Assuming that similar increase in cyclic GMP also occurs in the bronchial smooth muscle of these subjects, the high levels of cyclic GMP formed may lead to inhibition of cyclic AMP phosphodiesterase preventing hydrolysis of cyclic AMP and thereby maintaining relaxation of bronchial

smooth muscle. In contrast, alpha and cholinergic stimulation does not produce a significant increase in cyclic GMP in asthmatic patients and this probably has an effect opposite to that observed in normal subjects[178]. It is possible that in asthmatic patients we are observing an increased hydrolysis of cyclic AMP through stimulation of cyclic phosphodiesterase due to low concentration of cyclic GMP as reported by Beavo et al[102, 103] resulting in bronchoconstriction. Such a phenomenon in mast cells would also explain increased mediator release following alpha and cholinergic stimulation as already reported[29].

CHAPTER I

INTRODUCTION

1.0 BRONCHIAL ASTHMA AND DEVELOPMENTS IN ALLERGY AND ANAPHYLAXIS

1.1 Historical

A number of ancient Greek writings as early as the fifth century B.C. described symptoms resembling asthma. Areteus[1] in the second century A.D. gave a graphic description of asthma. In none of these early references, however, was there any sharp distinction between various forms of dyspnoea.

The first Renaissance physician to write about asthma was Geralomo Gordano (1501 - 1576 A.D.). He was called from Italy to Edinburgh to treat John Hamilton, Archbishop of St. Andrews. Gordano observed the Archbishop for six weeks before diagnosing asthma. His treatment included diet, exercise and purging to decrease the secretions of mucous in the throat and substitution of a bed of unspun silk for one of feathers. Happily for Gordano, John Hamilton recovered, but only lived long enough to be hanged by the Scottish Reformers[2].

In 1607 Joannes Baptista van Helmont[3] described nervous or spasmodic dyspnoea as a distinct entity and later Thomas Willis (1681)[4] clearly emphasised the spasmodic nature of asthma. Sir John Floyer[5] in 1698 published his classic treatise on asthma and assigned the cause of asthma to a "contracture of muscular fibres of bronchi".

The concept of hyper-sensitivity to hair dust was first suggested by Robert Bree (1811)[6] and described in detail in his book "Disordered Respiration". It remained, however, for Salter (1859)[7] to place this concept of hyper-sensitivity on more firm clinical grounds. He suggested that the asthmatic attack was caused by spastic contraction of the circular muscle around the small bronchi and it was due to excitomotory or reflex action. Salter by his perfect logic and clinical observations also suggested that the bronchi of asthmatic patients were hyper-sensitive and factors such as fog, cold air, emotion and exercise could cause bronchospasm in these patients. He said, "I believe it possible that asthma is sometimes produced by particular materials admixed with blood in the lungs, and that therefore it is so far humoral, but that these particular materials - whether absorbed unchanged, as alcohol, ethers and saline solutions, or the result of healthy digestion, or of perverted digestion - have nothing particular in them, but the essence of the disease in these cases, as well as in others, consists of a morbid sensitiveness and irritability of the pulmonary nervous system". In aetiological factors he stressed heredity, which he was able to trace in 40% of his patients. Since the muscular spasm did not explain all the clinical and experimental phenomena in asthma, the concept of obstruction of small bronchi from within was elaborated by other prominent nineteenth century physicians like Laennec, Ramadge and Trousseau.

1.2 Allergy and Anaphylaxis

In 1839 Francois Magendie[8] reported first definite work on anaphylaxis in dogs and he observed that dogs died suddenly if injected repeatedly with egg albumin. Flexner (1894)[9] confirmed these results. Hericourt and Richet (1898 - 1903)[10] performed many experiments on induced hyper-sensitiveness in animals and Portier and Richet (1902)[11] termed this phenomenon as anaphylaxis. Otto (1907)[12] demonstrated that anaphylaxis in guinea pigs depended on an antibody and he showed that it was possible to passively sensitise animals by transference of serum from a sensitised animal.

In 1906 Von Pirquet[13] coined the term "allergy" indicating an altered reaction or abnormal response and he originally used the term to designate the state of heightened sensitiveness to tuberculin but subsequently it included hyper-sensitiveness represented by hay fever and asthma. Coca (1923)[14] recognising the need for a term which would distinguish such clinical manifestations from other types of hyper-sensitiveness proposed the term "atopy".

The first definite suggestion that human hyper-sensitiveness - hay fever - was related to anaphylaxis in animals was made by Wolff-Eisner (1906)[15] and in 1910 Meltzer[16] and Karl Kossler independently suggested that asthma was a phenomenon of local anaphylaxis in the lungs. In 1912 Schloss[17] reported food sensitiveness in a child, demonstrated by skin tests, and from this point on, the use

of skin tests for diagnosis of sensitivity to foods and other substances developed rapidly.

1.3 Atopy and Reaginic Antibody

Prausnitz and Kustner[18] in 1921 first demonstrated the existence of a specific factor in the serum of an atopic subjects (Kustner, who was sensitive to fish) by passive transfer to the skin of a normal unsensitised person (Prausnitz). This specific antibody-like factor could not be shown by precipitation, complement fixation or by passive anaphylaxis in guinea pigs. This heat-labile antibody like substance, found especially in the sera of atopic persons, was called reagin by Coca.

The investigations to find other and better means of measuring human reagins and to isolate and characterise them has been an exciting development in recent years. The chief advances concerning atopy were the identification by Ishizaka (1966)[19] of the Immunoglobulin E (IgE) as the elusive reaginic antibody and the development of its quantitative measurement by Johansson and his colleagues (1967)[20].

Immunoglobulin E is a normal constituent of human serum which is present at 1/40000th concentration of Immunoglobulin G. It has the capacity to sensitise cells for long periods of time for subsequent triggering action of antigen. Atopic subjects have six times as much IgE as normal subjects, and in allergic asthma 63% of patients have raised IgE compared with 5% in non-atopic or intrinsic asthma[20]. Ishizaka[21] has further shown that the IgE

production occurs in the mucosal lymphoid cells and this may explain why atopic subjects are readily sensitised by this route, and may also throw light on possibility of local IgE sensitisation, without serological and skin sensitivity, in some subjects at present regarded as non-atopic or intrinsic.

The clinical characterisation of an individual as an atopic person depends on the presence of several features including characteristic symptomatology, the existence of skin sensitivity of immediate weal and flare type, and a family history of atopic disease. Eosinophilia is common to all atopic disorders, and the level of circulating eosinophils parallels the severity of patients' symptoms[22]. The familial clustering of atopic diseases suggests that an atopic tendency may be inherited[23]. The genetic predisposition does not seem to determine the organ or tissue which is involved. Similarly, there is a great variability in the allergens to which an atopic person will develop sensitivity.

1.4 Diseases of Reagin-Mediated Hyper-Sensitivity

Reagin-mediated hyper-sensitivity is also termed the immediate hyper-sensitivity or the type I allergic reaction[24]. The classical diseases of the type I allergy are seasonal and perennial allergic rhinitis and asthma, anaphylaxis, certain cases of urticaria and angioedema, atopic dermatitis, certain cases of food and drug reactions and bee sting hyper-sensitivity.

The changes characteristic of the type I allergic reaction are initiated by the interaction of a specific antigen with homologous IgE antibody bound to the tissue mast cells or circulating basophils[25]. The tissue changes include increase in vascular permeability, smooth muscle contraction, mucous gland hyper-secretion, leucotaxis and especially eosinophilotaxis, and irritation of sensory nerve endings. These tissue changes in the reagin-mediated allergic reaction can be accounted for by one or more of pharmacologically active substances (chemical mediators) released during the reaction.

1.5 Chemical Mediators Released in the Type I Allergic Reaction in Man

The presence of histamine in the mast cells[26] and its release from the mast cell granules during the reagin-mediated reaction was first demonstrated by Mota[27] and later confirmed by Austen and Humphrey[28]. Histamine and slow reacting substance of anaphylaxis (S.R.S.-A.) are also released from passively sensitised human lung on exposure to antigen[29]. Although the formation of bradykinin in allergic human lung has not been demonstrated, this substance is of interest as it is found in the nasal and bronchial secretions of patients with hay fever or asthma[30]. In addition bradykinin forming enzyme is released in guinea pigs during anaphylactic reaction[31, 32]. Serotonin (5 hydroxytryptamine) although a major mediator of anaphylaxis in certain animal species[29] is probably not important in allergic reaction in Man[33].

A recent addition to the ranks of chemical mediators is an eosinophilic chemotactic factor of anaphylaxis, E.C.F.-A.[34] and Prostaglandin F_2 alpha[35]. Prostaglandin F_2 alpha is found in human lung and is released in patients with asthma during bronchial challenge with appropriate antigen[36].

Acetylcholine, the cholinergic neurotransmitter, although not released during allergic reaction, has also been considered an important mediator in asthma because of its profound effect on bronchial smooth muscle in asthmatic patients[37].

1.6 Bronchial Hyper-Reactivity to Chemical Mediators in Asthma

Weiss, Robb and Blumgart[38] in 1929 first reported that asthmatic patients developed bronchospasm following an administration of a small dose of histamine which did not affect normal subjects. Curry[39] investigated this phenomenon of bronchial hyper-reactivity to histamine and acetylcholine in asthma and Tiffeneau[37] was first to quantitatively measure bronchial hyper-reactivity to acetylcholine and histamine in asthmatic patients. In recent years, with the discovery of new chemical mediators released in the type I allergic reaction, the bronchial hyper-reactivity in asthmatic patients has been demonstrated to bradykinin[40] and Prostaglandin F_2 alpha[41, 42]. This bronchial hyper-reactivity has been reported to remain constant in individual patients and is known to persist for many years even in the absence of active asthma[43, 44]. The cause of bronchial hyper-reactivity is

still uncertain. The beta adrenergic theory put forward by Szentivayni[45] answers many facets of atopy and bronchial hyper-reactivity in asthma.

2.0 DEFINITION OF ASTHMA

Bronchial asthma should be considered as intermittent reversible obstructive lung disease and differs from chronic bronchitis and emphysema where airways obstruction is chronic and largely irreversible. Asthmatic patients complain of wheezing, dyspnoea or tightness in the chest and cough productive of thick tenacious sputum. Adequate therapy with bronchodilator drugs or corticosteroids reverses the airways obstruction in these patients. It was previously thought that lung structure and gas exchange function is normal in asthmatic patients in symptom-free periods, however, recent studies have demonstrated persistent ventilation-perfusion abnormalities, mild hypoxaemia and significant degree of peripheral airways obstruction in asymptomatic patients.

On clinical grounds, skin testing together with the measurement of IgE, two main types of asthma can be broadly defined. First is the extrinsic or atopic asthma which begins early in life and is often associated with atopic eczema or allergic rhinitis. A patient with extrinsic asthma is symptomatic on exposure to a specific allergen, has positive skin tests and bronchial challenge reactions and frequently the serum IgE level is elevated. Second type is the perennial or intrinsic asthma which usually begins later in life and is often associated with nasal polyps. In a patient with intrinsic asthma no allergic aetiology can be detected by any currently available techniques and the serum IgE level is not elevated.

Respiratory tract infections predispose to asthmatic attacks in both types but more frequently in the intrinsic group. The family history of allergic diathesis tends to be more common among the extrinsic group.

Except for the partial separation of extrinsic and intrinsic groups on the basis of serum IgE levels and skin tests, there are at present no other immunological or biochemical parameters useful in classifying asthma or in understanding its underlying pathogenic mechanism. Bronchial hyper-reactivity and variability of airway calibre is the hallmark of asthma. In recent years, evidence has accummulated that the imbalance of autonomic neural control of the airways[45] gives rise to the airways hyper-reactivity in asthma.

The autonomic mechanisms in asthma form the main theme of this thesis and hence a short historical review, together with the present concept of biochemical nature of autonomic receptors and their role in bronchial asthma, is described in the following section.

3.0 THE AUTONOMIC NERVOUS SYSTEM, ATOPY AND ASTHMA

3.1 Autonomic Receptors

The two divisions of the autonomic nervous system are quite distinct in many of their anatomical and physiological characteristics. The neurohumoral transmitter of all preganglionic autonomic fibres, all postganglionic parasympathetic fibres and a few postganglionic sympathetic fibres is acetylcholine, whereas the neurohumoral transmitter in the majority of the sympathetic postganglionic fibres is noradrenaline.

As early as 1905, Langley[46] suggested that most substances then thought to act upon nerve endings acted instead upon "the receptive substance" of the cells of the organs innervated. A year later Dale referred to this concept to explain his observation that ergot alkaloids prevented the excitation actions of adrenaline but had no effect on its inhibitory actions. The adjectives "adrenergic" and "cholinergic" were introduced by Dale[47] (1933) to designate nerve fibres that release the sympathetic transmitter and acetylcholine respectively. The receptor concept was not accepted generally at the time because of the difficulty in explaining the diverse effects of sympathetic stimulation to a single transmitter on one kind of receptor. It was thought for some years that two mediators (Sympathin E and Sympathin I, both derived from a single substance "Sympathin") must be involved[48]. The proper explanation that two types of receptors, only one of which was blocked by Dale's ergot preparation, rather than

two mediators was put forward by Ahlquist[49] in 1948.

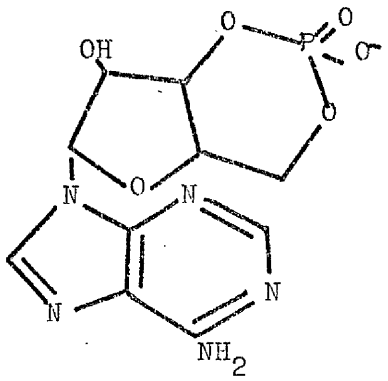
Ahlquist's main conclusions were based on a detailed comparison of the effectiveness of five sympathetic amines in cats, dogs, rats and rabbits. He found that various responses fell into two categories. For one noradrenaline was the most effective compound, and isoprenaline the least; whereas for the other isoprenaline was the most effective, with noradrenaline the least. Ahlquist suggested that these two types of responses reflected the actions mediated by two distinct receptors which he designated as the alpha and beta receptors. Soon after Ahlquist's paper it was shown that adrenergic blocking drugs abolished only the actions mediated by alpha receptors but subsequent development of agents such as dichloroisoproterenol and propranolol that block actions of beta receptors with comparable selectivity further confirmed Ahlquist's conclusion.

The possibility that there may be more than one kind of beta receptor is both of theoretical and practical importance. Lands and his associates[50] who compared the effectiveness of 15 sympathomimetic amines in causing bronchodilatation, vasodepression, cardiac stimulation and increased lipolysis. The relative potencies of the various compounds were such that the effects on the heart and lipolysis were mediated by a receptor (type B-1) of a kind somewhat different to the type (B-2) involved in both bronchodilatation and vasodepression.

Although the adrenergic and cholinergic receptors have had these names for many years we are only just beginning to get acquainted with their fundamental nature.

3.2 Adenyl Cyclase - Cyclic AMP System

It has recently been shown that the membrane bound adenyl cyclase is the second messenger and a mediator of actions of many hormones and neurohormones including catecholamines[51]. According to this concept a hormone, the first messenger, reacts with a receptor in a target cell membrane, resulting in the activation of membrane bound adenyl cyclase. In presence of Mg^{++} ions, the activated adenyl cyclase catalyses the formation of a cyclic nucleotide from adenosine triphosphate. This cyclic nucleotide, adenosine 3'5' monophosphate (cyclic AMP) is a nucleotide of adenylic acid with phosphate groups diesterified at Carbons 3' and 5' of the ribose moiety. Cyclic AMP is the intracellular messenger and is of central importance in the regulation of cellular function[52]. Cyclic AMP is hydrolysed by cyclic phosphodiesterase, specific for mononucleotide 3'5' bonds, to an inactive nucleotide, 5' AMP. The beta adrenergic responses of catecholamines are mediated through activation of adenyl cyclase and increase in cyclic AMP levels.



CYCLIC AMP

3.3 Adenosine Triphosphatase (ATPase)

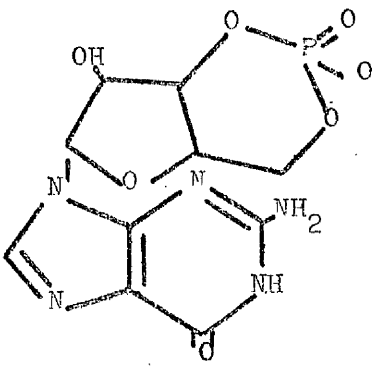
Although the exact biochemical nature of the alpha receptor is still uncertain, Belleau[53] proposed on the theoretical ground that membrane bound adenosine triphosphatase (ATPase) would represent an ideal enzyme for alpha adrenergic modulation. This hypothesis is supported by the observation that divalent cationic dependent ATPase activity in cell preparations can be stimulated by alpha adrenergic agonist and inhibited by alpha receptor blocking drugs[54, 55]. A recent advance has been the finding that alpha receptor activation causes changes in the ionic permeability of the cell membranes of several tissues[56]. It is likely that ionic selectivity of permeability caused by alpha receptor activation may vary between tissues. In the longitudinal muscle of the intestine mainly K^+ ions are concerned, so that the membrane potential rises and electrical activity is inhibited and in other smooth muscle cells e.g. arteries and veins depolarisation occurs. It has been suggested, however, that alpha receptor stimulation

may initiate contraction in certain smooth muscles by releasing Ca^{++} ions from an intracellular store, possibly in the sarcoplasmic reticulum[57].

An important but unsettled question is whether changes in cyclic AMP underlie alpha receptor mediated responses. One possibility which has been suggested is that alpha effects may result from a fall in the levels of cyclic AMP, and some evidence to support this suggestion has been reported[58, 59].

3.4 Guanyl Cyclase - Cyclic GMP System

It is now generally accepted that cholinergic and vagal responses are mediated by activation of guanyl cyclase and the formation of guanosine 3'5' monophosphate (cyclic GMP) which is the intracellular messenger[60, 61]. There is also evidence that guanyl cyclase is also activated by alpha adrenergic stimulation[62]. Several substances such as acetylcholine, bradykinin, prostaglandin $F_2\alpha$ and insulin are also known to increase cyclic GMP formation[63]. Cyclic GMP, like cyclic AMP, is hydrolysed in the cell by cyclic phosphodiesterase which may have varying affinities for respective cyclic nucleotides[64, 65]. The intracellular concentration of cyclic GMP and cyclic AMP is, therefore, a balance between the hormonal driving forces causing their formation and the rate of hydrolysis by phosphodiesterases within the cell. Guanyl cyclase activity has been demonstrated in many tissues including human lung and lymphocytes[66].



CYCLIC GMP

3.5 Autonomic Imbalance in Asthma

Salter (1859)[7] was first to suggest that spasmodic contraction of the muscle of bronchi was due to "a morbid sensitiveness and irritability of the pulmonary nervous system". Eppinger and Hess[67] in 1909 suggested that asthma was a result of parasympathetic over-activity and Pottenger (1928)[68] felt that in addition to the vagus overtone, there was also a deficiency in certain sympathotonic glands such as adrenals and thyroid, and that specific and certain non-specific stimuli only acted as precipitating factors in disorganised neurocellular mechanisms. Gudehus (1933)[69] and Handa (1934)[70] regarded bronchial asthma as a vagus neurosis of respiratory organs and believed that endocrine glands played a dominant role. The concept of autonomic imbalance in asthma was not generally accepted then because of failure or minimal effect of atropine or vagus section in the treatment of asthma[71].

3.6 The Beta Adrenergic Theory of Atopic Abnormality in Asthma

Over the past three decades the interest in the autonomic nervous control of the airways in asthma has been revived. The significant advance in our present knowledge of autonomic nervous system in asthma has been to produce an experimental animal counterpart of atopic state. A meaningful analogy to the bronchial hyper-reactivity in asthma has been the finding that certain strains of mice and rats when sensitised with Bordetella pertussis vaccine acquire hyper-sensitivity to histamine, serotonin and bradykinin[72, 73] and also to less specific stimuli such as cold air, changes in atmospheric pressure and respiratory irritants[74]. These animals also show a reduced beta receptor sensitivity to catecholamines and a reversal of normal adrenergic activity. In addition, there is a marked eosinophilia and an enhanced antibody formation[75]; these antibodies exhibit many of the features peculiar to atopic reagins and are thought to represent its animal counterpart. Subsequently, it has been shown that a similar state can be produced in experimental animals following beta adrenergic blockade with dichloroisoproterenol[45].

In Man, it has long been known that asthmatic patients are somehow more tolerant to adrenaline than normal subjects and this impression has gained experimental support by observation of reduced beta adrenergic responsiveness to catecholamines in asthmatic subjects[76, 77]. This beta receptor resistance in asthmatic patients is

reported to increase with the severity of asthma[78]. In status asthmaticus, the majority of patients become "fast" to adrenaline and this adrenaline fastness cannot be explained by tachyphylaxis as bronchial preparations under experimental conditions do not develop tachyphylaxis on repeated exposure to sympathomimetic amines[79].

Based on these observations in animal experiments and Man, Szentivayni[45] postulated that asthma is not an immunological disease but a unique pattern of bronchial hyper-reactivity to a broad spectrum of stimuli and results from the reduced functioning of the beta adrenergic receptor system and that the adrenergic neurotransmitters are released in the face of relatively unavailable beta effector system. The resultant adrenergic imbalance deprives the bronchial tree from its normal counter-regulatory control. This hypothesis is supported by observation in patients with hay fever and allergic rhinitis without previous history of asthma who develop bronchial hyper-reactivity to methacholine and antigen inhalation following beta adrenergic blockade with propranolol[80]. In addition, beta adrenergic blockade with propranolol has also been shown to precipitate bronchospasm in asthmatic patients[81]. According to Szentivayni[45], in asthma the reduced beta receptor activity would be primarily relegated to the lung rendering this organ the so called "target organ", whereas deficient beta function in other tissues could result, for example, in atopic dermatitis or allergic rhinitis.

Although Szentivayni put forward a unitarian concept to explain bronchial hyper-reactivity and atopic state in asthma, his conclusions are mainly based on observations in animal experiments. These observations are important, however, the inferences derived have to be regarded with caution firstly because of species differences and secondly because of failure to produce chronic unrelievable asthmatic state in animals. In addition, pharmacological β blockade in non-atopic subjects has failed to induce bronchial hyper-reactivity in these subjects[82]. Further, Szentivayni without much experimental evidence ventured to postulate that sympathetic imbalance in patients with asthma may result from increased alpha adrenergic activity of the airways. In 1968 when he put forward his hypothesis there was some evidence of presence of alpha adrenergic receptors in animal airways, however the existence of such receptors and their function in human bronchial tree was still in doubt.

3.7 Alpha Receptors in Mammalian Bronchial Tree

Dixon and Ransom (1912)[83] showed that stimulation of the cervical sympathetic fibres in cat could be followed either by bronchodilatation or bronchoconstriction depending upon the strength and the frequency of electrical stimulus used; they identified certain fine nerve fibres with a high threshold of excitability which were constrictor in function. Hebb (1941)[84] described bronchoconstriction after stimulation of stellate ganglion. More recently, Castro de la Mata et al[85] showed that the

bronchial smooth muscle in dogs contained alpha receptors which could be stimulated to cause bronchoconstriction. Takagi et al[86] have also reported both alpha and beta adrenergic receptors in guinea pig tracheo-bronchial tree and these observations have been confirmed[87]. The existence of sympathetic bronchoconstrictor receptors in human airways has been more doubtful until Kerr et al[88] in 1970 reported that histamine induced bronchoconstriction in patients with asthma could be inhibited by alpha receptor blocking drugs, phenoxybenzamine and phentolamine. This was probably the first indirect evidence of the presence of alpha receptors in the human airways. Recently Prime et al (1972)[89] and Simonsson et al (1972)[90] have shown that alpha receptor stimulation can cause bronchoconstriction in Man, however the significance of alpha adrenergic activity in human airways, and especially in patients with asthma, has not yet been fully investigated.

3.8 Cyclic AMP, Cyclic GMP and the Type I Allergic Reaction

The capacity of adrenaline to suppress the antigen induced wheal-and-flare reaction in an allergic subject and histamine release from sensitised guinea pig lung was described forty years ago[91, 92], but the mechanism was not appreciated until Sutherland described cyclic AMP formation following beta stimulation. Groth and his associates[93] reported that adrenaline reduces the amount of histamine released from guinea pig heart by anaphylactic challenge. Subsequently, Lichtenstein and Margolis[94] found that

adrenaline acting through beta receptors inhibited the release of histamine from peripheral blood leucocytes of allergic subjects when incubated with ragweed antigen. Assem and Schild[95], Ishizaka et al[96] and Orange, Austen and Austen[97] extended these studies to human lung. These workers found an inverse relationship between the tissue levels of cyclic AMP and the amount of histamine and S.R.S.-A. released. Beta agonists, Prostaglandin E_2 and methyl-xanthines and histamine itself increased intracellular levels of cyclic AMP and inhibited mediator release. Alpha stimulation, with phenylephrine or with adrenaline in the presence of propranolol, reduced the level of cyclic AMP and increased the amount of mediator release[98].

Conversely, cholinergic agonists and 8 bromocyclic guanosine monophosphate enhanced histamine release, though they had no effect on levels of cyclic AMP[98].

Thus, in mast cells, as in airways, it appears that the balance between cholinergic and beta adrenergic, as well as the balance between alpha and beta adrenergic activities, may play a critical homoestatic role.

3.9 Cyclic AMP, Cyclic GMP and the Bronchial Smooth Muscle

Catecholamines stimulate intracellular formation of cyclic AMP in bronchial smooth muscle and cause relaxation[99]. Phosphodiesterase inhibitors act by preventing the hydrolysis of intracellular cyclic AMP. The methyl-xanthines are the best known compounds with this property and asthma is frequently treated with Theophylline

compounds[100].

Several substances such as acetylcholine, bradykinin and prostaglandin F_2 alpha, which cause bronchoconstriction in Man, are known to increase cyclic GMP formation[100]. The suggestion that cyclic GMP may be increased in tissues of asthmatics, thus facilitating bronchoconstriction and mediator release was made by Polson et al[101], who have shown that manipulations producing an asthma-like condition in rodents are associated with an increase in the levels of cyclic GMP in their lungs.

Lewis et al[60] have shown that the effect of cyclic GMP on smooth muscle function is dose dependent; low concentration producing tracheal smooth muscle contraction whereas higher concentration produces a dose dependent relaxation. This action of cyclic GMP can be explained by the influence of cyclic GMP on cyclic AMP phosphodiesterase; in low concentration cyclic GMP stimulates cyclic phosphodiesterase, whereas in higher concentrations it inhibits cyclic AMP hydrolysis[102, 103].

It is possible to consider that at least in certain cells the concentrations of cyclic AMP and cyclic GMP may reflect the balance of beta adrenergic and cholinergic division of autonomic nervous system. The importance of such a relationship in asthma is evident. It has been suggested that cyclic AMP and cyclic GMP have a sort of reciprocal or Yin-Yang relationship in cell function[104]. Such a relationship may have a significant importance in the regulation of bronchomotor tone and chemical mediator

Table I BETA RECEPTOR SUBTYPES AND RESPONSES IN ASTHMA

Beta receptor subtype	Effects	Response in asthma
$Beta_1$	Cardiac Inotropic	-
	Chronotropic	Normal[76]
	Lipolysis and free fatty acid mobilisation	Normal[77]
$Beta_2$	Smooth muscle relaxation and vasodilatation	Diminished[76]
	Glycogenolysis	Diminished[76, 77]
	Eosinopenic	Diminished[105]

release in asthma.

3.10 Leucocyte Adenyl Cyclase Activity in Asthma

In addition to the above observations, an attempt has been made to study the cyclic nucleotide metabolism in the leucocytes of patients with asthma in relation to the activity of the disease. It is believed that the biochemical abnormality in asthma is not localised to target organs but is generalised (table I), and therefore studies on isolated viable leucocytes may provide a meaningful information[99].

In 1970 Scott[106] reported the presence of adenyl cyclase activity on the human leucocytes and this has promoted Smith and Parker (1970)[99] to examine the adenyl cyclase system in intact leucocytes and its stimulation by isoprenaline in patients with asthma. Contrasting results have been reported on the leucocyte adenyl cyclase activity in asthma. Logsdon et al[107] found that almost all their patients had a diminished leucocyte adenyl cyclase activity, whereas Gillespie et al[108] were unable to demonstrate any difference in leucocyte adenyl cyclase activity in asthmatic patients and normal subjects. Smith and Parker[99, 109] reported a diminished adenyl cyclase activity in patients with acute asthma but they could not demonstrate a significant difference in the leucocyte adenyl cyclase activity in patients in remission and normal subjects.

It still remains uncertain whether, in fact, the deficiency of beta receptor function or the adenyl cyclase activity is the underlying cause of bronchial hyper-

reactivity. If so, whether this defect is acquired or inherited and does the adenyl cyclase activity vary with the degree of airways obstruction.

3.11 Leucocyte ATPase Activity in Asthma

Coffey and his colleagues[54, 55] have shown that membrane bound divalent cationic dependent ATPase is activated by alpha stimulation and inhibited by alpha blocking drug, phentolamine. These workers[110] recently reported that Mg^{++} and Ca^{++} dependent ATPase activities were significantly increased in the leucocytes of asthmatic patients compared with sex-age matched normal subjects. ATPase activity in asthmatic patients could be significantly reduced by steroid therapy. In addition, they have proposed that the adrenergic imbalance in asthma is associated with reduced adenyl cyclase activity and enhanced ATPase activity. This in turn facilitates mediator release in the type I allergic reaction and also increases bronchomotor tone in asthmatic patients.

4.0 BRONCHIAL SMOOTH MUSCLE AND AIRWAYS OBSTRUCTION IN ASTHMA

4.1 Anatomy of Bronchial Smooth Muscle

Diseases associated with airways obstruction are extremely common and are a major cause of morbidity and mortality. It is important to know the structural arrangement of the bronchial muscle to understand the patho-physiology of obstructive airways disease. Varniers (1779)[111] was first to describe the ability of normal bronchi to contract. Reisseins (1882)[112] made an anatomical study of the bronchial musculature and described the bronchial smooth muscle. He pointed out that airways smooth muscle was so arranged that it allows easy adaptation of rhythmic changes in airways dimensions which form an integral part of breathing. Toldt (1888)[113] gave one of the earliest and more detailed descriptions of the anatomy of bronchial musculature. He outlined that the muscle fibres in the bronchi are arranged in lattice-like form (gitterformige), and dispelled earlier descriptions of its forming a completely closed muscular tube[114, 115]. Miller (1921)[116] confirmed Toldt's description and suggested that the muscle bands formed a network as 'geodesic', that is lying along the shortest mural pathway between any two points. He claimed that these geodesic bands prevent tangential motion and provide the greatest amount of strength, and at the same time allow the greatest amount of extension and contraction of the airways.

The present prevalent view is that the bronchial smooth muscle extends from the trachea down to the alveolar

ducts[117]. In the bronchioles and alveolar ducts the muscle is thicker relative to the diameter of the lumen than in the larger airways[118, 119, 120]. Thus changes in bronchomotor tone affect the diameter of the small airways more than that of large ones because of the greater muscle mass of the small airways. The alveolar ducts contain smooth muscle fibres arranged in rings and spirals around the mouths of alveoli and the sphincter-like appearance of the smooth muscle around the atria has been described by various workers[119, 121, 122]. The narrowing due to muscle contraction at such a strategic site would be of functional significance and would lead to air-trapping.

4.2 Physiological Effects of Bronchoconstriction

The precise physiological function of the airway smooth muscle remains undetermined. Radford and Lefcoe (1955)[123] suggested that airway smooth muscle does not contribute significantly in modifying lung recoil but indicated that bronchoconstriction might result in a decrease of effective lung volumes by closing off bronchial segments. Olsen et al (1967)[124] found that constriction of bronchial smooth muscle increased both the circumferential and the longitudinal tensions in bronchi and they claimed that bronchoconstriction led to a decrease in bronchial compliance with the result that the airways became less distensible and also less compressible. It would seem to indicate that in the constricted state a greater compressing force is necessary to close the airway than in the relaxed state. Olsen attributed this to the presence of

cartilaginous plaques in the airway wall which are pulled into an overlapping position when bronchi are narrowed, thus increasing the rigidity of the airways. In bronchioles, where cartilage is absent, increase in the smooth muscle tone could increase collapsibility and protect against closure[125]. It is known that the stability of a pipe is related to the ratio of the thickness of its wall to its internal diameter: the greater this ratio the less collapsible the pipe is. Hence, although it is quite possible that maximal constriction of bronchial smooth muscle might lead to critical closure of the airways[126], a smaller muscle tone might well help in improving airway stability without producing complete obstruction in the normal airways. Widdicombe and Nadel (1963)[127] suggested that smooth muscle tone may normally help to adjust the dead space and airways resistance to values at which the mechanical work of breathing is minimal.

4.3 Physiological Effects of Bronchoconstriction in Asthma

For more than 30 years aerosol of histamine and cholinergic agonists have been known to cause vigorous bronchoconstriction in patients with asthma. Normal persons respond with slight transient bronchoconstriction detectable with sensitive techniques but response of patients with asthma is 100 to 1000 fold greater. Although this airways hyper-reactivity in asthma was long recognised by clinicians, it was Tiffeneau[37] who first measured physiologically the airways response to inhaled acetylcholine in asthmatic patients. He demonstrated that

asthmatic patients develop significant fall in Forced Expiratory Volume in 1 second (FEV_1) by inhaling small quantities of acetylcholine which failed to produce a similar fall in FEV_1 in normal subjects. In asthmatic patients this threshold of excitability can be reduced further by allergic reactions, bronchopulmonary infection, psychic trauma and inhaled irritants that stimulate a bronchoconstrictor response. In recent years, acute provocation tests in asymptomatic patients with asthma have been used to examine some of the functional abnormalities which occur during a spontaneous asthmatic episode. Cade et al[44] measured plethysmographic lung volumes, airways resistance, static elastic properties, dynamic compliance, together with standard spirometric lung volumes and gas mixing in young asymptomatic asthmatics before and after methacholine inhalation. The results of their study suggested that both the large and small airways respond to provocation with methacholine. The larger airways appear to respond faster than the smaller airways and the response of the smaller airways appears to be more prolonged.

It is now known that changes in airways resistance reflect changes in the large airways[128] whereas FEV_1 is determined mainly by the elastic recoil of the lung and the resistance of the airways upstream from the equal pressure points[128]. In addition, Bouhuys and Woestijne[129] have suggested that reduction in maximum expiratory flow rate with little change in airways resistance indicates peripheral airways obstruction whereas a marked change in

airways resistance with little effect on maximum expiratory flow rate and FEV_1 indicates obstruction in the central airways. These authors reported two types of physiological responses in cotton workers following inhalation of cotton dust; one group (the "flow-rate" responders) there was a reduction in maximum expiratory flow rate and FEV_1 with little change in airways resistance, whereas in the other group (the "conductance" responders) there was a rise in airways resistance but little change in maximum expiratory flow rate or FEV_1. Further, it was suggested that the difference in the site of airways obstruction induced by cotton dust may be due to an anatomical variation in the autonomic innervation in the bronchial tree.

The bronchial smooth muscle cells in patients with severe asthma show changes of hypertrophy and hyperplasia which extend from central airways to the terminal bronchioles[130, 131, 132, 133]. The physiological effect of bronchial smooth muscle contraction due to various pharmacological mediators will depend on the site of contraction in these patients. The smooth muscle contraction occurring predominantly in the peripheral airways will give rise reductions in flow rates, FEV_1, together with airways closure resulting in air-trapping, increase in static lung volumes and a diffusion defect. On the other hand, smooth muscle contraction in the large airways will lead to an increase in airways resistance without affecting the elastic properties of lungs or lung mechanics. Because the effect is different depending on

the site of constriction the methods that determine the site are important.

The obstruction in the peripheral airways is difficult to assess clinically and physiologically and this zone is considered a 'quiet' or 'silent' zone[125] where disease process may smoulder on undetected. In recent years, measurements of closing volume[134, 135] and frequency dependent compliance[136] have been described for early detection of peripheral airways obstruction. The site of obstruction in asthma is not known, although there is some evidence that in remission considerable obstruction may affect peripheral airways[137, 138] whereas during an acute attack the central airways are affected. If one imagines that a division into "flow-rate" and "conductance" responders, as suggested by Bouhuys[129] exists in asthmatic patients, then the patients whose airways constrict more readily at the peripheral may be at a greater risk of morbidity than a patient who is a "conductance" responder.

5.0 STATEMENT OF THE PROBLEM

The review of literature supports the hypothesis that the glycaemic, lactate, peripheral vasodilatation and eosinopenic responses to adrenaline administration are diminished in patients with asthma whereas cardiac and free fatty acid mobilization responses are not (table I). In light of Lands[50] classification of beta adrenergic receptors, it is apparent that beta-2 responses are the ones chiefly impaired in these patients. It still remains uncertain whether the diminished beta receptor function or the adenyl cyclase activity is the underlying cause of bronchial hyper-reactivity. If so, whether this defect is acquired or inherited and does the diminished adenyl cyclase activity vary with the severity of airways obstruction or is drug induced.

The other question which still remains unresolved is the presence of alpha adrenergic receptors in human airways and their role in the control of bronchomotor tone. There is a vast amount of literature on the presence of alpha adrenergic receptors in smooth muscles of blood vessels and their function in control of blood pressure. However, in the most recent editions of Physiology and Pharmacology textbooks[139, 140] there is a delightful blank so far as alpha receptors are concerned in the bronchial smooth muscle. The main theme of the first part of this thesis is

PART I (Physiological)

(a) To find more direct evidence of the presence of alpha adrenergic receptors in the human lung and their role

in the control of bronchomotor tone in normal subjects and asthmatic patients.

(b) In addition, to study the effect of alpha adrenergic blocking drugs on (i) histamine, (ii) methacholine, (iii) Prostaglandin F_2 alpha, (iv) allergen, and (v) exercise induced bronchoconstriction in patients with extrinsic asthma, and

(c) to assess the therapeutic significance of alpha receptor blocking drugs in the management of asthma.

(d) The place sodium cromoglycate in management of extrinsic bronchial asthma is now well established. Although it has been shown to be effective in preventing mast cell degranulation in the type I allergic reaction[141] and inhibiting both the allergen[142] and exercise induced[143] broncho-constriction the mechanism of its action is still uncertain. There is some suggestion that sodium cromoglycate is a cyclic phosphodiesterase inhibitor and may act by increasing the intracellular levels of cyclic AMP[144, 145]. The effect of sodium cromoglycate has been studied in Prostaglandin F_2 alpha and post-exercise bronchoconstriction to compare its effect to alpha receptor blocking drugs.

Site of Bronchoconstriction

It has been reported that nervously mediated bronchoconstriction may operate at a different site in the airways from humoral bronchoconstriction[146, 147]. Histamine or a histamine releaser (48/80) injected into the right atrium has been shown to cause constriction of peripheral airways and alveolar ducts without affecting the calibre of the larger conducting airways. Vagally mediated bronchoconstriction, on the other hand, causes bronchoconstriction in the larger airways, there being little effect in the peripheral airways[146, 147]. Bouhuys and Woestijne[129] have postulated that individual variations in airways response to histamine and hemp dust in cotton workers is principally determined by variations of sympathetic tone. According to this hypothesis a subject with peripheral airways bronchoconstriction or 'flow-rate response' may have relatively few sympathetic fibres in peripheral airways so that the beta adrenergic activity might be insufficient to counteract the bronchoconstrictive effect of histamine or hemp dust in these airways. Conversely, in a subject with conductance response, the sympathetic distribution might be predominantly to smaller airways. This raises a question whether asthmatic patients show such differing airways responses following various provocation tests, i.e. beta blockade with propranolol or inhalation of histamine, methacholine, Prostaglandin F_2 alpha or allergen challenge. To answer this question airways resistance and FEV_1 are measured

simultaneously during various provocation tests in patients with asthma and the sequence of these changes assessed.

PART II (Biochemical)

Leucocyte Adenyl Cyclase Activity in Asthma

There have been conflicting reports on the leucocyte adenyl cyclase activity in patients with asthma[107, 108, 109]. These differences are probably arising from patient selection and failure to assess the severity of airways obstruction at the time of leucocyte adenyl cyclase assay. A more detailed physiological and clinical assessment of patients to relate the leucocyte activity to the activity of asthma is required to elucidate the differences. The aim of Part II of the project is to study in detail

(a) the leucocyte adenyl cyclase activity in patients with extrinsic asthma in relation to their symptoms and the degree of airways obstruction. In addition,

(b) the effect of alpha receptor blocking drugs, phentolamine and thymoxamine, on isoprenaline stimulation of the leucocyte adenyl cyclase activity, and also

(c) Ouabain, a K^+ Na^+ activated ATPase inhibitor, on isoprenaline stimulation of the leucocyte adenyl cyclase activity.

Lymphocyte Guanyl Cyclase Activity in Asthma

Although Polson et al[101] have suggested that enhanced guanyl cyclase activity may increase mediator release and bronchoconstriction in asthma, studies of cyclic GMP in asthmatic patients are lacking. It has recently been reported that guanyl cyclase activity is present in various tissues including human lung and lymphocytes. In the

preliminary time-course experiments it was established that the lymphocyte guanyl cyclase activity measured was membrane bound. To investigate the proposed hypothesis

(a) lymphocyte guanyl cyclase activity and the response to alpha and cholinergic stimulation in asthmatic patients and normal subjects is investigated.

(b) In addition, the effect of alpha receptor blocking drug, thymoxamine, on guanyl cyclase activity is also studied.

CHAPTER II

METHODS

PART I (Physiological Experiments)

6.0 PATIENTS AND NORMAL SUBJECTS

Patients

Forty patients, aged between 11 - 43, with extrinsic bronchial asthma and reversible airways obstruction have been studied. These patients had positive skin tests to inhalant allergens such as house dust, house dust mite (Dermatophagoids pteronyssinus), grass pollens or feathers, a blood eosinophil count of over 500 cells/cu.mm. and an IgE level above 100 ng./ml. Sixteen patients had associated atopic diseases such as eczema, allergic rhinitis or hay fever, and in twelve patients there was a family history of atopic diseases. Detailed clinical data on ten of these patients, selected one from each section, is presented in the Appendix.

Some of the patients were on daily maintenance therapy with bronchodilators, sodium cromoglycate or corticosteroids either given orally or as aerosol (beclamethasone dipropionate). All therapy was discontinued for at least 24 hours before each experiment.

Normal Subjects

Twenty two normal subjects, aged between 19 - 43 years, have also been studied as a control group. These subjects were volunteers, they had no respiratory disease, and no personal or family history of bronchial asthma or atopic disease.

Informed Consent

The procedure of experiments was explained to all patients and normal subjects, and an informed consent was obtained in each case. In the study of exercise induced asthma, which is more common in children, I have included patients who are below 18 years of age. In these patients, the procedure involved was explained to their parents and informed consents were obtained from them.

7.0 AIRWAYS MECHANICS

7.1 Measurement of FEV_1 and Airways Resistance

A number of methods of quantitatively expressing the rate of delivery of the forced vital capacity are used in assessing air flow obstruction in asthma. The most popular is to measure the volume expired in the first second (FEV_1) with a spirometer and kymograph. Forced Expiratory Volume in 1 second (FEV_1) is a much more sensitive index of severity of airways obstruction than vital capacity. In subjects with good motivation, the readings obtained are reproducible and show less than 10% variability.

I used a dry air wedge spirometer manufactured by Vitalograph Limited, Buckingham, England. This spirometer is portable and is easy to operate. The tracing obtained on a calibrated chart is clear and can be kept for future reference. The calibration of the spirometer is checked periodically using one litre plastic syringe.

The forced expiratory manoeuvre was explained and demonstrated to each participant. Only after ascertaining that the subject fully understood the procedure, and that he had good motivation, was he included in any of the experiments described. Three tracings of FEV_1 were recorded at each step of the experiment and the highest value of FEV_1 was taken as the result for the event. The vitalograph chart is calibrated at Ambient Temperature Saturated water vapour pressure (ATPS) and therefore requires only the temperature correction to convert the FEV_1 at Body Temperature Saturated water vapour pressure

(BTPS).

The resistance of the airways to the air flow through them depends on the radius, length and number of airways. The body plethysmography affords a good technique for measuring airways resistance (Raw) and thoracic gas volume (Vtg). The principle and design of a constant volume body plethysmograph has been described in detail by Dubois and his colleagues[148, 149].

The body plethysmograph (Fig. 1) which I used is a constant volume type and was constructed by the Department of Regional Physics and Bio-Engineering, Greater Glasgow Health Board. It is a 650 litre airtight wooden box with a perspex window. The flow rate is measured by a Fleish pneumotachograph (range 0 - 50 litre) and a micromanometer pressure transducer (Greer M_6 Mercury Electronics (Scotland) Limited; range $\pm$ 10 mm. H_2O). Pressure changes within the box are measured by a similar micromanometer (range $\pm$ 3 mm. H_2O), and both signals are amplified and displayed on the Y and X axis of a Lanscope Mk II oscilloscope. Pressure on the mouth side of the shutter is measured by a third Greer M_6 pressure transducer (range $\pm$ 30 cm. H_2O) and recorded on the Y axis of the same scope spot.

A subject sits in the body plethysmograph and as soon as the door is closed the pressure within the box rapidly rises as the air inside is warmed and humidified by the subject. During this initial period the box is vented periodically to the room outside by means of a solenoid operated valve until the pressure drift with the door closed

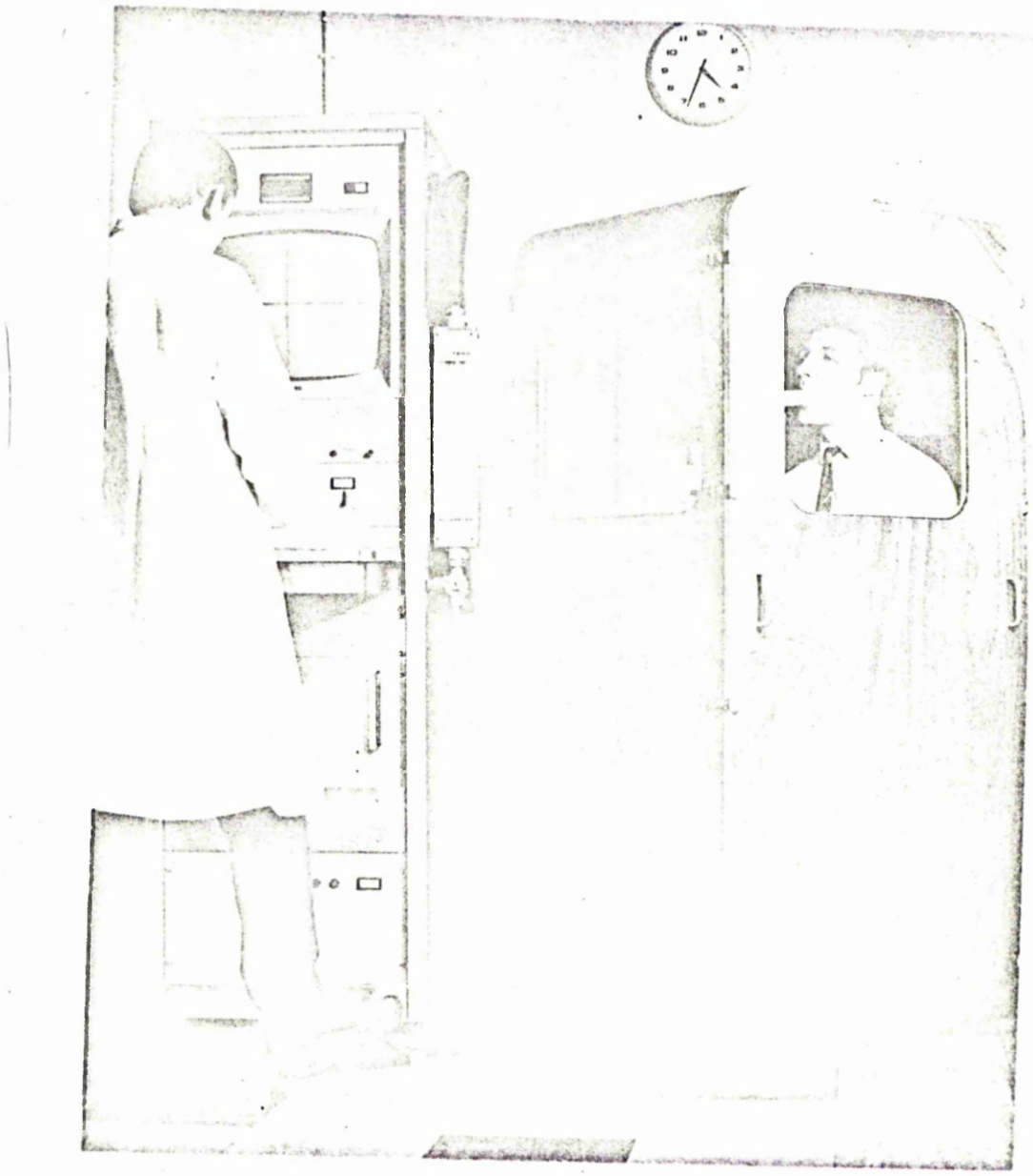


Fig. 1. Constant volume body plethysmograph.

is so slight that it does not interfere with the measurements. The subject then applies a nasal clip and pants shallowly through the pneumotachograph at approximately two cycles per second and a flow rate of 0.5 litre per second. During the panting manoeuvre the slope of the line generated on the oscilloscope is air flow divided by box pressure. This is rapidly aligned by a moving graticule on the oscilloscope face. The angular movement of the graticule is integrated so that the tangent for a given angle can be read directly on a visual display ($\tan \theta_1$). This modification simplifies the test procedure and numerous estimations can be carried out in a short period. A foot switch is then operated to occlude the pneumotachograph while the subject is panting, and also to alter the signal on the Y axis from air flow to mouth pressure. The slope now displaced on the oscilloscope is mouth pressure divided by box pressure and the tangent of this angle is read by the same technique ($\tan \theta_2$). Raw can be calculated by dividing the flow rate by mouth pressure, i.e. the mouth pressure $(\frac{\tan \theta_2}{\tan \theta_1})$. The resistance of pneumotachograph at a flow rate of 0.5 litre per second is 0.48 litres/cm.H_2O sec. and this value is subtracted from the total resistance to give Raw of the subjects. The thoracic gas volume (Vtg) is calculated from the mouth pressure measurement ($\tan \theta_2$) as outlined by Dubois et al (1956)[148].

The result of Raw is expressed as Specific Airways Conductance (SGaw) which is derived by dividing the

reciprocal of Raw by the thoracic gas volume at which Raw is measured ($SGaw = \frac{1}{Raw \times Vtg}$). This index is useful in comparing individual subjects of differing size and is now generally accepted as a standard method of expressing airways resistance.

The technique of panting was demonstrated by a trained technician to each subject on his first attendance. He was asked to practice panting until satisfactory and reproducible slopes were recorded. A mean of four recordings was taken to give SGaw for each step of the experiment. The calibration of signals on the oscilloscope were checked daily. The calibration factors of the body plethysmograph are as follows:

Flow : 200 litres/min. = 10 cm. deflection on Y axis of oscilloscope (Pressure range $\pm$ 10 mm. H_2O).

Mouth Pressure: 200 mm. H_2O = 10 cm. deflection Y axis (Pressure range $\pm$ 30 cm. H_2O).

Box Pressure : 50 ml. injected at 2 cycles/sec. = 10 cm. deflection X axis (Pressure range $\pm$ 3 mm. H_2O).

7.2 Methods of Nebulisation

Drugs given by inhalation were nebulised with a Wright's nebuliser using compressed air at a flow rate of 8 litres per minute and cylinder pressure not less than 10 lbs. per sq. in. A Wright's nebuliser has a capacity of 15 ml. and 8 - 16 ml. of water can be nebulised per hour.

As this volume varies between nebulisers, the rate of nebulisation of the three nebulisers used during this study were calibrated and the time taken to nebulise 1 ml. of normal saline was marked on each. A short vinyl tubing connected the nebuliser to a face mask (polymask or Hudson mask). The length of the tubing was kept short so that a subject could hold the nebuliser in front of his face and this also avoided excessive condensation of the aerosol along the length of the tubing.

The subject wore a comfortable mask and was asked to breathe deeply and evenly through a widely open mouth. The volume of drug nebulised was calculated from the difference in the volume of drug before and after nebulisation. By this method it is only possible to measure the amount of drug nebulised. The exact amount of drug going into the lung is difficult to assess as this has been known to vary greatly, and even with the best of techniques the amount reaching the lungs does not exceed 10%. Some amount of drug is also absorbed from buccopharyngeal mucosa and from gastrointestinal tract. Sodium cromoglycate and phenoxybenzamine were administered through a spinhaler (Fisons Pharmaceutical Limited).

7.3 Drugs Used

Histamine dihydrochloride and methacholine chloride were obtained in powder form and dissolved in distilled water to give a concentration of 200 ugm. per ml. and 2.5 mgm. per ml. respectively. These solutions were made up fresh before the experiments.

Stock drugs used were 0.5% phenylephrine hydrochloride solution (Boots Pure Drug Company Limited, U.K.), Propranolol (I.C.I. Limited, U.K.), 1.5% thymoxamine hydrochloride (W. R. Warner & Company Limited), phenoxybenzamine powder (Smith, Kline & French Laboratories Limited), 0.5% phentolamine (CIBA Laboratories Limited), sodium cromoglycate (Fisons Pharmaceutical Limited), 0.06% atropine sulphate (Antigen Limited, Ireland), Prostaglandin F_2 alpha (Armour Pharmaceutical Company Limited), allergen inhalation test solutions (Bencard Division, Beecham Research Laboratories Limited, U.K.) and 1% Isoprenaline solution (B.P.C.). Prostaglandin F_2 alpha was diluted to give a final concentration of 50 ugm. per ml., and the antigen solution was diluted in water to give a concentration of 500 protein nitrogen units (pnu) per ml.

8.0 ALPHA RECEPTORS IN ASTHMA

8.1 Response to Phenylephrine After Blockade of Alpha and Beta Adrenergic Receptors

Six patients, aged between 15 - 23 years, with extrinsic bronchial asthma and five normal subjects, aged between 19 - 26 years, were investigated. After recording the baseline FEV_1 and SGaw each subject inhaled 5 mgm. of phenylephrine hydrochloride solution (0.5%) through a Wright's nebuliser. FEV_1 and SGaw measurements were repeated 5 minutes after the end of inhalation. All subjects were then given propranolol orally. Normal subjects received 120 mgm. while asthmatic patients received 20 - 30 mgm. FEV_1 and SGaw were recorded 45 and 60 minutes after propranolol administration. At 60 minutes phenylephrine inhalation was repeated and thereafter the FEV_1 and SGaw were recorded at 2, 5 and 10 minutes. Asthmatic patients inhaled 80 ugm. of isoprenaline aerosol at the end of the test and FEV_1 and SGaw were recorded 10 minutes later.

The procedure described above was repeated in the six asthmatic patients. At the time they were given propranolol each patient inhaled 10 mgm. of phenoxybenzamine hydrochloride dispensed in a capsule with 20 mgm. of lactose from a spinhaler. In four patients the test was repeated after inhalation of 15 mgm. of thymoxamine through a Wright's nebuliser. Thymoxamine was not tolerated by two further patients because of its bitter taste and throat irritation.

The effect of 5 mgm. of phenylephrine hydrochloride on FEV_1 and SGaw after prior beta blockade with 5 mgm. of propranolol was studied in ten more patients with extrinsic asthma. After recording the baseline FEV_1 and SGaw, 5 mgm. of propranolol was given intravenously to each patient and FEV_1 and SGaw measured at 5, 10, 15 and 20 minutes. At 20 minutes, 5 mgm. of phenylephrine hydrochloride was given by inhalation and thereafter the FEV_1 and SGaw were recorded at 2, 4, 6 and 8 minutes. Asthmatic subjects inhaled 80 ugm. of isoprenaline aerosol at the end of the test and FEV_1 and SGaw were recorded 10 minutes later. Similar procedure was carried out in five normal subjects but these subjects received 10 mgm. of propranolol intravenously.

8.2 Response to Phenylephrine After Prior Beta and Cholinergic Blockade

It has been suggested that propranolol induced bronchoconstriction in asthmatic patients may be mediated through vagal over-activity[121]. Under such a situation it can be argued that the bronchoconstriction induced by phenylephrine in presence of beta blockade is due to a non-specific effect of this drug on irritant cholinergic receptors in the airways, and not due to its specific alpha receptor activity. To clarify this further, six patients with extrinsic bronchial asthma were investigated. The test procedure was similar to the one described in the previous experiment. After establishing the baseline SGaw, each subject inhaled 1.2 mgm. of atropine sulphate

from a Wright's nebuliser. SGaw was measured at 2, 5 and 10 minutes. At 10 minutes, each patient received 5 mgm. of propranolol intravenously and the test procedure thereafter has been described above.

8.3 Thymoxamine in Histamine Induced Bronchoconstriction

In 1970, I reported that histamine induced bronchoconstriction can be inhibited by alpha receptor blocking drugs, phentolamine and phenoxybenzamine. However, both these drugs have additional effects and criticism was raised at the time that the observed effects could have resulted from antihistaminic and atropine-like activities of phentolamine and phenoxybenzamine.

In recent years a new alpha receptor blocking drug, thymoxamine, has been introduced in clinical practice. The pharmacology of thymoxamine was described by Birmingham and Szolcsayni (1965)[150] and its relation to phentolamine by Brownlee (1966)[151]. It is the most specific alpha receptor blocking drug and is without the mixed action seen with other alpha receptor blocking drugs, such as phenoxybenzamine and phentolamine. Our observations of alpha receptor blockade on histamine induced bronchoconstriction have been confirmed by others using thymoxamine[152, 153]. To confirm these observations eight patients with extrinsic bronchial asthma, aged between 15 - 30 years, were investigated.

Histamine test

After establishing the baseline FEV_1, each subject inhaled normal saline for 2 minutes through a Wright's

nebuliser. FEV_1 was recorded at 5 and 10 minutes after placebo inhalation. At 10 minutes, each patient inhaled 200 ugm. of histamine dihydrochloride through a Wright's nebuliser. FEV_1 was recorded at 2 minutes after inhalation and thereafter at regular intervals for 30 minutes.

The test procedure described above was repeated in the eight asthmatic patients. On this occasion, each patient inhaled 15 mgm. of thymoxamine hydrochloride (1.5% solution) through a Wright's nebuliser instead of normal saline.

8.4 Thymoxamine in Methacholine Induced Bronchoconstriction

Although acetylcholine, the cholinergic neuro-transmitter, is not released during allergic reaction, it has profound effects on the airways of asthmatic patients[37]. According to Szentivayni[45] bronchial hyper-reactivity to chemical mediators and acetylcholine results from a functional imbalance between the alpha and beta adrenergic receptors in the airways. In order to assess the role of alpha receptors on the bronchial hyper-reactivity to cholinergic agents, I studied eight patients, aged between 15 - 30 years, with extrinsic asthma.

Methacholine inhalation test

The procedure for this test was exactly the same as for the histamine inhalation test. Instead of histamine the patient inhaled 800 ugm. of methacholine chloride (2.5% solution) through a Wright's nebuliser. The test procedure was repeated in the eight patients after inhalation of 15 mgm. of thymoxamine hydrochloride through

a Wright's nebuliser. FEV_1 was measured to assess the effects.

8.5 Thymoxamine, Atropine and Sodium Cromoglycate in Prostaglandin F_2 alpha Induced Bronchoconstriction

In recent years there has been an increasing interest in the role of prostaglandin F_2 alpha in the pathogenesis of asthma. Prostaglandin F_2 alpha is released from mammalian lungs during anaphylactic reaction and by various chemical and mechanical stimuli[35, 154]. Prostaglandin F_2 alpha is a potent bronchoconstrictor to which patients with asthma are exquisitely sensitive. Recent biochemical work has suggested that the effect of prostaglandin F_2 alpha may be mediated through guanyl cyclase system, which is known to be activated by cholinergic agents[60, 61] and also by alpha receptor stimulation[62]. In light of these observations, I studied the effect of prostaglandin F_2 alpha by inhalation on FEV_1 and SGaw in six patients, aged between 15 - 37 years, with extrinsic bronchial asthma, and tested the effect of thymoxamine, atropine and sodium cromoglycate (SCG) on prostaglandin F_2 alpha induced bronchoconstriction in these patients.

Procedure

After establishing the baseline FEV_1 and SGaw, each patient inhaled 0.5 ml. of prostaglandin F_2 alpha (50 ugm. per ml.) solution through a Wright's nebuliser. FEV_1 and SGaw measurements were recorded 2 minutes after the inhalation, and thereafter at 5 minute intervals for 25 minutes.

The test procedure was repeated three times in all patients. Ten minutes before the prostaglandin F_2 alpha inhalation, each received 1.2 mgm. of atropine sulphate or 40 mgm. of sodium cromoglycate or 15 mgm. of thymoxamine hydrochloride. Atropine sulphate and thymoxamine were given by inhalation through a Wright's nebuliser and sodium cromoglycate was inhaled using a spinhaler.

8.6 Thymoxamine in Allergen Induced Bronchoconstriction

It has recently been shown that alpha receptor stimulation enhances histamine release from sensitised human leucocytes in the type I allergic reaction. To extend this experimental observation to in vivo situation, I studied the effect of thymoxamine given intravenously and by inhalation on allergen induced bronchoconstriction in ten patients, aged between 18 - 46 years, with extrinsic bronchial asthma.

Allergen inhalation test

Standard solutions of house dust or pollen extract (500 protein nitrogen units per ml.) were used. After recording the baseline SGaw, each patient received 2 ml. of sterile normal saline intravenously. Five minutes later he inhaled an appropriate allergen solution through a Wright's nebuliser until he developed symptoms of airways obstruction. SGaw was recorded 5 minutes after the inhalation and thereafter at regular intervals for 60 minutes. On a different day (allowing at least three days between tests), the allergen inhalation test was repeated in each patient after intravenous administration of

thymoxamine (0.1 mgm. per kg. body weight). In two patients (No. 3 and 10 - Table XLVIII), allergen challenge was repeated after inhalation of 15 mgm. of thymoxamine (1.5%) through a Wright's nebuliser. In one patient (No. 10) the dose of allergen inhaled after thymoxamine was doubled.

8.7 Thymoxamine in Post-Exercise Bronchoconstriction

Exercise induced bronchoconstriction is a well recognised phenomenon in bronchial asthma[155] and in some patients exercise may act as the predominant or even the only precipitating stimulus of bronchoconstriction. The mechanism of exercise asthma still remains uncertain. One possibility is that noradrenaline, a powerful alpha receptor agonist, released during strenuous exercise may give rise to alpha stimulation and lead to broncho-constriction.

To test this hypothesis, thirteen patients, aged between 11 - 23 years, with extrinsic bronchial asthma who developed post-exercise bronchoconstriction were studied. In addition, the effect of sodium cromoglycate was compared with that of thymoxamine.

Exercise test

It has recently been reported that high efficiency negative work, namely running at near maximal work loads for 8 minutes, proves as a more potent stimulus for bronchoconstriction than other patterns of exercise tests[156]. Using this method, exercise tests were carried out in symptom-free periods. Each test consisted of

steady state exercise of running on an inclined treadmill (10^{o}) for up to 8 minutes. The speed of the treadmill was adjusted so that the patient's pulse rate at the end of exercise was at least 180 per minute. FEV_1 was recorded at 2 minutes after exercise and thereafter at regular intervals for the next 30 minutes.

In these thirteen patients, the test was repeated after inhalation of saline, thymoxamine and sodium cromoglycate. The order of drug treatment was randomised. In the control test, the patient inhaled normal saline through a Wright's nebuliser. The exercise test was repeated in each patient on different days after inhalation of 15 mgm. of thymoxamine hydrochloride solution (1.5% solution) or 40 mgm. of sodium cromoglycate through a spinhaler 15 minutes before exercise.

8.8 Alpha Receptor Blocking Drugs Alone and in combination with Isoprenaline on the SGaw

In vitro biochemical studies (discussed in Part II), it was noted that diminished beta receptor function in the leucocytes could be restored towards normal by alpha receptor blocking drugs, thymoxamine and phentolamine.

To investigate the therapeutic implication of this biochemical observation, I studied the effect of alpha receptor blocking drugs, thymoxamine and phentolamine, in ten patients, aged between 17 - 43 years, with extrinsic bronchial asthma. Each patient inhaled normal saline, 15 mgm. of thymoxamine (1.5%), 0.5 ml. of isoprenaline

(1%) or a mixture of 0.5 ml. of isoprenaline and 1 ml. of thymoxamine through a Wright's nebuliser. The order of inhalations was randomised. SGaw was measured before the inhalations and at 5 minute intervals for 30 minutes after each treatment. In three patients, who developed bronchospasm after thymoxamine administration, the test was repeated after inhalation of 5 mgm. of phentolamine (0.5%).

9.0 PHYSIOLOGICAL EFFECTS OF BRONCHOCONSTRICTION

9.1 The Site of Bronchoconstriction and the Relationship of Response to Initial Bronchomotor Tone

It is known that airways resistance predominantly reflects changes in large airways whereas FEV_1 is determined mainly by the elastic recoil of the lung and the resistance of the peripheral airways[129]. To study the effects of histamine, methacholine, allergen challenge, propranolol and prostaglandin F_2 alpha on the airways of asthmatic patients FEV_1 and SGaw were measured simultaneously after various provocation tests. The dose of bronchoactive agent used and procedure of inhalation has been described in the previous section. The number of patients studied in each group is as follows:

No. of Patients	Age range	Bronchoactive agent	Dose
16	15 - 37 years	Histamine dihydrochloride	200 ugm.
15	15 - 37 years	Methacholine chloride	800 ugm.
15	15 - 44 years	House dust or grass pollen	150 pnu
12	15 - 30 years	Propranolol	5 mgm. IV
7	15 - 23 years	Prostaglandin F_2 alpha	8 ugm.

(pnu : Protein nitrogen units)

Analysis of data

1. The mean percentage fall in FEV_1 and SGaw induced by each agent was plotted against time to assess time sequence of airways responses.

2. To investigate whether the absolute fall in FEV_1 and SGaw had any relationship to the baseline FEV_1 and SGaw, the changes were plotted against baseline values and correlation factors calculated. To do this the results of 65 tests with various agents were pooled.

CHAPTER III

RESULTS

Physiological Experiments

10.0 ALPHA RECEPTORS IN ASTHMA

10.1 Response to Phenylephrine After Prior Blockade of Alpha and Beta Receptors

This investigation was designed to study the effect of alpha stimulation on the airways of patients with extrinsic bronchial asthma and normal subjects. The mean results are given in tables II and III. In six patients, first phenylephrine produced a significant bronchodilatation and an increase in the mean FEV_1 and SGaw by 10% and 26% respectively ($P < .005$). Following oral propranolol, the mean FEV_1 fell by 18% and the mean SGaw by 51% at 60 minutes ($P < .05$). Phenylephrine inhalation repeated at 60 minutes produced bronchoconstriction and a further fall in the mean FEV_1 by 17% and the mean SGaw by 19%. The fall in FEV_1 and SGaw observed at 2, 5 and 10 minutes remained significant throughout the test as compared to readings at 60 minutes before the second phenylephrine inhalation ($P < .005$ and $< .001$). Isoprenaline inhalation at the end of the investigation increased the mean FEV_1 by 22% and the mean SGaw by 17%, however, the mean FEV_1 was still 12% and the mean SGaw 53% below the baseline recordings (Figs. 2 and 3).

In five normal subjects the mean FEV_1 did not change significantly after inhalation of phenylephrine nor was there any significant change in FEV_1 and SGaw produced by beta adrenergic blockade ($P > .10$). In each subject, after

120 mgm. of propranolol, repeat inhalation had little effect on FEV_1 or SGaw (tables IV and V, Fig. 4).

As in the control experiment, the first inhalation of phenylephrine produced bronchodilatation and an increase in the mean FEV_1 and SGaw by 9% and 29% respectively. After propranolol administration and phenoxybenzamine or thymoxamine the mean FEV_1 fell by 10% and the mean SGaw by 19% at 60 minutes. The mean fall in FEV_1 and SGaw effected by propranolol, phenoxybenzamine or thymoxamine was smaller than that observed when propranolol was given alone. However, the difference in the results was not statistically significant ($P > .10$). Phenylephrine inhalation repeated at 60 minutes produced bronchodilatation and an increase in the mean FEV_1 by 8% and the mean SGaw by 14%. The response to phenylephrine in the presence of alpha and beta blockade was significant compared to the effect of phenylephrine in the presence of beta blockade alone. Isoprenaline inhalation at the end of the test increased the mean FEV_1 by 5% and the mean SGaw by 22%. The mean FEV_1 was 3% and the mean SGaw 17% above baseline recordings (Figs. 2 and 3, tables VI and VII).

Additional experiments

The effect of alpha stimulation on the FEV_1 and SGaw was studied in a further 10 patients with extrinsic asthma and 5 normal subjects. The results of this investigation are given in tables VIII and IX.

Beta adrenergic blockade with 5 mgm. of propranolol given intravenously produced a significant fall in the mean FEV_1 and SGaw in 10 patients with extrinsic asthma ($P < .005$ and $< .001$), the fall in the mean FEV_1 and SGaw being 20% and 41% respectively at 20 minutes after propranolol administration. Phenylephrine inhalation at 20 minutes produced bronchoconstriction and a further fall in the mean FEV_1 by 15% and the mean SGaw by 27% (Fig. 5, $P < .001$). Isoprenaline inhalation at the end of the investigation increased the mean FEV_1 by 15% and the mean SGaw by 28%, however, the mean FEV_1 was still 19% and the mean SGaw 31% below the baseline readings.

In the 5 normal subjects 10 mgm. of propranolol given intravenously failed to cause a significant change in the mean FEV_1 and SGaw, and phenylephrine by inhalation at 20 minutes also failed to cause a significant change in both the FEV_1 and SGaw (table X, Fig. 6).

10.2 Response to Phenylephrine After Prior Blockade of Beta and Cholinergic Receptors

There is a possibility that bronchoconstriction caused by phenylephrine in the previous investigation could have resulted from stimulation of irritant cholinergic receptor in the airways[158]. To rule out this possibility airways response to phenylephrine after prior beta and cholinergic blockade was studied in six patients with extrinsic bronchial asthma.

Table II **Effect of phenylephrine and isoprenaline on FEV_1 after prior beta blockade with propranolol in 6 patients with extrinsic asthma**

Change in FEV_1 (litres)								
n = 6	Baseline	$Phenylephrine_1$	Propranolol		$Phenylephrine_2$			Isoprenaline
			45 min.	60 min.	2 min.	5 min.	10 min.	10 min. later
Mean	2.83	3.10	2.36	2.33	1.83	1.92	1.98	2.47
SEM	0.14	0.13	0.25	0.26	0.28	0.25	0.32	0.29
t test		4.28	2.66	2.02	3.84	4.59	3.01	
P		0.005	0.05	0.05	0.01	0.005	0.025	

Individual data published (Patel & Kerr, 1973)[157].

Table III Effect of phenylephrine and isoprenaline on SGaw after prior beta blockade with propranolol in 6 patients with extrinsic asthma

Change in SGaw (litres/cm.H_2O sec. litre)								
n = 6	Baseline	Phenylephrine$_1$	Propranolol		Phenylephrine$_2$			Isoprenaline
			45 min.	60 min.	2 min.	5 min.	10 min.	10 min. later
Mean	0.165	0.209	0.094	0.081	0.052	0.050	0.056	0.094
SEM	0.029	0.027	0.015	0.011	0.011	0.009	0.013	0.023
t test		4.13	2.18	2.87	5.34	5.26	3.00	
P		0.005	0.05	0.025	0.001	0.001	0.025	

Individual data published (Patel & Kerr, 1973)[157].

Table IV Effect of phenylephrine on FEV_1 after prior beta adrenergic blockade with propranolol in 5 normal subjects

Change in FEV_1 (litres)							
n = 5	Baseline	Phenylephrine$_1$	Propranolol		Phenylephrine$_2$		
			45 min.	60 min.	2 min.	5 min.	10 min.
Mean	3.83	3.82	3.74	3.72	3.75	3.72	3.77
SEM	0.58	0.60	0.58	0.58	0.57	0.57	0.59
t test		0.60	1.25	1.37	0.65	0.39	0.98
P		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Individual data published (Patel & Kerr, 1973)[157].

Table V **Effect of phenylephrine on SGaw after prior adrenergic blockade with propranolol in 5 normal subjects**

Change in SGaw (litres/cm.H_2O sec. litre)							
n = 5	Baseline	Phenylephrine$_1$	Propranolol		Phenylephrine$_2$		
			45 min.	60 min.	2 min.	5 min.	10 min.
Mean	0.276	0.283	0.259	0.271	0.282	0.272	0.294
SEM	0.019	0.022	0.012	0.017	0.015	0.019	0.023
t test		1.45	0.45	0.62	0.59	0.10	0.94
P		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Individual data published (Patel & Kerr, 1973)[157].

Table VI **Effect of phenylephrine and isoprenaline on FEV_1 after prior alpha and beta adrenergic blockade in 6 patients with extrinsic asthma**

Change in FEV_1 (litres)								
n = 10	Baseline	$Phenylephrine_1$	Propranolol plus phenoxybenzamine or thymoxamine		$Phenylephrine_2$			Isoprenaline
			45 min.	60 min.	2 min.	5 min.	10 min.	10 min. later
Mean	2.64	2.88	2.46	2.38	2.50	2.53	2.60	2.73
SEM	0.17	0.17	0.28	0.23	0.23	0.23	0.24	0.23
t test			1.13	0.92	6.55*	4.41*	4.84*	2.10
P			N.S.	N.S.	0.001	0.001	0.001	0.05

* The effect of phenylephrine in presence of alpha and beta receptor blockade is compared to the effect of phenylephrine in the presence of beta blockade alone at 60 min.

Individual data published (Patel & Kerr, 1973)[157].

Table VII **Effect of phenylephrine and isoprenaline on SGaw after prior alpha and beta adrenergic blockade in 6 patients with extrinsic asthma**

Change in SGaw (litres/cm.H_2O sec. litre)								
n = 10	Baseline	Phenylephrine$_1$	Propranolol plus phenoxybenzamine or thymoxamine		Phenylephrine$_2$			Isoprenaline
			45 min.	60 min.	2 min.	5 min.	10 min.	10 min. later
Mean	0.131	0.169	0.116	0.106	0.118	0.124	0.124	0.153
SEM	0.025	0.026	0.026	0.022	0.026	0.026	0.028	0.028
t test			0.75	1.01	2.43*	2.33*	2.92*	2.45
P			N.S.	N.S.	0.025	0.025	0.01	0.025

* The effect of phenylephrine in presence of alpha and beta blockade is compared to the effect of phenylephrine in the presence of beta blockade alone at 60 min.

Individual data published (Patel & Kerr, 1973)[157].

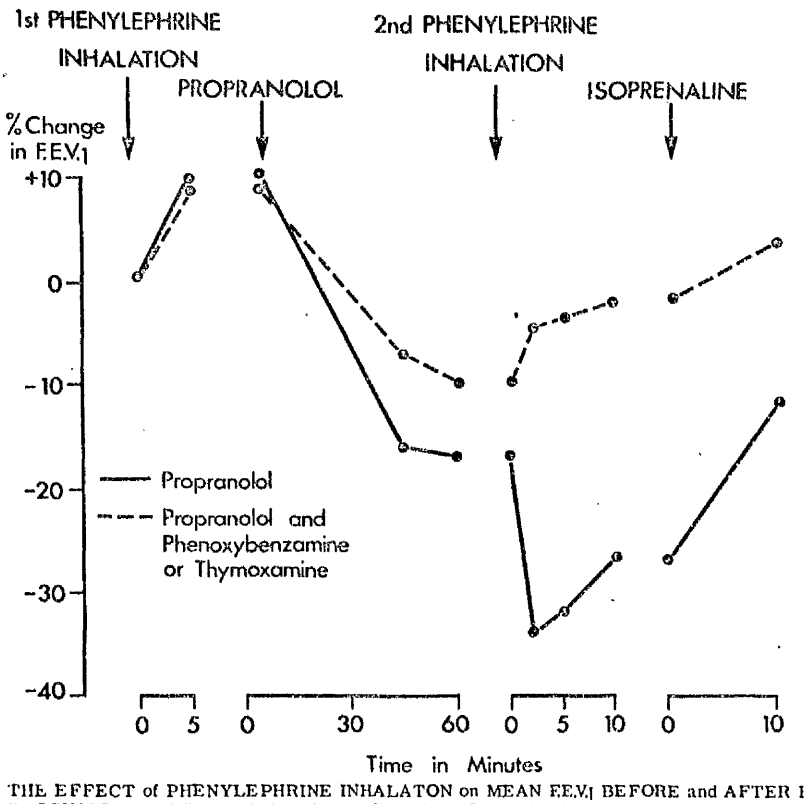


Fig. 2 THE EFFECT of PHENYLEPHRINE INHALATON on MEAN FEV.1 BEFORE and AFTER BETA ADRENERGIC BLOCKADE In SIX PATIENTS with EXTRINSIC BRONCHIAL ASTHMA.
THE PHENYLEPHRIE EFFECT is COMPLETELY INHIBITED by PRIOR ADMINISTRATION of PHENOXBENZAMINE or THYOXAMINE

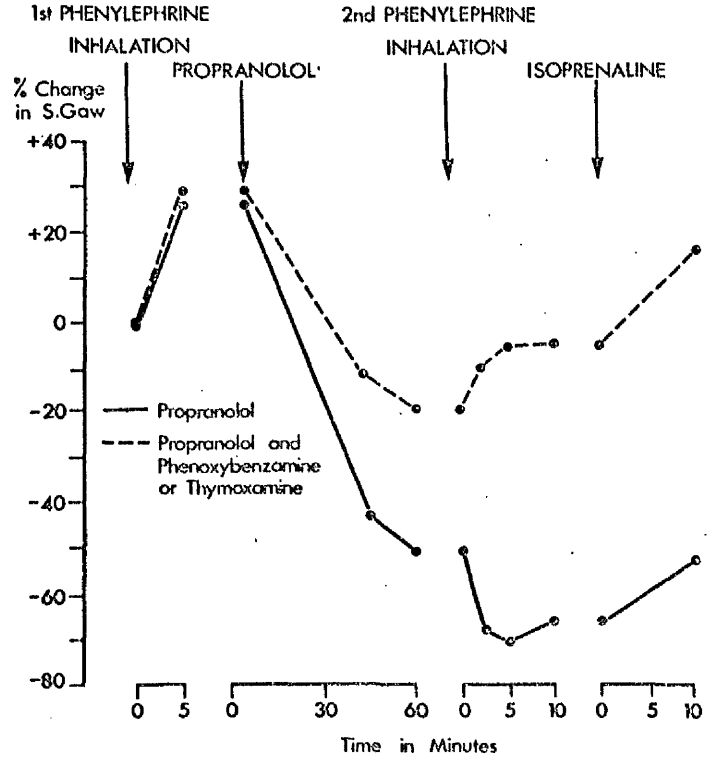


Fig. 3 EFFECT of PHENYLEPHRINE INHILATION on MEAN SGAW BEFORE and AFTER BETA ADRENERGIC BLOCKADE In SIX PATIENTS with EXTRINSIC BRONCHIAL ASTHMA THE PHENYLEPHRINE EFFECT is COMPLETELY INHIBITED by PRIOR ADMINISTRATION of PHENOXYBENZAMINE or THYMOXAMINE.

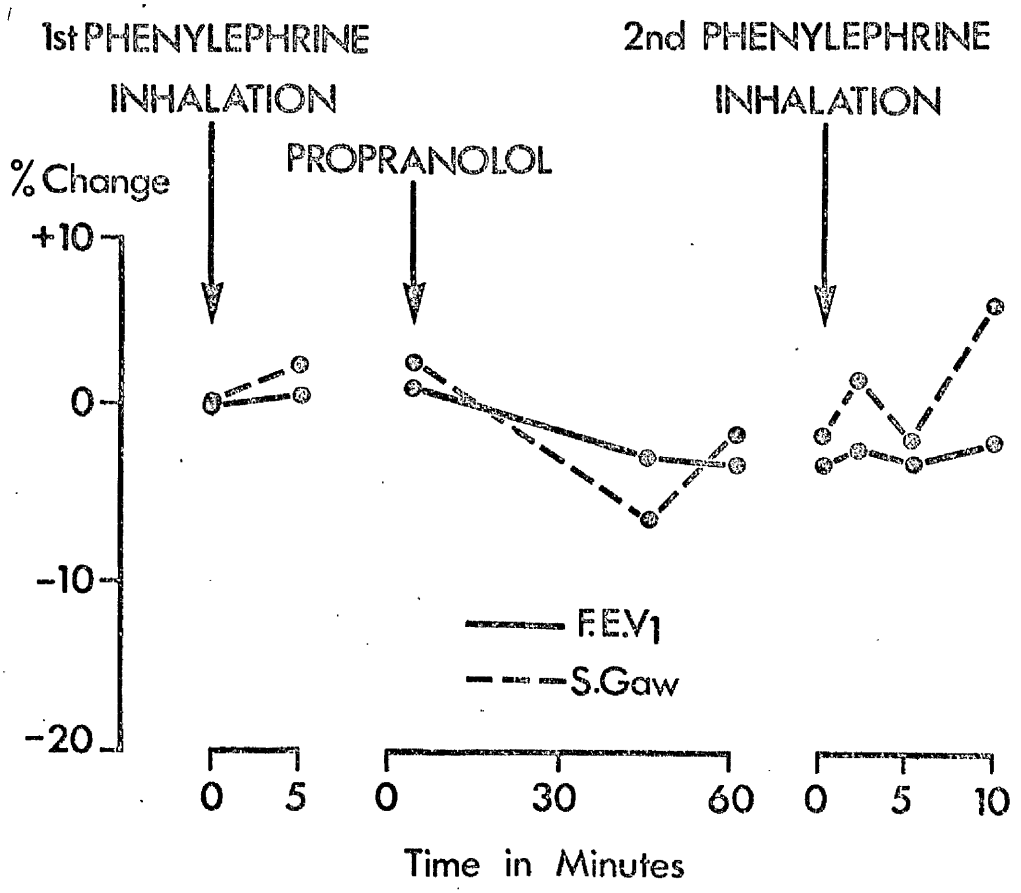


Fig.4 EFFECT of PHENYLEPHRINE INHALATION on MEAN F.E.V.1 and SGAW BEFORE AND AFTER BETA-ADRENERGIC BLOCKADE IN 5 NORMAL SUBJECTS.

Table VIII **Effect of phenylephrine and isoprenaline on FEV_1 after prior beta blockade with propranolol in 10 patients with extrinsic asthma**

n = 10	FEV_1 in litres									
		Propranolol 5 mgm. I.V.				Phenylephrine 5 mgm. by inhalation				Isoprenaline
	Baseline	5'	10'	15'	20'	2'	4'	6'	8'	
Mean	3.12	2.56	2.51	2.54	2.50	2.09	2.04	2.02	2.07	2.52
SEM	0.15	0.15	0.12	0.15	0.16	0.18	0.16	0.15	0.15	0.19
t test		3.00	3.44	3.59	3.62	4.99	5.59	5.06	5.47	
P		.02	.005	.005	.005	.001	.001	.001	.001	

Individual data given in Table XXXVII.
Clinical details of Patient No. 1 (Mr. R. F.) given in Appendix, Page 143.

Table IX **Effect of phenylephrine and isoprenaline on SGaw after prior blockade with propranolol in 10 patients with extrinsic asthma**

n = 10	SGaw (litres/cm.H_2O sec. litre)									
		Propranolol 5 mgm. I.V.				Phenylephrine 5 mgm. by inhalation				Isoprenaline
	Baseline	5'	10'	15'	20'	2'	4'	6'	8'	10 min. later
Mean	0.150	0.097	0.092	0.086	0.088	0.053	0.057	0.056	0.062	0.103
SEM	0.015	0.016	0.017	0.014	0.013	0.011	0.011	0.011	0.010	0.015
t test		4.67	4.02	5.53	5.30	6.51	5.45	3.16	3.57	
P		.01	.01	.001	.001	.001	.001	.02	.01	

Individual data given in Table XXXVIII.

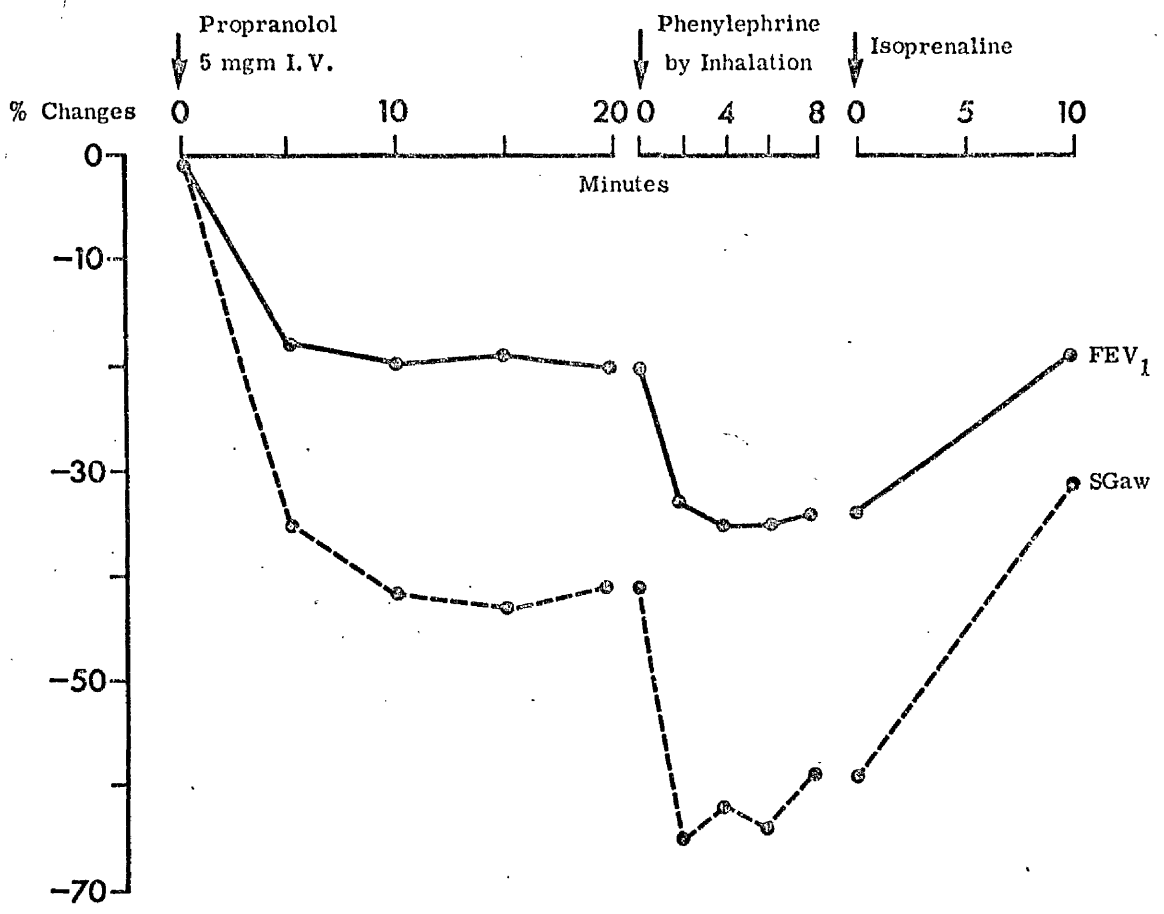


Fig. 5. Effect of phenylephrine inhalation on mean FEV_1 and SGaw after beta adrenergic blockade in 10 patients with extrinsic asthma.

Table X (a) Effect of phenylephrine on FEV_1 after prior beta blockade with propranolol in 5 normal subjects

n = 5	FEV_1 in litres								
		Propranolol 10 mgm. I.V.				Phenylephrine 5 mgm. by inhalation			
	Baseline	5 min.	10 min.	15 min.	20 min.	2 min.	4 min.	6 min.	8 min.
Mean	3.94	3.89	3.86	3.85	3.87	3.91	3.92	3.90	3.97
SEM	0.34	0.32	0.34	0.31	0.32	0.32	0.30	0.31	0.32
t test		1.60	1.98	2.00	1.57	0.89	0.93	0.81	1.98
P		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Individual data given in Table XXXIX.

Table X (b) Effect of phenylephrine on SGaw after prior beta blockade with propranolol in 5 normal subjects

n = 5	SGaw (litres/cm.H_2O sec. litre)								
		Propranolol 10 mgm. I.V.				Phenylephrine 5 mgm. by inhalation			
	Baseline	5 min.	10 min.	15 min.	20 min.	2 min.	4 min.	6 min.	8 min.
Mean	0.224	0.226	0.219	0.225	0.219	0.221	0.228	0.224	0.232
SE	0.011	0.011	0.011	0.010	0.011	0.009	0.007	0.009	0.006
t test		0.45	0.58	0.22	0.81	0.45	1.18	0.75	1.42
P		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Individual data given in Table XL.

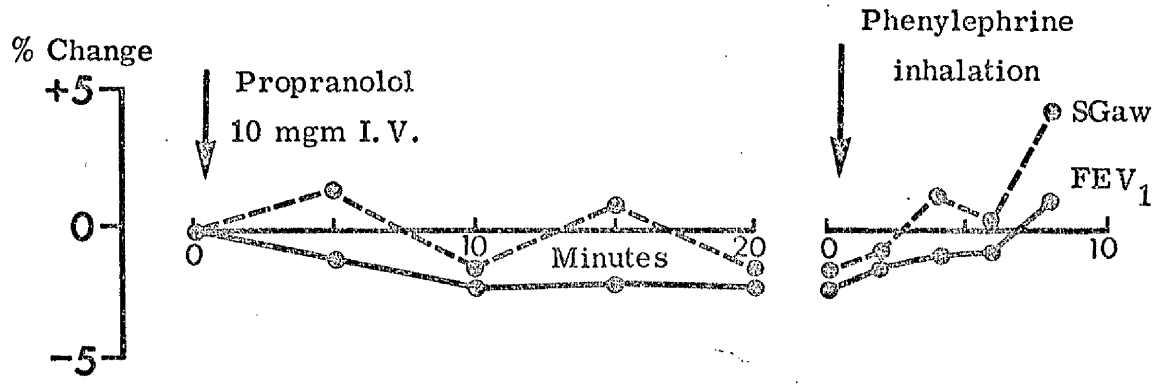


Fig. 6. Effect of phenylephrine inhalation on the mean FEV_1 and SGaw after prior beta adrenergic blockade in 5 normal subjects.

The results of this investigation are given in table XI. In six patients, atropine by inhalation produced a significant bronchodilatation and an increase in the mean SGaw by 53% at 10 minutes. After propranolol administration, the mean SGaw fell by 10% at 20 minutes. Phenylephrine inhalation in the presence of beta and cholinergic blockade caused bronchoconstriction and a fall in the mean SGaw by 33%. The fall in SGaw observed at 2, 4, 6 and 8 minutes remained highly significant throughout the test as compared to readings at 20 minutes before phenylephrine inhalation ($P < .001$). Isoprenaline inhalation at the end of the investigation increased the mean SGaw by 41% (Fig. 7).

10.3 Thymoxamine in Histamine Induced Bronchoconstriction

The results of this investigation are given in table XII. Histamine dihydrochloride given by inhalation caused a significant fall in the mean FEV_1 in 10 patients with extrinsic asthma. The maximal fall in FEV_1 was 40% and occurred at 2 minutes after the inhalation. Thereafter there was a gradual restitution in the FEV_1 in the following 30 minutes. In presence of alpha blockade with thymoxamine, histamine inhalation produced a smaller fall in FEV_1 as compared to the fall in FEV_1 produced by histamine alone. The maximal fall in FEV_1 was 20% at 2 minutes and thereafter there was gradual restitution in the FEV_1 in the following 30 minutes (Fig. 8). The inhibiting effect of thymoxamine on histamine induced fall in the FEV_1 was statistically

significant ($P < .05$). The results of this investigation suggest that alpha blockade can inhibit histamine induced bronchoconstriction in patients with asthma.

10.4 Thymoxamine in Methacholine Induced Bronchoconstriction

The results of this investigation are given in table XIII. In eight patients, 800 ugm. of methacholine chloride, a powerful cholinergic agonist, produced a significant fall in the mean FEV_1. The maximal fall in the mean FEV_1 was 41% and occurred at 2 minutes (Fig. 9). The fall in FEV_1 was highly significant ($P < .001$). In the following 30 minutes there was a gradual restitution in the FEV_1.

In these eight patients, thymoxamine given by inhalation did not produce a significant change in the mean FEV_1 and methacholine inhalation 10 minutes later produced a maximal fall in the mean FEV_1 of 47% at 2 minutes. There was no significant difference between the maximal fall in FEV_1 produced by methacholine alone and that produced by methacholine after pre-treatment with thymoxamine ($P > .10$). These results suggest that thymoxamine had no effect on methacholine induced bronchoconstriction (Fig. 9).

10.5 Thymoxamine, Atropine and Sodium Cromoglycate in Prostaglandin F_2 Alpha Induced Bronchoconstriction

The detailed results of changes in FEV_1 and SGaw produced by prostaglandin F_2 alpha and the effect of various drug treatments in six patients are given in tables XIV and XV. Prostaglandin F_2 alpha inhalation produced maximal fall in the mean FEV_1 and SGaw of 32% and 53%

respectively which occurred at 5 minutes after inhalation. The falls in FEV_1 and SGaw were highly significant ($P < .01$ and $P < .001$). In 25 minutes thereafter there was a gradual restitution in both the FEV_1 and SGaw (Figs. 10 and 11).

In these six patients atropine sulphate by inhalation increased the mean FEV_1 and SGaw by 9% and 67% respectively and the bronchodilatation produced by atropine was significant ($P < .025$). Prostaglandin inhalation 10 minutes later produced a fall in the mean FEV_1 and SGaw of 10% and 20% respectively. There was a statistically significant difference between the maximal falls in FEV_1 and SGaw produced by prostaglandin F_2 alpha alone and that produced by prostaglandin F_2 alpha after pre-treatment with atropine ($P < .05$). These results suggest that prior inhalation of atropine sulphate partially inhibited the bronchoconstriction induced by prostaglandin F_2 alpha.

Sodium cromoglycate and thymoxamine inhalations did not produce a significant change in the mean FEV_1 and SGaw. Further, both these drugs failed to inhibit prostaglandin F_2 alpha induced bronchoconstriction ($P > .10$, tables XIV and XV, Figs. 10 and 11).

10.6 Thymoxamine in Allergen Induced Bronchoconstriction

The results of this investigation are given in table XVI. In ten patients with extrinsic bronchial asthma, allergen inhalation produced a significant fall in the mean SGaw ($P < .01$). The maximal fall in the mean SGaw

was 59% at 15 minutes and thereafter there was a gradual restitution in the SGaw (Fig. 12).

Following intravenous thymoxamine, a smaller fall in the mean SGaw was observed. The maximal fall in the mean SGaw was 35% at 25 minutes. The inhibition obtained with thymoxamine was statistically significant ($P < .025$). The effect of intravenous thymoxamine on allergen induced bronchoconstriction varied greatly in individual patients. In six patients (Nos. 1, 2, 3, 5, 7 and 10, tables in the Appendix) the allergen provoked bronchospasm was completely or partially inhibited whereas in four patients (Nos. 4, 6, 8 and 9, tables XLVI and XLVII) thymoxamine had no effect in this respect.

Thymoxamine given by inhalation also inhibited allergen provoked bronchoconstriction in two patients (Nos. 3 and 10, tables XLVI and XLVII) and in one of these patients this protection was maintained even when the dose of allergen inhaled was doubled (Figs. 13 and 14).

Thymoxamine given intravenously did not cause a significant fall in blood pressure in any of the patients and none complained of any side-effects.

10.7 Thymoxamine and Sodium Cromoglycate in Post-Exercise Bronchoconstriction

The results are given in table XVII. In thirteen patients the maximal fall in the mean FEV_1 after treadmill exercise was 35% and occurred at 5 minutes. Thereafter there was a gradual restitution in FEV_1 over the next 25

minutes (Fig. 16). The fall in FEV_1 was highly significant ($P < .001$).

When the patients were pre-treated with thymoxamine, the maximal fall in the mean FEV_1 was 5% and occurred 5 minutes after exercise. Thereafter the FEV_1 returned to the baseline value over the next 25 minutes. The fall in mean FEV_1 was statistically significant ($P < .05$). However, when the falls in FEV_1 induced by exercise in the control test and after thymoxamine treatment were compared, the inhibitory effect of thymoxamine on post-exercise bronchoconstriction was found to be highly significant ($P < .01$, Fig. 16).

When the exercise test was repeated after inhalation of sodium cromoglycate, the maximal fall in mean FEV_1 was 10% and occurred 5 minutes after exercise. Thereafter the mean FEV_1 returned to the baseline value over the next 25 minutes. The fall in mean FEV_1 at 5 minutes was significant ($P < .025$). However, when the falls in FEV_1 induced by exercise in the control test and after sodium cromoglycate treatment were compared, the inhibitory effect of sodium cromoglycate on post-exercise bronchoconstriction was found to be highly significant ($P < .01$).

The inhibitory effect of thymoxamine and sodium cromoglycate on post-exercise bronchoconstriction in patients with bronchial asthma was comparable and no statistical difference was found between the two drug treatments (t test = 0.3, $P > .10$, Fig. 16).

10.8 Alpha Receptor Blocking Drugs Alone and in combination with Isoprenaline on SGaw

The results of this investigation are given in table XVIII. Saline inhalation did not cause a significant change in SGaw. The maximal increase in the mean SGaw was 45% after thymoxamine or phentolamine inhalation, however, this increase was significantly less than the increase in mean SGaw achieved with isoprenaline, 122%. When isoprenaline and thymoxamine were administered together the mean SGaw increased by 200% from the baseline value of SGaw (Figs. 17 and 18). There was a highly significant difference between the bronchodilatation achieved with isoprenaline alone and that achieved with isoprenaline plus thymoxamine or phentolamine ($P < .001$). The results of this investigation support the biochemical work reported in Part II that alpha receptor blocking drugs potentiate the effect of beta receptor stimulants.

Table XI **Effect of phenylephrine and isoprenaline after prior beta and cholinergic blockade in six patients with extrinsic asthma**

n = 6	SGaw (litres/cm.H_2O sec. litre)										
		Atropine	Propranolol 5 mgm. I.V.				Phenylephrine 5 mgm. by inhalation				Isoprenaline
	Baseline	10 min. later	5 min.	10 min.	15 min.	20 min.	2 min.	4 min.	6 min.	8 min.	10 min. later
Mean	0.110	0.170	0.159	0.157	0.159	0.157	0.123	0.127	0.124	0.121	0.166
SEM	0.008	0.012	0.011	0.016	0.021	0.019	0.016	0.015	0.013	0.011	0.018
t test		8.16	2.19	2.89	1.04	1.45	4.63	4.08	4.04	3.18	
P		.001	.05	.025	N.S.	N.S.	.001	.001	.001	.025	

Individual data given in Table XLI.

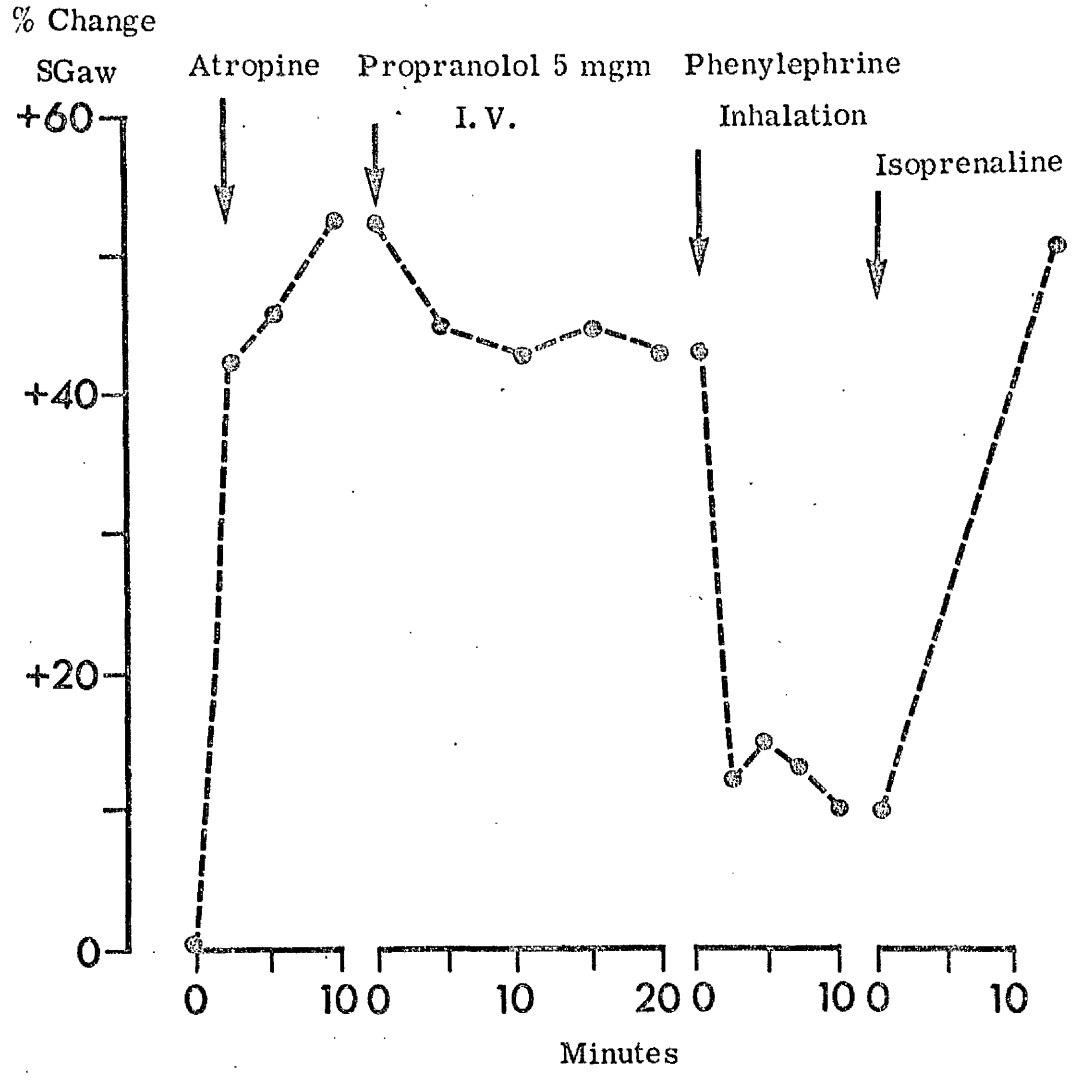


Fig. 7. Effect of phenylephrine inhalation on the mean SGaw after cholinergic and beta adrenergic blockade in 6 patients with extrinsic bronchial asthma.

Table XII Effect of thymoxamine on histamine induced fall in FEV_1 in 10 patients with extrinsic asthma

n = 10	Baseline	After histamine (200 ugm.) by inhalation				
		2 min.	5 min.	10 min.	15 min.	30 min.
Control test Mean FEV_1	2.97	1.77	2.05	2.25	2.50	2.75
SEM	0.27	0.22	0.25	0.25	0.24	0.26
Thymoxamine Mean FEV_1	2.95	2.36	2.42	2.64	2.73	2.77
SEM	0.24	0.23	0.23	0.26	0.27	0.26
t test	0.21	2.74	2.03	2.42	1.39	0.11
P	N.S.	.025	.05	.05	N.S.	N.S.

Individual data given in Table XLVI.
Clinical details of Patient No. 2 (Mr. D. C.) given in Appendix, Page 144.

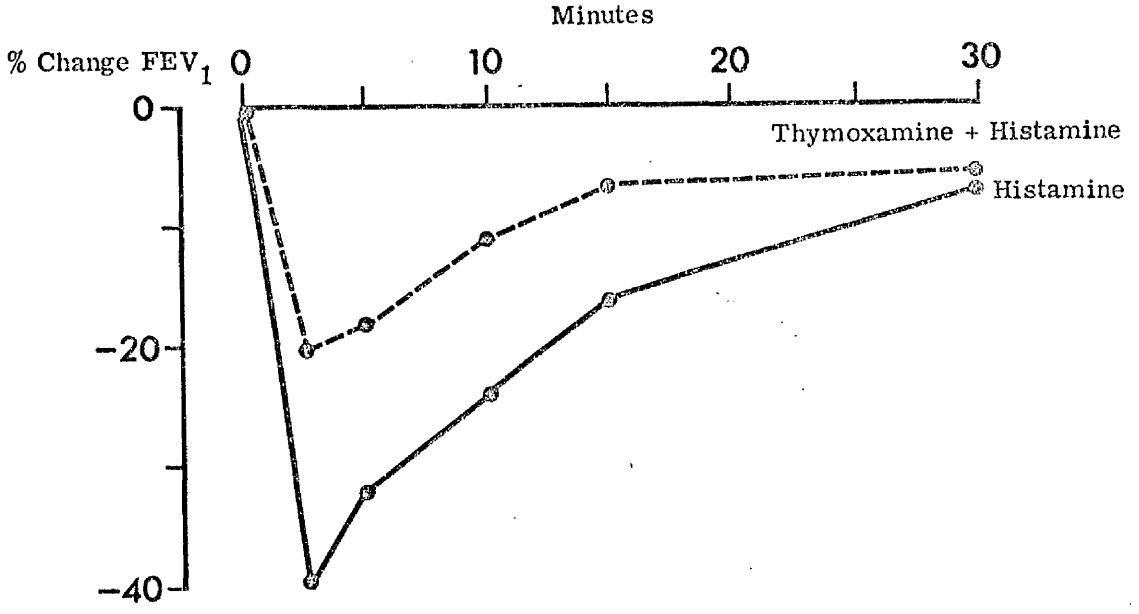


Fig. 8. Effect of thymoxamine on histamine induced fall in the mean FEV_1 in 8 patients with asthma. The histamine induced bronchoconstriction was partially inhibited by alpha blockade with thymoxamine.

Table XIII Effect of methacholine 800 ugm. on FEV_1 after prior blockade with thymoxamine in 8 patients with extrinsic asthma

n = 8	Baseline		After methacholine inhalation				
			2 min.	5 min.	10 min.	15 min.	30 min.
Control test Mean FEV_1	3.09		1.81	1.81	2.10	2.14	2.53
SEM	0.29		0.43	0.43	0.38	0.36	0.35
	B	A					
Thymoxamine Mean FEV_1	3.03	3.04	1.61	1.76	1.97	2.10	2.46
SEM	0.31	0.33	0.45	0.43	0.40	0.38	0.35
t test	0.15	0.63	1.50	0.37	0.87	0.30	0.49
P	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

B: Before thymoxamine.

A: After thymoxamine.

For individual data see Appendix Table XLVII.

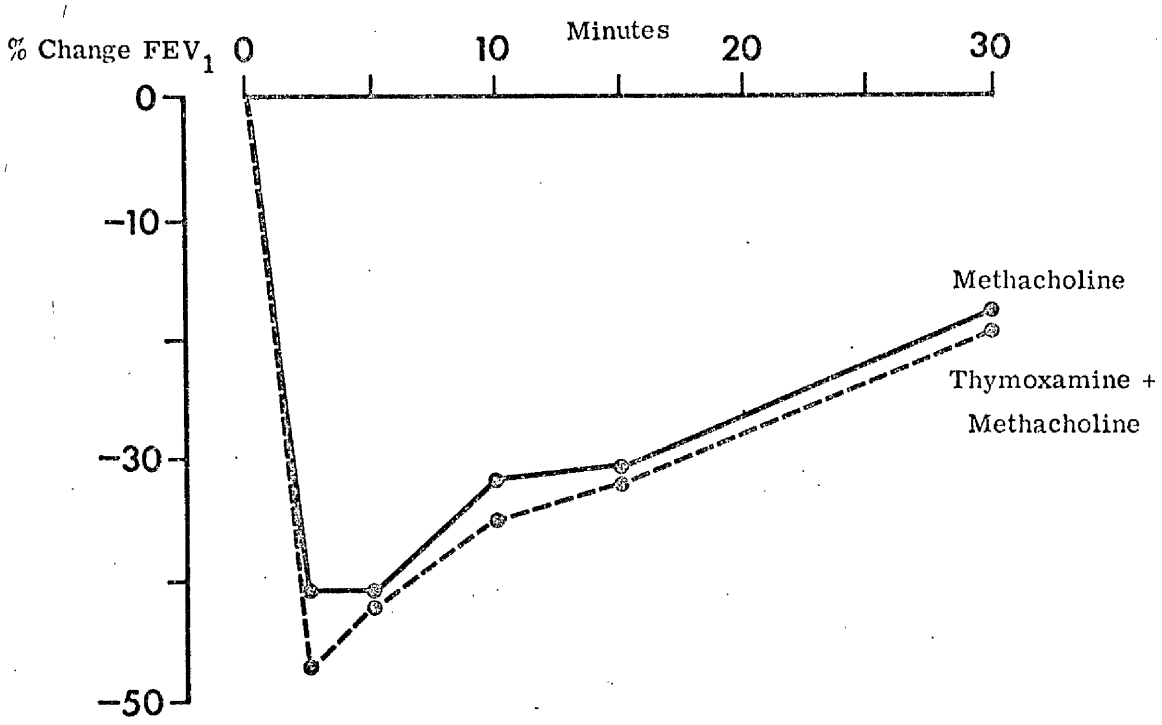


Fig. 9. Effect of thymoxamine on methacholine induced fall in the mean FEV_1 in 8 patients with extrinsic asthma. Thymoxamine failed to inhibit methacholine induced bronchoconstriction in these patients.

Table XIV The effect of atropine, sodium cromoglycate and thymoxamine on Prostaglandin $F_2\alpha$ induced fall in FEV_1 (litres) in 6 patients with extrinsic bronchial asthma

No.	Age	Sex	$PGF_2\alpha$		Atropine + $PGF_2\alpha$			SCG + $PGF_2\alpha$			Thymoxamine + $PGF_2\alpha$		
			Baseline	PGF_2	Baseline		PGF_2	Baseline		PGF_2	Baseline		PGF_2
					B	A		B	A		B	A	
1.	15	M	3.20	2.30	2.90	3.45	3.22	3.30	3.30	2.00	3.27	3.35	2.75
2.	29	M	3.42	2.00	3.45	3.60	3.25	3.25	3.30	2.35	3.37	3.25	2.60
3.	23	F	3.00	2.55	2.55	2.62	2.25	3.15	3.20	2.30	3.15	3.00	2.00
4.	37	M	2.80	1.62	2.25	2.20	2.00	2.40	2.60	1.55	2.90	3.00	1.70
5.	29	M	2.60	2.00	4.50	5.07	4.96	2.70	2.65	1.87	2.50	2.50	2.20
6.	24	M	4.80	4.00	2.40	2.60	2.00	4.85	4.90	3.60	4.75	4.40	3.15
Mean			3.30	2.41	3.00	3.26	2.95	3.28	3.33	2.28	3.32	3.25	2.40
SEM			0.32	0.34	0.35	0.42	0.47	0.35	0.34	0.29	0.31	0.26	0.22
P				.001		.025	.05		N.S.	N.S.		N.S.	N.S.

B: Before drug treatment.

A: After drug treatment.

Clinical details of Patient No. 3 (Mr. A. H.) given in Appendix, Page 145.

Table XV The effect of atropine, sodium cromoglycate and thymoxamine on Prostaglandin $F_2\alpha$ induced fall in SGaw (litre/sec./cm.H_2O/litre) in 6 patients with extrinsic bronchial asthma

No.	PGF_2	Atropine + $PGF_2\alpha$			SCG + $PGF_2\alpha$			Thymoxamine + $PGF_2\alpha$		
	Baseline	PGF_2	Baseline		PGF_2	Baseline		PGF_2	Baseline		PGF_2
			B	A		B	A		B	A	
1.	0.134	0.109	0.072	0.187	0.195	0.137	0.144	0.093	0.194	0.154	0.072
2.	0.068	0.023	0.120	0.142	0.072	0.074	0.078	0.038	0.071	0.081	0.040
3.	0.212	0.116	0.153	0.200	0.108	0.242	0.246	0.099	0.246	0.231	0.090
4.	0.128	0.053	0.122	0.170	0.107	0.121	0.126	0.056	0.130	0.138	0.048
5.	0.084	0.033	0.056	0.078	0.077	0.083	0.081	0.036	0.078	0.072	0.056
6.	0.282	0.091	0.104	0.279	0.286	0.289	0.285	0.108	0.162	0.166	0.074
Mean	0.151	0.071	0.105	0.176	0.141	0.158	0.160	0.072	0.147	0.140	0.063
SEM	0.033	0.016	0.015	0.027	0.034	0.036	0.035	0.013	0.028	0.024	0.008
P		.01		.025	.05		N.S.	N.S.		N.S.	N.S.

B: Before drug treatment.

A: After drug treatment.

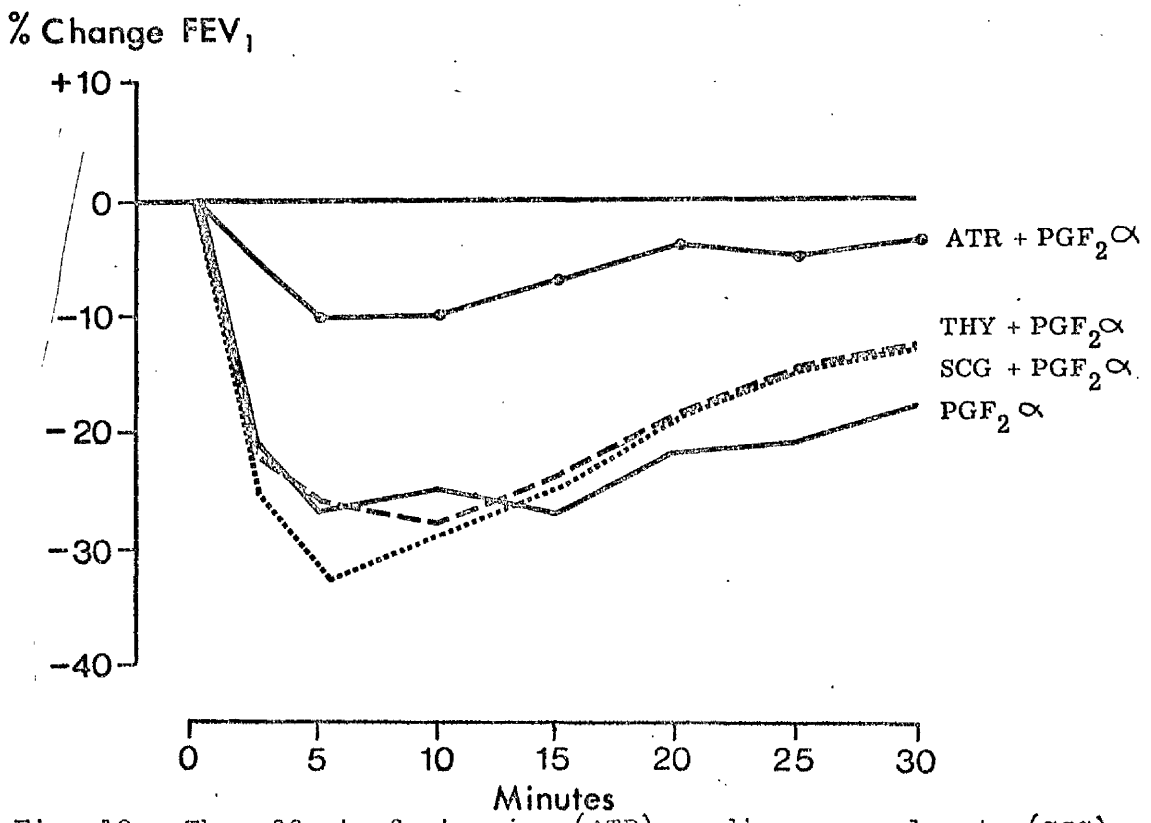


Fig. 10. The effect of atropine (ATR), sodium cromoglycate (SCG) and thymoxamine (THY) on $PGF_2\alpha$ induced fall in the mean FEV1 in 6 patients with asthma.

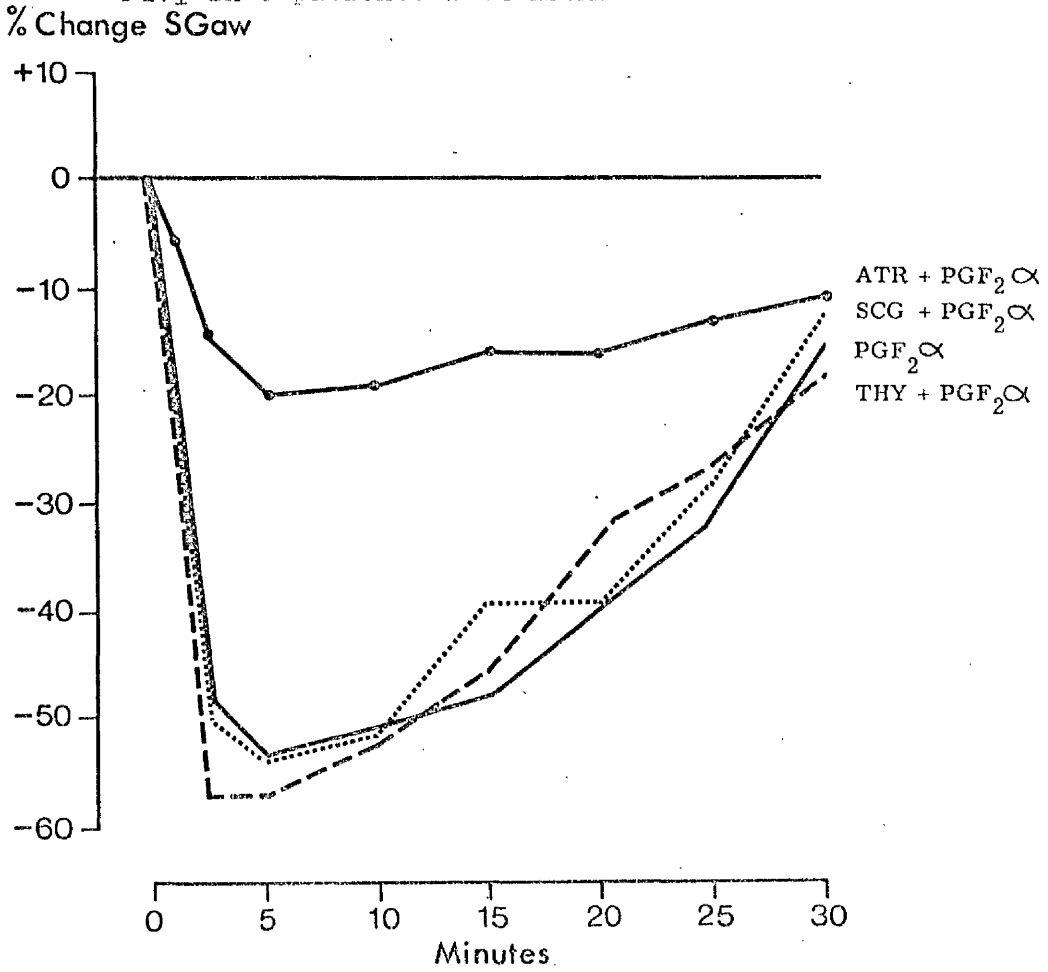


Fig. 11. The effect of ATR, SCG and THY on $PGF_2\alpha$ induced fall in the mean SGaw in 6 patients with asthma.

Table XVI Effect of thymoxamine on allergen induced fall in SGaw in 10 patients with extrinsic asthma

n = 10	Baseline	After allergen challenge						
		5 min.	10 min.	15 min.	25 min.	35 min.	45 min.	60 min.
Control test Mean SGaw	0.150	0.070	0.066	0.061	0.061	0.069	0.092	0.123
SEM	0.020	0.014	0.015	0.012	0.011	0.012	0.014	0.018
Thymoxamine Mean SGaw	0.142	0.123	0.101	0.101	0.092	0.098	0.107	0.120
SEM	0.019	0.028	0.022	0.022	0.020	0.022	0.022	0.024
t test		2.44	1.83	1.97	2.46	2.63	0.08	0
P		.025	.05	.05	.025	.025	N.S.	N.S.

Individual data given in Tables XLVIII and XLIX.
Clinical details of Mr. A. H. and Miss R. S. given in Appendix, Pages 145 and 149 respectively.

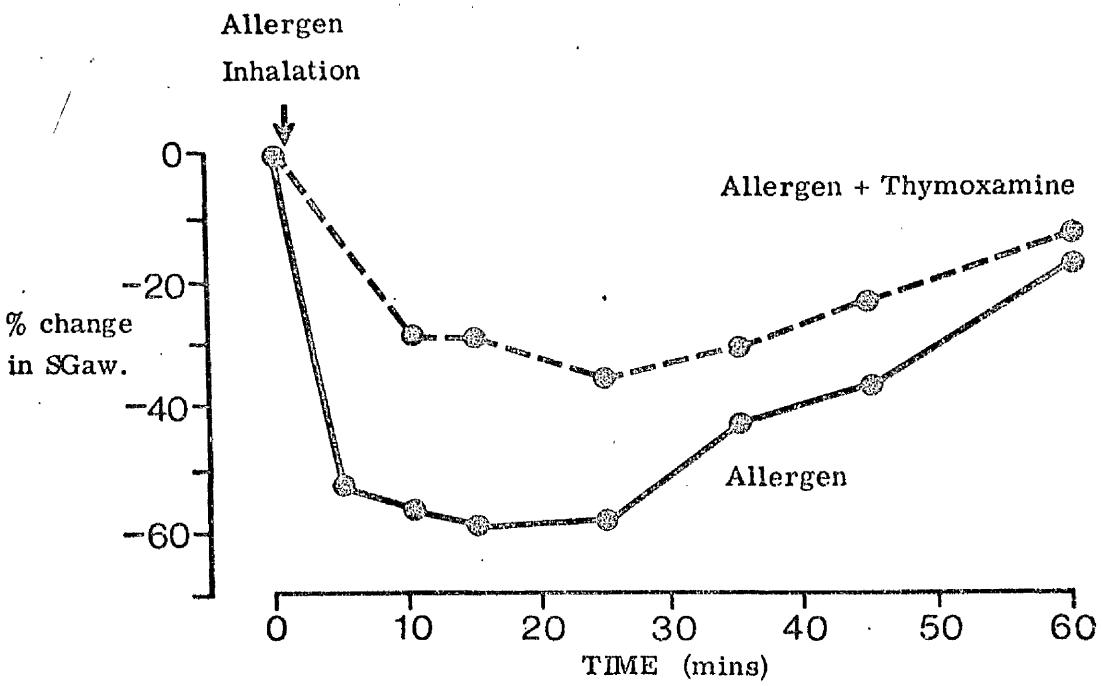


Fig. 12. Effect of thymoxamine on allergen induced fall in the mean SGaw in 10 patients with asthma.

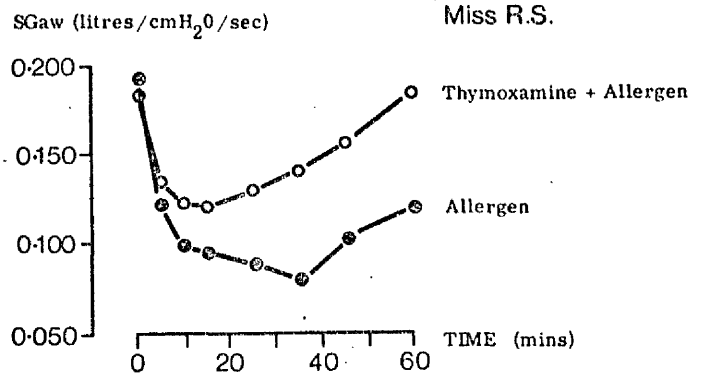


Fig. 14. Effect of thymoxamine by inhalation on allergen induced fall in SGaw in patient No. 10 (table XLVII).

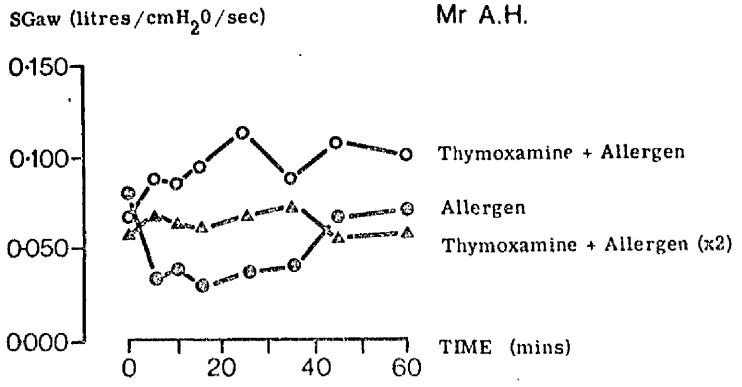


Fig. 13. Effect of thymoxamine by inhalation on allergen induced fall in the SGaw in patient No. 3 (table XLVII).

Table XVII Effect of thymoxamine and sodium cromoglycate on post-exercise bronchoconstriction in 13 patients with extrinsic bronchial asthma

n = 13		Baseline		After exercise (mins.) 2	5	10	15	20	25	30
Control test	Mean FEV_1		2.49	1.76	1.62	1.73	1.91	1.98	2.04	2.12
	SEM		0.20	0.24	0.20	0.18	0.14	0.15	0.16	0.16
	t test			5.26	8.90	5.93	5.61	3.47	3.39	2.54
	P			.001	.001	.001	.001	.005	.005	.025
		B	A							
Thymoxamine	Mean FEV_1	2.31	2.30	2.19	2.11	2.12	2.24	2.26	2.28	2.29
	SEM	0.15	0.14	0.20	0.17	0.17	0.19	0.18	0.20	0.21
	t test		0.23	0.857	1.88	1.53	0.441	0.360	0.151	0.525
	P		N.S.	N.S.	.05	N.S.	N.S.	N.S.	N.S.	N.S.
Sodium cromoglycate	Mean FEV_1		2.41	2.28	2.17	2.28	2.32	2.35	2.41	2.38
	SEM		0.19	0.23	0.21	0.21	0.22	0.21	0.21	0.20
	t test			1.19	2.46	1.14	0.81	0.54	0	0.27
	P			N.S.	.025	N.S.	N.S.	N.S.	N.S.	N.S.

Clinical details of Miss E. P. and Mrs. E. H. given in Appendix, Pages 147 and 156 respectively.

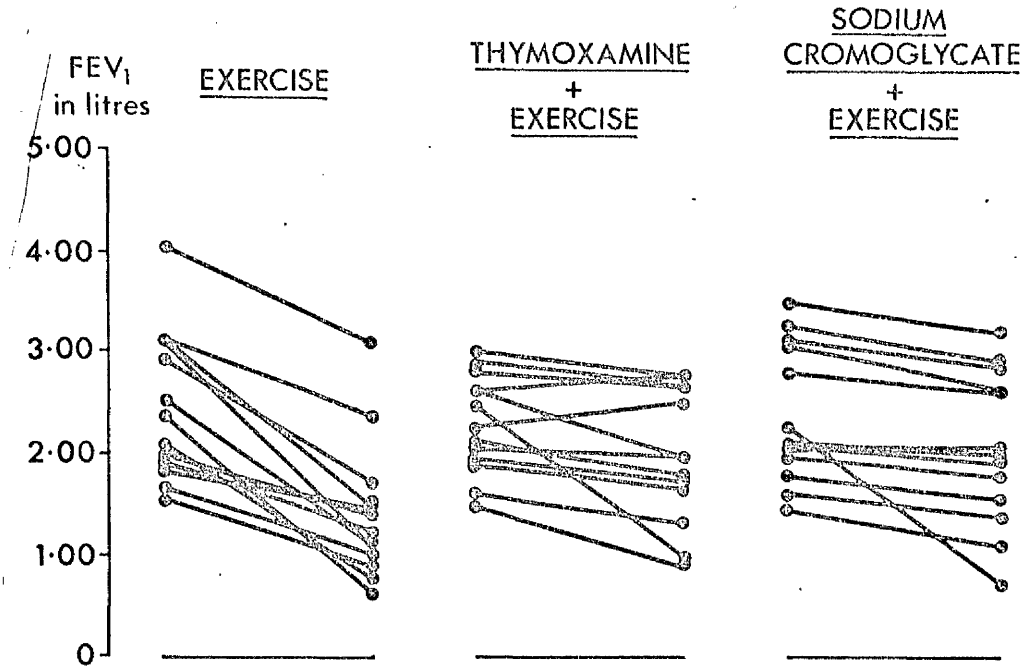


Fig. 15. Values of FEV_1 before and after treadmill exercise in 13 patients with asthma and the effect of thymoxamine and sodium cromoglycate.

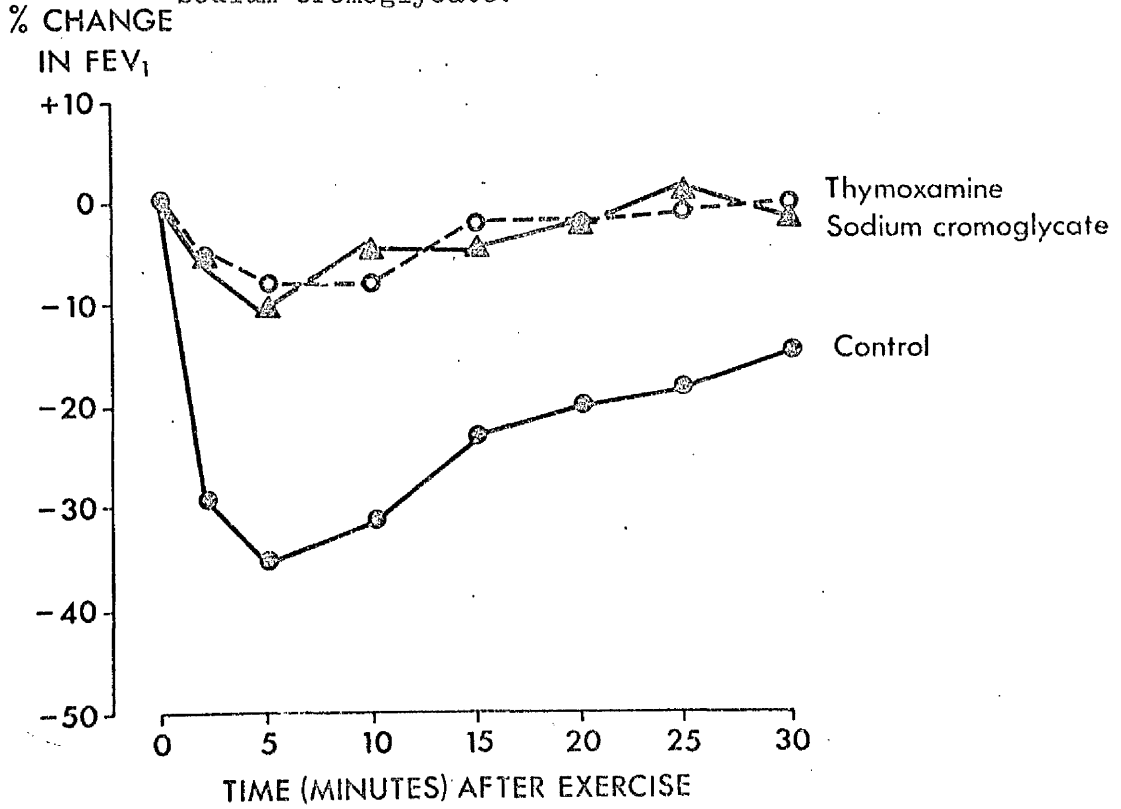


Fig. 16. Effect of thymoxamine and sodium cromoglycate on the mean fall in FEV_1 after treadmill exercise. Post-exercise bronchoconstriction was inhibited by thymoxamine and sodium cromoglycate.

Table XVIII **Effect of saline, thymoxamine or phentolamine, isoprenaline and isoprenaline + thymoxamine or phentolamine on SGaw in 10 patients with extrinsic asthma**

		Baseline	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.
Saline (n = 10)	Mean SGaw	0.090	0.089	0.087	0.091	0.090	0.093	0.093
	SEM	0.010	0.009	0.008	0.010	0.010	0.009	0.009
	t test		0.18	0.67	0.40	0	0.72	1.01
	P		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Thymoxamine or Phentolamine (n = 13)	Mean SGaw	0.068	0.094	0.096	0.098	0.098	0.094	0.096
	SEM	0.008	0.014	0.015	0.015	0.016	0.014	0.015
	t test		2.65	2.90	3.14	2.78	2.98	3.03
	P		.025	.01	.01	.025	.01	.01
Isoprenaline (n = 10)	Mean SGaw	0.074	0.148	0.158	0.164	0.164	0.166	0.162
	SEM	0.013	0.013	0.016	0.020	0.020	0.014	0.019
	t test		7.61	6.57	5.22	5.27	5.15	5.05
	P		.001	.001	.001	.001	.001	.001
Thymoxamine or Phentolamine + Isoprenaline (n = 13)	Mean SGaw	0.065	0.162	0.170	0.176	0.191	0.193	0.194
	SEM	0.008	0.014	0.016	0.013	0.014	0.012	0.014
	t test		8.94	8.13	9.74	10.92	12.76	11.00
	P		.001	.001	.001	.001	.001	.001

Individual data given in Table L.

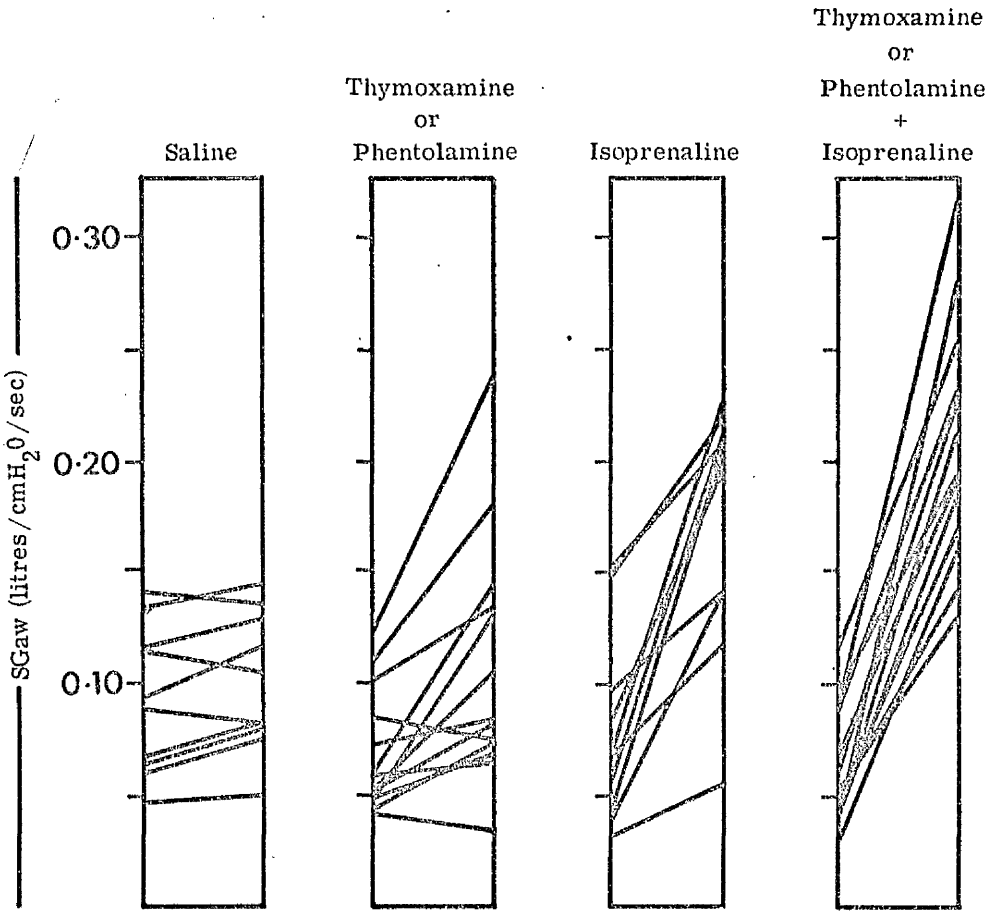


Fig. 17. Values of SGaw after saline, thymoxamine or phentolamine, isoprenaline and isoprenaline + thymoxamine or phentolamine in 10 patients with asthma.

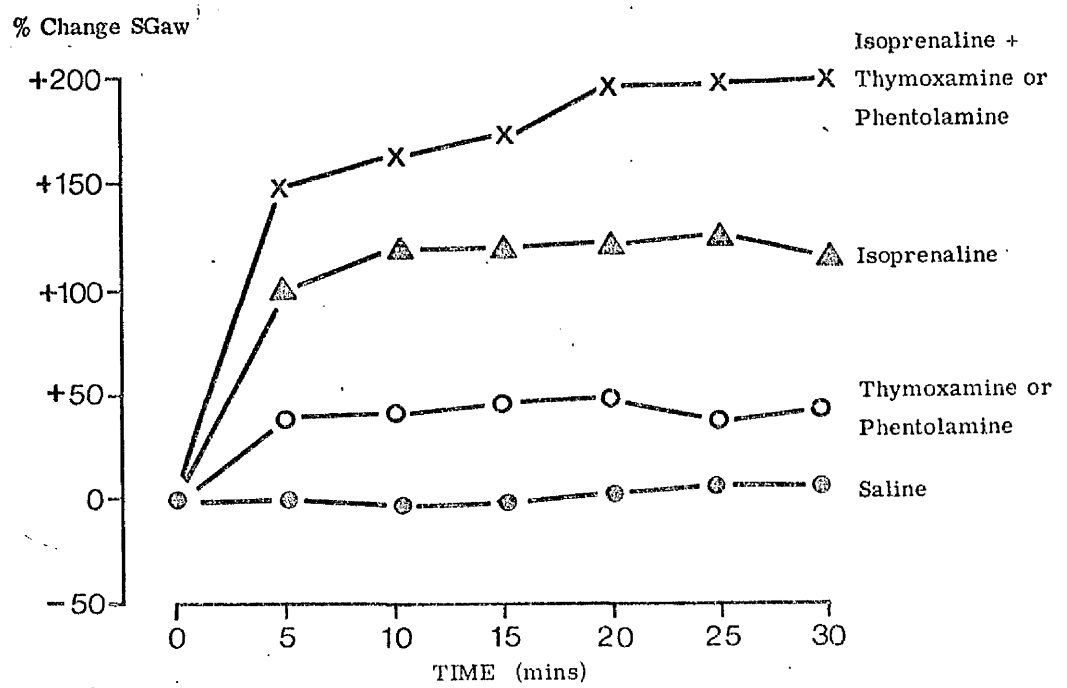


Fig. 18. Effect of saline, thymoxamine or phentolamine, isoprenaline and isoprenaline + thymoxamine or phentolamine on the mean SGaw in 10 patients with asthma.

11.0 PHYSIOLOGICAL EFFECTS OF BRONCHOCONSTRICTION

11.1 Site of Bronchoconstriction

As summarised in the method section, 65 provocation tests with different bronchoactive agents were carried out. The time-response relationship of fall in FEV_1 and SGaw to these agents are given in figures 19, 20, 21 and 22. The maximal fall in FEV_1 and SGaw and the recovery in both these assessments thereafter occurred simultaneously. In these 65 provocation tests, no differentiation into 'flow' or 'conductance' responders as proposed by Bouhuys et al[129] could be made. These results suggest that broncho-constriction induced by histamine, methacholine, allergen challenge, beta blockade and prostaglandin F_2 alpha occurs simultaneously in the central and peripheral airways.

11.2 Relationship of Response to Initial Bronchomotor Tone

On combining the results of the fall in SGaw produced by histamine, methacholine, allergen challenge, beta blockade and prostaglandin F_2 alpha in the 65 provocation tests (tables XIX - XXIII) and relating the change to the baseline value of SGaw, a significant correlation was observed ($r = 0.76$, t test = 9.21, $P < .001$, Fig. 23). The patient who had the highest baseline SGaw had the greatest fall in SGaw following any of the provocation manoeuvres. These results are in accord with Starling's law of heart muscle[159] and can be derived mathematically (Page 162).

On the other hand, the fall in FEV_1 produced by histamine, methacholine, allergen challenge, beta blockade and prostaglandin F_2 alpha did not relate to baseline value of FEV_1 ($r = 0.02$, t test = 0.08, $P > .10$, Fig. 24).

Table XIX

Maximum fall in FEV_1 and SGaw produced by histamine inhalation in 16 patients with asthma

No.	Age	Sex	FEV_1		SGaw	
			Baseline	Change	Baseline	Change
1.	18	F	2.40	1.00	0.087	0.066
2.	23	M	3.70	1.05	0.090	0.050
3.	20	M	3.05	1.45	0.079	0.065
4.	37	M	1.30	0.45	0.044	0.013
5.	15	M	3.20	1.20	0.068	0.038
6.	19	M	3.30	1.30	0.083	0.055
7.	21	F	2.70	1.65	0.092	0.071
8.	22	M	2.60	1.60	0.093	0.066
9.	30	F	2.60	0.25	0.207	0.160
10.	33	M	2.25	0.85	0.188	0.074
11.	31	M	3.20	0.40	0.179	0.082
12.	19	F	2.65	1.15	0.103	0.085
13.	29	M	2.40	0.95	0.082	0.061
14.	28	M	2.70	0.75	0.054	0.031
15.	21	M	4.40	1.70	0.185	0.154
16.	33	M	3.00	1.80	0.065	0.043
Mean			2.84	1.10	0.106	0.070
SEM			0.17	0.12	0.013	0.009

Table XX

Maximum fall in FEV_1 and SGaw produced by methacholine inhalation in 15 patients with asthma

No.	Age	Sex	FEV_1		SGaw	
			Baseline	Change	Baseline	Change
1.	18	F	2.50	1.55	0.115	0.050
2.	23	M	3.85	1.65	0.188	0.160
3.	20	M	3.15	1.95	0.113	0.069
4.	17	M	2.70	0.85	0.108	0.064
5.	15	M	2.90	1.70	0.312	0.161
6.	20	M	3.70	2.70	0.123	0.045
7.	21	F	2.70	1.50	0.136	0.108
8.	33	F	2.25	0.45	0.141	0.099
9.	31	M	3.25	1.40	0.167	0.141
10.	30	M	3.70	1.45	0.053	0.037
11.	28	M	3.05	0.85	0.075	0.052
12.	21	M	2.50	1.15	0.103	0.034
13.	20	M	4.75	0.30	0.308	0.176
14.	33	F	2.40	1.20	0.220	0.159
15.	37	M	1.90	1.10	0.116	0.060
Mean			3.02	1.32	0.152	0.094
SEM			0.19	0.15	0.020	0.013

Table XXI

Maximum fall in FEV_1 and SGaw produced by allergen challenge in 15 patients with asthma

No.	Age	Sex	FEV_1		SGaw	
			Baseline	Change	Baseline	Change
1.	20	M	4.70	0.70	0.235	0.154
2.	23	M	4.20	0.80	0.189	0.108
3.	21	F	2.75	1.55	0.144	0.115
4.	19	F	3.50	0.60	0.213	0.117
5.	15	M	2.40	0.60	0.077	0.052
6.	37	F	1.90	0.30	0.109	0.059
7.	19	F	2.30	0.60	0.132	0.044
8.	23	F	2.90	1.50	0.227	0.167
9.	19	M	2.90	1.85	0.066	0.055
10.	27	M	2.45	1.30	0.105	0.091
11.	30	M	3.35	0.95	0.082	0.063
12.	39	M	1.70	0.70	0.085	0.063
13.	20	M	2.75	1.95	0.062	0.047
14.	21	M	2.60	0.90	0.098	0.051
15.	18	F	2.20	1.10	0.076	0.048
Mean			2.84	1.02	0.127	0.082
SEM			0.21	0.13	0.016	0.010

Clinical details of Patient No. 8 (Mrs. J. H.) given in Appendix, Page 154.

Table XXII

Maximum fall in FEV_1 and SGaw produced by 5 mgm. of propranolol given intravenously in 12 patients with asthma

No.	Age	Sex	FEV_1		SGaw	
			Baseline	Change	Baseline	Change
1.	19	M	3.15	0.25	0.122	0.039
2.	21	F	3.20	0.60	0.189	0.062
3.	20	M	3.40	0.45	0.100	0.044
4.	15	M	3.05	1.05	0.165	0.102
5.	23	M	4.20	2.05	0.212	0.149
6.	19	F	2.80	0.70	0.104	0.050
7.	30	M	3.00	0.55	0.098	0.036
8.	19	F	3.30	0.25	0.153	0.044
9.	31	M	2.60	0.90	0.126	0.086
10.	21	F	2.30	0.80	0.133	0.072
11.	27	F	2.40	0.10	0.201	0.083
12.	20	M	4.80	0.60	0.312	0.144
Mean			3.18	0.69	0.160	0.076
SEM			0.21	0.15	0.018	0.011

Table XXIII

Maximum fall in FEV_1 and SGaw produced by Prostaglandin $F_2\alpha$ in 7 patients with asthma

No.	Age	Sex	FEV_1		SGaw	
			Baseline	Change	Baseline	Change
1.	15	M	3.20	1.95	0.134	0.044
2.	30	M	3.20	1.65	0.068	0.048
3.	21	F	3.00	0.95	0.212	0.143
4.	33	M	2.80	1.78	0.128	0.093
5.	28	M	2.60	0.63	0.084	0.051
6.	21	M	4.25	1.00	0.215	0.143
7.	23	M	4.80	1.10	0.282	0.191
Mean			3.41	1.29	0.160	0.102
SEM			0.31	0.19	0.030	0.022

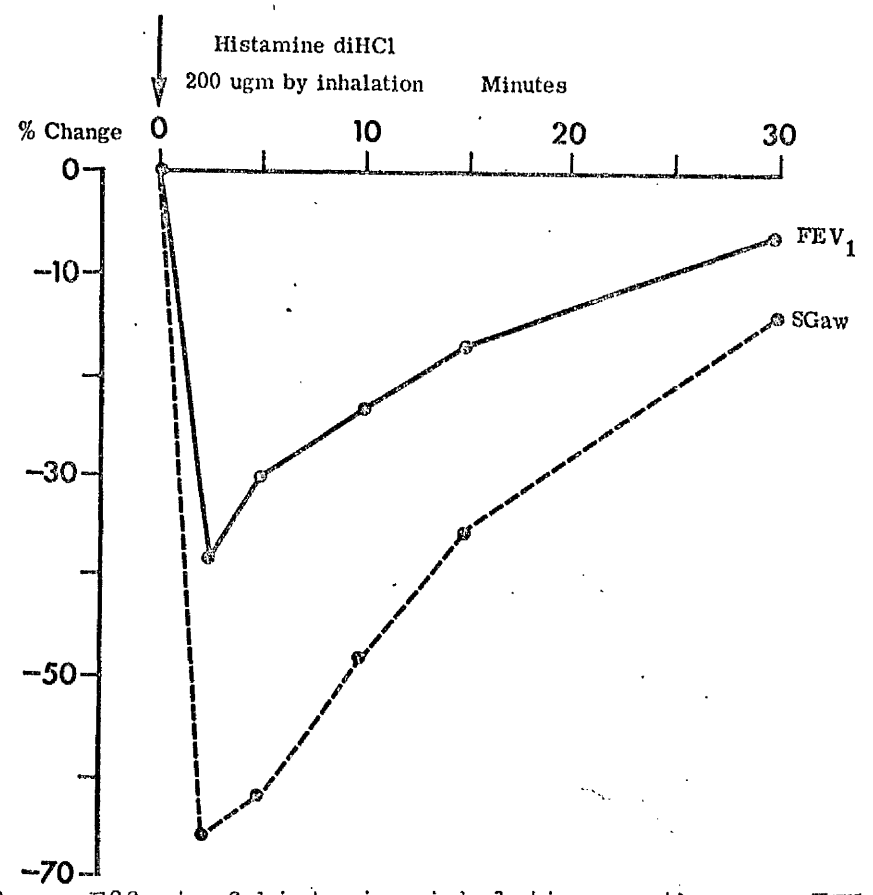


Fig. 19. Effect of histamine inhalation on the mean FEV_1 and SGaw in 16 patients with asthma.

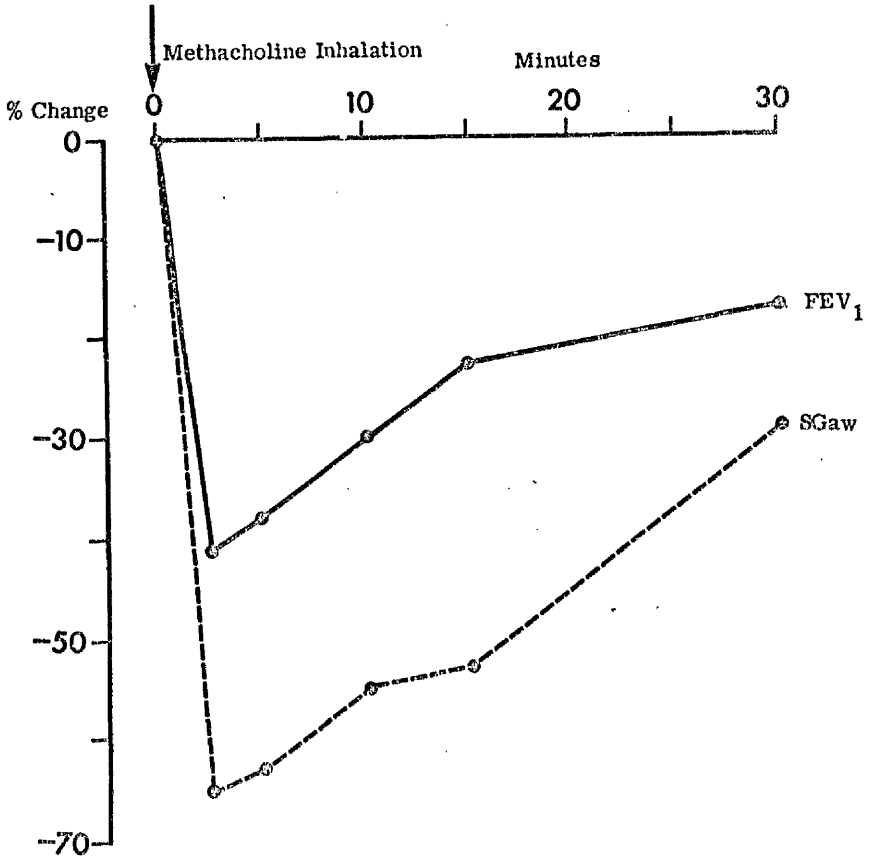


Fig. 20. Effect of methacholine inhalation on the mean FEV_1 and SGaw in 15 patients with asthma.

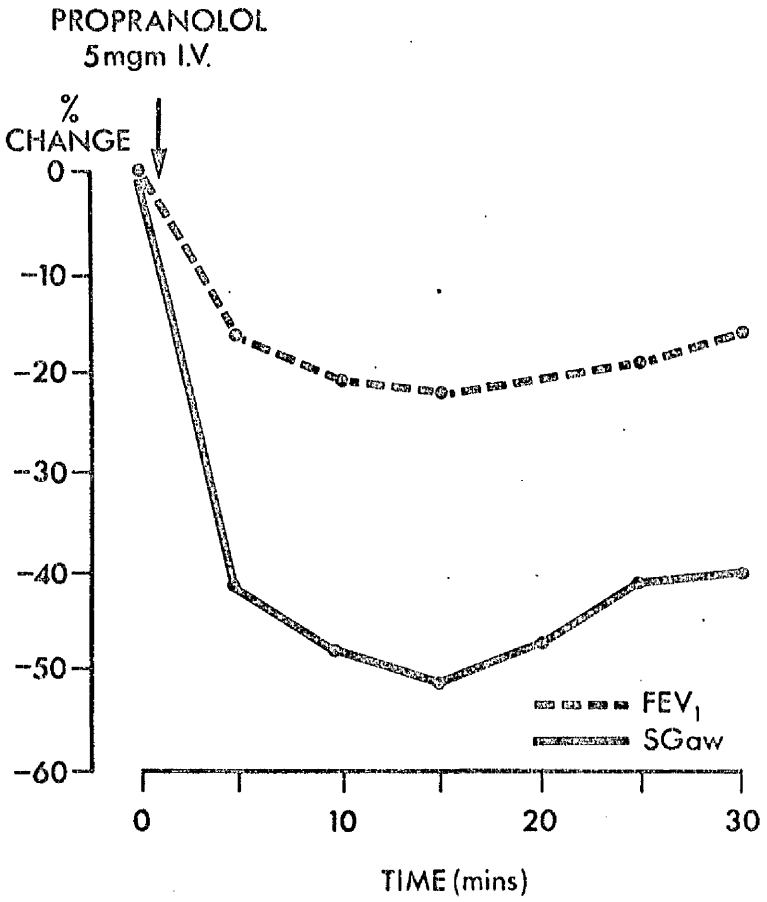


Fig. 21. Effect of intravenous propranolol (5 mgm.) on the mean FEV_1 and SGaw in 12 patients with asthma.

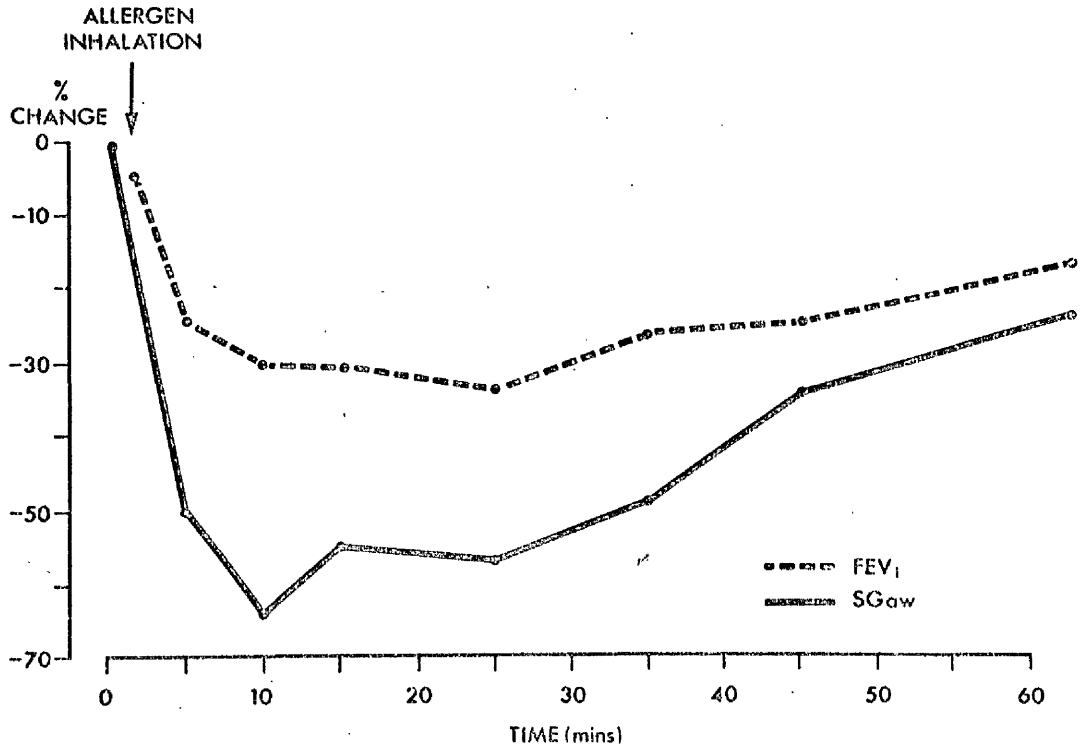


Fig. 22. Effect of allergen challenge on the mean FEV_1 and SGaw in 15 patients with asthma.

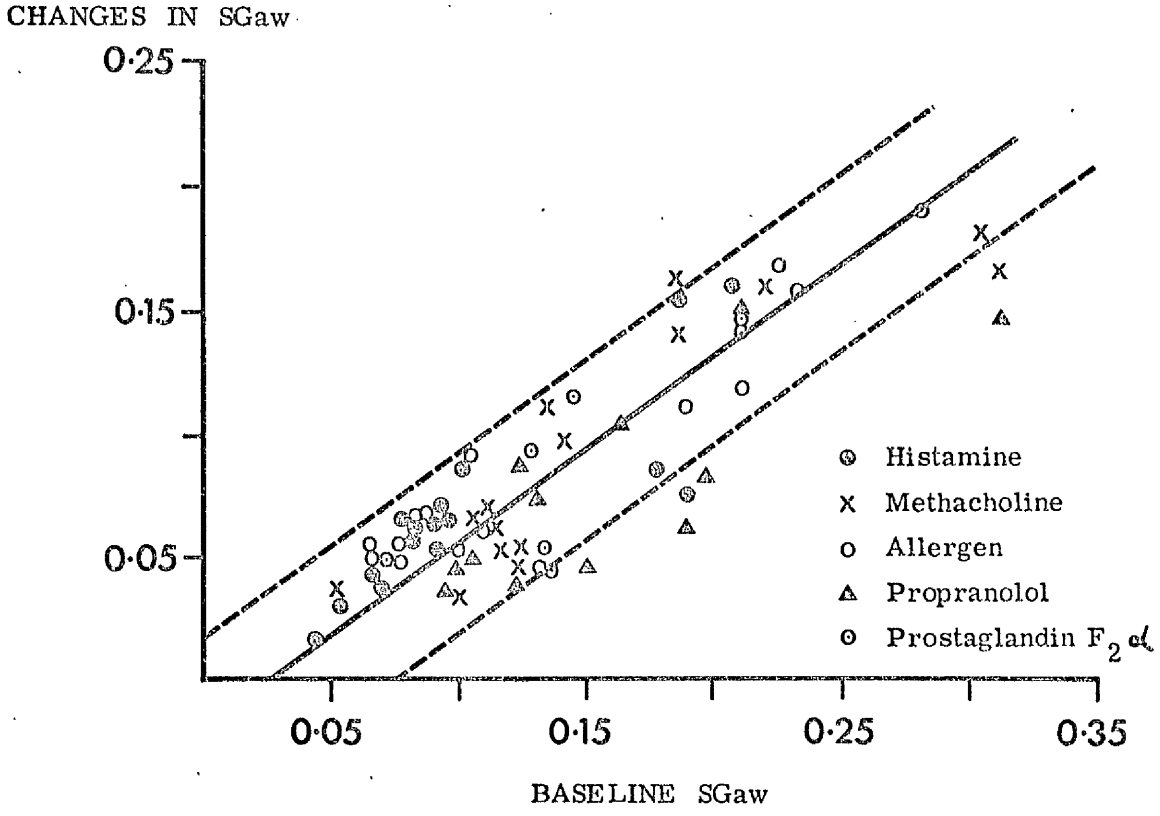


Fig. 23. Relationship between the baseline values of SGaw and the changes in SGaw produced by histamine, methacholine, allergen challenge, propranolol and prostaglandin F_2 in 65 provocation tests.

($r = 0.76$, student's $t = 9.21$, $P < .001$, $y = 0.586x + 0.003$).

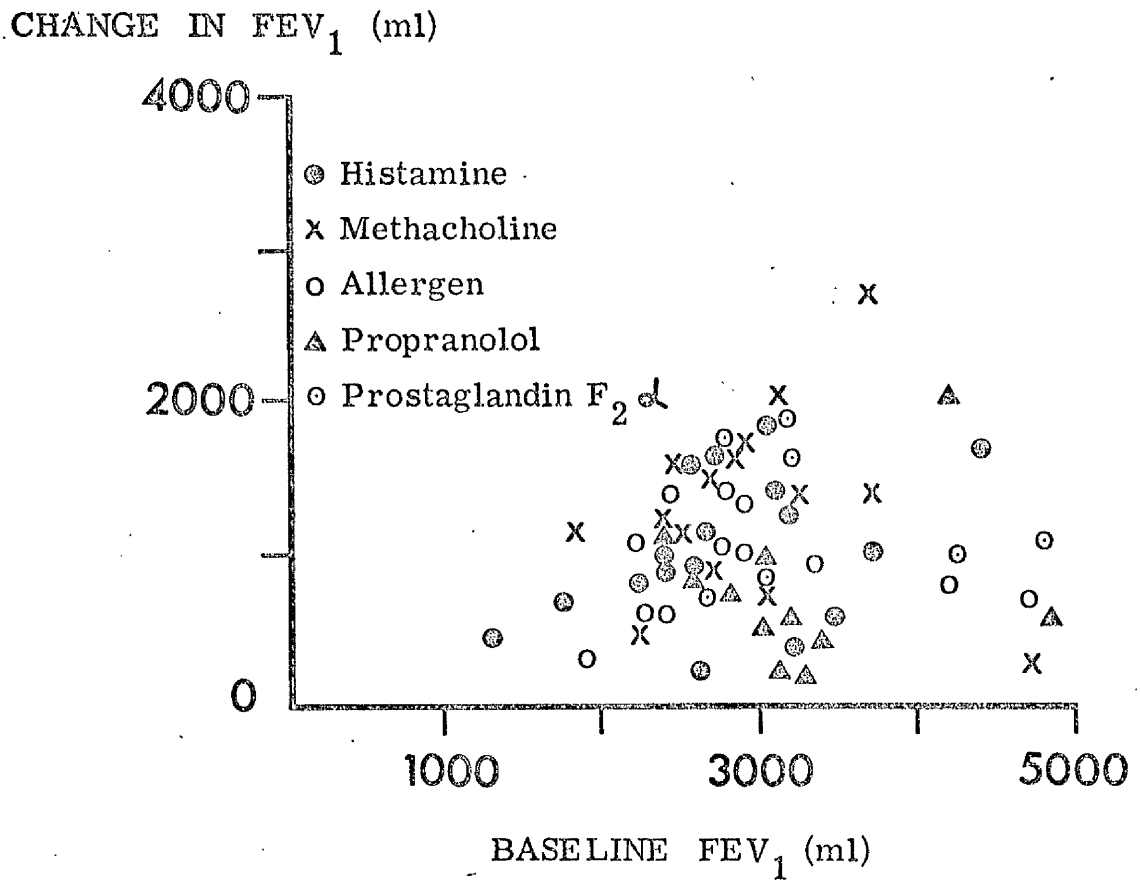


Fig. 24. Relationship between the baseline values of FEV_1 and the changes in FEV_1 produced by histamine, methacholine, allergen, propranolol and prostaglandin F_2 in 65 provocation tests.

($r = 0.02$, student's $t = 0.08$, $P > .10$).

CHAPTER IV

DISCUSSION

12.0 ROLE OF ALPHA RECEPTORS IN ASTHMA

12.1 Airways Response to Beta Blockade

A small dose of propranolol given orally or intravenously produced a significant fall both in the FEV_1 and SGaw in asthmatic patients, whereas a large dose of propranolol did not affect the FEV_1 and SGaw significantly in normal subjects. These observations are in agreement with similar results reported previously[81, 160]. Statistically, asthmatic patients and normal subjects belong to two different populations[157].

The control of bronchomotor tone is complex. It is directly under the autonomic neural influence through the vagus and sympathetic nerve supply and in addition to these are humoral factors which include histamine, 5 hydroxy-tryptamine, S.R.S.-A., prostaglandins and possibly other influences yet unknown. The extent to which all or some of these factors are active in a given subject at any one time is bound to vary and more so in asthmatic patients.

In most mammalian species vagal stimulation has been known to cause bronchoconstriction and prevention of these responses by atropine indicate that they are mediated by cholinergic fibres. Pharmacologically it has been shown that parasympathomimetic agents, such as acetylcholine and methacholine, cause an increase in resistance to air flow and it has therefore been inferred that the vagal

stimulation also causes bronchoconstriction in Man as in other mammals[161, 162]. In most animals and in Man there is some resting bronchomotor tone in eupnoea under normal conditions, mediated through the vagus nerves. Vagotomy, vagal cooling and atropine administration have been shown to increase the diameter of larger airways and increase anatomical dead space[117].

The effect of sympathetic nerve stimulation on lung mechanics is less certain than that of vagus, but generally it is thought to cause relaxation of bronchial calibre and decrease in resistance to air flow. Indirect pharmacological evidence on action of sympathetic nervous system on bronchi comes from use of adrenaline and isoprenaline in both animals and Man[163]. From the action of isoprenaline it appears that sympathomimetic broncho-dilatation depends on adrenergic beta stimulation. The existence of resting beta receptor adrenergic broncho-dilator activity in normal man is doubtful[117, 164] as both beta stimulation or beta blockade has failed to cause a significant change in the bronchial calibre in normal subjects[157, 160] and the effect of propranolol in normal subjects reported here would support this hypothesis.

The presence of alpha receptors in mammalian lungs with bronchoconstrictor activity has been postulated[84, 85, 86, 87]. Pharmacologically, alpha receptor stimulation in presence of beta blockade has been reported to cause bronchoconstriction in Man[89, 90, 157]. However, in normal

subjects alpha receptor stimulation does not cause bronchoconstriction[157] and it is unlikely that alpha adrenergic activity in the airways plays a significant role in the control of bronchomotor tone in these subjects.

Thus, it can be postulated that in normal subjects the mechanisms operating through alpha and beta adrenergic receptors, receptors at parasympathetic nerve endings and receptors for humoral mediators such as histamine, 5 hydroxytryptamine, bradykinin, prostaglandins and possibly through other receptors yet to be defined are so balanced that the combined effect of these mechanisms favours bronchodilatation.

In contrast, patients with asthma develop marked bronchoconstriction following beta blockade with propranolol. This suggests that there is an increased beta adrenergic activity at rest, the reduction of which by pharmacological blockade[81], infection or possibly other means can unmask an underlying state of bronchoconstriction. However, it is not known whether this enhanced activity is due to sympathetic nervous tone or to circulating catecholamines. It has been suggested that the cholinergic and alpha adrenergic activities are also possibly increased in asthmatic patients[165] and both cholinergic and alpha stimulation has been shown to increase bronchomotor tone[37, 39] and also to enhance mediator release in the type I allergic reaction[98]. Thus, the balance between the beta receptor activity (bronchodilator) and the cholinergic and

alpha activities (bronchoconstrictor) in asthmatic patients is much more subtle compared to normal subjects and any change in balance greatly alters the bronchial calibre. It is likely that beta blockade with propranolol in asthmatic patients results in unopposed vagal or alpha adrenergic over-activity or both favouring broncho-constriction. The failure of atropine to abolish completely the rise in airways resistance following propranolol administration[166] would suggest that alpha adrenergic activity in presence of beta blockade may be partly responsible for bronchoconstriction. Further, alpha receptor blockade has been shown to modify propranolol induced bronchoconstriction in asthmatic patients[157, 167] suggesting an increased alpha adrenergic activity in presence of beta blockade.

In addition, the distribution of autonomic influence in the central and peripheral airways in asthmatic patients is also important because of varying physiological responses to site of bronchoconstriction. Vagally mediated bronchoconstriction has been reported to cause selective bronchoconstriction in larger airways, there being little change in calibre of peripheral airways[146, 147]. Histamine, on the other hand, has been shown to cause constriction of peripheral airways and alveolar ducts without affecting the calibre of the larger conducting airways[117]. These variations in site of bronchoconstriction has been attributed to variations in sympathetic tone of the

airways[129]. Propranolol administration to asthmatic patients produced a significant fall both in the FEV_1 and SGaw suggesting that airways obstruction occurred simultaneously in the peripheral and central airways. These results suggest that beta blockade in asthmatic patients causes bronchoconstriction in the central and peripheral airways and similar observations have been reported following alpha receptor and cholinergic stimulation in asthmatic patients[157]. These observations therefore suggest that balance of autonomic influences mediated through alpha, beta and cholinergic receptors in asthmatic patients does not vary in the central and peripheral airways as has been proposed in normal subjects [129].

12.2 Beta Blockade Theory of Bronchial Hyper-Reactivity

Szentivayni in 1968[45] put forward an unitarian hypothesis to explain bronchial hyper-reactivity and atopic state in asthma. He proposed that increased irritability of the bronchial tree in asthma results from diminished beta receptor response to catecholamines and a relative in alpha adrenergic activity. Although his hypothesis has provided a great impetus to re-examine autonomic imbalance in asthma, most of his conclusions were derived from animal experiments. Mice given pertussis vaccine became hyper-sensitive to histamine and other mediators of anaphylaxis[73, 74, 75]. In addition, this was associated with eosinophilia, diminished response to beta stimulation and an enhanced

response to alpha stimulation[45, 75].

In contrast to observations in animal experiments, Zaid and Beall[82] have failed to induce methacholine or histamine hyper-sensitivity in normal subjects after beta adrenergic blockade with propranolol. Zaid and Beall's observations were confirmed by me in five normal subjects. In these subjects 10 mgm. of propranolol given intravenously did not cause a significant change in either the FEV_1 or SGaw and 200 ugm. of histamine dihydrochloride by inhalation in the presence of beta blockade also failed to affect the bronchial calibre significantly in these subjects (Figs. 25 and 26). These observations suggest that bronchial hyper-reactivity akin to that observed in asthmatic patients cannot be induced experimentally in normal subjects after beta blockade. Undoubtedly the observations in animal experiments are important, the inferences derived have to be regarded with caution because of species differences and failure to reproduce chronic unrelievable asthmatic state in animals. Further, Szentivayni ventured to propose that this autonomic imbalance could result in relative increase in alpha adrenergic activity of the airways when the existence of such receptors in the human airways was still in doubt and their function yet uncertain.

The diminished beta receptor responses observed in asthmatic patients are related to the severity of asthma. Inoue (1967)[78] reported that diminished metabolic responses to adrenaline administration increased with the severity of

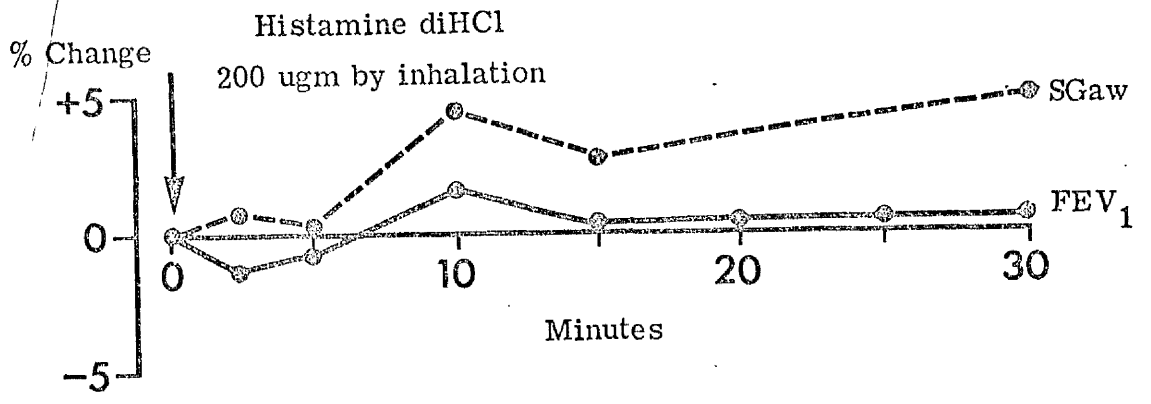


Fig. 25. Effect of histamine inhalation on the mean FEV_1 and SGaw in 7 normal subjects. Histamine failed to cause a significant change in the FEV_1 and SGaw in these patients.
Tables XLI and XLII.

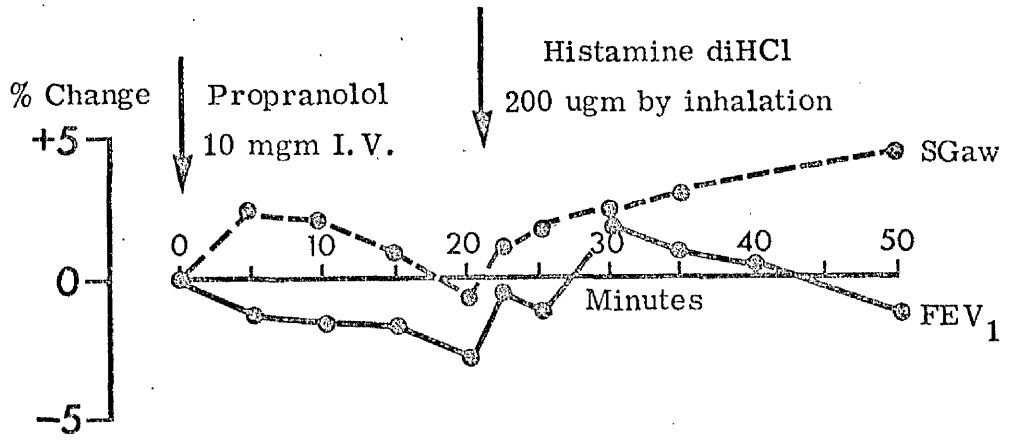


Fig. 26. Effect of histamine on the mean FEV_1 and SGaw after prior beta blockade in 5 normal subjects. Propranolol failed to cause any change in both the FEV_1 and SGaw and histamine by inhalation also failed to affect bronchial calibre significantly in these subjects.
Tables XLIII and XLIV.

asthma whereas asthmatic patients in remission could not be distinguished from normal subjects in this respect[78, 109, 168, 169]. It is probably true that the diminished beta receptor observed in patients with acute asthma may reflect a failing counter-regulatory mechanism rather than be considered the cause of asthma[169]. Further, the biochemical observations suggest that the beta receptor system is maximally stimulated and further stimulation becomes increasingly difficult[168]. Secondly, an increased rate of metabolism of catecholamines to 3 methoxy derivatives which are weak beta-adrenergic blockers[170] may also result in diminished response to sympathomimetic amines in these patients. The latter possibility still remains to be investigated.

12.3 Response to Phenylephrine After Blockade of Alpha and Beta Adrenergic Receptors

Although the presence of excitatory alpha adrenergic receptors in the respiratory tract of various animal species has been demonstrated[84, 85, 86, 87], the existence of such receptors in human bronchi has been more doubtful. In 1970, we[88] reported that histamine induced broncho-constriction in asthmatic patients could be inhibited by alpha receptor blocking drugs, phenoxybenzamine and phentolamine, suggesting that there are alpha adrenergic receptors in the human bronchial tree. However, it has been suggested that the effect we had observed was unlikely to be wholly due to alpha receptor blocking properties of phenoxybenzamine and phentolamine as both these agents are

known to have additional effects which include atropine-like and antihistaminic actions. About this time a more selective alpha receptor blocking drug, thymoxamine, became available for clinical use. Unlike phenoxybenzamine and phentolamine, thymoxamine is devoid of additional effects and is highly specific in its alpha blocking actions in isolated organs[151]. Our observations have been confirmed both by Prime et al[152] and Gaddie et al[153] using thymoxamine. At least this was the first indirect evidence of the presence of alpha adrenergic receptors in human airways.

In an attempt to find more direct evidence of the presence of alpha receptors in human airways, the effect of phenylephrine inhalation after prior beta and alpha blockade was studied in patients with bronchial asthma and normal subjects. Phenylephrine is a powerful alpha receptor stimulant with little effect on the beta receptor. A direct action on the receptor accounts for a greater part of its effects, only a small part being due to its ability to release noradrenaline[171]. In 16 patients with extrinsic bronchial asthma, phenylephrine given by inhalation in the presence of beta blockade, caused a significant fall in both the FEV_1 and SGaw (Figs. 2, 3 and 5) whereas phenylephrine had no effect on the airway calibre of ten normal subjects (Figs. 4 and 6). These observations in asthmatics provide a more direct evidence of the presence of alpha adrenergic receptors in the human bronchial tree and

that stimulation of these receptors can cause broncho-constriction in patients with asthma. But unlike Prime and his colleagues[89], I have been unable to demonstrate alpha adrenergic activity in the airways of normal subjects. In six asthmatic patients, the phenylephrine effect could be completely inhibited by alpha receptor blocking drugs, phenoxybenzamine and thymoxamine, suggesting that the effect of phenylephrine was specifically on the alpha adrenergic receptors (Figs. 2 and 3).

However, it has been suggested that beta adrenergic blockade in patients with asthma may lead unopposed parasympathetic activity which in turn causes broncho-constriction[166]. If this is so, a possibility arises that the phenylephrine induced bronchoconstriction in presence of beta blockade could have resulted from stimulation of 'irritant' cholinergic receptors in the airways[158]. The effect of phenylephrine after pre-treatment with atropine was studied in six patients with asthma.

12.4 Response to Phenylephrine After Blockade of Beta and Cholinergic Receptors

In six patients, atropine given by inhalation produced a significant bronchodilatation and rise in SGaw suggesting resting bronchomotor tone mediated by cholinergic activity as suggested by Widdicombe and Sterling (1970)[117]. In presence of cholinergic blockade with atropine, propranolol induced bronchoconstriction was

partially inhibited. Further, phenylephrine inhalation in presence of beta and cholinergic blockade produced a significant fall in SGaw (Fig. 7). The result of this investigation further suggests that the phenylephrine effects in patients with asthma is mediated specifically through alpha adrenergic receptors and cannot be due to a non-specific effect on 'irritant' cholinergic receptors[158].

12.5 Thymoxamine in Histamine Induced Bronchoconstriction

In a previous study, we had reported that alpha receptor blocking drugs, phenoxybenzamine and phentolamine, could inhibit histamine induced bronchoconstriction. However, both these drugs have additional effects and it is difficult to assess whether the inhibition observed was wholly due to alpha receptor blocking property of these drugs. Bianco et al[152] and Gaddie et al[153] have confirmed our findings using thymoxamine which is more specific in its alpha receptor blocking action and is devoid of additional effects seen with phenoxybenzamine and phentolamine[151]. I confirmed our observation using thymoxamine in eight patients with asthma (Fig. 8). Thymoxamine administered by inhalation effectively inhibited histamine induced fall in the FEV_1 in eight patients with extrinsic asthma.

The effect of histamine on bronchial smooth muscle is complex. It may act directly or by a delayed reflex action[172]. Although it is well established that patients with extrinsic asthma are hyper-sensitive to inhaled or

intravenously administered histamine[37, 38, 39, 88], the mechanism by which this hyper-sensitivity is produced still remains uncertain. One possibility is that this may be the sequelae of diminished beta receptor responsiveness.

The alpha and beta adrenergic receptors are believed to control ionised calcium concentration in the environment of contractile protein of myofibrils[173, 174] and histamine response of smooth muscle is dependent on calcium ions[175]. In addition, it has recently been shown the ionotropic and relaxing effect of cyclic AMP on cardiac muscle is mediated by modulation of rate of Ca^{++} binding with sarcoplasmic reticulum[176]. It can therefore be postulated that beta receptor agonists by increasing the Ca^{++} binding in the sarcoplasmic reticulum of bronchial smooth muscle fibrils cause relaxation whereas alpha receptor agonists may have a reverse effect[177].

Alpha receptor blocking drugs which are known to enhance cyclic AMP formation[107, 168] may increase Ca^{++} binding in the sarcoplasmic reticulum of bronchial smooth muscle and thus inhibit the histamine effect on the bronchial smooth muscle in asthmatic patients.

12.6 Thymoxamine in Methacholine Induced Bronchoconstriction

Although acetylcholine, the cholinergic neuro-transmitter, is not released in the reagin-mediated allergic reaction, it might be considered a mediator because of its profound effects on bronchial physiology[37]. In addition, cholinergic agonists have been shown to enhance histamine release from human lung[98].

Thymoxamine as expected failed to inhibit methacholine induced fall in FEV_1 in eight patients with asthma (Fig. 9). It is now established that cholinergic agonists, like acetylcholine and methacholine, activate guanyl cyclase and lead to an increase in cyclic GMP formation[63]. Guanyl cyclase-cyclic GMP is the second messenger system for cholinergic responses. Cyclic GMP formation is not influenced by thymoxamine in asthmatic patients[178] (Part II of this thesis) and therefore it is unlikely for thymoxamine to have any effect on methacholine induced broncho-constriction. Atropine which inhibits cyclic GMP formation effectively inhibits methacholine induced broncho-constriction in asthmatic patients and normal subjects (personal observations).

12.7 Thymoxamine, Atropine and Sodium Cromoglycate in Prostaglandin F_2 Alpha Induced Bronchoconstriction

Prostaglandin F_2 alpha is a potent bronchoconstrictor in Man. The presence of prostaglandin F_2 alpha in human lung and its release during type I allergic reaction and in response to various chemical and mechanical stimuli[42, 179] has led some workers to postulate that locally formed prostaglandin F_2 alpha may play an important part in pathogenesis of bronchial asthma[36, 42]. In addition, Paterson et al[179] have also suggested that prostaglandin F_2 alpha may be involved in exercise induced asthma.

In six patients with asthma atropine inhalation partially inhibited prostaglandin F_2 alpha induced fall in

the FEV_1 and SGaw whereas thymoxamine and sodium cromoglycate had no effect in this respect (Figs. 10 and 11). The failure of sodium cromoglycate to inhibit prostaglandin F_2 alpha induced bronchoconstriction together with the observations of its inhibitory effect in allergen[142] and exercise provoked asthma[143] would suggest that the release of prostaglandin F_2 alpha locally in the lung may not be a primary factor in the pathogenesis of asthma. In addition, indomethacin administration which markedly reduces the total body production of prostaglandin F_2 alpha does not completely inhibit allergen provoked asthma in Man or anaphylactic reaction in guinea pigs[180, 181].

The studies of cyclic nucleotide systems suggest that the effects of prostaglandin F_2 alpha like cholinergic agonists is mediated through guanyl cyclase-cyclic GMP[100]. The partial inhibition of prostaglandin F_2 alpha induced bronchoconstriction by atropine as observed in this investigation is consistent with this hypothesis. Although there is evidence that alpha stimulation increases cyclic GMP formation[62], it is unlikely that prostaglandin F_2 alpha acts on the alpha receptors in the lung as thymoxamine failed to alter prostaglandin F_2 alpha induced bronchoconstriction in asthmatic patients (Figs. 10 and 11). The evidence so far suggests that prostaglandin F_2 alpha together with other chemical mediators, such as histamine, bradykinin, serotonin and S.R.S.-A. is released during the

type I allergic reaction. Prostaglandin F_2 alpha is a very potent bronchoconstrictor to which asthmatic patients are exquisitely sensitive and it may act by stimulating cholinergic receptors. It would be wrong to consider prostaglandin F_2 alpha as the main factor in pathogenesis of asthma until we further clarify the cause of airways hyper-reactivity which seems to be the primary defect in asthma.

12.8 Thymoxamine in Allergen Induced Bronchoconstriction

In ten patients with asthma, allergen induced bronchoconstriction was inhibited by thymoxamine given intravenously (Fig. 12). In two of these patients the thymoxamine by inhalation also inhibited allergen provoked bronchoconstriction and in one patient this protection was maintained even when the dose of allergen inhaled was doubled (Figs. 13 and 14).

Lichtenstein and De Bernado[182] have shown that cyclic AMP inhibits mediator release and that the adenyl cyclase system is involved in the first stage of the type I allergic reaction which is not calcium dependent. There are two possibilities for the observed effects in this investigation. Firstly, that thymoxamine acts by increasing cyclic AMP[107, 168] and thereby prevents the release of pharmacologically active substance from mast cells following allergen challenge or that thymoxamine does not inhibit mediator release but alters the bronchial smooth muscle response to these mediators, by altering the

bronchomotor tone. It is now known that reagin-mediated allergic reaction releases significant quantities of prostaglandin F_2 alpha in addition to histamine, S.R.S.-A., bradykinin and 5 hydroxytryptamine[29]. The variable responses recorded after allergen challenge in presence of alpha blockade with thymoxamine could be accounted for by the failure of thymoxamine to inhibit the effect of prostaglandin F_2 alpha on the airways[183] suggesting that the dominant effect is on the bronchomotor tone rather than the mediator release.

12.9 Thymoxamine and Sodium Cromoglycate in Post-Exercise Bronchoconstriction

A significant inhibition of post-exercise bronchoconstriction was observed in 12 out of 13 patients following thymoxamine inhalation, and in the same 12 patients a statistically comparable inhibition was also obtained with sodium cromoglycate (Fig. 16). However, in one patient neither drug had any effect on post-exercise bronchoconstriction. Further, atropine sulphate given by inhalation also failed to inhibit exercise induced bronchospasm in this patient. These results contrast with the observations of Sly et al[184] who failed to inhibit post-exercise bronchoconstriction using phentolamine. Phentolamine is a short acting drug and it may produce inadequate and transient blockade when given intravenously [185].

A wide variety of humoral mediators have been suggested as the cause of exercise induced asthma. Histamine levels

have been found to be normal or unrelated to the degree of post-exercise bronchoconstriction[186, 187]. Further, antihistaminic drugs have failed to inhibit post-exercise bronchoconstriction[184, 188]. The role of serotonin as a bronchoconstrictor in Man is disputed[33] and its antagonist, methysergide, does not alter exercise response[189]. Local prostaglandin release has been suggested[179] and the demonstration of this release in guinea pig lungs in response to minor mechanical stimuli would support this view[154]. However sodium cromoglycate does not inhibit prostaglandin F_2 alpha induced bronchoconstriction in asthmatic patients[183]. The effect of sodium cromoglycate on prostaglandin F_2 alpha induced bronchoconstriction, together with the observation of its beneficial effect in exercise asthma[143], would suggest that the release of prostaglandin F_2 alpha locally in the lung may not be the primary factor in exercise induced bronchospasm.

Noradrenaline and adrenaline are released during strenuous exercise[190, 191], and this release of catecholamines is greatly enhanced by prior beta blockade with propranolol or oxprenolol[192]. A similar enhanced release of catecholamines may also occur in asthmatic patients who show diminished beta responsiveness to catecholamines. The observations of this investigation suggest that increased alpha adrenergic activity in the presence of diminished beta receptor responsiveness could be the mechanism of post-exercise bronchoconstriction in

these subjects. In addition, the report by Jones[193] that a proportion of normal subjects develop post-exercise bronchoconstriction in the presence of prior beta blockade with propranolol would further suggest that alpha adrenergic stimulation could be the cause of post-exercise bronchoconstriction in these subjects.

It has recently been suggested that sodium cromoglycate acts by inhibition of cyclic phosphodiesterase and leads to an increase in the levels of cyclic AMP[144, 145]. It is possible that like alpha receptor blocking drugs which increase cyclic AMP formation on stimulation with isoprenaline[107, 168] (reported in Part II of the thesis), sodium cromoglycate may also increase cyclic AMP level via inhibition of cyclic phosphodiesterase. The increase in cyclic AMP levels may modify the bronchomotor tone and alter the response to exercise in asthmatic patients.

12.10 Alpha Receptor Blocking Drugs Alone and in Combination with with Isoprenaline on SGaw

In ten patients with asthma, thymoxamine or phentolamine increased SGaw by 45%, this improvement was, however, significantly less than that achieved with isoprenaline alone. When isoprenaline and thymoxamine or phentolamine were administered together the mean SGaw increased by 200% from the baseline value of SGaw. There was a highly significant difference between the bronchodilatation achieved with isoprenaline alone and that achieved with isoprenaline plus thymoxamine or phentolamine (Figs. 17 and 18).

The above observations are supported by the biochemical studies on the leucocyte adenyl cyclase activity in asthmatic patients[168] (reported in Part II). In acute asthma, the leucocyte adenyl cyclase activity shows diminished responsiveness to stimulation with isoprenaline [168, 169]. This abnormal adenyl cyclase response in acute asthma can be restored towards normal by alpha receptor blocking drugs[107, 168], thymoxamine and phentolamine.

Thus, in the airways, as in leucocytes, it appears that the balance between alpha and beta adrenergic receptors may play a critical role in the control of cell function. In bronchial smooth muscle alpha receptor blockade restores the balance in favour of beta adrenergic receptors and hence the bronchodilator response to catecholamines. The use of alpha receptor blocking drugs may be another line of attack of this distressing disease as well as a means of better understanding of the mechanism which underlie bronchial hyper-reactivity in asthma.

13.0 PHYSIOLOGICAL EFFECTS OF BRONCHOCONSTRICTION

13.1 Site of Airways Obstruction

It has been reported that nervously mediated bronchoconstriction may operate at a different site in the airways from humoral bronchoconstriction[146, 147]. Histamine, on the other hand, has been reported to cause constriction of peripheral airways and alveolar ducts without affecting the calibre of larger conducting airways. Vagally mediated bronchoconstriction, on the other hand, causes bronchoconstriction in the larger airways there being little effect in the peripheral airways[146]. Bouhuys and Woestijne[129] have postulated that individual variations in airways response to histamine and hemp dust in cotton workers is principally determined by variation of sympathetic tone. According to this hypothesis a subject with peripheral airways obstruction demonstrated by fall in FEV_1 and flow rate ('flow rate response') may have relatively few sympathetic fibres in peripheral airways so that the beta adrenergic activity might be insufficient to counteract the bronchoconstriction effect of histamine or hemp dust in these airways. Conversely, in a subject with conductance response (fall in SGaw) the sympathetic distribution might be predominantly to smaller airways. To explore a similar possibility in patients with asthma, the airways responses as assessed by FEV_1 (peripheral airways) and SGaw (larger airways) during various provocation tests were analysed.

In 65 provocation tests, there was a good time relationship between the maximal fall in FEV_1 and SGaw produced by histamine, methacholine, propranolol, prostaglandin F_2 alpha and allergen challenge (Figs. 19 - 22). These results suggest that the airways obstruction occurs simultaneously in the central and peripheral airways in these patients, and it is difficult to demonstrate different sites of airways obstruction as has been shown in dogs following vagal stimulation[194] and as suggested in Man by Bouhuys and Woestijne[129]. Although the balance between sympathetic and parasympathetic divisions of autonomic nervous system in the airways may vary in dogs[194] and also in normal subjects[129, 195], the observations reported here suggest that the diminished beta receptor function may be present in both the central and peripheral airways in asthmatic patients and this would explain marked bronchoconstrictor response to various agents at both these sites.

13.2 Relationship of Response to Initial Bronchomotor Tone

The change in SGaw produced by histamine, methacholine, propranolol, prostaglandin F_2 alpha and allergen challenge depends on the baseline (initial) SGaw (Fig. 23). The greatest change in SGaw was elicited when the baseline SGaw was highest, i.e. maximum bronchoconstriction in response to any of the above agents occurred when the airways were maximally dilated. In contrast, the change in FEV_1 did not relate to the baseline value of FEV_1. This lack of correlation between the change in FEV_1 and the baseline

FEV_1 as reported here and previously[44] may be due to collapse and the closure of the peripheral airways during forced expiration manoeuvres. The collapse and closure of the peripheral airways is accentuated during maximal expiratory effort and remains a major cause of flow limitations[196]. Further, the FEV_1 is determined by the properties of upstream segment of airway which contributes a small proportion to the total airways resistance. Airways closure causes air trapping, leads to changes in lung volumes and accounts for the disparity in the results of lung volumes measured by body plethysmography and helium dilution method during spontaneous asthma[197]. On the other hand, the airways conductance is the measurement of airways obstruction in the central airways which do not collapse readily. The changes in SGaw may therefore provide a better assessment of the change in bronchomotor tone during provocation tests. The results of this investigation are in accord with in vitro studies on the guinea pig tracheal smooth muscle in which it has been shown that the muscle contraction is dependent on the inherent tone of the muscle[87]. Astin[198] has shown that patients with chronic bronchitis who had highest initial airways resistance had the greatest increase in airways resistance after propranolol administration whereas in this study asthmatic patients who had the lowest airways resistance (highest SGaw) had the greatest increases in airways resistance after propranolol administration.

The differing airways response to beta blockade in patients with asthma and chronic bronchitis may be due to underlying inherent differences in the bronchial reactivity and this may be of considerable clinical importance in classifying patients with obstructive airways disease.

13.3 Airways Closure

Recently Dolfuss et al[199] described a simple test using Xe^{133} to determine the lung volume at which ventilation ceases in the dependent lung zones as a result of airways closure. The volume termed the closing volume increases linearly with age in adults[200]. Other workers have described closing volume in disease and as a simple test to detect air flow obstruction in peripheral airways of less than 1 mm. in diameter[134, 135].

The closing volume, using single breath nitrogen test[201], was measured in eight patients with asymptomatic extrinsic bronchial asthma and ten normal subjects. The procedure and results are not included in the method and results sections of this thesis; however, the data of this investigation is given in the Appendix (tables LIII - LVI). The closing volume was significantly increased in asthmatic patients as compared to the closing volume observed in normal subjects. The closing volume in asthmatic patients decreased to normal values following salbutamol inhalation whereas propranolol caused a significant increase in the closing volume in these patients. The results of this investigation suggest that changes in the smooth muscle tone

in the terminal bronchioles, which is largely dependent on the sympathetic nervous activity of the airways, can significantly affect airways closure in patients with bronchial asthma.

Recently there has been a renewed interest in the observation of Huber and Koessler[130] that bronchial smooth muscle in asthmatic patients show changes of hypertrophy and hyperplasia. Hossain[133] noted a three fold increase in the number of smooth muscle cells and the absolute volume of muscle in necropsy specimens of bronchi of patients with asthma compared with that found in a normal airway. This change of hypertrophy and hyperplasia extend from the central airways to the terminal bronchioles. The increase in closing volume reported here and previously[202] suggests that bronchial smooth muscle contraction in terminal bronchioles with peripheral airways obstruction is present in many patients with asthma in remission. The influence of bronchomotor tone on airways closure may be more pronounced in asthmatic subjects because of an increase in absolute muscle volume and the bronchial hyper-reactivity to adrenergic activity. The cause of the hypertrophy and hyperplasia of bronchial smooth muscle cells in asthma is unknown. If these changes result from a latent airways obstruction in asymptomatic patients then an early detection and treatment may help in breaking the vicious cycle which leads to chronic muscular changes and chronic asthma.

P A R T II

Biochemical

CHAPTER V

METHODS

PART II (Biochemical Experiments)

14.0 ADENYL CYCLASE AND GUANYL CYCLASE SYSTEMS IN ASTHMA

14.1 Patients and Control Subjects

Fourteen out-patients, aged from 17 to 42 years, were studied. They were further divided into two groups. The first consisted of seven patients with active asthma as assessed by a history of daily wheezing, breathlessness on moderate exercise, clinical and spirometric evidence of airways obstruction, and the amount of bronchodilator and steroid therapy required for the relief of symptoms. The second group consisted of seven asthmatic patients in remission. Ten healthy adults, aged from 19 to 45 years, were studied as a control group. All therapy in asthmatic patients was discontinued for at least 24 hours before the experiments. Samples of venous blood were collected between 9 and 10 a.m. to avoid circadian variations. Blood films from each patient and control subject were made for differential leucocyte count. The FEV_1 was measured in asthmatic patients within a few minutes of blood collection.

14.2 Leucocyte Adenyl Cyclase Assay

Reagents

The reagents used were as follows: adenosine triphosphate (ATP), adenosine diphosphate (ADP), 5' adenosine monophosphate (5'-AMP), 3'-5' cyclic adenosine

Table XXIV Composition of buffered culture medium

Constituents	Concentration
NaCl	0.120 mol/litre
KCl	5.0 mmol/litre
$CaCl_2$	0.6 mmol/litre
$MgCl_2$	1.0 mmol/litre
Glucose	10.0 mmol/litre
human albumin	0.3 g/litre
TRIS HCl	25mM
human AB rhesus-positive serum	20% (v/v) adjusted to pH 7.40

monophosphate (cyclic AMP), adenosine, adenine, human serum albumin, theophylline, Ouabain, octahydrate, DL isoprenaline hydrochloride (all purchased from Sigma Chemical Company), phentolamine mesylate (Ciba Laboratories Limited), thymoxamine hydrochloride (W. R. Warner & Company, Eastleigh), and ^{3}H-adenine (15 Ci/mmol) (Radio Chemical Centre, Amersham). The scintillation grade chemicals naphthalene, 2, 5-diphenyloxazole (PPO), toluene, 1, 4-dioxan, and 2-ethoxyethanol and also the Whatman 3MM chromatography paper were purchased from BDH Chemicals Limited.

Preparation of leucocytes

Leucocytes were prepared from 40 ml. of whole blood according to the dextran sedimentation technique as described by Logsdon et al (1972)[107], and the cells were resuspended in a buffered culture medium (table XXIV) so that 2 ml. of a suspension contained between 150 and 200 x 10^{6} cells. The cell count was carried out in a Neubauer's Chamber.

Adenyl cyclase assay

Leucocyte adenyl cyclase assay was performed by the following modification of the intact cell method described by Humes et al (1969)[203]. Two ml. of a suspension of the leucocytes in the buffered culture medium described were incubated with shaking at 37^{o}C. for two hours with ^{3}H-adenine (1 u Ci/5 x 10^{6} cells) in a siliconised conical centrifuge tube. After incubation the cells were

centrifuged at 150 gm. for five minutes and the supernatant fluid removed. The cells were then washed three times with fresh culture medium to remove as completely as possible the extracellular labelled adenine and were resuspended in a fresh medium to give a concentration of about 10 x 10^6 cells/ml., 0.5 ml. quantities of which were then added to siliconised tubes and once again incubated in a shaking metabolic water bath at 37°C. for 15 minutes to allow temperature equilibration to occur. Then 0.5 ml. of a solution of 20 mmol. theophylline/l. dissolved in buffered culture medium was added to the cell suspension. The various drug treatments were carried out by adding appropriate agents at the same time as theophylline. These drug treatments are given in table XXV. The cells were incubated for a further five minutes and the reaction was terminated by boiling the reaction tubes for three minutes. A non-radioactive carrier solution, 0.1 ml. containing 5 mmol/l. each of 3'-5' cyclic AMP, ATP, ADP, 5'-AMP, adenosine and adenine, was then added and after thorough mixing the tubes were centrifuged. All incubations were performed in duplicate and the use of AB rhesus-positive serum helped to prevent aggregation of cells. Aliquots of 40 ml. of the protein free supernatant solution were spotted on a Whatman 3MM paper and subjected to chromatography for 14 hours in a solvent system consisting of 1M ammonium acetate (pH 7.50) and ethanol (30:75) which effectively separates 3'-5' cyclic AMP from

other nucleotides and purine bases[107]. The Rf values of these nucleotides and purine bases are given in table XXVI. After drying the chromatogram the positions of the spots were located under ultraviolet light and identified by means of markers in addition to mobilities. The areas corresponding to 3'-5' cyclic AMP, adenine plus adenosine, and ATP plus ADP plus 5'-AMP were cut out with scissors and placed in separate vials. The nucleotides and purine bases were eluted from the paper with 1 ml. of 10 mM TRIS HCl (pH 7.40) and 15 ml. of a scintillation fluid, constituents of which are given in table XXVII. The vials were then counted in a Beckman scintillation counter with a counting efficiency of 30% for ^{3}H.

14.3 Analysis of Data

Following the procedure devised by Logsdon et al (1972)[107] adenyl cyclase activity was calculated as the ratio of ^{3}H cyclic AMP to the total activity found in ATP, ADP, 5'-AMP, cyclic AMP, adenosine, and adenine expressed as percentage. This method avoids differences in the uptake of ^{3}H adenine by the cells in different sample tubes. The percentage of cyclic AMP formed in the control tubes containing only theophylline was taken as the control for each experiment. The percentage cyclic AMP formed after each drug treatment was then divided by the control value for that experiment so that each drug treatment was expressed as a percentage of the control value, thus making possible comparison between the various treatments in each

Table XXV Different drug treatments of the leucocyte adenyl cyclase activity

Control experiments	Theophylline alone.
Beta adrenergic stimulation	Isoprenaline 10^{-6}M and 10^{-4}M.
Alpha adrenergic blockade	Thymoxamine 2 x 10^{-4}M or Phentolamine 2 x 10^{-4}M.
Alpha blockade	Thymoxamine 2 x 10^{-4}M or Phentolamine 2 x 10^{-4}M.
+	+
Beta stimulation	Isoprenaline 10^{-4}M.
ATPase inhibitor	Ouabain 2 x 10^{-4}M.
ATPase inhibitor + beta stimulation	Ouabain 2 x 10^{-4}M + Isoprenaline 10^{-4}M.

Table XXVI Mobility data for nucleotides and purines in adenyl cyclase assay

	Rf. values
ATP	0.05
ADP	0.05
5'-AMP	0.13
3'-5' cyclic AMP	0.42
adenosine	0.51
adenine	0.59

Table XXVII Composition of Scintillation Fluid

2'-5 diphenyloxazole	4 g.
naphthalene	80 g.
1,4-dioxan	300 ml.
2-ethoxyethanol	300 ml.
toluene to make up to 1 litre.	

experiment and between experiments. Results from separate experiments were pooled and group means calculated plus or minus the standard error of the mean. Students t test was used to determine the significance of drug effects.

14.4 Lymphocyte Guanyl Cyclase Assay

Patients and control subjects

Twelve patients, aged from 14 to 44 years, with extrinsic bronchial asthma were studied. They were further divided into two groups; six patients with active asthma and six patients were in remission. The criteria for dividing these patients have already been described. Ten healthy adults, aged from 19 to 45 years, were studied as a control group. All therapy in asthmatic patients was discontinued for at least 24 hours before the experiments. Samples of venous blood were collected between 8.30 and 9.30 a.m. to avoid circadian variations. The FEV_1 was measured in asthmatic patients within a few minutes of blood collection.

Reagents

Reagents used were guanosine triphosphate (GTP), guanosine diphosphate (GDP), 5'-guanosine monophosphate (5'-GMP), 3'5' cyclic guanosine monophosphate (cyclic GMP), guanosine, guanine, human albumin, DL propranolol hydrochloride, L-noradrenaline (all purchased from Sigma Chemical Company), acetylcholine (Cal Biochem), thymoxamine hydrochloride (W. R. Warner & Company), Ficoll (Pharmacia Fine Chemicals, Sweden), hypaque 45% (Winthrop Laboratories,

Surrey), ^{3}H-guanine, 1 Ci/mmol (Radio Chemical Centre, Amersham). The scintillation grade chemicals, 2, 5-diphenyloxazole (PPO), toluene, 1-4 dioxan, and 2 ethoxyethanol and also the Whatman 3MM chromatography paper were purchased from BDH Chemicals Limited.

Preparation of lymphocytes

Lymphocytes were prepared from 40 ml. of whole blood. This volume of blood was carefully layered over an equal volume of ficoll-hypaque and then centrifuged with 400 gm. at the interface for 20 minutes as described by Harris et al[204]. The red cells and granulocytes were spun down and the lymphocytes appeared as a narrow white layer immediately below the supernatant ficoll-hypaque interface. The lymphocyte layer was carefully removed and resuspended in the buffer solution (pH 7.40), the constituents of which are given in table XXIV. After centrifuging this suspension for 5 minutes at 70 gm. the supernatant was discarded and the procedure was repeated. Finally, the lymphocyte pellet was resuspended in 1.5 ml. of buffer prior to incubation with ^{3}H-guanine at 37^{o}C. This method produced a highly purified preparation of lymphocytes.

Guanyl cyclase assay

The procedure for guanyl cyclase assay was as described previously for the leucocyte adenyl cyclase assay. Briefly, the procedure involved the incubation of lymphocytes with ^{3}H-guanine (1 u Ci/5 x 10^{6} cells) at 37^{o}C. for two hours followed by stimulation of the washed,

resuspended cells for 5 minutes with the appropriate drugs. The various drug treatments are given in table XXVIII. The reaction was terminated by boiling and 0.1 ml. of non-radioactive carrier solution containing 5mM each of 3'5' cyclic GMP, GTP, GDP, 5' AMP, guanosine and guanine was added. Chromatographic separation of nucleotides and purine bases after 18 hours of development are given in table XXIX. The areas corresponding to 3'5' cyclic GMP, guanine plus guanosine and GTP plus GDP plus 5' GMP were cut out and placed in separate vials. The nucleotides and purine bases were eluted from the paper with 1 ml. of water and 15 ml. of scintillation fluid, constituents of which are given in table IV. The scintillation counting was done on a Packard S.S. scintillation counter with 30% efficiency for ^{3}H.

Analysis of data

The guanyl cyclase activity was calculated as the ratio of ^{3}H cyclic GMP to the total activity found in GTP, GDP, 5' GMP, cyclic GMP, guanosine and guanine expressed as a percentage. The method for expressing results has been described previously[107, 168].

Table XXVIII Different drug treatments of the lymphocyte guanyl cyclase activity

Control	Nil
Beta adrenergic blockade	Propranolol 2 x 10^{-4}M
Alpha adrenergic stimulation	Propranolol 2 x 10^{-4}M + Noradrenaline 10^{-4}M
Alpha adrenergic blockade	Thymoxamine 2 x 10^{-4}M
Cholinergic stimulation	Acetylcholine 10^{-4}M
Alpha blockade + Cholinergic stimulation	Thymoxamine 2 x 10^{-4}M + Acetylcholine 10^{-4}M

Table XXIX Mobility data for nucleotides and purines in guanyl cyclase assay

	Rf. values
GTP	0.03
GDP	0.04
5'-GMP	0.11
3'-5' cyclic GMP	0.34
guanosine	0.45
guanine	0.51
hypoxanthine	0.53

CHAPTER VI

RESULTS

Biochemical Experiments

15.0 LEUCOCYTE ADENYL CYCLASE ACTIVITY

15.1 Response to Isoprenaline in Normal Subjects, Asthmatic Patients in Remission and Patients with Active Asthma

The results of each group of subjects are shown in table XXX together with the statistical evaluation. There was a significant difference in the mean percentage value of ^{3}H cyclic AMP in the control (or basal) level between the active asthmatic group (1.48 ± 0.21) and the asthmatic group in remission (0.64 ± 0.08, $P < .005$) but not between the active asthmatic group and the normal group (1.15 ± 0.26). The difference between the normal group and the asthmatics in remission was also not significant ($P > .10$).

In normal subjects isoprenaline at $10^{-6}M$ and also $10^{-4}M$ evoked significant increases in leucocyte adenyl cyclase activity 61% ($P < .02$) and 44% ($P < .005$) respectively. In the asthmatic remission group significant increases in leucocyte adenyl cyclase activity were observed, that is 64% ($P < .005$) and 93% ($P < .001$). However, no significant increase in enzyme activity was observed in the acute asthmatic group when the cells were stimulated with $10^{-6}M$ and $10^{-4}M$ isoprenaline.

In the asthmatic group, when the basal or control level of adenyl cyclase activity was plotted against the increase in activity of enzyme on stimulation with 10^{-4}M isoprenaline (Fig. 26), there was a significant inverse correlation between these two parameters ($r = 0.82$, t test = 4.79, $P < .001$). These results suggest that in patients with acute asthma, the leucocyte adenyl cyclase activity is maximally stimulated and further stimulation becomes increasingly difficult.

The leucocyte adenyl cyclase response to 10^{-4}M isoprenaline (percentage increase over the control value) in individual patients did not relate with the changes in FEV_1 and SGaw produced by propranolol administration or allergen challenge. Further, no correlation was observed between the total circulating reagins (IgE) and the control adenyl cyclase activity or its responsiveness to isoprenaline. The results of this investigation have been published and a copy of the paper is included in the Appendix[169].

15.2 Effect of Thymoxamine and Phentolamine on the Leucocyte Adenyl Cyclase Response to Isoprenaline

The results of each group of subjects are given in table XXXI. Thymoxamine 2×10^{-4}M produced no increase in the three groups examined, but in combination with isoprenaline 10^{-4}M the increased activity in all three groups was highly significant. Similarly, phentolamine at 2×10^{-4}M alone evoked no change in any group, but in

combination with 10^{-4}M isoprenaline a highly significant increase was obtained in all three groups.

15.3. Effect of K^+ Na^+ Activated ATPase Inhibitor, Ouabain, on the Leucocyte Adenyl Cyclase Response to Isoprenaline

The results of each group of subjects are given in table XXXII. Ouabain 2 x 10^{-4}M caused a marginally significant increase in ^{3}H-cyclic AMP in the asthmatic patients in remission but no change in active asthmatic or normal groups. The combination of Ouabain 2 x 10^{-4}M and isoprenaline 10^{-4}M significantly stimulated leucocyte adenyl cyclase activity in all three groups.

Table XXX The leucocyte adenyl cyclase response to isoprenaline in normal subjects, asthmatic patients in remission and patients with acute asthma

	Control value	Isoprenaline 10^{-6}M	Isoprenaline 10^{-6}M
Normal subjects (n = 10)		% increase	% increase
Mean	1.15	61.1	44.3
SEM	0.26	23.2	11.8
P		.02	.005
Asthmatic patients in remission (n = 7)			
Mean	0.64	164.0	92.6
SEM	0.08	18.2	11.1
P		.005	.001
Patients with acute asthma (n = 7)			
Mean	1.48	10.0	12.4
SEM	0.21	8.5	10.6
P		0.30	0.20

The control value represents the basal level of incorporation of ^{3}H-adenine into cyclic AMP and the results of the drugs are given as percentage increase from the basal or control value.

Individual data published (Alston, Patel & Kerr, 1974)[169].

Table XXXI The effect of thymoxamine and phentolamine on the leucocyte adenyl cyclase response to isoprenaline in normal subjects and patients with asthma

	THYMOXAMINE 2×10^{-4}		PHENTOLAMINE 2×10^{-4}	
Normal subjects (n = 10)	ALONE	+ ISOPRENALINE 10^{-4}M	ALONE	+ ISOPRENALINE 10^{-4}M
	% increase	% increase	% increase	% increase
Mean	12.3	81.5	9.8	107.8
SEM	7.9	16.5	10.8	27.5
P	0.10	0.001	0.40	0.005
Asthmatic patients in remission (n = 6)				
Mean	19.5	163.1	28.8	189.2
SEM	9.0	16.5	11.0	21.0
P	0.10	0.001	0.20	0.001
Patients with acute asthma (n = 5)				
Mean	3.3	85.5	32.0	74.3
SEM	10.5	15.0	22.0	12.0
P	0.80	.001	0.20	.001

The results of the drugs are given as percentage increase from the basal or control value.

Individual data published (Alston, Patel & Horr. 1974)[162].

Table XXXII The effect of K^+Na^+ activated ATPase inhibitor, Ouabain, on the leucocyte adenyl cyclase response to isoprenaline in normal subjects and patients with asthma

	Ouabain 2×10^{-4}M	
Normal subjects (n = 10)	Alone	+ Isoprenaline 10^{-4}M
Mean	-10.9	+54.4
SEM	13.3	13.3
P	0.25	0.005
Asthmatic patients in remission (n = 6)		
Mean	39.1	103.6
SEM	16.3	13.7
P	0.05	.001
Patients with acute asthma (n = 5)		
Mean	8.2	50.2
SEM	13.0	15.7
P	0.60	0.02

The results of the drugs are given as percentage increase from the basal or control value.

Individual data published (Alston, Patel & Kerr, 1974)[168].

Table XXXIII Mean FEV_1 in patients with acute asthma and asthmatic patients in remission

	Active Group (n = 6)	Remission Group (n = 6)
Mean FEV_1	53.2%	85.3%
SEM	7.11	6.24
P		.01

Clinical details of Mr. I. W. (Active Asthma) given in Appendix, Page 152.
Clinical details of Miss E. G. (Asthma in Remission) given in Appendix, Page 158.

16.0 LYMPHOCYTE GUANYL CYCLASE ACTIVITY

16.1 Response to Propranolol and Propranolol + Noradrenaline

Results of this investigation are summarised in table XXXIV. Propranolol alone at 2×10^{-4}M did not evoke any significant difference in cyclic GMP levels in all three groups. However, propranolol at 2×10^{-4}M in combination with noradrenaline 10^{-4}M produced a very significant increase in guanyl cyclase activity in normals, 94% ($P < .001$), but no significant increase in either the active asthmatic group or asthmatic patients in remission.

16.2 Response to Acetylcholine, Thymoxamine and Thymoxamine + Acetylcholine

Results of this investigation are given in table XXXV. Acetylcholine alone at 10^{-4}M produced a significant increase in the lymphocyte guanyl cyclase activity in normal group, 47% ($P < .02$), but no significant change in either the remission group or patients with active asthma.

Thymoxamine alone at 2×10^{-4}M produced a significant increase in the enzyme activity in the normal group, 50% ($P < .01$) and also in the remission group, 21% ($P < .02$), but no significant change in the active group. Thymoxamine at 2×10^{-4}M in combination with acetylcholine 10^{-4}M produced a highly significant increase in the guanyl cyclase activity in the normal group, 104% ($P < .001$), but no significant change in either the remission group or patients with active asthma.

Table XXXIV The lymphocyte guanyl cyclase response to noradrenaline after prior beta blockade with propranolol in normal subjects and patients with asthma

	Control value	Propranolol 2×10^{-4}M	
Normal subjects (n = 10)		Alone % increase	+ Noradrenaline 10^{-4}M % increase
Mean	3.35	22.4	94.0
SEM	0.52	19.0	26.5
P		.10	.001
Asthmatic patients in remission (n = 6)			
Mean	5.11	12.7	35.2
SEM	1.01	16.6	25.2
P		N.S.	N.S.
Patients with acute asthma (n = 6)			
Mean	4.70	-16.7	- 6.3
SEM	1.26	23.8	21.6
P		N.S.	N.S.

The control value represents basal level of incorporation of ^{3}H-adenine into cyclic GMP and the results of the drugs are given as percentage increase from the basal or control value.
Individual data published (Haddock, Patel, Alston & Kerr, 1975)[178].

Table XXXV The lymphocyte guanyl cyclase to acetylcholine, thymoxamine, and thymoxamine + acetylcholine in normal subjects and patients with asthma

	Acetylcholine 10^{-4}M	Thymoxamine 2 x 10^{-4}M	
Normal subjects (n = 8)	% increase	Alone % increase	+Acetylcholine 10^{-4}M % increase
Mean	46.5	50.6	103.8
SEM	17.8	23.4	23.6
P	0.02	0.01	.001
Asthmatic patients in remission (n = 4)			
Mean	58.8	21.3	60.7
SEM	33.0	6.44	38.8
P	N.S.	0.025	N.S.
Patients with active asthma (n = 6)			
Mean	- 1.0	0.2	-24.2
SEM	17.5	16.5	13.0
P	N.S.	N.S.	N.S.

The results of the drugs are given as percentage increase from the basal or control value.

Individual data published (Haddock, Patel, Alston & Kerr, 1975)[178].

Table XXXVI Comparison of the patients suffering from acute asthma with those in remission

Asthmatic Condition	Mean FEV_1 as percentage of predicted VC	No. of Patients	Increase in cyclic AMP in response to 10^{-4} mol Isoprenaline/l. in terms of control value of 100% with 10 mmol Theophylline/l.
Acute	56%	7	112.4 ± 10.6
In remission	67%	7	192.6 ± 11.1

Clinical details of one of the patients with acute asthma, Mr. M. S., given in Appendix, Page 151.
Clinical details of one of the patients in remission, Mr. A. H., given in Appendix, Page 145.

CHAPTER VII

DISCUSSION

17.0 LEUCOCYTE ADENYL CYCLASE ACTIVITY

17.1 Response of Leucocyte Adenyl Cyclase to Isoprenaline

The increase in leucocyte ^{3}H cyclic AMP in the active or "acute" asthmatic group on stimulation with $10^{-4}M$ isoprenaline was not significant (12.4%) and was associated with a mean FEV_1 of 56% for the group. This contrasts with the 93% increase in ^{3}H cyclic AMP in the remission group with a mean FEV_1 of 67% (table XXXVI). These findings are in agreement with the clinical division of the patients into those suffering from active or "acute" asthma and from asthma in remission. These results are in accord with the observations of Parker and Smith[109] who found not only was there a diminished responsiveness of leucocyte adenyl cyclase to isoprenaline stimulation associated with a more severe degree of airways obstruction but, in addition, two of their asthmatic patients showed a waxing and waning in this activity with remission and exacerbations of the illness. The results of Logsdon et al[107] who found most of their asthmatic patients had reduced responsiveness to isoprenaline, and those of Gillespie et al[108], who found that most of their asthmatic patients showed no difference from normals in this respect, are difficult to explain, but it should be noted that these authors did not report measurements of airways obstruction in their patients.

The significant differences in the control values of adenyl cyclase activity in active asthmatic group and those

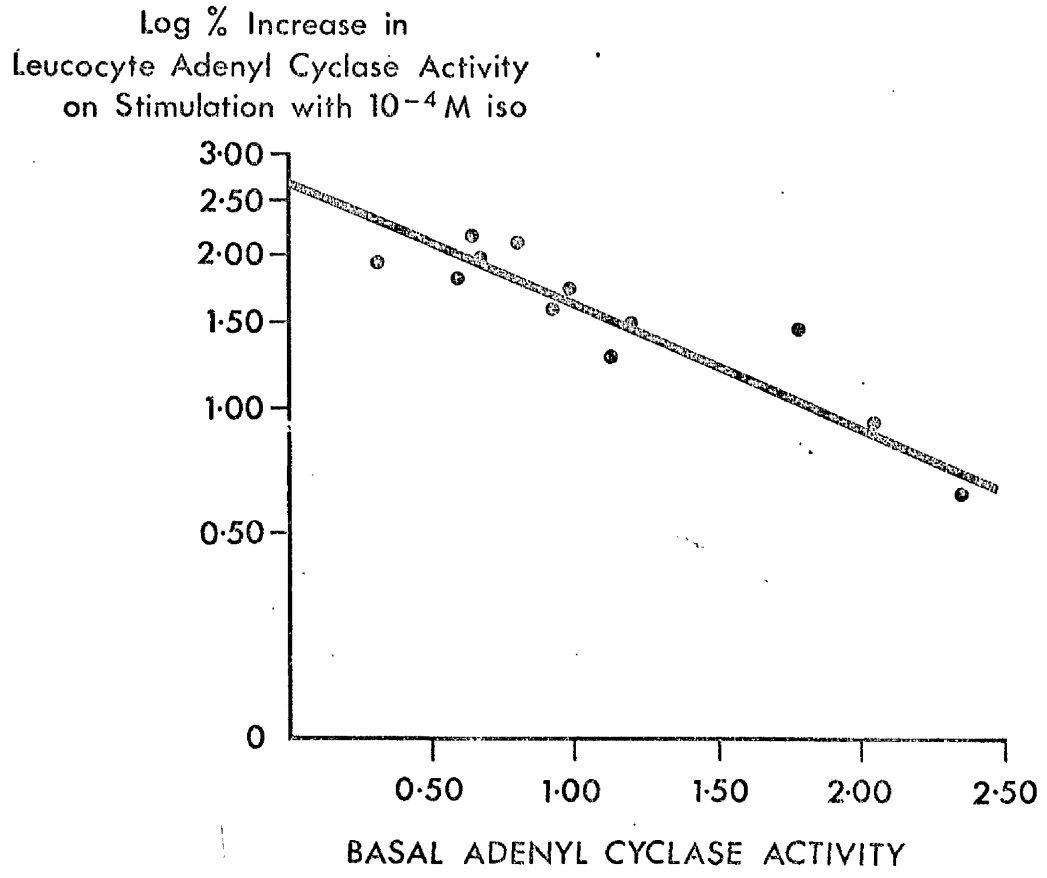


Fig. 27. Relationship between the control (basal) leucocyte adenyl cyclase activity and $\log_{10}$ per cent increase in this activity in response to 10^{-4}M isoprenaline in patients with asthma.

($r = 0.82$, student's $t = 4.79$, $P < .001$).

Individual data published (Patel, Alston and Kerr, 1974)[169].

in remission suggest that in active asthma the leucocyte adenyl cyclase may be almost maximally stimulated by circulating endogenous catecholamines and that further stimulation with sympathomimetic amines becomes increasingly difficult, resulting in adrenaline fast state (Fig. 27). This diminished beta receptor responsiveness in acute asthma is generalised and may also explain the diminished metabolic responses to adrenaline administration in some patients with asthma.

It is difficult to postulate whether the diminished beta receptor responsiveness observed in some patients with asthma is an inherent abnormality or whether it results from the severity of the disease. The presence of this abnormality in patients with acute asthma and inverse relationship of enzyme activity to the basal levels of adenyl cyclase activity suggests that this enzyme system is maximally stimulated by endogenous factors under the stress of the disease and further stimulation becomes increasingly difficult. In addition, increased metabolisation of catecholamines to 3 methoxyadrenaline and 3 methoxy-noradrenaline, which are weak beta blockers[170], could also account for diminished beta responsiveness in these patients and this possibility still remains to be investigated.

17.2 Effect of Alpha Blocking Drugs and Ouabain on the Leucocyte Adenyl Cyclase Response to Isoprenaline

In Part I of this thesis the presence of alpha receptors with bronchoconstrictor activity has been demonstrated in

human lung. The effect of alpha receptor blocking drugs was therefore studied in the experimental system. It is clear that phentolamine and thymoxamine both restore the responsiveness of leucocyte adenyl cyclase activity to isoprenaline towards normal in acute asthmatic groups. Moreover, Ouabain, a potent inhibitor of Na^+ and K^+ activated ATPase[205], also restores the adenyl cyclase responsiveness to isoprenaline towards normal in acute asthmatic groups. The similarity of the action of Ouabain to that of the alpha blocking drugs supports the view that there is increased alpha receptor activity in acute asthma, and that the membrane bound enzyme Na^+ K^+ activated ATPase is closely associated with alpha receptor activity[110]. Coffey et al[110] have demonstrated with broken cell preparations that there is increased ATPase activity in the leucocytes of asthmatic children, and that this activity can be reduced towards normal by treatment with hydrocortisone, and also that latter restores the responsiveness of the cells to isoprenaline stimulation. This increased ATPase activity in leucocytes of asthmatic patients cannot be attributed to treatment with broncho-dilator drugs since these drugs show no effect on the leucocyte ATPase activity of normal subjects treated with sympathomimetic amines[110].

The above observations would imply that the therapeutic approach to patients with acute asthma would be to stimulate their adenyl cyclase cyclic AMP system through the combined

use of beta adrenergic agonist and alpha receptor blocking drugs and indeed such an approach is feasible in management of patients with resistant airways obstruction[206] (Figs. 17 and 18).

17.3 Relationship of Leucocyte Adenyl Cyclase Activity and Airways Response to Beta Blockade and Allergen Challenge

In 13 patients the leucocyte adenyl cyclase response to isoprenaline (percentage increase over the control value) in individual patients did not correlate with the changes in FEV_1 and SGaw produced by propranolol administration or allergen challenge. Further, no correlation was observed between the total circulating reagins (IgE) and the control adenyl cyclase activity or its responsiveness to isoprenaline.

However, when the basal or control level of adenyl cyclase activity was plotted against the percentage increase in the activity of enzyme on stimulation with 10^{-4}M isoprenaline (Fig. 27) there was a significant inverse correlation between the two parameters. The individual data of this investigation was published in Clinical Allergy[169] and the reprint of the paper is included in the Appendix.

Assuming that the beta adreno-receptor dysfunction in asthma is generalised and also reflected in the peripheral leucocytes, the results of this investigation suggest that the phenomenon of bronchial hyper-reactivity and IgE production may not be related to diminished beta receptor

function. In normal subjects beta receptor blockade does not induce histamine or methacholine hyper-reactivity (Figs. 24 and 25) whereas in certain animal species beta adrenergic blockade with dichloroisoproterenol has been shown to induce histamine hyper-sensitivity and a state resembling atopy in these animals[45]. Undoubtedly the observations in animal experiments are important, their significance in the aetiology of bronchial asthma has to be regarded with caution because of species differences and failure to reproduce chronic unrelievable asthmatic state in animals. The diminished beta receptor function in patients with acute asthma may reflect a failing counter-regulatory mechanism rather than be considered the cause of asthma.

In vitro experiments it has been shown that beta stimulation is associated with inhibition of mediator release from mast cells and leucocytes[193] and with broncho-dilatation. Patients with acute asthma being defective in response to beta stimulation are prone to enhanced immunological induced release of chemical mediators and subsequent bronchoconstriction. Both alpha adrenergic and cholinergic mechanisms have recently been implicated in both enhanced mediator release[98] and bronchoconstrictive mechanisms[178]. In so far as these mechanisms appear to be enhanced in their activity in asthma, it seems reasonable to speculate that the enzyme systems mediating these influences will be found to be increased in their activity. The alpha adrenergic ATPase relationship has been proposed

by Belleau[53] and evidence to support this hypothesis has been put forward by Coffey et al[54, 55]. Similarly, cholinergic cyclic GMP relationship has been observed in a number of tissues including lymphocytes and lung[66]. The cyclic GMP has been proposed to have an opposing influence to the cyclic AMP[63]. It may be that the beta adrenergic defect observed in the cells of asthmatic patients may well result from more primary imbalances in the membrane ATPase and guanyl cyclase cyclic GMP system. The effect of alpha receptor blocking drugs and Na^+ K^+ activated ATPase inhibitor, Ouabain, would support this hypothesis. However to confirm that guanyl cyclase activity is also increased in patients with asthma the lymphocyte guanyl cyclase response to alpha and cholinergic stimulation was studied in patients with asthma and normal subjects.

18.0 LYMPHOCYTE GUANYL CYCLASE ACTIVITY

18.1 Guanyl Cyclase Response to Alpha and Cholinergic Stimulation and the Effect of Thymoxamine on this Response

The early work on the distribution and sub-cellular location of guanyl cyclase indicates that in most tissues studied it occurred mainly in the membrane-free cytoplasm of the cell in contrast with adenyl cyclase which is present mainly in the plasma membrane[207, 208]. However, Rudland et al[209] have recently demonstrated that stimulation of guanyl cyclase activity by fibroblast growth factor (FGF) in BALB/C 3 TB cells in tissue culture was due almost entirely to an enzyme activity located on the plasma membrane fraction. Since the concern was to explore cell receptor activities a method which measured plasma membrane guanyl cyclase activity seemed more likely to give significant results. Figure 28 shows that stimulation of lymphocytes with noradrenaline in presence of propranolol produced a considerable stimulation of guanyl cyclase activity which reached maximum between 5 and 10 minutes and returned towards baseline by about 15 minutes. This time course activity experiment is comparable in the degree of stimulation and duration of effect to the stimulation of membrane bound leucocyte adenyl cyclase activity with isoprenaline[109]. Further, addition of phosphodiesterase inhibitor, theophylline, to the medium increased the basal level of lymphocyte guanyl cyclase activity in both the normal and asthmatic subjects without altering the overall

pattern of response. These observations suggest that the lymphocyte guanyl cyclase activity measured was membrane bound.

It has been suggested that guanyl cyclase is activated by acetylcholine[60, 61] and also by alpha stimulation[62]. In normal subjects alpha stimulation with noradrenaline + propranolol and cholinergic stimulation with acetylcholine produced a very significant increase in cyclic GMP formation. Unexpectedly, thymoxamine also produced a significant although a lesser increase in cyclic GMP production in the normal subjects which makes it difficult to explain action of alpha receptor blocking drugs on this basis. However, Illiano et al[210] in their studies on isolated fat cells found that cholinergic agonist, atropine, also caused a slight increase in cyclic GMP formation. In contrast, the only significant stimulatory drug effect on cyclic GMP production in asthma patients' cells was observed with thymoxamine in the remission group. Acetylcholine alone and in combination with thymoxamine and alpha stimulation with noradrenaline did not evoke a significant increase in cyclic GMP formation in either the active asthmatic group or patients in remission. These observations are the reverse of what might have been expected as it has been reported with experimental animals that increased levels of cyclic GMP are associated with a more severe degree of anaphylaxis[101]. However, Lewis et al[60] have shown that the effect of cyclic GMP on smooth muscle function is dose

dependent; low concentrations producing tracheal smooth muscle contraction whereas higher concentrations produce a dose dependent relaxation. This effect of cyclic GMP on smooth muscle can be explained by the influence of cyclic GMP on cyclic AMP phosphodiesterase as suggested by Beavo et al[102, 103]. Using particulate preparations of cyclic phosphodiesterase from various tissues it has been shown that low concentrations of cyclic GMP stimulated hydrolysis of cyclic AMP whereas with higher concentrations of cyclic GMP there was an inhibition of cyclic AMP hydrolysis (Fig. 29). Similar findings have been reported for rat lymphocytes[211], and concentration of cyclic GMP required to demonstrate this phenomenon is within the physiological range[103].

The lymphocyte guanyl cyclase activity in normal subjects and patients with asthma can be explained in light of the relationship of cyclic GMP to cyclic AMP. In normal subjects alpha adrenergic and cholinergic stimulation cause a significant rise in cyclic GMP levels which may lead to inhibition of cyclic phosphodiesterase preventing hydrolysis of cyclic AMP and thereby maintaining relaxation of bronchial smooth muscle. On the other hand, in acute asthma alpha and cholinergic stimulation does not produce a significant rise in cyclic GMP level. In this situation, a low intracellular concentration of cyclic GMP increases the hydrosis of cyclic AMP through stimulation of cyclic AMP phosphodiesterase resulting in increased bronchomotor

tone and bronchoconstriction (Fig. 30). The high basal levels of adenyl cyclase activity[168, 169], and the responsiveness to isoprenaline stimulation of adenyl cyclase in acute asthma supports this hypothesis. Asthmatic patients in remission demonstrate cyclic GMP responses midway between those found in normal subjects and active asthma. The factors depressing cyclic GMP response in acute asthma and confirmation that cyclic phosphodiesterase activity is increased requires further investigation.

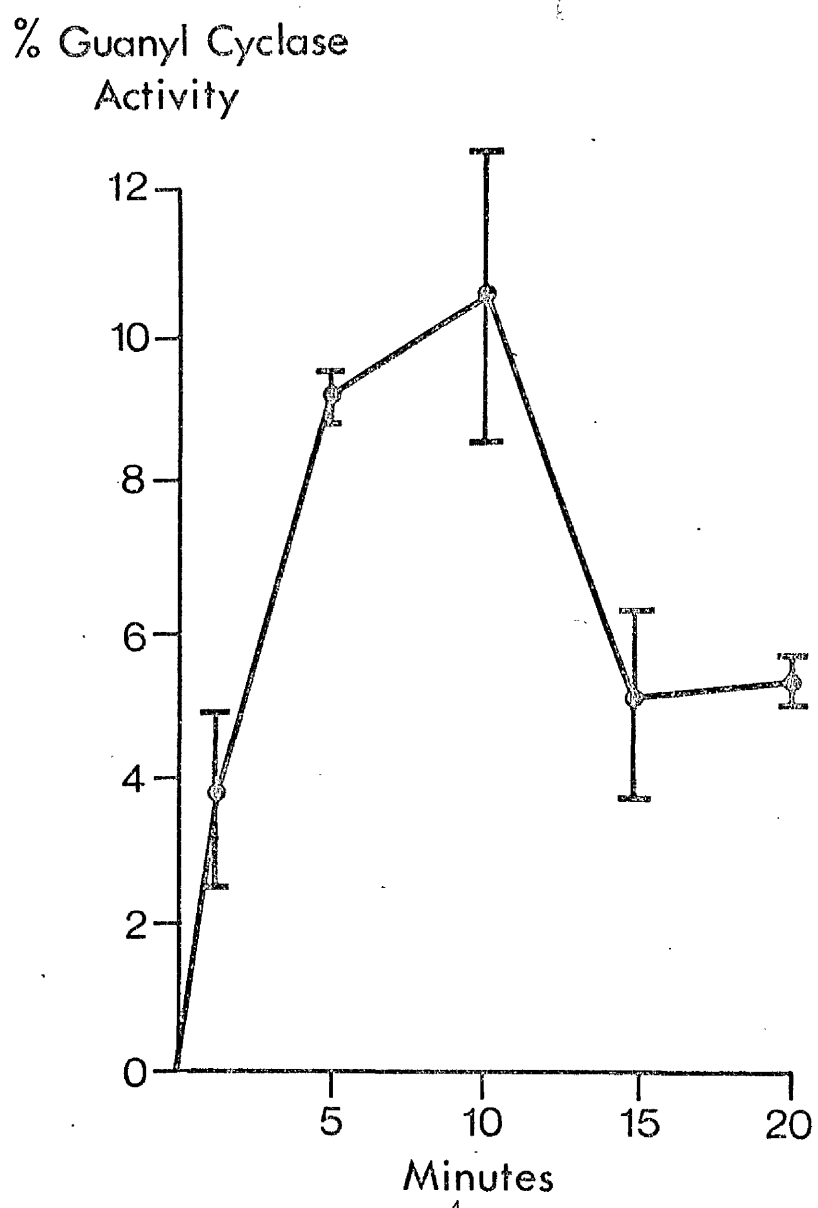


Fig. 28. The noradrenaline (10^{-4}M) stimulation of guanyl cyclase in lymphocytes of normal subjects in the presence of propranolol (2 x 10^{-4}M) expressed as a function of time (± SEM).

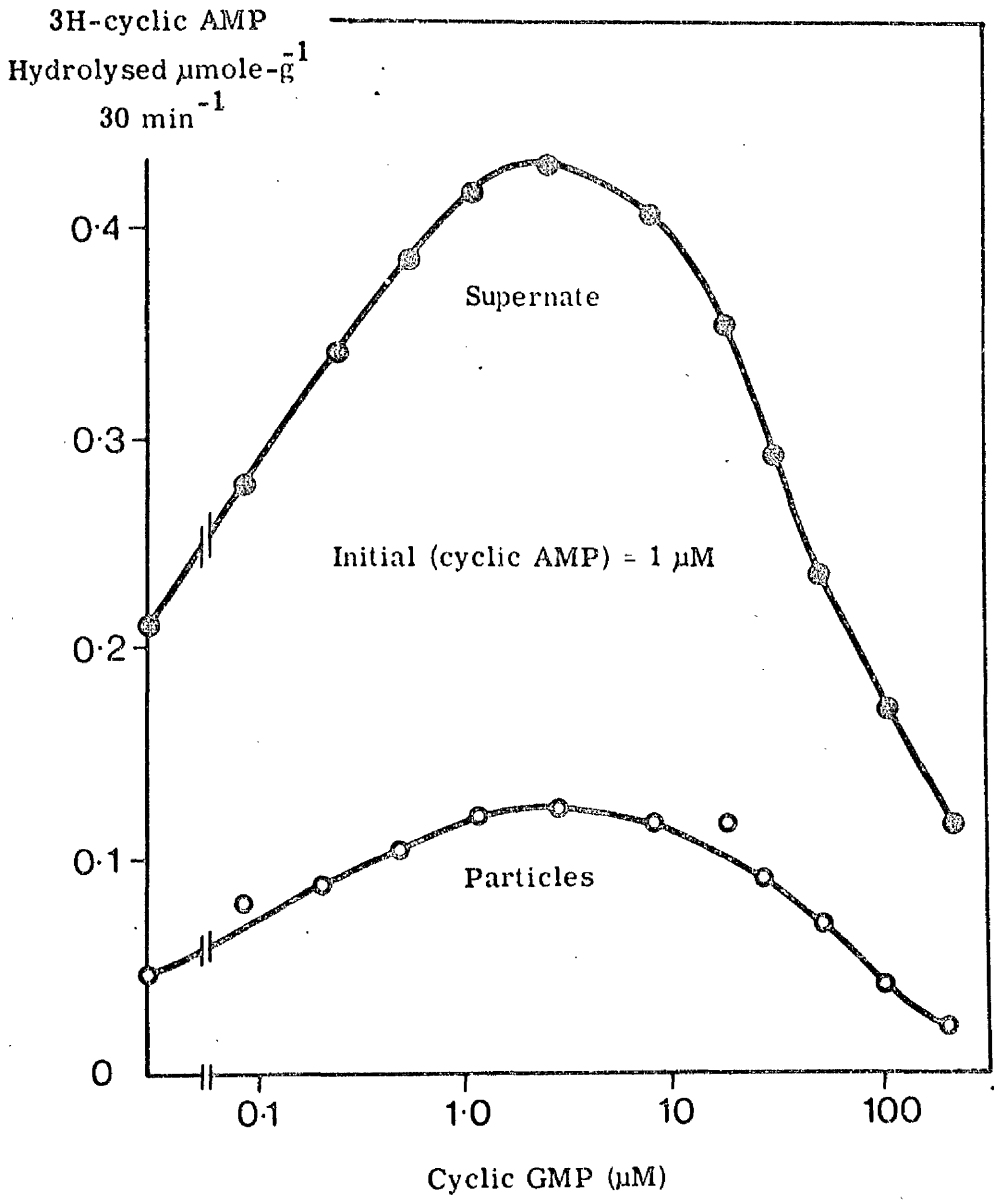


Fig. 29 Effect of increasing concentrations of Cyclic GMP on the hydrolysis of 3H-cyclic AMP by rat liver supernatant and particulate fractions (Beavo, Hardman & Sutherland, 1970)

RELATIONSHIP of CYCLIC 3'5'AMP and 3'5'GMP

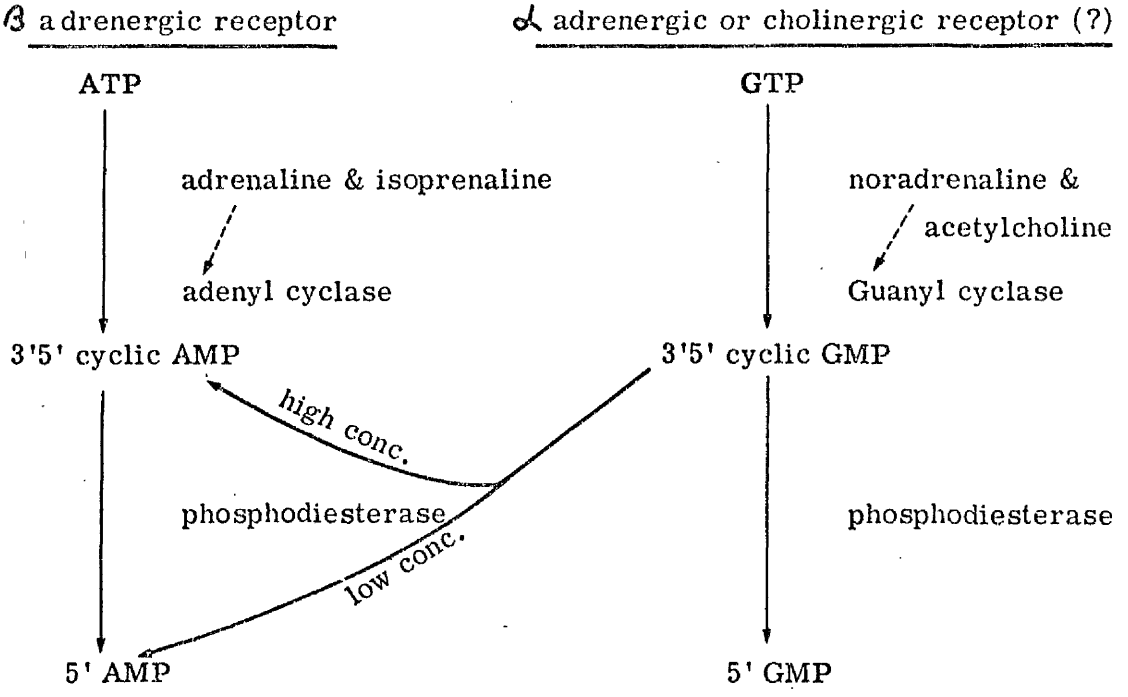


Fig. 30. The proposed relationship of cyclic GMP to cyclic AMP in control of bronchomotor tone. Activation of guanyl cyclase by acetylcholine or noradrenaline increases the concentration of cyclic GMP. High concentration of cyclic GMP inhibits cyclic AMP phosphodiesterase preventing the hydrolysis of cyclic AMP to 5' AMP, thus maintaining normal bronchomotor tone. In asthma, the low levels of cyclic GMP stimulate cyclic AMP phosphodiesterase and promote the hydrolysis of cyclic AMP to 5' AMP thus increasing bronchomotor tone.

CORRELATION BETWEEN PHYSIOLOGICAL AND BIOCHEMICAL OBSERVATIONS

In Man pharmacological stimulation of beta adrenergic receptors in the airways causes bronchodilatation whereas cholinergic stimulation induces bronchoconstriction[117]. In addition, observations reported here confirm the presence of alpha adrenergic receptors in the human airways which when stimulated cause bronchoconstriction[157]. It may therefore be postulated that the state of bronchomotor tone in an individual depends on the balance between bronchodilator beta adrenergic and bronchoconstrictor cholinergic and alpha adrenergic activities. In non-asthmatic subjects, the balance of different autonomic divisions is so adjusted that pharmacological stimulation or blockade of any division does not change the bronchial calibre significantly. This balanced autonomic control of the airways in normals may also explain an attenuated or absence of response to pharmacologically active substances such as histamine, bradykinin, S.R.S.-A., acetylcholine and prostaglandins on the airways of these subjects[37, 39, 42, 157].

In contrast, the patients with asthma show a great variability in bronchomotor tone, an abnormality which has been recognised for many years and considered to be the hallmark of asthma. Furthermore, pharmacological stimulation of the beta adrenergic receptors in the airways causes marked bronchodilatation whereas cholinergic stimulation causes bronchoconstriction in these patients.

In addition, the investigations reported in Part I of this thesis confirm that alpha adrenergic receptors stimulation in asthmatic patients can cause significant bronchoconstriction in both the central and peripheral airways. In contrast to normal subjects, the balance between the bronchodilator beta adrenergic and bronchoconstrictor alpha adrenergic and cholinergic activities in the airways is more critical in asthmatic subjects and a change in any one division greatly influences the bronchial calibre.

Although Prime et al[89] and Simonsson et al[90] have demonstrated more directly the presence of alpha adrenergic receptors in human airways, its role in control of bronchomotor tone had not been investigated. Unlike Prime et al[89], I was unable to demonstrate bronchoconstrictor alpha adrenergic activity in normal subjects even after beta adrenergic blockade with large doses of propranolol. This observation would indicate that alpha adrenergic activity, like beta and cholinergic activities[117], contributes little in the control of bronchomotor tone in normal subjects. In contrast, asthmatic patients develop significant bronchoconstriction following alpha stimulation with phenylephrine. In patients with acute asthma, there is diminished beta receptor responsiveness to catecholamines[76, 77] which increases with severity of asthma[78]. In such a situation, the minor alpha receptor stimulating properties of catecholamines may become dominant and cause bronchoconstriction by stimulating alpha receptors. This phenomenon may well explain the

adrenaline fastness or reversal commonly seen in status asthmaticus.

In various provocation tests, alpha blockade with thymoxamine effectively inhibited histamine, exercise[214] and allergen[215] induced bronchospasm whereas it had no effect on prostaglandin F_2 alpha[195] or mechacholine induced bronchoconstriction. The inhibitory effect of thymoxamine in histamine, allergen or exercise induced bronchoconstriction may be mediated by ability of alpha receptor blocking drugs to restore beta receptor responsiveness to catecholamines[107, 168]. Although the overall inhibition of histamine, allergen or exercise induced bronchoconstriction was statistically significant, there was wide individual variation in these patients suggesting inherent differences in airways sensitivity to different mediators released in the type I reaction. For instance, in a patient in whom thymoxamine effectively blocked allergen induced asthma may reflect predominance of histamine hyper-reactivity which is inhibited by alpha blockade whereas in a patient in whom thymoxamine had no effect in this respect may reflect predominance of hyper-reactivity to other spasmogens such as prostaglandin F_2 alpha which are not inhibited by alpha blockade[195]. The failure of thymoxamine to inhibit methacholine and prostaglandin F_2 alpha induced bronchoconstriction is easy to explain as both these agents have been shown to activate cholinergic receptors through guanyl cyclase cyclic GMP system[100]

which is unaffected by thymoxamine[178]. In addition, inhibition of methacholine and prostaglandin F_2 alpha by atropine is consistent with this hypothesis.

It is difficult to postulate whether the autonomic imbalance in asthma is a local phenomenon relegated to the lung or is mediated from higher centres. The influence of emotion and psyche on the bronchomotor tone in patients with asthma would suggest that higher centres may be involved. In addition, differences in metabolic responses to epinephrine administration in normal subjects and patients with asthma[76, 77, 78] indicate that the biochemical abnormality observed in asthma is more generalised than previously thought.

The autonomic receptors have been visualised as specialised receptive organs on the cell membrane since they were first described by Dale in 1933[47]. Our understanding of the biochemical nature of these receptors is just beginning to develop. The presence of enzymatic autonomic receptor activities on the membranes of peripheral leucocytes has provided a meaningful material for more basic and fundamental research into biochemical abnormalities in asthma. The leucocyte adenyl cyclase (now identified with beta receptor function) stimulation with isoprenaline is diminished in patients with acute asthma whereas response of this enzyme system in patients in remission does not show any significant difference from normal subjects[109, 168, 169]. The high basal levels of

adenyl cyclase activity and its inversely related response to isoprenaline in acute asthma[169] would seem to reflect maximal stimulation of this enzyme system by endogenous factors so that further stimulation becomes increasingly difficult. This observation would support previously reported refractoriness to adrenaline administration[76, 77] in asthmatic patients which is known to increase with the severity of asthma[78]. It is unlikely that the diminished adenyl cyclase activity in asthma is drug induced as has been suggested by Connolly and co-workers[212]. In normal subjects long term oral medication with sympathomimetic amines has failed to produce similar phenomenon[109], and secondly the patients studied in the present investigation were off simple bronchodilator drugs and steroids for at least 24 hours before the blood samples were collected for enzymatic assays. The airways bronchoconstrictor response to beta blockade with propranolol in asthmatic patients can be explained in light of biochemical observations. In patients with asthma, the adenyl cyclase activity is increased, and this is required to maintain broncho-dilatation and to counteract bronchoconstricting influences mediated by cholinergic, alpha adrenergic and possibly local hormonal activities. Propranolol which inhibits adenyl cyclase[216] leads to reduction in the beta receptor function of the airways and hence bronchoconstriction.

The possibility of enhanced cholinergic mechanisms in asthmatic patients has also been postulated[101, 165]. In

contrast to my expectation, the lymphocyte guanyl cyclase response, an enzyme system known to mediate cholinergic[60], [61] and possibly alpha adrenergic responses[62], was depressed in asthmatic group as a whole and no distinction could be made on the basis of clinical state of the disease as was possible in the adenyl cyclase study. It appears that guanyl cyclase cyclic GMP abnormality in asthma may be a primary defect which in turn modifies adenyl cyclase activity. Alpha and cholinergic stimulation both cause bronchoconstriction in Man, and this response is grossly exaggerated in asthmatic patients. In guanyl cyclase studies, alpha and cholinergic stimulation have been shown to cause a significant increase in cyclic GMP formation in lymphocytes of normal subjects. Assuming that similar increase in cyclic GMP also occurs in the bronchial smooth muscle of these subjects, the high levels of cyclic GMP formed may lead to inhibition of cyclic AMP phosphodiesterase preventing hydrolysis of cyclic AMP and thereby maintaining relaxation of bronchial smooth muscle. This observation would explain the absence of bronchoconstrictor response on alpha and cholinergic stimulations in normal subjects. In contrast, alpha and cholinergic stimulation does not produce a significant increase in cyclic GMP formation in asthmatic patients and this would have an effect opposite to that observed in normal subjects. It is possible that in asthmatic patients we are observing an increased hydrolysis of cyclic AMP through stimulation of

cyclic phosphodiesterase as reported by Beavo et al[102, 103] resulting in bronchoconstriction. Such a phenomenon in bronchial smooth muscle would explain bronchoconstrictor response to alpha and cholinergic stimulation and in mast cells increased mediator release by alpha and cholinergic agonists as already reported by Kaliner et al[98].

It is my belief that the answer to the pathogenesis of bronchial hyper-reactivity and atopy in asthma may lie in the relationship of nucleotide enzymatic activities on the bronchial and mast cell membranes and further research in this field may provide further understanding and better management of this disease.

Therapeutic Implications

Biochemical and physiological evidence is presented which suggest that alpha receptor blocking drugs potentiate the effect of beta agonists. A combined administration of thymoxamine and isoprenaline certainly causes greater and more prolonged bronchodilatation as compared to bronchodilatation achieved with isoprenaline alone. In addition, combined therapy has proven of value in patients with chronic labile airways obstruction who had previously failed to respond to salbutamol alone. The use of alpha blocking drugs may prove a new approach in management of asthma. However, the wide clinical application of such a form of therapy may be limited at present because of short duration of effect and hypotension caused by alpha receptor blocking drugs when given intravenously and local irritant effect when inhaled.

A P P E N D I X

Clinical and Lung Function Data

of 10 Patients with Asthma

Patient No. 1.

Mr. R. F.

Age : 18 years.

Occupation: Accounts-clerk.

History:

This young man gave a history of episodic asthma and hay fever since early childhood. There were no seasonal variations in his asthma, however, his attacks were mainly nocturnal. There was no history of exercise induced asthma.

Family History:

Parents alive.

Two brothers and one sister - sister suffers from asthma.

Therapy:

Sodium cromoglycate one spincapsule four times a day and one Franol tablet at night.

Investigations:

Haemoglobin	:	97%.
Total W.B.C. count	:	7,600/mm^3.
Absolute eosinophil count	:	694/mm^3.
Total serum IgE level	:	343 ng/ml.
Skin tests	:	House dust ++
		Dermatophagoides Pteronyssinus ++
		B_2 Grasses +++
		Feathers (mixed) ++
Pulmonary function tests	:	V.C. 3.80
		FEV_1 3.15

Patient No. 2.

Mr. D. C.

Age : 20 years.

Occupation: Welder in shipyard.

History:

This patient gave a history of atopic eczema and asthma since early childhood. His attacks of asthma were fairly mild and there was no seasonal variation. He denied history of exercise induced asthma.

Family History:

Two sisters and two brothers - one brother suffers from asthma.

Therapy:

Sodium cromoglycate one spincapsule four times a day + Salbutamol inhaler - 2 puffs when required.

Investigations:

Haemoglobin	:	108%.
Total W.B.C. count	:	7,450/mm^3.
Absolute eosinophil count	:	864/mm^3.
Total serum IgE level	:	808 ng/ml.
Skin tests	:	House dust ++
		Dermatophagoides Pteronyssinus ++
		B_2 Grasses ++++
		Dog hair +
Pulmonary function tests	:	V.C. 2.60
		FEV_1 1.75

Patient No. 3.

Mr. A. H.

Age : 30 years.

Occupation: Lecturer at Technical College.

History:

This patient gave a history of episodic asthma and hay fever since early childhood. His attacks of asthma were fairly mild but tended to become more frequent between June and September. There was no history of exercise induced asthma.

Family History:

No siblings.

Mother suffers from asthma.

Therapy:

Isoprenaline 5 mgm. sublingually.

Required this medication occasionally.

Investigations:

Haemoglobin	:	98%.
Total W.B.C. count	:	5,500/mm^3.
Absolute eosinophil count	:	836/mm^3.
Total serum IgE level	:	467 ng/ml.
Skin tests	:	House dust ++
		Dermatophagoides Pteronyssinus ++
		B_2 Grasses ++
		Feathers -ve
		Dog hair +
Pulmonary function tests	:	V.C. 6.43 (5.60)
		FEV_1 3.87
		F.R.C. 4.50 (4.25)

R.V. 2.50 (2.17)

T.L.C. 8.93 (7.77)

Patient No. 4.

Miss E. P.

Age : 16 years.

Occupation: School girl.

History:

This patient gave a history of recurrent respiratory tract infection associated with wheezing in her childhood. In the last two to three years her asthma had been fairly mild but her main problem was exercise induced bronchospasm which prevented her from taking part in physical activities at school.

Family History:

Two sisters - one suffers from asthma.

Mother also suffers from asthma.

Therapy:

Sodium cromoglycate one spincapsule three times a day + Salbutamol 4 mgm. orally - when required.

Investigations:

Haemoglobin	:	102%.
Total W.B.C. count	:	5,800/mm^3.
Absolute eosinophil count	:	720/mm^3.
Total serum IgE level	:	1,496 ng/ml.
Skin tests	:	House dust ++
		Dermatophagoides Pteronyssinus ++
		B_2 Grasses -
		Feathers -
Pulmonary function tests	:	V.C. 3.80 (3.30)
		FEV_1 2.45

F.R.C. 2.60 (1.90)

R.V. 1.55 (0.90)

T.L.C. 5.35 (4.30)

$D_L CO$ 25.9 (25.0) ml. CO/min/mm.Hg.

Patient No. 5.

Miss R. S.

Age : 23 years.

Occupation: Primary school teacher.

History:

This patient gave a history of atopic eczema and episodic asthma since early childhood. Since moving to Glasgow two years ago, her asthmatic attacks were more frequent. There was no seasonal variation. She also gave a history of exercise induced bronchospasm.

Family History:

Two brothers - one brother has atopic eczema and asthma.

Therapy:

Sodium cromoglycate one spincapsule four times a day + Salbutamol inhaler 2 puffs when required.

Investigations:

Haemoglobin	:	94%.
Total W.B.C. count	:	7,620/mm^3.
Absolute eosinophil count	:	893/mm^3.
Total serum IgE level	:	1,110 ng/ml.
Skin tests	:	House dust ++
		Dermatophagoides Pteronyssinus +++
		B_2 Grasses +
		Feathers +
Pulmonary function tests	:	V.C. 4.40 (3.60)
		FEV_1 3.40
		F.R.C. 1.95 (2.67)

R.V. 1.00 (1.32)

T.L.C. 5.40 (4.92)

$D_L CO$ 19.0 (21.9) ml. CO/min/mm.Hg.

Patient No. 6.

Mr. M. S.

Age : 29 years.

Occupation: Engineer.

History:

This young man gave a history of atopic eczema, hay fever and asthma since early childhood. His attacks of asthma were episodic but tended to be more frequent in summer months. There was no history of exercise induced asthma.

Family History:

Two brothers - one brother suffers from atopic eczema.

Therapy:

Salbutamol inhaler - 2 puffs when required.

Investigations:

Haemoglobin	:	107%.
Total W.B.C. count	:	7,450/mm^3.
Absolute eosinophil count	:	825/mm^3.
Total serum IgE level	:	140 ng/ml.
Skin tests	:	House dust +++
		Dermatophagoides Pteronyssinus +++
		B_2 Grasses +++
		Feathers -
Pulmonary function tests	:	V.C. 4.94 (4.96)
		FEV_1 1.75
		F.R.C. 4.35 (3.74)
		R.V. 2.15 (1.87)
		T.L.C. 7.09 (6.83)

Patient No. 7.

Mr. I. W.

Age : 43 years.

Occupation: Wages clerk.

History:

This patient gave a history of atopic eczema and asthma since early childhood. In the last ten years his asthma had been fairly chronic and he required occasional steroid therapy. He also gave a history of exercise induced asthma.

Family History:

Patient was an adopted child and has no recollection of parents or siblings.

He has two sons and two daughters - none suffers from an atopic disease.

Therapy:

Sodium cromoglycate one spincapsule four times a day.

Salbutamol inhaler - 2 puffs four to six times a day.

Investigations:

Haemoglobin	:	104%.
Total W.B.C. count	:	8,600/mm^3.
Absolute eosinophil count	:	1,069/mm^3.
Total serum IgE level	:	13,140 ng/ml. (double checked)
Skin tests	:	House dust ++
		Dermatophagoides Pteronyssinus ++
		B_2 Grasses ++
		Aspergillus Fumigatus + - Arthus reaction absent.

Pulmonary function tests :	V.C.	3.00	(4.66)	
	FEV_1	1.52		
	F.R.C.	3.10	(3.74)	
	R.V.	2.49	(2.02)	
	T.L.C.	5.49	(6.68)	
	D_LCO (rest)	11.4	(19.6)	ml. CO/min/ mm.Hg.

Patient No. 8.

Mrs. J. H.

Age : 26 years.

Occupation: Housewife.

History:

This patient gave a history of hay fever for the last four years and this was associated with pollen asthma. She was fairly free of symptoms in the other times of the year. She also suffered from rheumatoid arthritis, however, there were no joint deformities and she was fairly mobile.

Family History:

One brother and one sister - sister suffers from hay fever.

Therapy:

Beclamethasone dipropionate aerosol 200 ugm. four times a day from May to September. Indomethacin 25 mgm. four times a day for rheumatoid arthritis.

Investigations:

Haemoglobin	:	88%.
Total W.B.C. count	:	4,650/mm^3.
Absolute eosinophil count	:	529/mm^3.
Total serum IgE level	:	280 ng/ml.
Skin tests	:	House dust -
		Dermatophagoides Pteronyssinus --
		B_2 Grasses ++
Pulmonary function tests	:	V.C. 2.65 (3.51)
		FEV_1 1.95
		F.R.C. 1.95 (2.67)

R.V.	1.16	(1.37)	
T.L.C.	3.80	(4.87)	
$D_L CO$ (rest)	18.7	(20.2)	ml. CO/min/ mm.Hg.

Patient No. 9.

Mrs. E. H.

Age : 25 years.

Occupation: Housewife.

History:

This patient gave a history of episodic attacks of asthma for two years. Her main problem was exercise induced asthma.

Family History:

No siblings.

No history of atopic disease in the family.

Therapy:

Sodium cromoglycate one spincapsule four times a day + Salbutamol inhaler when required.

Investigations:

Haemoglobin	:	92%.
Total W.B.C. count	:	7,620/mm^3.
Absolute eosinophil count	:	605/mm^3.
Total serum IgE level	:	not estimated.
Skin tests	:	House dust ++
		Dermatophagoides Pteronyssinus ++
		B_2 Grasses -
		Feathers -
Pulmonary function tests	:	V.C. 3.70 (2.99)
		FEV_1 2.40
		F.R.C. 3.25 (2.20)
		R.V. 2.02 (1.00)
		T.L.C. 5.74 (4.08)

$D_L CO$ (rest) 23.2 (19.8) ml. CO/min/mm.Hg.

Patient No. 10.

Miss E. G.

Age : 16 years.

Occupation: School girl.

History:

This girl gave a history of wheezing from early childhood. Her attacks were mild and episodic. There was no seasonal variation. In the last two years she complained of exercise induced asthma and her effort tolerance was limited to going up a flight of stairs.

Family History:

Two brothers - one suffers from asthma.

Therapy:

Sodium cromoglycate one spincapsule four times a day + Franol tablet when required.

Investigations:

Haemoglobin	:	89%.
Total W.B.C. count	:	4,670/mm^3.
Absolute eosinophil count	:	560/mm^3.
Total serum IgE level	:	280 ng/ml.
Skin tests	:	House dust ++
		Dermatophagoides Pteronyssinus +++
		B_2 Grasses +
		Feathers +
Pulmonary function tests	:	V.C. 3.60 (3.88)
		FEV_1 2.00
		F.R.C. 3.15 (2.90)

R.V.	1.87	(1.44)	
T.L.C.	5.47	(5.32)	
$D_L CO$ (rest)	16.9	(22.2)	ml. CO/min/ mm.Hg.

Abbreviations

F.R.C. : Functional Residual Capacity.

R.V. : Residual Volume.

T.L.C. : Total Lung Capacity.

D_LCO : Carbon Monoxide Transfer Factor.

Values in parenthesis are the predicted values for the patient.

Interpretation of Prick Tests

Reactions were assessed by the degree of erythema and the size of wheal produced (after allowance for any positive response to the glycero-saline control):

+++	Very sensitive	– Wheal more than 3 mm. diameter.
		– Flare more than 5 mm. diameter.
++	Moderately sensitive	– Wheal less than 3 mm. diameter.
		– Flare less than 5 mm. diameter.
+	Mildly sensitive	– Wheal insignificant.
		– Flare less than 3 mm. diameter.

Mathematical Derivation of Observations on Page 88

MATHEMATICAL DERIVATION OF OBSERVATIONS ON PAGES 88 AND 109

According to Starling's law of the heart, an increase in the diastolic volume of the ventricle results in a proportional increase in cardiac output, and during isotonic contraction of a single muscle fibre the degree of contraction is proportional to the initial length of the muscle fibre.

$$\text{Thus, } L \propto l \qquad \text{(i)}$$

where L is the initial length and l is the change in length produced by muscle contraction.

If the muscle fibre surrounds the circumference of a flexible tube, it will produce change in radius following contraction, and the above equation can be rewritten as

$$2\pi R \propto 2\pi r \qquad \text{(ii)}$$

where R is initial radius, r is change in radius.

$$\therefore R \propto r \qquad \text{(iii)}$$

The above relationship also holds true for isolated guinea-pig tracheal muscle (Everitt & Cairncross, 1969)[60].

According to Poiseuille's Law, airways resistance

$$(Raw) \propto \frac{8 \times L \times n}{\pi R^4} \qquad \text{(iv)}$$

$$\text{Airways Resistance (Raw)} \quad \frac{8 \times L \times n}{\pi R^4}$$

where L is the length of a bronchus
n is the viscosity of respiratory gases
and R is the radius of the bronchus.

In an individual patient, as the change in the length of an airway during bronchoconstriction is negligible, and as the viscosity of the respiratory gases also do not change

significantly, the above equation can therefore be rewritten as

$$\text{Raw} \propto \frac{k}{\pi R^4} \quad \left(\text{where } \frac{8 \times \text{Ln}}{\pi} \text{ is a constant } k\right)$$

Conductance (Gaw) which is $\frac{1}{\text{Raw}}$ would be

$$\frac{R^4}{k} \qquad \text{(v)}$$

and change in Gaw $\frac{r^4}{k}$ (vi)

where r is change in radius of the bronchus.

As $R \propto r$ (iii)

$$\therefore \frac{R^4}{k} \propto \frac{r^4}{k}$$

$\therefore$ Initial Gaw $\propto$ change in Gaw.

This contention is consistent with observations on pages 88 and 109.

Additional Tables

Table XXXVII **Effect of phenylephrine and isoprenaline on FEV_1 after prior beta blockade with propranolol in 10 patients with extrinsic asthma**

No.	Age	Sex	FEV_1 in litres								
			Baseline	Propranolol 5 mgm. I.V.				Phenylephrine by inhalation				Isoprenaline
				5' min.	10' min.	15' min.	20' min.	2' min.	4' min.	6' min.	8' min.	10 min. later
1.	17	F	2.50	2.50	2.45	2.45	2.30	2.30	2.30	2.30	2.25	2.45
2.	17	M	3.15	3.05	2.90	2.95	2.95	2.55	2.55	2.55	2.70	3.10
3.	23	F	3.20	2.75	2.60	2.60	2.70	1.90	1.70	1.85	1.85	2.30
4.	19	M	3.40	3.00	2.95	3.10	3.15	2.70	2.45	2.50	2.60	3.40
5.	15	M	3.05	2.00	2.30	2.25	2.00	1.40	1.45	1.40	1.60	2.45
6.	23	M	4.20	2.30	2.15	2.40	2.30	1.70	1.80	1.50	1.60	2.15
7.	17	F	2.80	2.60	2.50	2.10	2.15	1.55	1.85	2.00	2.10	2.60
8.	30	M	3.00	2.45	2.45	2.45	2.50	2.50	2.15	2.30	2.15	2.60
9.	19	F	3.30	3.25	3.05	3.30	3.20	2.90	2.80	2.45	2.60	2.95
10.	37	M	2.60	1.70	1.75	1.75	1.70	1.40	1.30	1.30	1.25	1.20
Mean	21.7		3.12	2.56	2.51	2.54	2.50	2.09	2.04	2.02	2.07	2.52
SEM	2.19		0.15	0.15	0.12	0.15	0.16	0.18	0.16	0.15	0.15	0.19
t test				3.00	3.44	3.59	3.62	4.99	5.59	5.06	5.47	
P				.02	.005	.005	.005	.001	.001	.001	.001	

Table XXXVIII **Effect of phenylephrine and isoprenaline on SGaw after prior blockade with propranolol in 10 patients with extrinsic asthma**

No.	SGaw (litres/cm.H_2O sec. litre)									
		Propranolol 5 mgm. I.V.				Phenylephrine Inhalation				Isoprenaline
	Baseline	5'	10'	15'	20'	2'	4'	6'	8'	10' later
1.	0.227	0.222	0.216	0.191	0.180	0.140	0.154	0.148	0.148	0.211
2.	0.122	0.083	0.083	0.078	0.084	0.058	0.059	0.059	0.069	0.096
3.	0.189	0.131	0.127	0.131	0.118	0.043	0.047	0.043	0.050	0.130
4.	0.100	0.063	0.063	0.056	0.063	0.034	0.039	0.044	0.052	0.124
5.	0.165	0.064	0.063	0.064	0.060	0.028	0.033	0.037	0.041	0.083
6.	0.212	0.072	0.063	0.068	0.073	0.048	0.050	0.048	0.054	0.074
7.	0.104	0.080	0.061	0.054	0.061	0.027	0.033	0.049	0.051	0.096
8.	0.098	0.079	0.076	0.062	0.071	0.047	0.053	0.063	0.072	0.088
9.	0.153	0.117	0.132	0.109	0.123	0.071	0.068	0.047	0.059	0.100
10.	0.126	0.058	0.040	0.046	0.045	0.032	0.031	0.026	0.024	0.026
Mean	0.150	0.097	0.092	0.086	0.088	0.053	0.057	0.056	0.062	0.103
SEM	0.015	0.016	0.017	0.014	0.013	0.011	0.011	0.011	0.010	0.015
t test		4.67	4.02	5.53	5.30	6.51	5.45	3.16	3.57	
P		.001	.001	.001	.001	.001	.001	.02	.01	

Table XXXIX Effect of phenylephrine on FEV_1 after prior blockade with propranolol in 5 normal subjects

No.	Age	Sex	FEV_1 in litres								
				Propranolol 10 mgm. I.V.				Phenylephrine 5 mgm. by inhalation			
			Baseline	5'	10'	15'	20'	2'	4'	6'	8'
1.	23	M	4.50	4.35	4.40	4.30	4.50	4.40	4.40	4.40	4.50
2.	19	F	2.95	3.00	2.95	2.95	3.00	2.95	3.00	3.00	3.10
3.	19	M	4.15	4.10	4.05	4.00	3.95	4.10	4.15	4.05	4.10
4.	21	M	3.35	3.30	3.20	3.35	3.30	3.40	3.45	3.40	3.40
5.	21	M	4.75	4.70	4.70	4.65	4.60	4.70	4.60	4.65	4.75
Mean			3.94	3.89	3.86	3.85	3.87	3.91	3.92	3.90	3.97
SEM			0.34	0.32	0.34	0.31	0.32	0.32	0.30	0.31	0.32
t test				1.60	1.98	2.00	1.57	0.89	0.93	0.81	1.98
P				N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Table XL Effect of phenylephrine on SGaw after prior blockade with propranolol in 5 normal subjects

No.	SGaw (litres/cm.H_2O sec. litre)								
		Propranolol 10 mgm. I.V.				Phenylephrine 5 mgm. by inhalation			
	Baseline	5'	10'	15'	20'	2'	4'	6'	8'
1.	0.232	0.221	0.188	0.232	0.214	0.225	0.222	0.235	0.228
2.	0.234	0.236	0.236	0.231	0.236	0.222	0.229	0.214	0.235
3.	0.192	0.188	0.193	0.187	0.182	0.189	0.209	0.203	0.212
4.	0.255	0.253	0.249	0.249	0.246	0.246	0.253	0.253	0.250
5.	0.206	0.234	0.225	0.224	0.216	0.222	0.229	0.214	0.235
Mean	0.224	0.226	0.219	0.225	0.219	0.221	0.228	0.224	0.232
SE	0.011	0.011	0.011	0.010	0.011	0.009	0.007	0.009	0.006
t test		0.45	0.58	0.22	0.81	0.45	1.18	0.75	1.42
P		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Table XLI **Effect of phenylephrine and isoprenaline after prior beta and cholinergic blockade in six patients with extrinsic asthma**

No.	Age	Sex	SGaw (litres/cm.H_2O sec. litre)												
				Atropine			Propranolol 5 mgm. I.V.				Phenylephrine 5 mgm. by inhalation				Isoprenaline
			Baseline	2'	5'	10'	5'	10'	15'	20'	2'	4'	6'	8'	10 min. later
1.	17	M	0.080	0.104	0.113	0.131	0.128	0.118	0.114	0.120	0.094	0.090	0.083	0.081	0.106
2.	23	M	0.120	0.176	0.182	0.172	0.169	0.156	0.152	0.147	0.130	0.128	0.121	0.126	0.137
3.	23	F	0.123	0.176	0.174	0.201	0.176	0.201	0.225	0.199	0.123	0.136	0.145	0.132	0.233
4.	17	F	0.130	0.212	0.205	0.205	0.196	0.204	0.222	0.227	0.190	0.194	0.173	0.159	0.202
5.	18	F	0.091	0.146	0.155	0.164	0.155	0.141	0.133	0.139	0.118	0.115	0.117	0.121	0.173
6.	30	M	0.116	0.123	0.134	0.148	0.131	0.121	0.109	0.109	0.080	0.096	0.107	0.108	0.148
Mean			0.110	0.156	0.161	0.170	0.159	0.157	0.159	0.157	0.123	0.127	0.124	0.121	0.166
SEM			0.008	0.016	0.014	0.012	0.011	0.016	0.021	0.019	0.016	0.015	0.013	0.011	0.018
t test				4.17	5.95	8.16	2.19	2.89	1.04	1.45	4.63	4.08	4.04	3.18	
P				.01	.001	.001	N.S.	.025	N.S.	N.S.	.001	.001	.001	.025	

Table XLII **Effect of histamine 200 ugm. by inhalation on FEV_1 in 7 normal subjects**

No.	Age	Sex	FEV_1 in litres					
				Histamine Inhalation				
			Baseline	2	5	10	15	30
1.	23	M	4.50	4.30	4.50	4.50	4.40	4.50
2.	21	F	3.35	3.25	3.30	3.40	3.35	3.35
3.	19	F	2.85	2.85	2.85	2.80	2.80	2.85
4.	21	M	5.25	5.35	5.45	5.50	5.50	5.50
5.	19	M	4.15	3.90	4.00	4.10	4.00	4.00
6.	21	M	3.35	3.50	3.35	3.50	3.45	3.40
7.	21	M	4.75	4.75	4.65	4.85	4.70	4.70
Mean			4.03	3.99	4.01	4.09	4.03	4.04
SEM			0.33	0.33	0.35	0.35	0.35	0.35
t test				0.72	0.24	0.12	0	0.22
P				N.S.	N.S.	N.S.	N.S.	N.S.

Table XLIII **Effect of histamine 200 ugm. by inhalation on SGaw in 7 normal subjects**

No.	SGaw (litres/cm.H_2O sec. litre)					
		Histamine Inhalation				
	Baseline	2	5	10	15	30
1.	0.176	0.181	0.176	0.201	0.181	0.177
2.	0.135	0.153	0.134	0.138	0.143	0.167
3.	0.168	0.169	0.148	0.181	0.150	0.152
4.	0.186	0.171	0.204	0.178	0.194	0.203
5.	0.196	0.193	0.189	0.203	0.213	0.212
6.	0.255	0.245	0.253	0.253	0.250	0.250
7.	0.206	0.221	0.222	0.222	0.230	0.227
Mean	0.189	0.190	0.189	0.197	0.194	0.198
SEM	0.014	0.012	0.016	0.014	0.015	0.013
t test		0.44	0.20	0.92	0.95	1.40
P		N.S.	N.S.	N.S.	N.S.	N.S.

Table XLIV Effect of 200 ugm. of histamine on FEV_1 after prior beta blockade with propranolol in 5 normal subjects

No.	Age	Sex	FEV_1 in litres									
				Propranolol 10 mgm. I.V.				Histamine Inhalation				
			Baseline	5'	10'	15'	20'	2'	5'	10'	15'	30'
1.	23	M	4.50	4.35	4.45	4.40	4.30	4.45	4.50	4.65	4.55	4.50
2.	19	F	2.95	3.00	2.95	2.95	3.00	3.05	3.00	2.95	3.05	3.00
3.	19	M	4.15	4.10	4.05	4.00	3.95	3.90	4.00	4.10	4.00	4.00
4.	21	M	3.35	3.30	3.20	3.35	3.30	3.50	3.35	3.50	3.45	3.40
5.	21	M	4.75	4.70	4.70	4.65	4.60	4.75	4.65	4.85	4.70	4.70
Mean			3.94	3.89	3.87	3.87	3.83	3.93	3.90	4.01	3.95	3.92
SEM			0.34	0.32	0.34	0.32	0.30	0.31	0.32	0.35	0.32	0.32
t test				1.57	1.75	1.34	1.24	1.24	1.06	0.72	1.19	1.72
P				N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Table XLV **Effect of 200 ugm. of histamine on SGaw after prior beta blockade with propranolol in 5 normal subjects**

No.	SGaw (litres/cm.H_2O sec. litre)									
		Propranolol 10 mgm. I.V.				Histamine Inhalation				
	Baseline	5'	10'	15'	20'	2'	5'	10'	15'	30'
1.	0.285	0.291	0.285	0.294	0.299	0.280	0.280	0.283	0.272	0.280
2.	0.234	0.236	0.236	0.231	0.236	0.246	0.250	0.240	0.247	0.249
3.	0.196	0.184	0.193	0.182	0.175	0.193	0.189	0.203	0.213	0.212
4.	0.255	0.253	0.249	0.249	0.246	0.245	0.253	0.253	0.250	0.250
5.	0.206	0.234	0.225	0.224	0.216	0.221	0.222	0.222	0.230	0.227
Mean	0.235	0.240	0.238	0.236	0.234	0.237	0.239	0.240	0.242	0.245
SEM	0.016	0.017	0.015	0.018	0.020	0.014	0.015	0.014	0.010	0.012
t test		0.95	0.68	0.45	0.42	0.25	0.68	1.02	1.40	1.80
P		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Table XLVI Effect of thymoxamine on histamine induced fall in FEV_1 in 10 patients with extrinsic asthma

No.	Age	Sex	FEV_1 in litres											
			Baseline		After histamine (200 ugm.) by inhalation									
					2 min.		5 min.		10 min.		15 min.		30 min.	
			B	A	B	A	B	A	B	A	B	A	B	A
1.	17	F	2.40	2.30	1.40	1.90	1.80	1.85	1.60	2.15	1.95	2.00	2.40	2.00
2.	23	M	3.75	3.30	2.70	2.70	3.20	3.00	3.00	3.05	3.00	2.90	3.60	2.90
3.	17	M	3.05	3.05	1.60	1.95	1.70	2.10	1.95	2.15	2.50	2.55	3.15	2.70
4.	37	M	1.30	1.35	0.85	1.00	1.00	1.05	1.30	1.10	1.40	1.10	1.30	1.20
5.	15	M	3.50	3.50	2.50	3.10	2.90	3.15	3.10	3.40	3.20	3.40	3.20	3.50
6.	15	M	3.20	3.40	2.00	2.10	2.25	2.20	2.80	3.00	3.00	3.40	3.00	3.40
7.	23	F	2.70	3.50	1.05	3.25	1.70	3.10	2.10	3.30	2.30	3.45	2.25	3.30
8.	23	M	4.40	3.95	2.70	2.85	3.15	3.05	3.50	3.50	3.90	3.65	4.20	3.90
9.	30	M	3.00	2.30	1.20	1.80	1.20	1.70	1.50	1.70	1.80	1.75	2.20	1.90
10.	28	F	2.40	2.80	1.70	2.90	1.55	3.00	1.65	3.00	1.90	3.05	2.20	2.90
Mean			2.97	2.95	1.77	2.36	2.05	2.42	2.25	2.64	2.50	2.73	2.75	2.77
SEM			0.27	0.24	0.22	0.23	0.25	0.23	0.25	0.26	0.24	0.27	0.26	0.26
t test				0.21		2.74		2.03		2.42		1.39		0.11
P				N.S.		.025		.05		.05		N.S.		N.S.

B: Before thymoxamine. A: After thymoxamine.

Table XLVII Effect of methacholine on FEV_1 after prior alpha blockade with thymoxamine in 8 patients with extrinsic asthma

NO.	AGE	SEX	FEV_1 in litres											
			Baseline		After methacholine inhalation									
					2 min.		5 min.		10 min.		15 min.		30 min.	
			B	A	B	A	B	A	B	A	B	A	B	A
1.	17	F	2.50	2.55	0.95	0.90	1.05	1.35	1.40	1.50	1.40	1.55	1.70	2.10
2.	23	M	3.85	4.10	2.20	1.90	2.30	1.50	2.35	2.00	2.15	2.05	3.35	2.30
3.	17	M	3.15	3.20	1.20	1.10	1.25	1.50	1.35	1.50	1.60	1.50	2.35	2.00
4.	15	M	2.90	2.75	1.70	0.80	1.20	1.15	1.75	1.70	1.70	2.00	2.40	2.70
5.	23	F	2.70	2.20	1.20	1.35	1.00	1.15	1.20	1.00	1.40	1.30	1.45	1.35
6.	21	M	2.50	2.50	1.35	0.70	1.70	1.05	2.60	1.50	2.60	1.70	2.50	2.45
7.	19	M	4.75	4.80	4.65	4.60	4.60	4.50	4.50	4.70	4.45	4.70	4.50	4.70
8.	37	F	2.40	2.25	1.20	1.50	1.40	1.85	1.65	1.85	1.85	2.00	2.00	2.05
Mean			3.09	3.04	1.81	1.61	1.81	1.76	2.10	1.97	2.14	2.10	2.53	2.46
SEM			0.29	0.33	0.43	0.45	0.43	0.43	0.38	0.40	0.36	0.38	0.35	0.35
t test				0.63		1.50		0.37		0.87		0.30		0.49
P				N.S.		N.S.		N.S.		N.S.		N.S.		N.S.

B: Before thymoxamine.

A: After thymoxamine.

Table XLVIII Effect of allergen challenge on SGaw

in 10 patients with extrinsic bronchial asthma

Change in SGaw (litres/cm.H_2O sec. litre)										
No.	Age	Sex	Allergen Inhalation							
			Baseline	5 min.	10 min.	15 min.	25 min.	35 min.	45 min.	60 min.
1.	19	M	0.235	0.142	0.129	0.114	0.091	0.081	0.116	0.132
2.	23	M	0.189	0.122	0.111	0.094	0.087	0.081	0.101	0.120
3.	21	F	0.144	0.034	0.030	0.029	0.042	0.045	0.054	0.140
4.	18	F	0.213	0.122	0.149	0.097	0.096	0.106	0.167	0.258
5.	46	F	0.109	0.063	0.060	0.063	0.066	0.063	0.063	0.093
6.	18	F	0.132	0.088	0.089	0.091	0.114	0.110	0.120	0.114
7.	23	F	0.227	0.066	0.038	0.068	0.060	0.127	0.145	0.146
8.	18	M	0.066	0.022	0.017	0.011	0.011	0.023	0.026	0.045
9.	27	M	0.105	0.024	0.014	0.022	0.019	0.029	0.047	0.094
10.	28	M	0.082	0.021	0.024	0.019	0.023	0.025	0.078	0.088
Mean			0.150	0.070	0.066	0.061	0.061	0.069	0.092	0.123
SEM			0.020	0.014	0.015	0.012	0.011	0.012	0.014	0.018

Table XLIX Effect of allergen challenge on SGaw after prior alpha blockade with thymoxamine in 10 patients with extrinsic bronchial asthma

Change in SGaw (litres/cm.H_2O sec. litre)								
No.	Allergen Inhalation							
	Baseline	5 min.	10 min.	15 min.	25 min.	35 min.	45 min.	60 min.
1.	0.245	0.237	0.179	0.178	0.107	0.101	0.133	0.202
2.	0.193	0.195	0.169	0.189	0.189	0.212	0.225	0.238
3.	0.183	0.207	0.188	0.147	0.104	0.105	0.126	0.122
4.	0.177	0.174	0.069	0.043	0.051	0.059	0.083	0.076
5.	0.079	0.081	0.088	0.095	0.095	0.101	0.098	0.097
6.	0.124	0.037	0.052	0.064	0.071	0.080	0.080	0.085
7.	0.179	0.204	0.179	0.200	0.200	0.220	0.221	0.231
8.	0.087	0.023	0.019	0.021	0.019	0.025	0.028	0.045
9.	0.067	0.020	0.027	0.026	0.022	0.026	0.027	0.035
10.	0.082	0.051	0.044	0.048	0.057	0.052	0.052	0.076
Mean	0.142	0.123	0.101	0.101	0.092	0.098	0.107	0.120
SEM	0.019	0.028	0.022	0.022	0.020	0.022	0.022	0.024
t test		2.44	2.63	1.83	1.97	2.46	0.08	0.00
P		.025	.025	.05	.05	.025	N.S.	N.S.

Table L Effect of saline, thymoxamine or phentolamine. isoprenaline and isoprenaline + thymoxamine or phentolamine on SGaw in 10 patients with extrinsic asthma

No.	Age	Sex	Saline		Thymoxamine or Phentolamine		Isoprenaline		Isoprenaline + Thymoxamine or Phentolamine	
			B	A	B	A	B	A	B	A
1.	27	M	0.064	0.069	0.040	0.029	0.036	0.136	0.039	0.161
2.	28	M	0.084	0.082	0.048	0.064	0.036	0.187	0.046	0.156
3.	19	M	0.060	0.068	0.053	0.085	0.055	0.216	0.042	0.211
4.	21	M	0.0138	0.103	0.102	0.143	0.065	0.206	0.038	0.264
5.	24	M	0.113	0.108	0.110	0.153	0.146	0.222	0.088	0.274
6.	18	M	0.062	0.060	0.082	0.068	0.065	0.114	0.056	0.130
7.	37	M	0.092	0.112	0.063	0.127	0.081	0.190	0.100	0.174
8.	23	F	0.132	0.129	0.125	0.232	0.129	0.218	0.121	0.252
9.	32	M	0.110	0.092	0.052	0.075	0.096	0.116	0.099	0.227
10.	41	M	0.040	0.042	0.042	0.048	0.027	0.030	0.035	0.141
1.*					0.046	0.112			0.064	0.204
2.*					0.058	0.062			0.049	0.141
6.*					0.069	0.079			0.068	0.188
Mean			0.090	0.087	0.068	0.098	0.074	0.164	0.065	0.194
SEM			0.010	0.008	0.008	0.015	0.013	0.020	0.008	0.014
t test				0.63		3.14		5.27		11.00
P				N.S.		.01		.001		.001

* Patients given phentolamine.

B: Before drug treatment.
A: After drug treatment.

Table LI Effect of Histamine Dihydrochloride (200 ugm.) by inhalation on FEV_1 in 16 patients with extrinsic asthma

NO.	AGE	SEX	FEV_1	Histamine Dihydrochloride (200 ugm.)				
			Control	2'	5'	10'	15'	30'
1.	17	F	2.40	1.40	1.80	1.60	1.95	2.40
2.	23	M	3.75	2.70	3.20	3.00	3.00	3.60
3.	17	M	3.05	1.60	1.70	1.95	2.50	3.15
4.	37	M	1.30	0.85	1.00	1.30	1.40	1.30
5.	15	M	3.20	2.00	2.25	2.80	3.00	3.00
6.	19	M	3.30	2.30	2.75	3.00	3.00	3.10
7.	21	F	2.70	1.05	1.70	2.10	2.30	2.25
8.	20	M	2.60	1.00	1.45	1.80	1.75	2.40
9.	14	M	2.60	2.45	2.35	2.40	2.55	2.50
10.	30	F	2.25	1.80	1.80	1.40	1.95	2.05
11.	30	M	3.20	2.80	3.00	3.00	3.15	3.25
12.	18	F	2.65	0.50	0.80	1.40	1.25	2.60
13.	28	M	3.35	2.50	2.40	2.70	2.80	3.10
14.	30	M	2.70	1.95	2.00	2.30	2.20	2.55
15.	23	M	4.40	2.70	3.15	3.50	3.90	4.20
16.	30	M	3.00	1.20	1.20	1.50	1.80	2.20
Mean			2.90	1.80	2.03	2.23	2.41	2.73
SEM			0.17	0.18	0.19	0.18	0.18	0.17
t test				9.04	6.82	6.54	5.00	3.43
P				.001	.001	.001	.001	.005

Table LII Effect of Histamine Dihydrochloride (200 ugm.) by inhalation on SGaw in 16 patients with extrinsic asthma

NO.	AGE	SEX	SGaw	Histamine Dihydrochloride (200 ugm.)				
			Control	2'	5'	10'	15'	30'
1.	17	F	0.087	0.021	0.028	0.029	0.035	0.081
2.	23	M	0.090	0.040	0.041	0.042	0.042	0.095
3.	17	M	0.079	0.014	0.027	0.035	0.037	0.081
4.	37	M	0.044	0.036	0.034	0.037	0.031	0.046
5.	15	M	0.068	0.030	0.041	0.071	0.073	0.076
6.	19	M	0.083	0.028	0.066	0.063	0.069	0.077
7.	21	F	0.092	0.021	0.030	0.054	0.059	0.063
8.	20	M	0.093	0.027	0.034	0.041	0.043	0.095
9.	14	M	0.207	0.092	0.047	0.065	0.114	0.111
10.	30	F	0.118	0.044	0.044	0.080	0.076	0.087
11.	30	M	0.179	0.097	0.102	0.147	0.194	0.172
12.	18	F	0.103	0.018	0.022	0.027	0.046	0.069
13.	28	M	0.082	0.021	0.024	0.023	0.044	0.080
14.	30	M	0.054	0.023	0.028	0.038	0.055	0.060
15.	23	M	0.185	0.031	0.043	0.067	0.106	0.164
16.	30	M	0.065	0.012	0.020	0.022	0.024	0.045
Mean			0.102	0.035	0.039	0.053	0.066	0.088
SEM			0.012	0.006	0.005	0.008	0.011	0.009
t test				8.00	6.20	5.16	4.97	2.15
P				.001	.001	.001	.001	.025

Table LIII Effect of methacholine (800 ugm.) by inhalation on FEV_1 in 15 patients with extrinsic asthma

No.	Age	Sex	FEV_1	Methacholine				
			Control	2'	5'	10'	15'	30'
1.	17	F	2.50	0.95	1.05	1.40	1.40	1.70
2.	23	M	3.85	2.20	2.30	2.35	2.15	3.35
3.	17	M	3.15	1.20	1.25	1.35	1.60	2.35
4.	16	M	2.70	1.85	2.40	2.35	2.35	2.50
5.	15	M	2.90	1.70	1.20	1.75	1.70	2.40
6.	19	M	3.70	1.00	1.20	1.40	1.00	2.10
7.	21	F	2.70	1.20	1.00	1.20	1.40	1.45
8.	30	F	2.25	2.00	1.70	2.10	2.15	2.20
9.	30	M	3.25	2.20	1.85	2.20	2.50	2.80
10.	28	M	3.70	2.25	2.45	2.60	2.80	3.00
11.	30	M	3.05	2.10	2.65	2.90	3.15	3.20
12.	21	M	2.50	1.35	1.70	2.60	2.60	2.50
13.	21	M	4.75	4.65	4.60	4.50	4.45	4.50
14.	31	F	2.40	1.20	1.40	1.65	1.85	2.00
15.	37	M	1.90	0.80	1.15	1.30	1.60	1.70
Mean			3.02	1.78	1.86	2.11	2.18	2.52
SEM			0.19	0.24	0.24	0.22	0.22	0.20
t test				7.50	5.95	5.11	4.23	4.20
P				.001	.001	.001	.001	.001

Table LIV Effect of methacholine (800 ugm.) by inhalation on SGaw in 15 patients with extrinsic asthma

No.	SGaw	Methacholine				
	Control	2'	5'	10'	15'	30'
1.	0.115	0.065	0.067	0.068	0.072	0.087
2.	0.188	0.028	0.030	0.036	0.026	0.068
3.	0.113	0.044	0.049	0.054	0.051	0.102
4.	0.108	0.024	0.034	0.048	0.050	0.125
5.	0.312	0.054	0.051	0.088	0.081	0.272
6.	0.123	0.078	0.079	0.085	0.092	0.090
7.	0.136	0.028	0.030	0.035	0.039	0.045
8.	0.141	0.042	0.045	0.054	0.059	0.095
9.	0.167	0.026	0.034	0.039	0.049	0.080
10.	0.053	0.016	0.019	0.026	0.030	0.040
11.	0.075	0.023	0.053	0.067	0.077	0.082
12.	0.103	0.069	0.070	0.098	0.086	0.101
13.	0.308	0.193	0.149	0.132	0.151	0.212
14.	0.220	0.061	0.090	0.125	0.125	0.126
15.	0.116	0.046	0.052	0.080	0.082	0.081
Mean	0.152	0.053	0.057	0.069	0.071	0.107
SEM	0.020	0.011	0.008	0.008	0.009	0.016
t test		6.10	5.66	4.93	4.90	4.05
P		.001	.001	.001	.001	.001

Table LV The effect of Salbutamol inhalation on airways mechanics in eight patients with extrinsic bronchial asthma

n = 8	B	A	P
V.C.	5.21 (0.30)	5.28 (0.29)	N.S.
R.V.	1.88 (0.20)	1.99 (0.22)	N.S.
$F.E.V_1$	3.20 (0.30)	3.75 (0.29)	.01
M.M.F.R.	2.18 (0.45)	3.34 (0.44)	.001
SGaw	0.085 (0.009)	0.242 (0.030)	.001
C.V./V.C.%	14.70 (1.12)	7.30 (1.08)	.001
C.C./T.L.C.%	36.00 (2.35)	31.70 (2.80)	.001

S.E.M. : Standard Error of the Mean in parentheses.
B : Before Salbutamol inhalation.
A : After Salbutamol inhalation.
V.C. : Vital capacity in litres.
R.V. : Residual volume in litres.
M.M.F.R. : Maximum mid-expiratory flow rate litres $second^{-1}$.
C.V. : Closing volume or Phase IV.
C.C. : Closing capacity = C.V. + R.V.
T.L.C. : Total lung capacity.

Table LVI The effect of Salbutamol inhalation on airways mechanics in ten normal subjects

n = 10	B	A	P
V.C.	4.91 (0.30)	4.97 (0.30)	.05
R.V.	1.27 (0.17)	1.33 (0.12)	N.S.
$F.E.V_1$	3.72 (0.22)	3.91 (0.24)	.01
M.M.F.R.	3.61 (0.35)	4.13 (0.35)	.001
SGaw	0.224 (0.017)	0.388 (0.056)	.005
C.V./V.C.%	8.70 (1.12)	7.80 (0.99)	N.S.
C.C./T.L.C.%	27.90 (3.57)	27.10 (1.61)	N.S.

Table LVII The effect of propranolol given intravenously on the airways mechanics in eight patients with extrinsic bronchial asthma

n = 8	B	A	P
V.C.	5.12 (0.32)	5.00 (0.34)	N.S.
R.V.	1.80 (0.21)	1.98 (0.22)	.05
$F.E.V_1$	3.27 (0.36)	3.04 (0.38)	.05
M.M.F.R.	2.60 (0.53)	2.09 (0.51)	.001
SGaw	0.183 (0.039)	0.086 (0.025)	.001
C.V./V.C.%	13.65 (0.87)	18.71 (1.14)	.001
C.C./T.L.C.%	35.30 (1.96)	41.80 (1.34)	.005

Table LVIII

The effect of propranolol given intravenously on the airways mechanics in nine normal subjects

n = 9	B	A	P
V.C.	5.02 (0.32)	5.01 (0.30)	N.S.
R.V.	1.28 (0.14)	1.23 (0.14)	N.S.
$F.E.V_1$	3.87 (0.27)	3.86 (0.29)	N.S.
M.M.F.R.	3.93 (0.45)	3.84 (0.49)	N.S.
SGaw	0.289 (0.049)	0.261 (0.054)	N.S.
C.V./V.C.%	8.20 (2.61)	9.10 (0.99)	N.S.
C.C./T.L.C.%	26.90 (1.68)	26.60 (1.81)	N.S.

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Publications

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Effect of Alpha-receptor Blocking Drugs and Disodium Cromoglycate on Histamine Hypersensitivity in Bronchial Asthma

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Summary: Twenty patients with extrinsic type bronchial asthma are shown to have a significant fall in vital capacity (V.C.) and forced expiratory volume in 1 second ($F.E.V._1$) after an intravenous infusion of 50μg. of histamine dihydrochloride. In 10 of these subjects the fall in V.C. and $F.E.V._1$ produced by intravenous histamine is inhibited by the alpha-receptor blocking drugs phentolamine and phenoxybenzamine injected before the histamine test. The inhalation of disodium cromoglycate in 10 subjects is also shown to inhibit the fall in V.C. and $F.E.V._1$ produced by the intravenous infusion of histamine. It is suggested that bronchial smooth muscle in asthmatic subjects has alpha-adrenergic receptor sites, and that a possible mechanism for the action of disodium cromoglycate is that it stabilizes the cell membrane, thereby altering calcium ion transport.

Introduction

In 1929 Weiss, Robb, and Blumgart reported that intravenously administered histamine produced a fall in ventilatory capacity of asthmatic subjects at dosage levels which did not affect the pulmonary ventilation in normal subjects. This hypersensitivity to histamine of the airways in asthmatic

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subjects has often been confirmed (Curry, 1946; Dowell, Kerr, and Park, 1966) and has been shown to persist for many years even in the absence of active asthma (Bouhuys *et al.*, 1960).

Certain animal species develop histamine hypersensitivity after an injection of a vaccine prepared from *Bordetella pertussis* organisms (Parfenjev and Goodline, 1948), and while investigating this phenomenon in the laboratory Fishel, Szentivanyi, and Talmage (1962) observed that in some species histamine hypersensitivity could be produced by the administration of the beta-adrenergic blocking drug dichloroisoproterenol. These authors went on to suggest that the histamine hypersensitivity was the result of a functional imbalance between the two types (alpha and beta) of adrenergic receptor systems, and Szentivanyi (1968) developed this hypothesis into a general theory to explain the atopic abnormality in bronchial asthma. In man the clinical importance of these experimental observations became evident when McNeill (1964) reported that propranolol, a beta-adrenergic receptor blocking drug, caused a fall in ventilatory capacity in asthmatic subjects which was not reversed by isoprenaline. McNeill and Ingram (1966), using a body plethysmograph, further demonstrated increased airways resistance in normal subjects when given propranolol. On the other hand, Zaid and Beall (1966) were unable to show increased bronchial sensitivity to histamine in normal subjects with beta-adrenergic receptor blockade.

Here we report on an investigation designed to assess the relationship of alpha-adrenergic receptors in bronchial smooth muscle to histamine hypersensitivity in patients with bronchial asthma. In addition, it is shown that disodium cromoglycate inhibits the hypersensitivity to histamine of bronchial smooth muscle in patients with asthma.

Patients and Methods

Patients with bronchial asthma of the extrinsic type and known to have fully reversible airways obstruction were investigated. These patients all had positive skin tests to inhalant antigens, such as house dust, the house dust mite, and grass pollens. In addition, they all had a blood eosinophilia of at least 700 cells/cu.mm. The histamine test was carried out in each subject at a time when they had minimal airways obstruction and had not required an oral bronchodilator drug in the preceding 12 hours. Informed consent was obtained for these procedures in every case.

Histamine Test.—The control test was carried out with the patients made comfortable in a sitting position during which

baseline levels were determined for the vital capacity (V.C.) and the forced expiratory volume in 1 second ($F.E.V._1$) in litres, a dry spirometer (Vitalograph) being used. During this period an intravenous infusion of normal saline was set up, and once steady state readings for the vital capacity and $F.E.V._1$ had been obtained the infusion was switched to a solution of 50 μg. of histamine dihydrochloride in 200 ml. of normal saline. This was administered over a period of 10 to 15 minutes. The vital capacity and $F.E.V._1$ measurements were continued at regular intervals for 40 minutes after the infusion of histamine had been completed. For the test infusion the procedure was carried out as above but immediately before the histamine drip was begun 5 mg. of phentolamine was injected intravenously into the arm not being used for the infusion. In three patients 10 mg. of phenoxybenzamine was administered in 150 ml. of normal saline intravenously over a period of two hours before the histamine drip.

Disodium Cromoglycate.—Ten patients had a histamine control test and were then put on disodium cromoglycate (without isoprenaline), three 20-mg. capsules being inhaled daily for a period of two weeks. Following this the histamine test was repeated, each subject inhaling 40 mg. of disodium cromoglycate 30 minutes before the test infusion. The investigation was randomized, some patients having the test infusion with phentolamine or disodium cromoglycate carried out first and having the control test repeated at a later date. Patients on disodium cromoglycate were asked to stop this preparation, and one week later the histamine test was repeated. In view of the known prolonged action of phenoxybenzamine this test was always carried out after the control investigation had been completed.

Results

Alpha-Adrenergic Blocking Drugs.—The results of the control test in which cases Cases 1 to 10 were given 50 μg. of histamine dihydrochloride intravenously are shown in Table I. The fall in V.C. and $F.E.V._1$ was observed in all 10 patients and was most pronounced at 10 minutes. There was considerable restitution of both V.C. and $F.E.V._1$ by 40 minutes (Fig. 1). For the test Cases 1 to 8 received 5 mg. of phentolamine intravenously and Cases 8 to 10 had 10 mg. of phenoxybenzamine intravenously before the histamine infusion. The results are shown in Table II. In these patients the fall in V.C. and $F.E.V._1$ due to histamine was completely inhibited (Fig. 1). A paired *t* test shows that there was no significant difference in the V.C. and $F.E.V._1$ at 0 time and 40 minutes, but at 5, 10, and 20 minutes there was a significant difference in both V.C. and $F.E.V._1$ when compared with the control test.

TABLE I.—*Results in 10 Asthmatic Subjects After 50μg. Histamine Dihydrochloride Intravenously*

Case No.	Age	Time in Minutes				
		0	5	10	20	40
		Change in V.C. (in Litres)				
1	22	3·65	2·55	2·45	2·70	3·35
2	26	3·30	3·00	2·95	3·00	3·30
3	30	4·55	4·45	4·00	3·60	3·75
4	20	3·00	2·75	1·60	2·85	2·95
5	34	3·25	2·50	2·50	2·85	3·20
6	19	3·50	3·10	2·55	3·40	3·35
7	53	2·50	1·10	1·50	1·60	2·15
8	23	3·00	2·75	2·60	2·90	2·82
9	28	2·35	2·20	2·00	1·80	2·40
10	26	4·00	4·05	3·65	3·70	3·90
		Change in F.E.V.$_1$ (in Litres)				
1		2·25	1·55	1·20	0·85	2·20
2		2·50	2·30	2·10	2·15	2·20
3		2·50	2·20	2·05	1·75	1·90
4		2·30	1·60	1·15	1·70	2·00
5		2·40	2·05	2·05	2·25	2·35
6		1·90	1·35	1·20	1·60	1·60
7		1·55	0·45	0·95	1·00	1·25
8		1·65	1·15	1·15	1·50	1·65
9		1·30	1·10	0·95	0·95	1·00
10		3·10	2·95	2·55	2·80	2·90

Disodium Cromoglycate.—The results of the control test in which Cases 11 to 20 were given 50 μg. of histamine dihydrochloride intravenously are shown in Table III. The fall in V.C. and F.E.V.$_1$ was observed in them all and was most pronounced at 20 minutes. There was considerable restitution of both V.C. and F.E.V.$_1$ by 40 minutes (Fig. 2). For the test, Cases 11 to 20 inhaled 40 mg. of disodium cromoglycate 30 minutes before the intravenous infusion of histamine. The results are shown in Table IV. In these 10 patients the fall in V.C. and F.E.V.$_1$ due to histamine was completely inhibited (Fig. 2). A paired *t* test shows that at 0 time there was no significant difference in the V.C. or F.E.V.$_1$ whereas between 15 and 30 minutes and 10 and 30 minutes for the V.C. and F.E.V.$_1$ respectively there was a significant difference when compared with the control test.

Discussion

The effect of histamine on smooth muscle may be produced by a direct action of the amine on bronchial smooth muscle or by a delayed reflex action (DeKock, Nadel, Zwi, Colebatch,

TABLE II.—*Results in 10 Asthmatic Subjects when 50 μg. Histamine Dihydrochloride is Infused After Phentolamine 5 mg. or Phenoxybenzamine 10 mg. Intravenously*

Case No.	Time in Minutes				
	0	5	10	20	40
	Change in V.C. (in Litres)				
1	3·00	2·95	3·25	2·90	3·15
2	3·15	3·10	3·25	3·00	3·10
3	4·70	4·35	4·50	4·15	4·50
4	2·80	2·95	2·95	3·10	2·95
5	3·20	2·95	2·95	2·35	2·90
6	3·45	3·35	3·30	3·70	3·80
7	2·50	2·25	2·65	2·50	2·55
8	3·15	3·10	3·10	3·20	3·25
*8	2·75	2·75	2·60	2·90	2·85
*9	2·65	2·65	2·70	2·70	3·05
*10	3·95	3·90	4·05	4·10	4·05
t =	0·79	3·11	5·90	2·71	1·99
	Change in F.E.V.$_1$ (in Litres)				
1	1·85	2·10	2·00	2·15	1·90
2	2·55	2·55	2·35	2·55	2·45
3	3·10	2·60	2·75	2·75	3·10
4	2·25	2·15	2·30	2·30	2·35
5	2·35	1·80	1·90	1·75	1·90
6	1·70	1·80	1·90	1·90	1·85
7	1·60	1·75	2·10	1·55	1·85
8	2·45	2·45	2·60	2·90	2·85
*8	1·95	1·90	1·80	2·25	2·15
*9	1·85	2·05	1·75	1·80	1·95
*10	2·95	2·90	2·90	3·10	3·15
t =	1·27	3·66	5·25	7·35	2·08

*Subjects given phenoxybenzamine. $t0{\cdot}05 = 2{\cdot}28$. $t0{\cdot}01 = 3{\cdot}169$.

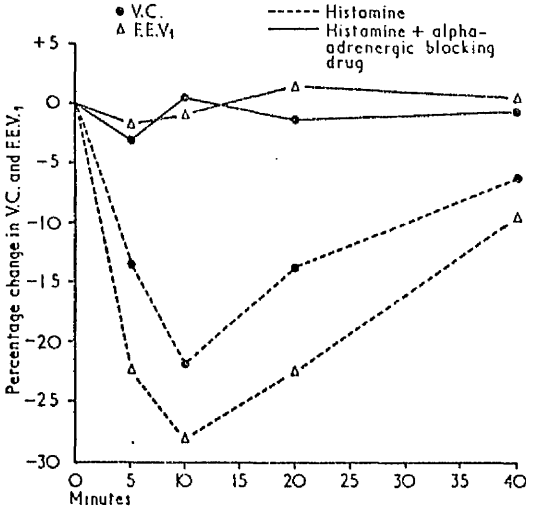

FIG. 1.—Mean fall in V.C. and F.E.V.$_1$ in 10 subjects after intravenous infusion of 50 μg. of histamine dihydrochloride. This fall in V.C. and F.E.V.$_1$ is completely inhibited by prior injection of phentolamine or phenoxybenzamine.

TABLE III.—*Results in 10 Asthmatic Subjects After 50 μg. Histamine Dihydrochloride Intravenously*

Case No.	Age	Time in Minutes								
		0	5	10	15	20	25	30	35	40
		Change in V.C. (in Litres)								
11 ..	33	3·20	3·20	3·20	2·90	2·90	2·70	2·80	2·80	
12 ..	15	3·75	3·10	3·30	3·40	3·50	3·70	3·50	3·70	3·80
13 ..	28	2·70	2·70	2·60	2·60	2·60	2·40	2·30	2·30	2·30
14 ..	20	2·60	2·40	2·30	2·30	2·00	1·75	2·30	2·20	2·20
15 ..	15	3·10	2·75	2·60	2·30	2·30	2·80	2·95	3·20	3·20
16 ..	31	3·75	3·10	3·20	3·00	2·40	3·30	3·20	3·25	3·35
17 ..	36	4·15	4·10	4·20	3·70	3·30	3·40	3·65	3·80	3·75
18 ..	31	3·25	3·10	2·50	2·30	1·90	2·45	2·35	2·45	2·45
19 ..	13	2·00	2·00	1·80	1·60	1·40	1·75	2·00	2·00	2·00
20 ..	14	4·00	3·90	3·60	3·70	3·60	3·60	3·90	4·00	4·00
		Change in F.E.V.$_1$ (in Litres)								
11 ..		2·25	2·00	1·90	1·80	1·85	1·90	1·75	1·80	
12 ..		2·40	1·85	1·90	1·20	1·25	1·20	1·50	2·20	2·20
13 ..		1·90	1·90	1·80	1·70	1·60	1·45	1·50	1·50	1·50
14 ..		1·10	0·80	0·70	0·90	0·90	1·00	1·00	1·10	1·10
15 ..		2·10	1·55	1·60	1·50	1·35	1·80	1·90	2·05	2·00
16 ..		2·05	1·90	1·90	1·80	1·45	1·80	1·90	1·80	1·65
17 ..		2·45	2·30	2·30	2·20	2·10	1·90	2·00	2·10	2·10
18 ..		1·25	1·00	0·90	0·80	0·80	0·90	0·95	0·90	0·90
19 ..		1·70	1·55	1·20	1·20	1·20	1·35	1·60	1·70	1·70
20 ..		2·70	2·60	2·20	2·00	1·95	2·70	2·70	2·70	2·70

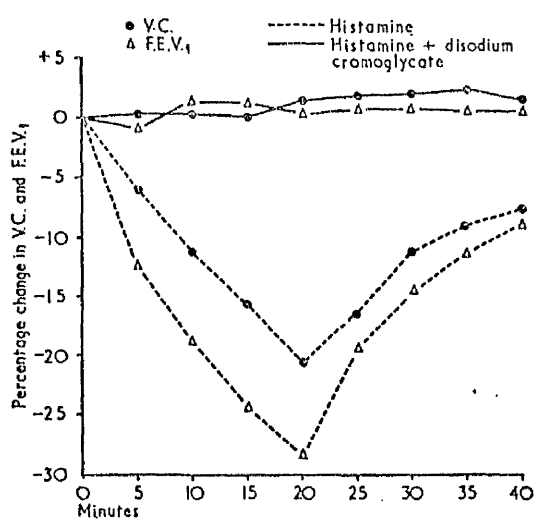

FIG. 2.—Mean fall in V.C. and F.E.V.$_1$ in 10 subjects after intravenous infusion of 50 μg. of histamine dihydrachloride. This fall in V.C. and F.E.V.$_1$ is completely inhibited by prior inhalation of disodium cromoglycate.

TABLE IV.—*Results in 10 Asthmatic Subjects when 50 µg. Histamine Dihydrochloride Intravenously is Infused after Inhalation of 40 mg. Disodium Cromoglycate*

Case No.	Time in Minutes								
	0	5	10	15	20	25	30	35	40
Change in V.C. (in Litres)									
11	2·55	2·60	2·60	2·60	2·60	2·65	2 65	2·70	
12	2·55	2·60	2·35	2·50	2·70	2·70	2·80	2·70	2·70
13	2·80	2·80	2·90	2·80	2·80	2·80	2·85	2·90	2·85
14	3·20	3·20	3·30	3·20	3·20	3·30	3·20	3·30	3·30
15	3·20	3·20	3 20	3·20	3·20	3·10	3 20	3·30	3·25
16	3·80	3·80	3·80	3·80	3·85	3·90	3·90	3 90	3·85
17	4·50	4·50	4 60	4·60	4·50	4·60	4·50	4·60	4·60
18	3·40	3·35	3·25	3·25	3·30	3·30	3 30	3·30	3·30
19	2·20	2·20	2·20	2·30	2·30	2·20	2·15	2·20	2·20
20	5·00	5·20	5·10	5·20	5·10	5·20	5·20	5·20	5·20
t =	0·15	1·59	1·76	2·62	9·10	2·39	2·54	2·04	1·82
Change in F.E.V.$_1$ (in Litres)									
11	1·35	1·35	1·35	1·40	1·30	1·45	1·30	1·45	
12	1·95	1·95	2·05	1·90	2·00	2·00	2·00	2·00	2·00
13	2·15	2·20	2·20	2·30	2·20	2·20	2·20	2·20	2·20
14	2·20	2·25	2·20	2·20	2·20	2·30	2·30	2·35	2·30
15	1·70	1·70	1·80	1·75	1·80	1·70	1·85	1·85	1·80
16	1·80	1·80	1·80	1·85	1·75	1·90	1 80	1·85	1·90
17	3·00	3·00	3·00	3 00	3·00	3·00	3·00	3 05	3·00
18	1·40	1·35	1·25	1·25	1·25	1·30	1·25	1·30	1·30
19	1·70	1·70	1·80	1 80	1·80	1·65	1·65	1·65	1·70
20	3·60	3·40	3·60	3·60	3·70	3·55	3·70	3·60	3·65
t =	0·96	1·81	2·40	3·03	3·32	2·89	2·84	1·94	2·35

t 0·05 = 2·26. t0·01 = 3·25.

and Olsen, 1966). Our investigation has again confirmed that patients with asthma have a bronchial smooth muscle which is hypersensitive to histamine. We have not been able to show a similar fall in ventilatory capacity to histamine by this method in patients with chronic bronchitis (unpublished observations). The mechanism of this histamine hypersensitivity in bronchial asthma is not clear.

Little attention has been paid to the possibility that bronchial smooth muscle has alpha-adrenergic receptor sites, but alpha-receptor sites do exist on the bronchial smooth muscle of certain animal species (Castro de la Mata, Penna, and Aviado, 1962; Everitt and Cairncross, 1969). Both phentolamine and phenoxybenzamine are classified as alpha-receptor blocking drugs. Phentolamine, which is an imidazole (as is histamine), has a direct effect on the adrenal medulla, releasing noradrenaline. During the test with phentolamine the patients experienced a mild tachycardia but so long as they remained seated during the test there was no fall in blood pressure. It is unlikely that the inhibition of the histamine effect on ventilatory capacity in these patients was mediated via the adrenal medulla. This view would seem to be con-

firmed by the results obtained with phenoxybenzamine. Though this drug gave rise to tachycardia, hypotension was not a problem during the test period, as the patients remained at rest. Further, phenoxybenzamine does not have a direct action on the adrenal medulla. Phenoxybenzamine has an antihistamine effect which cannot be dissociated from its alpha-adrenergic blocking activity (Goodman and Gilman, 1965). It is difficult to assess the many pharmacological effects which have been reported due to phentolamine and phenoxybenzamine. Nevertheless, our results suggest that the bronchial smooth muscle of patients with asthma have alpha-adrenergic receptor sites and that blockade of these sites with alpha-receptor antagonists alters the sensitivity of the bronchial smooth muscle to histamine.

Disodium cromoglycate was introduced for the treatment of patients with allergic bronchial asthma by Howell and Altounyan (1967). On inhalation disodium cromoglycate can inhibit the fall in V.C. and $F.E.V._1$ in patients with allergic asthma when challenged by inhalation of the appropriate antigen (Pepys, Hargreave, Chan, and McCarthy, 1968). This drug has also been shown to inhibit the fall in V.C. and $F.E.V._1$ in exercise-induced asthma (Davies, 1968). On the other hand, disodium cromoglycate has been reported not to affect the histamine response of human bronchial smooth muscle (Cox, 1967). We have investigated the effect of this drug on the phenomenon of histamine hypersensitivity in patients with bronchial asthma and have found disodium cromoglycate is a potent inhibitor of the fall in ventilatory capacity produced in these subjects by an intravenous infusion of histamine.

The mechanism by which disodium cromoglycate produces its effect in bronchial asthma remains obscure. The drug inhibits the release of pharmacologically active amines following the antigen-antibody reaction (Cox, 1967). The release of these amines after the antigen-antibody reaction in anaphylaxis is dependent on three factors: calcium, a heat-labile factor, and free sulphydryl groups (Mongar and Schild, 1957; Austen and Humphrey, 1961). Though in some animal species intravenous disodium cromoglycate does give rise to profound reflex cardiovascular changes (Cox, 1967), it is unlikely that disodium cromoglycate acts as an alpha-receptor blocking drug as cardiovascular effects have not been reported in man.

The alpha- and beta-adrenergic receptors are believed to control the ionized calcium concentration in the environment of the contractile protein of the myofibrils (Filo, Bohr, and Ruegg, 1965; Bohr, 1967), and the histamine response of smooth muscle is dependent on the concentration of ionized

calcium (Daniel, 1964). It can therefore be postulated that beta-receptor blocking drugs by increasing the ionized calcium of smooth muscle fibrils increase the histamine response, and that the alpha-receptor blocking drugs by lowering the ionized calcium inhibit the histamine response of bronchial smooth muscle. One explanation of our observations is that disodium cromoglycate stabilizes the cell membrane and this alters calcium ion transport. Such an effect would explain both the inhibition of amine release after the antigen-antibody reaction and the inhibition of histamine hypersensitivity of bronchial smooth muscle affected by this drug. Further investigation of the mechanism of action of disodium cromoglycate should lead to a better understanding of the nature of bronchial asthma.

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The airways response to phenylephrine after blockade of alpha and beta receptors in extrinsic bronchial asthma

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Summary

Phenylephrine, a powerful alpha receptor stimulant, has been shown to cause a significant fall in the FEV_1 and SGaw in six patients with extrinsic bronchial asthma after prior beta blockade with propranolol. In contrast, propranolol or phenylephrine after prior beta blockade failed to effect a significant change in the FEV_1 and SGaw in five normal subjects. The phenylephrine effect can be completely inhibited by alpha receptor blocking drugs, phenoxybenzamine and thymoxamine. These observations suggest that the bronchomotor tone in asthma is largely controlled by the sympathetic activity and that there are alpha receptors in the human airways which in the presence of beta blockade can be stimulated to give bronchoconstriction.

Introduction

Hyper-reactivity of the airways in patients with asthma was first reported in 1929 (Weiss, Robb & Blumgart, 1929). This phenomenon has frequently been confirmed (Curry, 1946; Dowell, Kerr & Park, 1966) and has been shown to be present for many years even in absence of active asthma (Bouhuys *et al.*, 1960). Fishel, Szentivayni & Tamalge (1962) postulated that the bronchial hyper-reactivity in asthma is due to the functional imbalance in the neural control of the small airways. Asthmatic subjects may develop bronchoconstriction following beta adrenergic blockade with propranolol (McNeill, 1964; Richardson & Sterling, 1969) and contrasts with normal subjects where beta adrenergic blockade has failed to effect a significant change in the ventilatory capacity or airways resistance (Zaid & Beall, 1966; Richardson & Sterling, 1969; Astin, 1972). The beta receptor is membrane bound adenyl cyclase (Robison, Butcher & Sutherland, 1967) and studies on leucocyte adenyl cyclase in patients with extrinsic asthma have shown a diminished response to stimulation with isoproterenol (Logsdon, Middleton & Coffey, 1972; Parker & Smith, 1973).

Little is known about alpha adrenergic receptors in the human bronchial tree, although alpha receptors are known to be present in the airways of animals and give rise to bronchoconstriction on stimulation (Castro de la Mata, Penna & Aviado, 1962; Everitt & Cairncross, 1969). Histamine induced hyper-reactivity of the bronchial tree

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is inhibited by alpha receptor blockade (Kerr, Govindaraj & Patel, 1970; Bianco *et al.*, 1972; Gaddie *et al.*, 1972).

We have investigated the role of alpha and beta receptors in the human bronchial tree and the part they play in the control of bronchial smooth muscle tone. Change in the airways calibre produced by phenylephrine after prior blockade with propranolol, and phenoxybenzamine or thymoxamine was measured by Forced Expiratory Volume in 1 sec (FEV_1) and Specific Airways Conductance (SGaw) in normal subjects and patients with extrinsic bronchial asthma.

Table 1. Details of patients with extrinsic bronchial asthma

No.	Age	Sex	Eosinophil cells/mm^3	IgE (ng/ml)
1	21	F	893	1110
2	17	F	792	400
3	15	M	429*	1647
4	23	M	702	150
5	18	M	864	808
6	19	M	694	343

* On steroid therapy.

Patients and methods

Six patients with extrinsic bronchial asthma and reversible airways obstruction were investigated. These patients had positive skin tests to inhalant allergens, a blood eosinophilia of at least 600 cells/mm^3 and an IgE level above 100 ng/ml (details in Table 1). Disodium cromoglycate therapy was discontinued for 7–10 days and simple bronchodilator drugs stopped for 12–24 hr before the tests were carried out. The five normal subjects were volunteers, they had no respiratory disease and there was no personal or family history of bronchial asthma or atopic disease.

FEV_1 was measured on a Garthur Vitalograph spirometer. SGaw was measured with a constant volume body plethysmograph as described by Dubois, Bothelho & Comroe (1956). Conductance was estimated at a flow rate of 0·5 litre/sec during inspiration while subject panted shallowly at 2 cycles/sec. At the end of each run, thoracic gas volume was estimated by recording the mouth pressure at the end of expiration. The mean of four recordings was calculated to give the SGaw.

Response to phenylephrine before and after propranolol

After recording the baseline FEV_1 and SGaw, each subject inhaled 0·75–1·00 ml of 0·5% isotonic solution of phenylephrine hydrochloride through a Wright's nebulizer using compressed air at a flow rate of 8 litres/min. FEV_1 and SGaw measurements were repeated 5 min after the end of the inhalation. All subjects were then given propranolol orally. Normal subjects received 120 mg while asthmatic patients received 20–30 mg. FEV_1 and SGaw were recorded 45 and 60 min after propranolol administration. At 60 min phenylephrine inhalation was repeated and thereafter the FEV_1 and SGaw were recorded at 2, 5 and 10 min. Asthmatic subjects inhaled 80 μg of isoprenaline aerosol at the end of the test and FEV_1 and SGaw were recorded 10 min later.

Table 2. Effect of phenylephrine and isoprenaline on FEV_1 after prior beta blockade with propranolol in six patients with extrinsic asthma

				Change in FEV_1 (litres)					
No.	Dose of propranolol (mg)	Baseline	Phenylephrine$_1$	Propanolol		Phenylephrine$_2$			Isoprenaline
				45 min	60 min	2 min	5 min	10 min	10 min later
1	20	3·25	3·35	2·40	2·10	1·60	1·50	1·55	1·55
2	20	2·35	2·60	2·25	2·25	1·25	2·05	2·05	2·20
3	30	2·80	3·35	2·00	2·70	1·85	1·90	2·00	2·40
4	30	2·80	2·90	1·45	1·30	1·00	1·05	0·75	—
5	30	2·60	3·00	2·80	2·40	2·35	2·15	2·55	2·95
6	30	3·15	3·40	3·25	3·25	2·90	2·90	3·00	3·25
Mean		2·83	3·10	2·36	2·33	1·83	1·92	1·98	2·47
(S.E.)		(0·14)	(0·13)	(0·25)	(0·26)	(0·28)	(0·25)	(0·32)	(0·29)
		t	4·28	2·66	2·02	3·84	4·59	3·01	
		P	<0·005	<0·05	=0·05	<0·01	<0·005	<0·025	

Response to phenylephrine after beta and alpha adrenergic blockade

The procedure described above was repeated in the six asthmatic patients. At the time they were given propranolol each patient inhaled 10 mg of phenoxybenzamine hydrochloride dispensed in a capsule with 20 mg of lactose from a spinhaler (Fisons Ltd). In patients 2, 3, 4 and 5 the test was repeated after inhalation of 1·00 ml of 1·5% solution of thymoxamine through a Wright's nebulizer. Thymoxamine was not tolerated by two further patients (1 and 6) because of its bitter taste and throat irritation, and could not be given intravenously because of postural hypotension in the presence of prior beta blockade.

Table 3. Effect of phenylephrine and isoprenaline on SGaw after prior beta blockade with propranolol in six patients with extrinsic asthma

			Change in SGaw (l/cmH_2O sec)					
No.	Baseline	Phenylephrine$_1$	Propranolol		Phenylephrine$_2$			Isoprenaline
			45 min	60 min	2 min	5 min	10 min	10 min later
1	0·242	0·315	0·051	0·049	0·025	0·023	0·021	0·021
2	0·083	0·158	0·061	0·062	0·046	0·056	0·055	0·066
3	0·140	0·199	0·103	0·099	0·039	0·041	0·039	0·096
4	0·253	0·265	0·078	0·065	0·039	0·032	0·034	—
5	0·154	0·178	0·155	0·125	0·099	0·081	0·097	0·154
6	0·119	0·137	0·115	0·088	0·065	0·065	0·093	0·132
Mean	0·165	0·209	0·094	0·081	0·052	0·050	0·056	0·094
(S.E.)	(0·029)	(0·027)	(0·015)	(0·011)	(0·011)	(0·009)	(0·013)	(0·023)
	t	4·13	2·18	2·87	5·34	5·26	3·00	
	P	<0·005	<0·05	<0·025	<0·001	<0·001	<0·025	

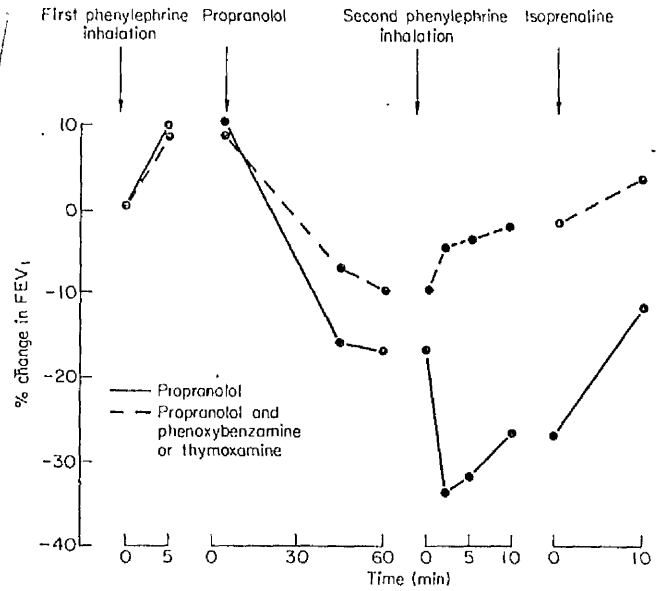

Fig. 1. The effect of phenylephrine inhalation on the mean FEV_1 in six patients with asthma before and after beta adrenergic blockade. The first phenylephrine inhalation caused an increase in the FEV_1. Following propranolol, the FEV_1 fell by 17% at 45 min and 18% at 60 min. In the presence of propranolol blockade, the second phenylephrine inhalation caused a further fall in the FEV_1. Thereafter isoprenaline inhalation increased the FEV_1 by 22%. When phenoxybenzamine or thymoxamine is given with propranolol there was a smaller fall in the FEV_1. The second phenylephrine inhalation produced an 8% increase in the FEV_1 at 10 min. Thereafter isoprenaline restored the FEV_1 above the baseline recording.

Results

Response to phenylephrine before and after beta blockade

In the six asthmatic patients (1–6) the mean FEV_1 increased by 10% and the mean SGaw by 26% after the first inhalation of phenylephrine. The changes were highly significant ($P<0{\cdot}005$). After propranolol the mean FEV_1 fell by 17% at 45 min and 18% at 60 min and the mean SGaw fell by 43% at 45 min and 51% at 60 min. The changes were significant ($P<0{\cdot}05$). Phenylephrine inhalation was repeated at 60 min, this caused a further fall in the mean FEV_1 by 17% and the mean SGaw fell by 19% (Tables 2 and 3 and Figs. 1 and 2). The fall in FEV_1 and SGaw observed at 2, 5 and 10 min remained significant throughout the test as compared to the readings at 60 min before the second phenylephrine inhalation ($P<0{\cdot}001$).

Following the inhalation of 80 μg of isoprenaline at the end of the investigation the mean FEV_1 increased by 22% and the mean SGaw by 17%, but the mean FEV_1 was still 12% and the mean SGaw 53% below the baseline recordings.

In the five normal subjects (7–11) the mean FEV_1 and SGaw did not change significantly after inhalation of phenylephrine nor was there any significant change in FEV_1 and SGaw produced by beta adrenergic blockade ($P>0{\cdot}10$). In each subject, after 120 mg of propranolol, repeat inhalation had little effect on FEV_1 or SGaw ($P>0{\cdot}10$) (Tables 4 and 5 and Fig. 3).

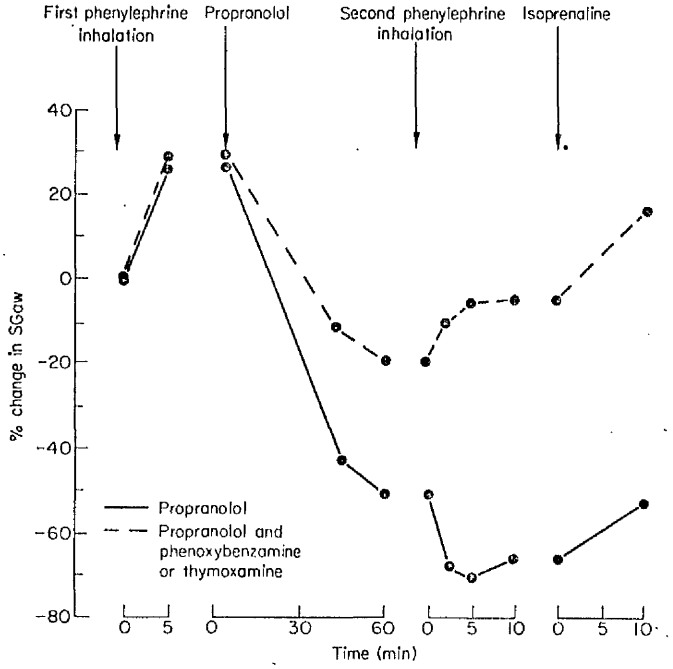

Fig. 2. The effect of phenylephrine inhalation on the mean SGaw in six patients with asthma before and after beta adrenergic blockade. The first phenylephrine inhalation caused an increase in the SGaw. Following propranolol, the SGaw fell by 43% at 45 min and 51% at 60 min. In the presence of propranolol blockade, the second phenylephrine inhalation caused a further fall in the SGaw. Thereafter isoprenaline inhalation increased the SGaw by 17%. When phenoxybenzamine or thymoxamine was given with propranolol there was a smaller fall in the SGaw. The second phenylephrine inhalation produced a 14% increase in the SGaw at 10 min. Thereafter isoprenaline restored the SGaw above the baseline recording.

Table 4. Effect of phenylephrine on FEV_1 after prior beta adrenergic blockade with propranolol in five normal subjects

			Change in FEV_1 (litres)						
No.	Age (years)	Sex	Baseline	Phenylephrine$_1$	Propranolol		Phenylephrine$_2$		
					45 min	60 min	2 min	5 min	10 min
7	26	F	2·55	2·55	2·55	2·60	2·50	2·45	2·55
8	25	M	4·50	4·50	4·45	4·45	4·50	4·55	4·70
9	26	F	3·30	3·25	3·10	3·00	3·20	3·20	3·00
10	23	M	5·80	5·85	5·70	5·65	5·60	5·50	5·60
11	19	F	3·00	2·95	2·90	2·90	2·95	2·90	3·00
Mean			3·83	3·82	3·74	3·72	3·75	3·72	3·77
(S.E.)			(0·58)	(0·60)	(0·58)	(0·58)	(0·57)	(0·57)	(0·59)
			t	0·60	1·25	1·37	0·65	0·39	0·98
			P	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S., not significant.

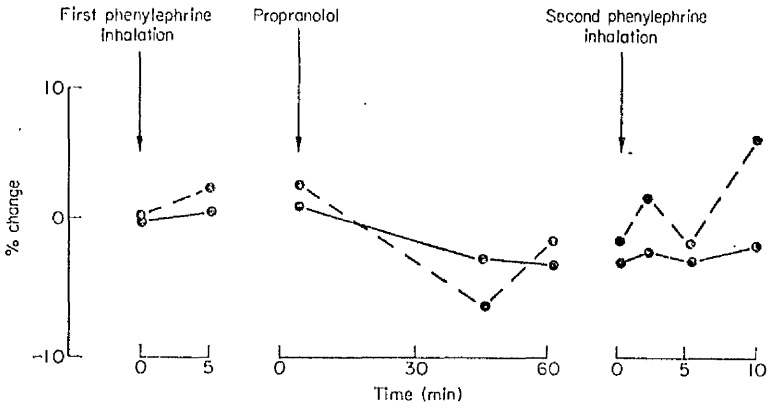

Fig. 3. The effect of phenylephrine inhalation on the mean FEV_1 (——) and SGaw (— — —) in five normal subjects before and after beta adrenergic blockade. The first phenylephrine inhalation failed to cause a significant change in the FEV_1 and SGaw. Following propranolol, the FEV_1 fell by 2% at 45 min and 3% at 60 min, and the SGaw fell by 6% at 45 min and 2% at 60 min. In the presence of propranolol blockade, the second phenylephrine failed to effect a significant change either in the FEV_1 or SGaw.

Response to phenylephrine following beta and alpha adrenergic blockade in asthmatic patients

The first inhalation of phenylephrine increased the mean FEV_1 by 9% and the mean SGaw by 29%. After propranolol administration and phenoxybenzamine or thymoxamine the mean FEV_1 fell by 7% at 45 min and 10% at 60 min and the mean SGaw fell by 11% at 45 min and 19% at 60 min. The mean fall in FEV_1 and SGaw effected by propranolol, and phenoxybenzamine or thymoxamine was smaller than that observed when propranolol was given alone, although the difference in the results is

Table 5. Effect of phenylephrine on SGaw after prior adrenergic blockade with propranolol in five normal subjects

			Change in SGaw (l/cmH_2O sec)				
No.	Baseline	$Phenylephrine_1$	Propranolol		$Phenylephrine_2$		
			45 min	60 min	2 min	5 min	10 min
7	0·245	0·248	0·238	0·261	0·256	0·265	0·281
8	0·336	0·358	0·300	0·336	0·306	0·315	0·336
9	0·230	0·244	0·230	0·230	0·213	0·213	0·230
10	0·300	0·316	0·268	0·272	0·277	0·315	0·360
11	0·269	0·253	0·258	0·258	0·258	0·256	0·265
Mean	0·276	0·283	0·259	0·271	0·282	0·272	0·294
(S.E.)	(0·019)	(0·022)	(0·012)	(0·017)	(0·015)	(0·019)	(0·023)
	t	1·45	0·45	0·62	0·59	0·10	0·94
	P	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S., not significant.

not statistically significant ($P>0{\cdot}10$). At 60 min the second phenylephrine inhalation was given, thereafter the mean rise in FEV_1 was 5% at 2 min, 6% at 5 min and 8% at 10 min, and the mean rise in SGaw was 9% at 2 min and 14% at 5 and 10 min. The response to phenylephrine in the presence of alpha and beta receptor blockade was significant as compared to the effect of phenylephrine in the presence of beta blockade alone ($P<0{\cdot}05$) (Tables 6 and 7 and Figs. 1 and 2).

Following inhalation of 80 μg of isoprenaline at the end of the investigation the mean FEV_1 increased by 5% and the mean SGaw by 22%. The mean FEV_1 was 3% and the mean SGaw 17% above baseline recordings.

Table 6. Effect of phenylephrine and isoprenaline on FEV_1 after prior alpha and beta adrenergic blockade in six patients with extrinsic asthma

			Change in FEV_1 (litres)					
No.	Baseline	Phenylephrine$_1$	Propranolol plus phenoxybenzamine or thymoxamine*		Phenylephrine$_2$			Isoprenaline
			45 min	60 min	2 min	5 min	10 min	10 min later
1	3·00	3·25	3·00	2·65	2·70	2·60	3·20	3·20
2	2·10	2·30	2·00	2·00	2·30	2·20	2·30	2·30
3	3·25	3·70	3·15	2·90	3·35	3·30	3·30	3·40
4	2·00	2·20	1·05	1·50	1·60	1·60	1·50	1·65
5	3·00	3·10	3·25	2·95	3·15	3·20	3·15	3·25
6	3·40	3·40	3·40	3·45	3·20	3·40	3·40	3·45
2*	2·20	2·25	1·50	1·95	2·05	1·95	2·20	2·25
3*	2·60	3·15	2·80	2·20	2·40	2·50	2·45	3·05
4*	1·95	2·40	1·30	1·15	1·20	1·35	1·30	1·50
5*	2·90	3·10	3·20	3·05	3·10	3·20	3·20	3·25
Mean	2·64	2·88	2·46	2·38	2·50	2·53	2·60	2·73
(S.E.)	(0·17)	(0·17)	(0·28)	(0·23)	(0·23)	(0·23)	(0·24)	(0·23)
		t	1·13	0·92	6·55	4·41	4·84	2·10
		P	N.S.	N.S.	<0·001	<0·001	<0·001	<0·05

N.S., not significant. *Subjects given thymoxamine.

Discussion

The relative alpha stimulating potencies of compounds acting on the alpha receptor is in the descending order; phenylephrine > noradrenaline > adrenaline > isoprenaline and is in the reverse order of their beta stimulating properties (Ahlquist & Levy, 1959; Furchgott, 1960). Phenylephrine is a powerful alpha receptor stimulant with little effect on the beta receptor. A direct action on the receptor accounts for the greater part of its effects, only a small part being due to its ability to release noradrenaline (Goodman & Gilman, 1970). Our observations of marked changes in the FEV_1 and SGaw in asthmatic patients following pharmacologically induced alteration in the sympathetic activity confirms the well-known variability in the bronchial calibre in these subjects, and contrasts with the absence of significant change in the FEV_1 and SGaw in normal subjects following similar pharmacological procedures. Statistically

the asthmatic and normal subjects belong to two different populations. Our results support Widdicombe's view (1966) that the sympathetic nervous system has a minor role to play in the control of bronchial calibre in normal subjects.

In the asthmatic subjects small doses of propranolol caused a significant fall in the FEV_1 and SGaw, and after beta blockade phenylephrine gave rise to further bronchoconstriction, whereas if these subjects had a combined beta and alpha blockade a lesser though not significant fall in the FEV_1 and SGaw occurred. Phenylephrine at this stage gave a reversed action with an increase in the FEV_1 and SGaw. These observations suggest that in asthmatic subjects there are alpha receptors in the airways which in the presence of beta blockade can be stimulated to give bronchoconstriction.

Table 7. Effect of phenylephrine and isoprenaline on SGaw after prior alpha and beta adrenergic blockade in six patients with extrinsic asthma

					Change in SGaw (l/cmH_2O sec)			
No.	Baseline	$Phenylephrine_1$	Propranolol plus phenoxybenzamine or thymoxamine*		$Phenylephrine_2$			Isoprenaline
			45 min	60 min	2 min	5 min	10 min	10 min
1	0·248	0·290	0·247	0·226	0·223	0·224	0·248	0·257
2	0·138	0·167	0·080	0·105	0·108	0·119	0·105	0·139
3	0·282	0·347	0·274	0·227	0·300	0·300	0·309	0·332
4	0·081	0·113	0·032	0·037	0·040	0·038	0·039	0·048
5	0·104	0·116	0·122	0·101	0·115	0·136	0·123	0·168
6	0·158	0·174	0·124	0·118	0·117	0·124	0·126	0·174
2*	0·080	0·131	0·056	0·073	0·071	0·068	0·067	0·087
3*	0·068	0·128	0·069	0·050	0·067	0·069	0·066	0·135
4*	0·065	0·091	0·027	0·021	0·031	0·037	0·032	0·047
5*	0·085	0·131	0·129	0·111	0·117	0·125	0·125	0·146
Mean	0·131	0·169	0·116	0·106	0·118	0·124	0·124	0·153
(S.E.)	(0·025)	(0·026)	(0·026)	(0·022)	(0·026)	(0·026)	(0·028)	(0·028)
		t	0·75	1·01	2·43	2·33	2·92	2·45
		P	N.S.	N.S.	<0·025	<0·025	<0·01	<0·025

N.S., not significant.
* Subjects given thymoxamine.

But unlike Prime *et al.* (1972) we have been unable to demonstrate alpha activity in non-asthmatic subjects. It should be noted that isoprenaline was only able to overcome the phenylephrine effect in the presence of beta blockade whereas isoprenaline restored the FEV_1 and SGaw above the baseline recordings in the presence of beta and alpha blockade. Alpha receptor blocking drugs modify the beta blockade produced by propranolol and this observation could have a therapeutic significance in the management of asthma.

Alpha receptor blocking drugs have been known to inhibit histamine hyper-reactivity of the airways in asthmatic subjects (Kerr *et al.*, 1970; Bianco *et al.*, 1972; Gaddie *et al.*, 1972) and in our experience histamine hyper-reactivity is consistently

associated with alpha receptor activity and contrasts with methacholine induced bronchoconstriction which is not inhibited by thymoxamine (personal observations).

We have no evidence as to whether alpha receptor activity as reported here is a genetic or an acquired function of the small airways. In either case the bronchomotor tone is dependent on the balance of activity between the beta and alpha receptors. Membrane bound adenyl cyclase is now recognized as the beta receptor (Robison *et al.*, 1967) and shows a subnormal response to isoproterenol during active asthma but approaches normal activity during remission (Parker & Smith, 1973). This suggests that the variability in the bronchial calibre is due to changes in the balance of sympathetic activity. The normal sympathetic neuro-transmitter is noradrenaline and during periods of diminished adenyl cyclase activity, increased noradrenaline would be available to activate the alpha receptors and give rise to bronchoconstriction. This effect has been shown to be inhibited by phenoxybenzamine and thymoxamine. Further observations on the nature of the alpha receptor will lead to a better understanding of the control of bronchial calibre in patients with asthma.

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Atropine, Sodium Cromoglycate, and Thymoxamine in $PGF_2\alpha$-induced Bronchoconstriction in Extrinsic Asthma

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Summary

In six patients with extrinsic bronchial asthma the inhalation of prostaglandin (PG) $F_2\alpha$ in a small dosage produced significant bronchoconstriction, whereas PGE_2 produced bronchodilatation. In these patients cholinergic blockade with atropine partially inhibited the $PGF_2\alpha$-induced bronchoconstriction, but the α-receptor-blocking drug thymoxamine and sodium cromoglycate did not. These results suggest that the effect of $PGF_2\alpha$ is mediated through cholinergic receptors in the airways, and this effect is grossly exaggerated in asthma. The failure to inhibit $PGF_2\alpha$-induced bronchoconstriction with sodium cromoglycate and the observation of an inhibitory effect of sodium cromoglycate in both allergic and exercise asthma suggest that locally formed $PGF_2\alpha$ may not be the main factor in the pathogenesis of bronchial asthma.

Introduction

Human lung contains prostaglandins (PG) of both the E and F series, E_2 and $F_2\alpha$ being the most abundant.[1,2] $PGF_2\alpha$, a potent bronchoconstrictor to which patients with bronchial asthma are highly sensitive,[3-5] is released from mammalian lungs during anaphylactic reactions[6] and by various chemical and mechanical stimuli.[7] Recently, a considerable increase in plasma levels of $PGF_2\alpha$ metabolites in asthmatic patients after allergen challenge was reported.[8] Based on these observations it was postulated that locally formed $PGF_2\alpha$ may play an important part in the pathogenesis of bronchial asthma.[5,8] PGE_2, on the other hand, causes bronchodilatation in man.[3,5]

PGE_2 activates adenyl cyclase, now identified with β-receptor function,[9-11] and its bronchodilator effect is mediated by an increase in cyclic adenosine monophosphate (cyclic AMP). Conversely, $PGF_2\alpha$ has been reported to activate guanyl cyclase and lead to the formation of cyclic guanosine monophosphate (cyclic GMP).[12] Guanyl cyclase activity has been found in various tissues, including human lung.[13] Cyclic GMP activates cholinergic responses,[14,15] and guanyl cyclase may also be activated by α-stimulation.[16] Cyclic GMP has been reported to have an opposing influence to cyclic AMP in regulating cell function, and according to Haddock *et al.*[17] the relationship of cyclic AMP and cyclic GMP in the lung may influence bronchomotor tone. In patients with asthma the normal balanced relationship of PGE_2 and $PGF_2\alpha$ may be altered, giving rise to variability in the bronchomotor tone.[18] In the light of the biochemical observations on prostaglandins I have studied the effects of PGE_2 and $PGF_2\alpha$ on forced expiratory volume in one second (FEV_1) and specific airways conductance (SGaw) in six patients with extrinsic bronchial asthma and tested the effects of atropine, the α-receptor-blocking drug thymoxamine, and sodium cromoglycate on $PGF_2\alpha$-induced bronchoconstriction in these patients.

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Patients, Materials, and Methods

Six patients aged 15 to 37 years with extrinsic bronchial asthma and reversible airways obstruction were studied. All reacted to prick tests with inhalant allergens and had a blood eosinophilia of over 500×10^6/l. Simple bronchodilators such as salbutamol and isoprenaline were stopped for at least 24 hours before the tests.

FEV_1 was recorded on a Garthur Vitalograph spirometer. Airways resistance (Raw) was measured in a constant-volume body plethysmograph at a flow rate of 0·5 l/s and a panting frequency of 2/s.[18] Conductance (Gaw), the reciprocal of airways resistance, was divided by the thoracic gas volume at which Raw was measured to give SGaw (s^{-1} kPa^{-1}). The mean of four recordings was calculated to give the SGaw for each step of the experiment.

Drugs.—A sterile aqueous solution of $PGF_2\alpha$ (as a tromethamine salt) 5 g/l was diluted with normal saline to give a concentration of 50 mg/l. Similarly, a stock solution of PGE_2 1 g/l was diluted with normal saline to give a concentration of 50 mg/l. Other drugs used were atropine sulphate (600 mg/l; Antigen Ltd.), thymoxamine hydrochloride (15 g/l; Warner & Co. Ltd.), and sodium cromoglycate (20 mg in powder form dispensed in a Spincapsule; Fisons Ltd.).

Procedure.—After establishing baseline values for FEV_1 and SGaw each patient inhaled about 0·5 ml of a $PGF_2\alpha$ solution through a Wright nebulizer using compressed air at a flow rate of 8 l/min. FEV_1 and SGaw were measured two minutes after inhalation and thereafter at five-minute intervals for 25 minutes. This test was repeated three times in all patients. Ten minutes before inhaling $PGF_2\alpha$ each patient inhaled 1·2 mg atropine sulphate or 40 mg sodium cromoglycate or 15 mg thymoxamine hydrochloride. Atropine sulphate and thymoxamine were inhaled through a Wright nebulizer, and sodium cromoglycate was inhaled using a Spinhaler. In all six patients the test was repeated after inhaling 0·5 ml of a PGE_2 solution through a Wright nebulizer. Placebo inhalations with normal saline were performed on each experimental day and the test procedure was carried out only when no significant change in FEV_1 and SGaw with normal saline was observed.

Results

Inhalation of $PGF_2\alpha$ produced maximum falls in the mean FEV_1 and SGaw of 27% and 53% respectively (tables I and II), which occurred five minutes after inhalation. These falls were highly significant ($P < 0.001$ and $P < 0.01$). Over the next 25 minutes the values gradually returned towards the baseline (figs. 1 and 2).

Inhalation of atropine sulphate increased the mean FEV_1 and SGaw by 9% and 68% respectively, the bronchodilatation produced being significant ($P < 0.025$) (tables I and II). $PGF_2\alpha$ inhalation 10 minutes later then reduced the mean FEV_1 and SGaw by 10% and 20% respectively. The maximum falls in FEV_1 and SGaw produced by $PGF_2\alpha$ differed significantly from those produced by $PGF_2\alpha$ after pretreatment with atropine sulphate ($P < 0.05$). This suggests that prior inhalation of atropine sulphate partially inhibited the bronchoconstriction induced by $PGF_2\alpha$.

Sodium cromoglycate inhalation did not produce a significant change in the mean FEV_1 or SGaw, and $PGF_2\alpha$ inhalation 10 minutes later produced maximum falls of 32% and 55% respectively. There was no significant difference between the maximum falls in FEV_1 and SGaw produced by $PGF_2\alpha$ alone and those produced after pretreatment with sodium cromoglycate ($P > 0.10$). This suggests that sodium cromoglycate had no effect on $PGF_2\alpha$-induced bronchoconstriction (tables I, II; figs. 1, 2).

Inhalation of thymoxamine produced no significant change in the mean FEV_1 or SGaw, and $PGF_2\alpha$ inhalation 10 minutes later produced maximum falls of 26% and 55% respectively. There was no significant difference between the maximum falls in FEV_1 and SGaw produced by $PGF_2\alpha$ alone and those produced by $PGF_2\alpha$ after pretreatment with thymoxamine ($P > 0.10$). This suggests that thymoxamine had no effect on $PGF_2\alpha$-induced bronchoconstriction

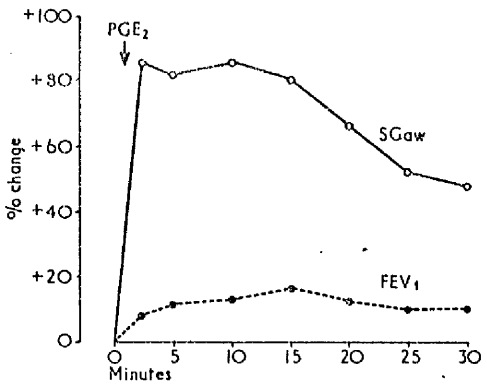

FIG. 3—Effect of PGE_2 inhalation on mean FEV_1 and SGaw in six patients with extrinsic bronchial asthma.

inhibited $PGF_2\alpha$-induced bronchoconstriction in these patients. In contrast, sodium cromoglycate and thymoxamine failed to change the mean FEV_1 and SGaw, and neither of these drugs had any effect on the $PGF_2\alpha$-induced bronchoconstriction. $PGF_2\alpha$ in some patients caused symptoms of upper airway irritation—cough, substernal tightness, and increased mucous secretion—similar to those experienced after methacholine inhalation.

$PGF_2\alpha$ is released from guinea-pig and rat lungs during anaphylactic reactions[6] and by various chemical and mechanical stimuli.[7] The release of $PGF_2\alpha$ during type I allergic reactions and the observation of increased sensitivity of asthmatic patients to inhaled $PGF_2\alpha$ led Mathé *et al.*[5] to postulate that endogenous, locally formed $PGF_2\alpha$ may play an important part in the pathogenesis of bronchial asthma. This view was supported by a report of about an eightfold increase in plasma levels of 15-keto-13,14-dihydroprostaglandin $F_2\alpha$, the main metabolite of $PGF_2\alpha$, in asthmatic patients after allergen challenge.[8] Local $PGF_2\alpha$ release in the lung has been suggested as the mechanism of exercise-induced asthma,[19] and the demonstration of this release in guinea-pig lungs in response to minor mechanical stimuli supports this view. The results of the present investigation, however, suggest that sodium cromoglycate does not inhibit $PGF_2\alpha$-induced bronchoconstriction in asthmatic patients.

This effect of sodium cromoglycate on $PGF_2\alpha$-induced bronchoconstriction together with the observations of its inhibitory effect in allergen-provoked[20] and exercise-provoked[21] asthma suggest that the release of prostaglandins locally in the lung may not be the primary factor in the pathogenesis of asthma. In addition, indomethacin administration, which considerably reduces the total body production of $PGF_2\alpha$, does not completely inhibit allergen-provoked asthma in man or anaphylactic reactions in guinea-pigs.[22]

The studies on cyclic nucleotide systems suggest that the effects of PGE_2 are mediated through β-adrenergic receptors, whereas $PGF_2\alpha$ may activate cholinergic receptors. The partial inhibition of $PGF_2\alpha$-induced bronchoconstriction by atropine as reported here is consistent with this hypothesis. α-Blockade with thymoxamine failed to inhibit the $PGF_2\alpha$-induced bronchoconstriction in these patients with asthma, which suggests that $PGF_2\alpha$ does not stimulate α-receptors in the lung. In man, cholinergic or vagal stimulation causes bronchoconstriction, which is grossly exaggerated in asthma.[23] The evidence so far suggests that $PGF_2\alpha$ together with other chemical mediators such as histamine, bradykinin, serotonin, and SRS-A are released during the type I allergic reaction. $PGF_2\alpha$ is a potent bronchoconstrictor to which asthmatic patients are highly sensitive, and it may act by stimulating cholinergic receptors. Airways hyperreactivity to chemical mediators released in the type I allergic reaction is now well recognized in patients with asthma. Though Szentivayni[24] suggested that this hyperreactivity in asthma may result from an imbalance between the α- and β-adrenergic receptors in the lung, the evidence to support this theory is not conclusive.[25] It would be wrong to consider $PGF_2\alpha$ as the main factor in the pathogenesis of asthma until we further clarify the cause of airways hyperreactivity, which appears to be the primary defect in asthmatic patients.

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Effect of alpha receptor blocking drug, thymoxamine, on allergen induced bronchoconstriction in extrinsic asthma

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Summary

In ten patients with extrinsic bronchial asthma, allergen provoked bronchospasm was significantly inhibited by the alpha receptor blocking drug thymoxamine given intravenously. In two of these patients thymoxamine by inhalation also effectively inhibited allergen induced bronchoconstriction. It is suggested that thymoxamine may be acting either by increasing intracellular levels of cyclic AMP and thus inhibiting mediator release following allergen challenge or by modifying the airways response to these mediators by altering the bronchomotor tone. The variable responses recorded after allergen challenge in presence of alpha blockade with thymoxamine suggests that the dominant effect is on the bronchomotor tone rather than the mediator release.

Introduction

It has recently been shown that the reagin mediated release of histamine and SRS-A can be inhibited by catecholamines which activate the membrane bound adenyl cyclase (now identified with beta receptor function) and lead to an increase in the cyclic adenosine monophosphate (cyclic AMP) formation (Lichtenstein & De Bernado, 1971; Orange, Austen & Austen, 1971). In acute asthma, it has been shown that the diminished leucocyte adenyl cyclase response to isoprenaline can be restored towards normal by alpha receptor blocking drugs, phentolamine and thymoxamine (Logsdon *et al.*, 1973; Alston, Patel & Kerr, 1974). Further, it has been reported that alpha adrenergic stimulation augment histamine and SRS-A release from human lung and isolated rat mast cells (Kaliner, Orange & Austen, 1972; Coffey & Middleton, 1973). The above observations have lead us to study the effect of thymoxamine, the most specific alpha receptor blocking drug available, on allergen induced bronchoconstriction in ten patients with extrinsic asthma.

Patients and methods

Ten patients, aged between 18 and 46 years, with extrinsic bronchial asthma and reversible airways obstruction were investigated. All patients had positive prick tests to inhalant allergens and a blood eosinophil count of over 500 cells/mm^3. Sodium

cromoglycate therapy was discontinued for 7–10 days and simple bronchodilator drugs stopped 12–24 hr before the tests.

Airways resistance (Raw) was measured with the help of a constant volume body plethysmograph (Dubois, Bothelho & Comroe, 1956) at a flow rate of 0·5 l/sec while the patient panted shallowly at 2 cycles/sec. Conductance, the reciprocal of Raw, was divided by the thoracic gas volume at which Raw was measured to give the Specific Airways Conductance (SGaw). The mean of four recordings was calculated to give the SGaw.

Allergen inhalation test

Standard solutions of house dust or pollen extract (500 protein nitrogen u/ml) were used. After recording the baseline SGaw, each patient inhaled an appropriate allergen solution through a Wright's nebulizer until he developed symptoms of airways obstruction. SGaw was recorded 5 min after the inhalation and thereafter at regular intervals for 60 min. On a different day (allowing at least 3 days between the tests), the allergen inhalation test was repeated in each patient after intravenous administration of thymoxamine (0·1 mgm/kg body weight). In two patients (nos 3 and 10) allergen challenge was repeated after inhalation of 15 mgm of thymoxamine (1·5%) through a Wright's nebulizer. In one patient (no. 10) the dose of allergen inhaled after thymoxamine was doubled.

Results

Allergen inhalation produced a significant fall in the mean SGaw in ten patients with extrinsic bronchial asthma ($P<0·01$). The maximal fall in the mean SGaw was 59% at 15 min and thereafter there was a gradual restitution in the SGaw (Table 1 and Fig. 1).

Following intravenous thymoxamine, a smaller fall in the mean SGaw was observed. The maximal fall in the mean SGaw was 35% at 25 min. The overall inhibition

Table 1. Effect of allergen challenge on SGaw in ten patients with extrinsic bronchial asthma

			Change in SGaw (l/cmH_2O sec. l)							
No.	Age	Sex	Allergen inhalation							
			Baseline	5 min	10 min	15 min	25 min	35 min	45 min	60 min
1	19	M	0·235	0·142	0·129	0·114	0·091	0·081	0·116	0·132
2	23	M	0·189	0·122	0·111	0·094	0·087	0·081	0·101	0·120
3	21	F	0·144	0·034	0·030	0·029	0·042	0·045	0·054	0·140
4	18	F	0·213	0·122	0·149	0·097	0·096	0·106	0·167	0·258
5	46	F	0·109	0·063	0·060	0·063	0·066	0·063	0·063	0·093
6	18	F	0·132	0·088	0·089	0·091	0·114	0·110	0·120	0·114
7	23	F	0·227	0·066	0·038	0·068	0·060	0·127	0·145	0·146
8	18	M	0·066	0·022	0·017	0·011	0·011	0·023	0·026	0·045
9	27	M	0·105	0·024	0·014	0·022	0·019	0·029	0·047	0·094
10	28	M	0·082	0·021	0·024	0·019	0·023	0·025	0·078	0·088
Mean			0·150	0·070	0·066	0·061	0·061	0·069	0·092	0·123
s.e. mean			0·020	0·014	0·015	0·012	0·011	0·012	0·014	0·018

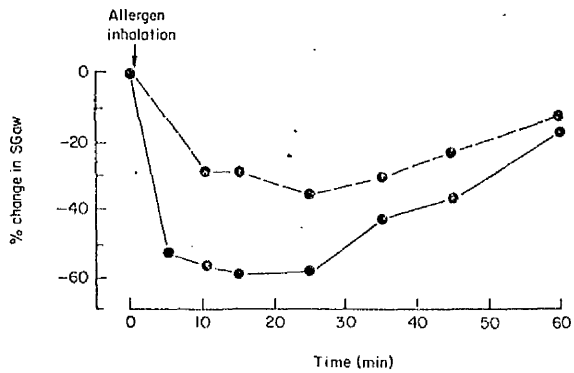

Fig. 1, The effect of intravenous thymoxamine on allergen induced fall in the SGaw in ten patients with extrinsic bronchial asthma. The fall in SGaw was partially inhibited by thymoxamine. ●——●, Allergen; ○— — —○, allergen + thymoxamine.

obtained with thymoxamine in these ten patients was statistically significant ($P<0{\cdot}025$, Tables 1 and 2, Fig. 1). However the effect of intravenous thymoxamine on allergen induced bronchoconstriction varied greatly in individual patients. In three patients (nos 2, 5 and 7), the allergen provoked bronchospasm was completely inhibited whereas in four patients (nos 4, 6, 8 and 9) thymoxamine had no effect in this respect. In the remaining three patients (nos 1, 3 and 10) thymoxamine partially inhibited allergen induced bronchoconstriction.

Thymoxamine given by inhalation also inhibited allergen provoked bronchoconstriction in two patients (nos 3 and 10) and in one of these patients (no. 10) this

Table 2. Effect of allergen challenge on SGaw after prior beta blockade with thymoxamine in ten patients with extrinsic bronchial asthma

	Change in SGaw (l/cmH_2O sec. l)							
No.						Allergen inhalation		
	Baseline	5 min	10 min	15 min	25 min	35 min	45 min	60 min
1	0·245	0·237	0·179	0·178	0·107	0·101	0·133	0·202
2	0·193	0·195	0·169	0·189	0·189	0·212	0·225	0·238
3	0·183	0·207	0·188	0·147	0·104	0·105	0·126	0·122
4	0·177	0·174	0·069	0·043	0·051	0·059	0·083	0·076
5	0·079	0·081	0·088	0·095	0·095	0·101	0·098	0·097
6	0·124	0·037	0·052	0·064	0·071	0·080	0·080	0·085
7	0·179	0·204	0·179	0·200	0·200	0·220	0·221	0·231
8	0·087	0·023	0·019	0·021	0·019	0·025	0·028	0·045
9	0·067	0·020	0·027	0·026	0·022	0·026	0·027	0·035
10	0·082	0·051	0·044	0·048	0·057	0·052	0·052	0·076
Mean	0·142	0·123	0·101	0·101	0·092	0·098	0·107	0·120
s.e. mean	0·019	0·028	0·022	0·022	0·020	0·022	0·022	0·024
t test		2·44	2·63	1·83	1·97	2·46	0·08	0·00
		0·025	0·025	0·05	0·05	0·025	N.S.	N.S.

protection was maintained even when the dose of allergen inhaled was doubled (Figs 2 and 3).

Thymoxamine given intravenously did not cause a significant fall in blood pressure in any of the patients and none complained of any side effects. However, thymoxamine by inhalation precipitates transient bronchospasm in some patients due to its local irritant effect on the airways.

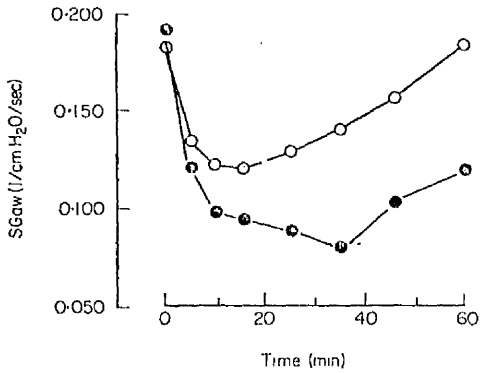

Fig. 2. The effect of thymoxamine by inhalation on allergen induced fall in the SGaw in patient no. 3. Thymoxamine effectively inhibited allergen induced bronchoconstriction. ●——●, Allergen; ○——○, allergen + thymoxamine.

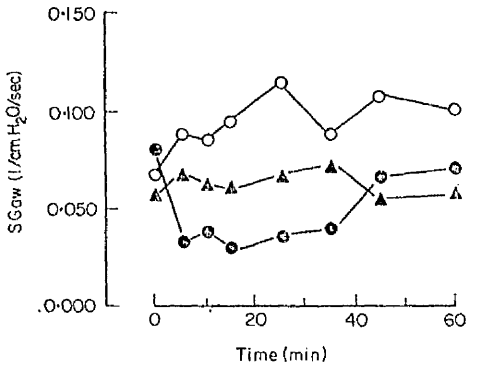

Fig. 3. The effect of thymoxamine by inhalation on allergen induced fall in the SGaw in patient no. 10. Thymoxamine effectively inhibited allergen induced bronchoconstriction. This protection was maintained even when the dose of allergen inhaled was doubled. ○——○, Thymoxamine + allergen; ●—— ●, allergen; ▲——▲, thymoxamine + allergen (× 2).

Discussion

It is now generally accepted that cyclic AMP functions as a second messenger for many hormonal actions including catecholamines (Robison, Butcher & Sutherland, 1971). The catecholamines activate membrane bound adenyl cyclase leading to accumulation of intracellular cyclic AMP. Cyclic AMP inhibits mediator release (histamine and SRS-A) from mast cells and is active in the first stage of type I allergic reaction which is not calcium dependent (Lichtenstein & De Bernado, 1971). Alpha receptor agonists have been shown to increase the release of histamine and SRS-A

in the type I allergic reaction (Kaliner *et al.*, 1972; Coffey & Middleton, 1973). These observations suggest that thymoxamine is acting by cyclic AMP formation (Logsdon *et al.*, 1973; Alston *et al.*, 1974) and in our experiments leading to inhibition of mediator release following allergen challenge.

Although the results are statistically significant, the wide variation in the responses following allergen challenge suggest that the action of thymoxamine may not be to inhibit the release of pharmacologically active substances but rather to modify the airways response to mediators released by altering bronchomotor tone. Patients with extrinsic asthma are hyper-reactive to chemical mediators released in the type I allergic reaction (Curry, 1946; Tiffeneau, 1955; Varonier & Panzani, 1968; Mathe *et al.*, 1973). This hyper-reactivity remains constant in individual patients (Cade & Pain, 1971) and persists for many years even in the absence of active asthma (Bouhuys *et al.*, 1970). It has been postulated that bronchial hyper-reactivity is due to a functional imbalance between the alpha and beta adrenergic receptors and results from a diminished beta receptor function in the lung (Szentivayni, 1968). Alpha adrenergic receptors have been demonstrated in human lung and stimulation of these receptors can cause bronchoconstriction (Prime *et al.*, 1972; Patel & Kerr, 1973). It has recently been shown that alpha receptor blocking drugs, including thymoxamine, inhibit histamine induced bronchoconstriction in patients with asthma (Kerr, Govindaraj & Patel, 1970; Bianco *et al.*, 1972). The type I allergic reaction is now known to release significant quantities of Prostaglandin $F_2\alpha$ (Green, Hedqvist & Svanborg, 1974), in addition to histamine, SRS-A, bradykinins and 5-hydroxytryptamine. The variable responses recorded after allergen challenge in presence of alpha blockade with thymoxamine could be accounted for by the observation that thymoxamine does not inhibit the effect of Prostaglandin $F_2\alpha$ on the airways (Patel, 1975) suggesting that the dominant effect of thymoxamine is on bronchomotor tone rather than on the mediator release. The effect of thymoxamine in the management of patients with extrinsic asthma requires further investigations to define its place in the treatment of asthma.

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THE EFFECT OF THYMOXAMINE AND SODIUM CROMOGLYCATE ON POST - EXERCISE BRONCHOCONSTRICTION IN ASTHMA.

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SUMMARY

Of the 22 patients with extrinsic bronchial asthma, 13 patients developed post-exercise bronchoconstriction after treadmill exercise whereas in 9 patients treadmill exercise had no effect on the ventilatory capacity. No statistical difference in the resting lung volumes and CO transfer factor was found between the two groups. A significant inhibition of post-exercise bronchoconstriction was observed in 12 out of 13 patients following thymoxamine or sodium cromoglycate inhalation.

In some asthmatic patients who show diminished beta receptor responsiveness to catecholamines, noradrenaline released during exercise could have a marked alpha agonistic effect giving rise to bronchoconstriction. Inhibition of post-exercise bronchoconstriction by alpha blockade with thymoxamine suggests that increased alpha adrenergic activity in the presence of diminished beta receptor function could be the mechanism of post-exercise bronchoconstriction in these patients. Sodium cromoglycate, with its cyclic phosphodiesterase inhibiting action, may act by increasing the levels of cyclic AMP and restoring the beta receptor responsiveness to catecholamines.

INTRODUCTION

Exercise induced bronchoconstriction is a well recognised phenomenon in bronchial asthma (Jones et al, 1963), and in some patients exercise may act as the predominant or even the only precipitating stimulus of bronchoconstriction. Using more sensitive methods it has been shown that vigorous exercise can cause a small but significant reduction in bronchial calibre of normal subjects (McNeill et al, 1966; Fisher et al, 1970; Anderson et al, 1971; Lefcoe et al, 1971). Inhibition of post-exercise airways obstruction by sympathomimetic amines (Jones et al, 1963; McNeill et al, 1966; Rebuck & Read, 1968; Crompton, 1968) suggests that it results from contraction of bronchial smooth muscle.

Recently there has been an increasing interest in the presence of alpha adrenergic receptors in the human bronchial tree and their role in the control of bronchomotor tone. Alpha adrenergic stimulation has been reported to cause bronchoconstriction in animals and man (Castro de la Mata et al, 1962; Everitt & Cairncross, 1969; Simonsson et al, 1971; Prime et al, 1972; Patel & Kerr, 1973). The hyper-reactivity of airways in patients with bronchial asthma may be due to increased alpha adrenergic activity (Falliers et al, 1971; Patel & Kerr, 1973). Noradrenaline, a powerful alpha receptor agonist, is released by strenuous exercise (Vendsalu, 1960; Kozlowski et al, 1973) and this release is enhanced if there is hypoxia and metabolic acidosis. In patients with bronchial asthma there is evidence of diminished beta receptor responsiveness to catecholamines (Inoue, 1967; Middleton & Finke, 1968; Parker & Smith, 1973; Alston et al, 1974). In this situation, post-exercise release of noradrenaline may give rise to alpha stimulation and lead to bronchoconstriction. However, Sly et al (1967) have shown that alpha adrenergic blockade with phentolamine has no effect on post-exercise bronchoconstriction. Phentolamine is a short acting alpha receptor blocking drug and it may produce inadequate blockade when given intravenously. Although sodium cromoglycate has been reported to be effective in preventing post-exercise bronchoconstriction (Muittari & Kreus, 1969), its mode of action in exercise induced bronchospasm still remains uncertain.

We have investigated the effect of thymoxamine, a specific alpha receptor blocking drug, on post-exercise bronchoconstriction in 13 patients with bronchial asthma. The effect of thymoxamine is compared to sodium cromoglycate in these patients, and the possible mode of action of both these drugs is discussed.

PATIENTS AND METHODS

Twenty two patients with extrinsic bronchial asthma and reversible airways obstruction who complained of dyspnoea or wheezing after moderate exertion were investigated. All patients had positive skin tests to inhalant allergens and a blood eosinophil count of over 500 cells per mm^3. Three patients were on sodium cromoglycate and others used simple bronchodilator drugs like salbutamol aerosol for relief of acute symptoms. Sodium cromoglycate was discontinued for 7-10 days and simple bronchodilator drugs stopped at least 24 hours before the tests.

Pulmonary Function Assessment

Total lung capacity (TLC) and Residual Volume (RV) were measured by helium dilution method in a closed circuit using a Godart Pulmotest. Vital Capacity (VC) and Forced Expiratory Volume 1 sec. (FEV_1) were measured with the help of a Garthur Vitalograph spirometer. All lung volumes were corrected for body temperature, pressure and saturated with water vapour (BTPS). Predicted values of lung volumes for adults were taken from Bates et al (1971) and those for children from Cotes (1965).

The resting gas transfer factor (D_LCO) was measured by the steady state method using carbon monoxide and an end tidal sampler (Bates et al, 1962). Predicted values for D_LCO at rest were taken from Bates et al (1971) and Cotes (1965).

Exercise test

Exercise tests were carried out in symptom free periods and after the preliminary pulmonary assessment had been completed. Each test consisted of steady state exercise of running on an inclined treadmill (10^o) for up to 8 minutes. The speed of treadmill was adjusted so that the patient's pulse rate at the end of exercise was at least 180 per min. FEV_1 was recorded at 2 minutes after exercise and thereafter at regular intervals for the next 30 minutes.

In thirteen patients (1 - 13) who developed post-exercise bronchoconstriction, the test was repeated after inhalation of saline, thymoxamine and sodium cromoglycate. The order of drug treatment was randomised and the observer (AMM) recording the FEV_1 after exercise was

unaware of the drug treatment. In the control test, the patient inhaled normal saline through a Wright's nebulizer using compressed air at a flow rate of 8 litres per minute. On a different day, the exercise test was repeated after inhalation of 15 mgm. of thymoxamine hydrochloride solution (1.5% aqueous solution) through a Wright's nebulizer. Thymoxamine has a bitter taste and causes transient bronchoconstriction in some patients which usually settles within 5-10 minutes. At a later date, the exercise test was repeated in each patient after inhalation of 40 mgm. of sodium cromoglycate through a spinhaler 15 minutes before the exercise.

RESULTS

The anthropometric and lung function data are given in table 1. Although all patients with bronchial asthma complained of dyspnoea on moderate exertion, only thirteen patients (1 - 13) developed a significant fall in FEV_1 after treadmill exercise. There was no statistical difference in the resting lung volumes and transfer factor (D_LCO) at rest between the patients who developed post-exercise bronchoconstriction and those patients in whom treadmill exercise had no effect on the ventilatory capacity.

In thirteen patients (1 - 13) the maximal fall in the mean FEV_1 after treadmill exercise was 35% and occurred at 5 minutes. Thereafter there was a gradual restitution in FEV_1 over the next 25 minutes (Fig. 1). The fall in FEV_1 was highly significant ($P < .001$).

When the patients were pretreated with thymoxamine, the maximal fall in mean FEV_1 was 5% and occurred 5 minutes after exercise. Thereafter the FEV_1 returned to the baseline value over the next 25 minutes. The fall in mean FEV_1 was statistically significant ($P < .05$). However, when the falls in FEV_1 induced by exercise in the control test and after thymoxamine treatment were compared, the inhibitory effect of thymoxamine on post-exercise bronchoconstriction was found to be highly significant (t test = 2.80, $P < .01$).

When the exercise test was repeated after inhalation of sodium cromoglycate, the maximal fall in mean FEV_1 was 10% and occurred 5 minutes after exercise. Thereafter the mean FEV_1 returned to the

minutes was significant ($P < .025$). However, when the falls in FEV_1 induced by exercise in the control test and after sodium cromoglycate treatment were compared, the inhibitory effect of sodium cromoglycate on post-exercise bronchoconstriction was found to be highly significant (t test = 3.0, $P < .01$).

The inhibitory effect of thymoxamine and sodium cromoglycate on post-exercise bronchoconstriction in patients with bronchial asthma was comparable and no statistical difference was found between the two drug treatments (t test = 0.3, $P > .10$).

DISCUSSION

Recently it has been shown that form, intensity and duration of exercise, and time intervals at which observations are made, are critical in assessing the effect of exercise on airways obstruction. It has been reported that high efficiency negative work, namely running at near maximal work loads for eight minutes, provides a more potent stimulus for bronchoconstriction than other patterns of exercise tests (Anderson et al, 1971; Fitch & Morton, 1971). Although all the patients with asthma in the present investigation complained of dyspnoea on moderate exertion, only 13 patients developed a significant fall in FEV_1 after treadmill exercise. Nine of the patients examined did not develop post-exercise bronchoconstriction, although in one patient (No. 19) post-exercise bronchoconstriction was demonstrated two years previously. There was no statistical difference in the resting lung volumes and CO transfer factor between the patients who developed post-exercise bronchoconstriction and those patients in whom treadmill exercise had no effect. Exercise response was measured by a fall in FEV_1, and if more sensitive method of measuring airways obstruction had been used, such as mid-expiratory flow rate, a higher incidence of post-exercise bronchoconstriction may have been obtained (McFadden & Linden, 1972; Paterson et al, 1973).

A significant inhibition of post-exercise bronchoconstriction was observed in 12 out of 13 patients following thymoxamine inhalation, and in these 12 patients a statistically comparable inhibition of post-exercise bronchoconstriction was also obtained with sodium cromoglycate. However, in one patient (No. 10) neither drug had any effect on post-exercise bronchoconstriction. Further, atropine sulphate given by inhalation also failed to inhibit exercise induced bronchospasm in this patient.

The mechanism of exercise induced bronchoconstriction in asthma remains uncertain. A wide variety of humoral mediators have been suggested. Histamine levels have been found to be normal or unrelated to the degree of post-exercise bronchoconstriction (Granerus et al, 1971; Mathews & Pan, 1970). Further, antihistaminic drugs have failed to

inhibit post-exercise bronchoconstriction (McNeill et al, 1966; Sly et al, 1967). The role of serotonin as a bronchoconstrictor in man is disputed (Michelson et al, 1958) and its antagonist, methysergide, does not alter the exercise response (Quanjer et al, 1971). Local prostaglandin release in the lung has been suggested, and the demonstration of this release in guinea-pig lungs in response to minor mechanical stimuli (Piper & Vane, 1971) would support this view. However, sodium cromoglycate does not inhibit prostaglandin induced bronchospasm in asthmatic patients (Smith, 1974; personal observations). The effect of sodium cromoglycate on prostaglandin induced bronchoconstriction, together with the observation of its beneficial effect in exercise induced asthma, would suggest that the release of prostaglandin locally in the lung may not be the primary factor in exercise induced bronchospasm.

Noradrenaline and adrenaline are released during strenuous exercise, and this release of catecholamines is greatly enhanced by prior adrenergic blockade with propranolol or oxprenolol (Irving et al, 1974). A similar enhanced release of catecholamines may also occur in asthmatic patients who show diminished beta responsiveness to catecholamines. Of the 13 patients with exercise induced airways obstruction, 12 showed a significant inhibition of post-exercise bronchoconstriction following thymoxamine inhalation. These observations suggest that increased alpha adrenergic activity in the presence of diminished beta receptor responsiveness could be the mechanism of post-exercise bronchoconstriction in these patients. The report by Jones (1972) that a proportion of normal subjects develop post-exercise bronchoconstriction in the presence prior beta blockade with propranolol would further suggest that alpha adrenergic stimulation by catecholamines could be the cause of post-exercise bronchoconstriction in these subjects. Recently, work on adenyl cyclase (identified with beta receptor function) has shown a diminished responsiveness of this enzyme system to catecholamines in some patients with asthma (Parker & Smith, 1973; Alston et al, 1974). In this situation, noradrenaline and adrenaline would have a marked alpha agonistic effect giving rise to

bronchoconstriction (Patel & Kerr, 1973). It has recently been suggested that sodium cromoglycate acts by inhibition of cyclic phosphodiesterase (Taylor et al, 1974), and leads to an increase in the levels of cyclic adenosine monophosphate (cyclic AMP). These observations are consistent with the hypothesis that post-exercise bronchoconstriction is caused by alpha adrenergic activity in the airways, and can be inhibited by alpha receptor blocking drugs and sodium cromoglycate.

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NO.	AGE (Yrs.)	SEX	HEIGHT (Cm.)	WEIGHT (Kgm.)	FEV_1 (litres)	FVC (litres)	RV (litres)	TLC (litres)	D_LCO (ml.CO/min./mm.Hg.)
1	13	M	160	64.2	2.32	3.70 (3.30)	0.77 (0.90)	4.47 (4.30)	31.2 (25.0)
2	14	M	140	39.0	1.56	1.76 (2.30)	0.65 (0.74)	2.50 (3.00)	19.0 (20.0)
3	11	M	137	40.9	1.61	2.27 (2.15)	0.62 (0.83)	3.10 (2.80)	16.1 (19.0)
4	11	M	152	50.0	2.03	2.72 (2.92)	0.75 (0.80)	3.47 (3.72)	17.2 (23.0)
5	13	F	156	56.3	1.95	2.98 (3.10)	0.72 (0.86)	3.70 (4.00)	14.9 (23.0)
6	15	F	160	61.3	2.45	3.80 (3.30)	1.55 (0.90)	5.35 (4.30)	25.9 (25.0)
7	11	F	145	44.0	2.27	3.02 (2.50)	0.93 (0.70)	3.95 (3.30)	11.1 (21.0)
8	23	F	159	70.0	3.40	4.40 (3.60)	1.00 (1.32)	5.40 (4.92)	19.0 (21.9)
9	15	M	174	59.0	2.78	4.61 (4.20)	0.97 (1.10)	5.58 (5.40)	21.0 (29.0)
10	21	F	167	80.9	1.64	3.09 (3.88)	2.07 (1.44)	5.16 (5.32)	17.5 (22.2)
11	11	M	144	30.9	1.32	2.18 (2.50)	0.86 (0.70)	3.04 (3.20)	18.1 (20.0)
12	19	F	165	78.63	2.67	3.74 (4.62)	0.61 (1.42)	4.35 (6.04)	19.7 (24.5)
13	21	M	174	70.0	3.31	4.78 (5.26)	1.13 (1.72)	5.91 (6.98)	27.7 (25.2)
14	27	F	165	62.3	2.59	3.71 (3.68)	1.65 (1.54)	5.36 (5.22)	19.1 (19.7)
15	11	M	135	34.0	1.70	2.45 (2.10)	0.60 (0.60)	3.05 (2.70)	18.9 (18.0)
16	9	M	122	26.0	1.35	1.80 (1.83)	0.50 (0.45)	2.30 (2.05)	12.0 (14.0)
17	10	M	135	27.2	2.05	2.50 (2.10)	0.70 (0.60)	3.20 (2.70)	18.0 (17.5)
18	9	M	155	27.2	1.43	1.62 (1.83)	0.77 (0.51)	2.39 (2.39)	16.5 (15.5)
19	15	M	168	50.0	3.24	4.65 (3.40)	1.03 (0.95)	5.68 (4.40)	26.9 (25.0)
20	12	M	155	46.3	2.45	2.75 (3.10)	0.70 (0.90)	3.45 (4.00)	17.5 (24.0)
21	12	M	144	40.9	1.62	2.43 (2.60)	0.55 (0.70)	2.98 (3.30)	13.8 (20.0)
22	21	F	158	40.9	1.33	2.47 (3.60)	2.04 (1.32)	4.51 (4.92)	13.3 (21.9)

(Figures in parentheses are the predicted values for each patient)

e 2: Effect of Thymoxamine and Sodium Cromoglycate on post-exercise bronchoconstriction in 13 patients with extrinsic bronchial asthm

n = 13		BASELINE	AFTER EXERCISE (mins.) 2	5	10	15	20	25	30
CONTROL TEST	Mean FEV_1	2.49	1.76	1.62	1.73	1.91	1.98	2.04	2.12
	SEM	0.20	0.24	0.20	0.18	0.14	0.15	0.16	0.16
	t test		5.26	8.90	5.93	5.61	3.47	3.39	2.54
	P		<.001	<.001	<.001	<.001	<.005	<.005	<.025
		B A							
THYMOXAMINE	Mean FEV_1	2.31 2.30	2.19	2.11	2.12	2.24	2.26	2.28	2.29
	SEM	0.15 0.14	0.20	0.17	0.17	0.19	0.18	0.20	0.21
	t test	0.23	0.857	1.88	1.53	0.441	0.360	0.151	0.525
	P	N.S.	N.S.	<.05	N.S.	N.S.	N.S.	N.S.	N.S.
SODIUM CROMOGLYCATE	Mean FEV_1	2.41	2.28	2.17	2.28	2.32	2.35	2.41	2.38
	SEM	0.19	0.23	0.21	0.21	0.22	0.21	0.21	0.20
	t test		1.19	2.46	1.14	0.81	0.54	0	0.27
	P		N.S.	<.025	N.S.	N.S.	N.S.	N.S.	N.S.

FEV_1 = in litres

SEM = 1 Standard Error of the Mean

N.S. = Not Significant

B: before thymoxamine inhalation.

A: after thymoxamine inhalation.

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EFFECT OF PROSTAGLANDIN F_2 alpha ON LUNG MECHANICS IN EXTRINSIC ASTHMA

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SUMMARY

In normal subjects inhalation of $PGF_{2\alpha}$ produced two qualitatively different airways responses. In five subjects there was a significant fall in SGaw without change in maximum expiratory flow rates, FEV_1 or CV. In contrast, the remaining three subjects showed a significant fall in flow rates and FEV_1 together with a significant increase in CV while their SGaw was unaffected. $PGF_{2\alpha}$ inhalation in six asthmatic patients produced a significant fall in maximum expiratory flow rates, FEV_1 and SGaw. These patients showed a dual response with individual variability in magnitudes of changes. It is suggested that differing responses may reflect the balance between the sympathetic and parasympathetic nervous controls of the airways, and that the diminished beta receptor activity in asthmatic patients may account for heightened bronchoconstrictor response to inhaled $PGF_{2\alpha}$ both centrally and peripherally in the bronchial tree.

Prostaglandin $F_2\alpha$ ($PGF_2\alpha$), a potent bronchoconstrictor, is released from guinea-pig and rat lungs during anaphylactic reaction and by various chemical and mechanical stimuli (Edmonds, Berry & Wyllie, 1969; Piper & Vane, 1969). This local release of $PGF_2\alpha$ together with the exquisite sensitivity of asthmatic patients has led Mathe, Hedqvist, Holmgren & Svanborg (1973) to postulate that endogenous, locally formed $PGF_2\alpha$ may play an important part in the pathogenesis of bronchial asthma. This hypothesis is further supported by the report of Green, Hedgvist & Svanborg (1974) who have shown an eight fold rise in the plasma levels of $PGF_2\alpha$ metabolites in asthmatic patients following allergen challenge. In addition, local release of $PGF_2\alpha$ has been suggested as the mechanism of exercise induced asthma (Paterson, Ahmad & Lefcoe, 1973).

Despite the importance of $PGF_2\alpha$ in asthma, most previous studies of its effect in the human lung have been limited to the measurement of FEV_1 and airways conductance (Mathe et al, 1973; Patel, 1975). In order to obtain additional information on the effect of $PGF_2\alpha$ on lung mechanics, dynamic and static lung volumes, specific airways conductance and closing volume were measured before and after $PGF_2\alpha$ inhalation in eight normal subjects and seven patients with extrinsic asthma.

PATIENTS AND METHODS

Seven patients aged between 15 and 30 years with extrinsic asthma and reversible airways obstruction were studied. All patients had positive prick tests to inhalant allergens and a blood eosinophilia of over 500 cells mm. Simple bronchodilator drugs like salbutamol and isoprenaline were stopped for at least 24 hours before the tests.

The control group consisted of eight volunteers aged between 18 and 30 years. They had no respiratory disease and there was no personal or family history of bronchial asthma or atopic disease. Five subjects in this group (1,2,5,7 & 8) were light smokers. Informed consent was obtained in each case.

The static lung volumes were measured by helium dilution method in a closed circuit using Godart Pulmotest. Forced Expiratory Volume in 1 second (FEV_1), Maximum Mid-expiratory Flow Rate (MMFR) and Vital Capacity (VC) were recorded on a Godart Expirograph. All lung volumes were corrected to body temperature, pressure, saturated with water vapour (BTPS).

Airways resistance (Raw) was measured with a constant volume body plethysmograph (Dubois, Bothelho & Comroe, 1956) at a flow rate of 0.5 litre sec^{-1} and a panting frequency of 2 Hz. Conductance, the reciprocal of Raw, was divided by the thoracic gas volume at which Raw was measured to give specific airways conductance (SGaw). The mean of four recordings was calculated to give SGaw for the event.

Closing volume (CV) was measured by a single breath nitrogen test as modified by Anthonisen, Danson, Robertson & Ross (1969). The equipment circuit and procedure has been described previously by Buist & Ross (1973). Both the inspiratory and expiratory flow rates were kept under 0.5 litre sec^{-1} by close observation of the rate of movement of the pen recording the volume trace on spirometer

spirometer/

chart. Throughout the expiration, gas was sampled at the mouth and nitrogen concentration was estimated using a nitrogen meter (Godart Nitrograph). The volume change was recorded using a potentiometer connected to the spirometer pulley. The nitrogen concentration in the expired gas and the VC were recorded on Y and X axis respectively of an X-Y plotter. The volume at which the nitrogen concentration rose sharply from the alveolar plateau (or Phase III) has been termed the closing volume (or Phase IV) and is expressed as the fraction of VC. The term closing capacity (CC) is used for the sum of CV and RV and is expressed as the fraction of Total Lung Capacity (TLC).

DRUGS

A sterile stock solution of $PGF_2\alpha$ (as a tromethamine salt), 5mgm/ml., was diluted with normal saline to give a final concentration of 50ugm/ml.

PROCEDURE

After measuring static lung volumes, FEV_1, MMFR, SGaw and CV each subject inhaled 0.5 ml. of $PGF_2\alpha$ solution through a Wrights nebulizer using compressed air at a flow rate of 8 litres per minute. The measurements were repeated five minutes after inhalation. The whole procedure in each subject took thirty minutes to carry out. The test procedure was similar in asthmatic patients, however, satisfactory CV tracings could not be obtained in these patients because of marked bronchoconstrictor response to $PGF_2\alpha$.

$PGF_2\alpha$ inhalation produced coughing, retrosternal tightness in five normal subjects (1 - 5) whereas the remaining three subjects (6 - 8) complained of dyspnoea and wheezing. In contrast, all patients with asthma developed more marked and prolonged broncho-constrictor response following $PGF_2\alpha$ inhalation.

Lung function changes before and after $PGF_2\alpha$ inhalation in normal subjects are given in Table I and II. Two qualitatively different responses could be distinguished in these subjects depending on the symptoms. In five subjects (1 - 5) with coughing and retrosternal tightness there was a highly significant fall in SGaw, 38%, ($P<.001$) whereas FEV_1, MMFR, & CV/TLC% were unaffected. The second group of subjects (6 - 8) showed a significant fall in FEV_1, MMFR, together with a significant increase in both CV/VC% and CC/TLC%. The mean fall in FEV_1 and MMFR was 8.3% ($P<.01$) and 15% ($P<.025$) respectively; and the mean CV/VC% and CC/TLC% increased by 152.0% ($P<.01$) and 27.6% ($P<.01$) respectively from the baseline values. However, $PGF_2\alpha$ did not cause a significant change in the mean SGaw in these subjects (Table III). These differing airways responses did not relate to the smoking habits of the subjects, and time-course experiments in four of these subjects failed to show any relationship to the duration of $PGF_2\alpha$ effect on the bronchial tree.

$PGF_2\alpha$ inhalation in all asthmatic patients but one (9) produced a marked fall in FEV_1, MMFR and SGaw with a significant increase in RV(Table II). The mean fall in FEV_1, MMFR and SGaw was 17.5% ($P<.05$), 19.7% ($P<.05$) and 62.0% ($P<.001$) respectively. The mean RV increased by 37.0% ($P<.05$) after $PGF_2\alpha$ inhalation. Apart from one patient (9) who showed a conductance response, all other patients showed a dual response with individual variability in magnitudes of changes.

In contrast, airways resistance assesses airflow obstruction in central airways there being little contribution from the peripheral airways to total resistance. Thus, conductance-response would seem to reflect $PGF_2\alpha$ induced smooth muscle contraction in relatively large airways whereas as flow rate response to reflect smooth muscle contraction in the peripheral airways. This hypothesis is further supported by observation of a significant increase in the CV in the flow-rate responders suggesting airways closure and air trapping.

Bouhuys and Woestijne (1970) have postulated that individual variations in airways response to histamine or hemp dust is principally determined by variations of sympathetic tone of the airways. According to this hypothesis a subject with flow rate response may have relatively few sympathetic fibres in peripheral airways so that the β adrenergic activity might be insufficient to counteract the bronchoconstrictive effect of histamine or hemp dust in these airways. Conversely, in a subject with conductance response, the sympathetic distribution might be predominantly to the smaller airways. Similarly in dogs, vagal stimulation has been shown to cause bronchoconstriction either centrally or peripherally (Bates, Macklem & Christie, 1971). Reed (1974) has suggested that broncho-motor tone in man is dependent on the balance between the cholinergic and sympathetic divisions of the autonomic nervous system. The observations in man and in dog suggest that the autonomic balance between the sympathetic and parasympathetic system may vary in the different parts of bronchial tree and hence account for differing airways response to histamine, hemp dust, $PGF_2\alpha$ or vagal stimulation. It is now well recognised that patients with asthma show a diminished β receptor response to catecholamines (Cookson & Reed, 1963; Middleton & Finke, 1968; Alston, Patel & Kerr, 1974). Hence, a diminished β receptor function in both the central and peripheral

peripheral/

airways in asthmatic patients would explain the marked broncho-constrictor effect of $PGF_2\alpha$ at both these sites. In addition, the diminished beta adrenergic responsiveness reflects a failing counter-regulating mechanism against bronchoconstricing mechanism and probably accounts for airways hyper-reactivity in asthma (Patel, Alston & Kerr, 1974). In such a situation, $PGF_2\alpha$ released locally by specific and non specific stimuli would cause marked bronchoconstriction both in the central and peripheral airways in asthmatic patients with significant air trapping.

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Table 1.

Lung Function Changes in Response to Inhaled Prostaglandin $F_2\alpha$ in 8 Normal Subjects

No.	Age	Sex	FEV_1 (litres)		V.C. (litres)		MMFR litres sec^{-1}		SGaw cm H_2O^{-1} sec^{-1}		RV (litres)		CV/VC%		CC/TLC%	
			B	A	B	A	B	A	B	A	B	A	B	A	B	A
	30	M	4.71	4.88	6.66	6.68	3.68	4.31	0.265	0.203	1.89	1.53	6.7	8.8	27.3	25.8
*	35	F	2.87	2.73	4.24	3.98	1.77	1.81	0.211	0.143	1.16	1.55	4.9	8.9	25.3	34.5
	18	F	3.64	3.74	4.08	4.00	4.08	4.44	0.234	0.132	0.84	0.96	4.8	4.7	21.0	23.1
*	27	F	3.33	3.06	4.60	4.44	2.30	2.16	0.211	0.113	1.21	1.31	13.0	10.8	31.1	31.5
*	18	M	4.88	4.72	5.71	5.68	5.72	5.37	0.279	0.152	1.66	1.40	8.2	7.7	28.9	26.0
*	24	M	5.05	4.59	5.66	5.34	6.29	5.34	0.248	0.258	1.08	0.97	5.3	14.0	20.5	27.2
*	26	M	3.22	3.01	4.23	4.18	3.25	2.32	0.166	0.149	1.66	1.83	4.6	11.1	31.5	38.1
	28	M	4.36	3.97	5.65	5.70	3.90	3.56	0.138	0.118	1.80	2.19	4.7	11.8	27.7	36.3
Mean			4.01	3.84	5.10	5.00	3.86	3.66	0.219	0.158	1.46	1.47	6.5	9.7	26.7	30.1
SEM			0.30	0.30	0.33	0.30	0.54	0.45	0.017	0.017	0.11	0.14	1.0	1.00	1.5	2.0
P				<0.05		N.S.		N.S.		<0.025		N.S.		<0.05		=0.0

* cigarette smokers.

SEM = 1 standard error of mean.

N.S. not significant.

B: Before $PGF_2\alpha$ inhalation.

A: After $PGF_2\alpha$ inhalation.

able II.

Lung function changes in response to inhaled Prostaglandin $F_2\alpha$ in 7 patients with bronchial asthma

c	Age	Sex	FEV_1 (litres)		V.C. (litres)		MMFR litres sec^{-1}		SGa_w cm H_2O^{-1} sec^{-1}		R.V. litres	
			B	A	B	A	B	A	B	A	B	A
9	24	M	4.64	4.64	6.00	5.98	3.87	4.12	0.167	0.087	1.25	1.6
0	28	M	2.05	1.43	5.21	3.61	0.83	0.40	0.084	0.033	2.64	3.3
1	15	M	3.29	2.80	4.62	3.97	2.25	1.69	0.174	0.056	1.46	1.9
2	22	M	2.05	1.98	3.33	3.16	1.11	1.17	0.051	0.035	1.67	1.8
3	23	F	4.09	3.21	5.89	5.01	2.80	2.00	0.114	0.033	1.65	1.8
4	30	M	3.27	3.11	5.09	4.97	2.55	1.99	0.174	0.054	2.27	2.3
5	29	M	3.32	1.51	6.30	4.28	1.54	0.61	0.102	0.035	2.32	4.8
ean			3.24	2.67	5.21	4.42	2.13	1.71	0.124	0.048	1.89	2.5
E.			0.36	0.42	0.38	0.36	0.40	0.46	0.018	0.007	0.19	0.4
				< .05		< .05		< .05		< .01		< .0

E. = 1 standard error of mean.

= Before $PGF_{2\alpha}$ inhalation.

= After $PGF_{2\alpha}$ inhalation.

TABLE III

Lung function changes depending on symptoms following $PGF_2\alpha$ inhalation in normal subjects

	ΔFEV_1	ΔVC	$\Delta MMFR$	$\Delta SGaw$	ΔRV	$\Delta CV/VC\%$	$\Delta CC/TLC\%$
Subjects (1 – 5)	−0.06	−0.10	−0.02	−0.091	−0.09	+0.67	+1.19
with retrosternal	(0.08)	(0.05)	(0.20)	(0.012)	(0.15)	(1.07)	(2.19)
tightness	−1.5%	−2.0%	−1.0%	−38.0%	+6.6%	+8.9%	+4.5%
and coughing	N.S.	N.S.	N.S.	<.001	N.S.	N.S.	N.S.
Subjects (6 – 8)	−0.35	−0.11	−0.67	−.009	+0.17	+7.40	+7.32
with wheezing	(0.07)	(0.11)	(0.17)	(.009)	0.11	(0.50)	0.63
	−8.3%	−2.1%	−15.0%	−4.8%	+11.2	+152.0%	+27.6%
	<.01	N.S.	<.025	N.S.	N.S.	<.01	<.01

N.S. Not significant

Values in parenthesis = 1 standard error of mean

Δ Change