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THE AUTONOMIC NERVOUS SYSTEM AND BRONCHIAL ASTHMA

JEREMY MICHAEL KALLENBACH

A dissertation submitted to the Faculty of Medicine, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosopy.

Johannesburg, 1987

DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

J Kallenbach

Jeremy Michael Kallenbach

3 Sept 1987

Date

בס"ד

TO MY WIFE, RENEE

"..... ויהיו בעיניו כימים אחדים באהבתו אתה ;"

ABSTRACT

Autonomically mediated non-pulmonary responses to various stimuli were studied in asthmatic and non-asthmatic individuals. In addition, the circadian changes in a number of autonomic and hormonal variables affecting airway calibre and their relation to circadian changes in peak expiratory flow rate were compared in similar groups of subjects.

I. THE PARASYMPATHETIC COMPONENT OF THE AUTONOMIC NERVOUS SYSTEM:

This was studied by examining the heart rate variations induced by deep breathing (the magnitude of respiratory sinus arrhythmia), the Valsalva manoeuvre, and standing up from the recumbent position, all of which are parasympathetically mediated. The asthmatic patients had evidence suggesting enhanced parasympathetic neural output to the sino-atrial node, as manifested by a significantly greater magnitude of respiratory sinus arrhythmia than the normal control subjects. The heart rate responses to both the Valsalva manoeuvre and to standing up from the recumbent position were not, however, significantly different in asthmatic patients and normal subjects. No increase in the magnitude of respiratory sinus arrhythmia could be induced in normal subjects by breathing through an external resistance, suggesting that the finding was not a direct consequence of fluctuations in intrathoracic pressure related to airway obstruction. Non-asthmatic atopic subjects had a magnitude of

respiratory sinus arrhythmia which was intermediate between, but not significantly different from that of either the asthmatic patients or the normal control subjects.

A statistical analysis suggested the presence of a direct relationship between the magnitude of respiratory sinus arrhythmia and the degree of bronchial hyperreactivity in a group of asthmatic patients of varying severity. However, the suggestion of an enhancement of the parasympathetic neural output to the sino-atrial node in asthmatic patients could not be confirmed by a study of the "vagolytic" effect of atropine, which resulted in similar increments in the heart rates of both asthmatic and non-asthmatic subjects.

II. THE ALPHA-ADRENERGIC COMPONENT OF THE SYMPATHETIC NERVOUS SYSTEM:

As it was not possible to use a potent parenterally administered alpha-adrenergic agonist, the alpha-adrenergic component of the sympathetic nervous system was studied by examining the metabolic and cardiovascular responses to the alpha-2 agonist, clonidine. There was no difference in the responses of asthmatic and non-asthmatic individuals in the plasma growth hormone, glucose and potassium levels, or in the systolic and diastolic blood pressures following the administration of a 150 ug oral dose. A number of the asthmatic patients, however, exhibited marked plasma growth hormone hyperresponsiveness, and it was felt that they might possibly represent a subgroup of asthmatic patients in which alpha-

adrenergic hypersensitivity was an important pathogenetic mechanism. For this reason, the patterns of growth hormone secretion in asthmatic patients were investigated further.

The responses to exercise in the plasma growth hormone and prolactin levels, both of which appear to be alpha-adrenergically mediated, as well as those in the levels of adrenocorticotropic hormone (ACTH) and cortisol were studied. The asthmatic patients were each studied twice; following the administration of anti-asthmatic premedication which effectively prevented significant exercise-induced bronchospasm, and without premedication. The asthmatic patients appeared to have a diminished plasma cortisol response to exercise, although this was only statistically significant when exercise-induced bronchospasm was prevented by premedication. This finding did not appear to be explicable on the basis of corticosteroid therapy. There were no consistent differences in the other hormonal variables studied. Previous studies of the glucocorticoid response to exercise in asthmatic patients have yielded markedly conflicting results, and it is felt that confirmation of the present data is required before they can be considered in relation to the pathogenesis of asthma.

There was no difference between asthmatic and non-asthmatic subjects in the plasma growth hormone responses to exercise, and likewise, no significant difference in nocturnal growth hormone levels. A number of growth hormone "hyperresponders" and "hyporesponders" to the various stimuli studied appeared to be present among both the asthmatic and non-asthmatic subjects, and it is felt that the examination of growth hormone responses was not a

suitable method for the study of the alpha-adrenergic component of the autonomic nervous system.

III. THE BETA-ADRENERGIC COMPONENT OF THE SYMPATHETIC NERVOUS SYSTEM:

This was studied by examining the metabolic and cardiovascular responses of asthmatic and non-asthmatic subjects to the beta-2 agonist, salbutamol. The asthmatic patients had a significantly greater rise in plasma insulin levels than the normal control subjects following the intravenous administration of salbutamol. There were no consistent significant differences in the responses in the levels of plasma free fatty acids, glucose or potassium, the systolic and diastolic blood pressures, the pulse pressure or the heart rate.

No definite explanation can be given for the increased insulin response in the asthmatic patients, a surprising manifestation in them of beta-adrenergic hypersensitivity. The present findings emphasise that autonomic events occurring in the respiratory tract are not necessarily a reflection of those occurring in other remote sites, as well as the need for caution in the interpretation of data obtained from the autonomic studies in asthma.

IV. THE CIRCADIAN CHANGES IN AUTONOMIC AND HORMONAL VARIABLES IN RELATION TO CIRCADIAN CHANGES IN AIRWAY CALIBRE:

The mechanism of "morning dipping" in asthma was investigated by analysing circadian variations in sympathetic

"activity" as reflected by the plasma epinephrine and norepinephrine levels, the parasympathetic "activity" as reflected by the heart rate and magnitude of respiratory sinus arrhythmia, the plasma levels of cortisol and ACTH, and the serum neutrophil chemotactic activity in asthmatic patients and normal subjects.

Plasma cortisol levels varied significantly during the study in all groups of subjects. The midnight plasma cortisol levels were significantly lower in the asthmatic patients with significant "dipping" than in either those without significant "dipping" or the normal subjects. The midnight plasma cortisol levels of the "non-dippers" were not significantly different from those of the normal subjects. The "dippers" exhibited greater circadian variations in the cortisol levels than either the "non-dippers" or the normal subjects, whereas the magnitude of the circadian variations in the "non-dippers" resembled that of the normal subjects. There was a strong inverse correlation between the midnight plasma cortisol levels and the magnitude of the subsequent early morning fall in peak expiratory flow rate in the asthmatic patients.

There was no significant variation in the plasma epinephrine and norepinephrine level or in the serum neutrophil chemotactic activity during the study, nor any difference between the groups of subjects in any of these variables.

Although maximum, mean and minimum heart rate, and the magnitude of respiratory sinus arrhythmia exhibited a significant degree of variation during the study only in the asthmatics with

"dipping", there was no difference between the groups of subjects in any of these variables. None of the electrocardiographic variables correlated, moreover, with the magnitude of the fall in the peak expiratory flow rate in the asthmatic subjects. The magnitude of respiratory sinus arrhythmia appeared to diminish in the patients with "dipping", despite the simultaneous occurrence of bronchoconstriction, suggesting that the latter was not related to an increase in parasympathetic neural output.

Although the possibility could not be excluded that the lower midnight plasma cortisol levels observed in the asthmatic patients with "dipping" were related to therapy with beclomethasone dipropionate, the finding in them of normal plasma levels of ACTH mitigated against a depressant effect of the compound on hypothalamic-pituitary-adrenal function. The present findings suggest that the relation of the plasma cortisol level to the bronchospasm which occurs under various clinical and experimental conditions requires elucidation. However, the effect of beclomethasone dipropionate on hypothalamic-pituitary-adrenal function in adults similarly requires further investigation.

Some of the work described in this thesis has appeared in the following publications:

Kallenbach JM, Joffe BI, Seftel HC, Zwi S. Comparison of the metabolic responses to alpha-adrenergic stimulation in asthmatics and non-asthmatics after clonidine. S Afr Med J 1984; 65:131-134.

Kallenbach JM, Webster T, Dowdeswell R, Reinach SG, Scott Millar RN, Zwi S. Reflex heart rate control in asthma. Evidence of parasympathetic overactivity. Chest 1985; 87: 644-648.

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The studies of serum neutrophil chemotactic activity were performed by Professor R Anderson, head of the Department of Immunology, University of Pretoria.

Dr G Reinach, head of the Institute for Biostatistics of the Medical Research Council did the statistical analyses for the studies described in Chapters 8.2 and 8.5, and part of the statistical analysis for that described in Chapter 11. The remainder of the statistical analyses were done by Mr W van der Walt, Chief Statistician of the National Food Research Institute of the Council for Scientific and Industrial Research.

Although numerous colleagues assisted with the studies, Dr David Jankelow was most closely involved.

Mrs Oria Cohen typed the thesis and provided invaluable secretarial assistance, and Drs Philip Pincus and Gideon Naude assisted me with proof-reading.

PREFACE

The airways of asthmatic patients characteristically exhibit hyperreactivity to diverse bronchoconstrictor stimuli. Although the precise cause of the phenomenon remains uncertain, a considerable amount of experimental evidence suggests that it may be related to some abnormality in the autonomic control of airway calibre which results in a predisposition to bronchoconstriction. As the possibility exists that any such abnormality could be part of a generalised autonomic derangement, the object of this thesis was to compare various aspects of autonomic nervous function in groups of asthmatic and non-asthmatic subjects. A review of the autonomic nervous innervation of the airways and of the function and regulation of autonomic receptors is followed by a description of the investigations performed. The descriptions of the investigations pertaining to a particular component of the autonomic nervous system are preceded in each case by a review of the existing experimental evidence, linking it to the pathogenesis of bronchial asthma.

The studies presented in this thesis were completed by March, 1986 and the reviews by December, 1986.

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SECTION I

INTRODUCTION

CHAPTER 1

THE PATHOGENESIS OF BRONCHIAL HYPERREACTIVITY IN ASTHMA

The pulmonary airways of asthmatic patients characteristically exhibit hyperreactivity to numerous physical, chemical and pharmacological stimuli, the manifestation of which is the occurrence of reversible airway obstruction. The acuteness of onset of this response suggests that it is related mainly to smooth muscle contraction. The precise cause of airway hyperreactivity remains uncertain, despite being the subject of a considerable amount of research and speculation. A number of mechanisms, however, appear most likely to be responsible for the phenomenon (Boushey, 1980; Barnes, 1983). These are:

i. Diminished baseline airway calibre:

As the resistance to pulmonary airflow is inversely related to the fourth power of the airway radius, further constriction of an abnormally narrow airway results in a disproportionate increase in airway resistance when compared with the response obtained in a normal airway. The demonstration, however, of airway hyperreactivity in patients without pre-existing airway obstruction, a relatively common finding in asthmatic patients in remission, suggests that decreased baseline airway calibre is a contributory, rather than an independent factor.

ii. Abnormality of the airway epithelium:

Acute viral infections of the respiratory tract have been shown to cause temporary airway hyperreactivity in non-asthmatic subjects, possibly as a result of damage to the epithelium, which allows increased access to the underlying sensory receptors, or to the smooth muscle cells themselves,

of various irritative stimuli. In a similar manner, a number of inflammatory mediators, including the different products of arachidonic acid metabolism and the various compounds associated with IgE-mediated hypersensitivity reactions, may be important in perpetuating a state of abnormally increased airway "permeability" in asthmatic patients.

iii. Abnormality of airway smooth muscle:

Hypertrophy and hyperplasia of smooth muscle are known to occur in the airways of asthmatic patients. Whether these are primary abnormalities or the results of chronic smooth muscle contraction, they may contribute to airway hyperreactivity.

iv. Abnormality of cellular calcium ion transport:

It has been suggested that patients with asthma may have a generalised increase in the transport of calcium ions across cell membranes. This could result in an enhancement in the contractility of smooth muscle as well as of mucus secretion and mast cell mediator release, each of which may be involved in the pathogenesis of asthma, and are, moreover, critically calcium ion dependent.

v. Disorders in the autonomic control of airway calibre:

That airway hyperreactivity in asthma may be related to some disorder in the autonomic control of airway calibre which predisposes to airway narrowing, has long been a popular hypothesis. The tone of airway smooth muscle is finely modulated by both the sympathetic and the parasympathetic components of the autonomic nervous system.

In addition, a non-adrenergic non-cholinergic (NANC) system which has recently been shown to be present in human airways appears to be important in the control of airway calibre. While both cholinergic and alpha-adrenergic agonists stimulate the contraction of airway smooth muscle, beta-adrenergic agonists cause it to relax. The NANC system may be involved in both inhibitory and contractile smooth muscle responses. Overactivity of any of the stimulatory components, or underactivity of any of the inhibitory components of this autonomic regulatory system could theoretically predispose to airway narrowing and hyperreactivity.

Autonomic mechanisms are al o closely involved in the control of the secretions of the airway glands and of the release of mediators from mast cells, abnormalities of both of which may be important in the pathogenesis of bronchial asthma.

SECTION II

THE AUTONOMIC NERVE SUPPLY AND RECEPTORS OF THE RESPIRATORY TRACT

CHAPTER 2

PARASYMPATHETIC MECHANISMS

2.1 THE PARASYMPATHETIC INNERVATION OF THE RESPIRATORY TRACT

The parasympathetic responses occurring in the airways are mediated by the local release of acetylcholine from parasympathetic nerve endings. The parasympathetic efferent nerve supply to the respiratory tract arises from the dorsal motor nucleus of the vagus in the floor of the fourth ventricle of the brain, and runs in the vagal trunks (Widdicombe and Sterling, 1970). These preganglionic fibres synapse in ganglia located within the extrachondral and subchondral plexuses of the airway walls and in relation to the larger pulmonary blood vessels (Widdicombe, 1963; Spencer and Leof, 1964). The postganglionic fibres are distributed among the smooth muscle cells of the airways and a rich network of acetylcholinesterase-containing fibres is present from the central airways to the level of the terminal bronchioles (Partanen et al. 1982; Sheppard MN et al. 1983).

In the bovine trachea, postganglionic axons appear to be relatively sparse, with one axon occurring in relation to approximately ninety smooth muscle cells (Cameron and Kirkpatrick, 1977). The apparent lack of axons in direct proximity to muscle cells suggests the possibility that acetylcholine is released into the clefts between muscle bundles, resulting initially in the depolarisation of the more peripheral cells with subsequent excitation of the rest of the bundle (Cameron and Kirkpatrick, 1977). While axons appear to be similarly sparse in the human trachea, their density in the

smaller bronchi is considerably greater - one axon being related to approximately three muscle cells (Daniel et al. 1986). In addition to supplying the airway smooth muscle, parasympathetic fibres have been shown to terminate at the level of the mucous glands of the airways, where acetylcholinesterase-containing nerve fibres run around and between the bronchial glandular acini (Partanen et al. 1985).

Electrical stimulation of the vagal trunks results in a rapid increase in the resistance to airflow, the response being typically cholinergic with inhibition by atropine, and enhancement by cholinesterase inhibitors (Colebatch and Halmagyi, 1963; Olsen et al. 1965). The reaction occurs within a second, reaching a maximum within six seconds, the control value being, likewise, rapidly regained after the cessation of stimulation (Olsen et al. 1965). This time-course suggests the relation of the response to smooth muscle contraction, rather than to an increase in glandular mucus secretion, and rapid freezing of the airway during vagal stimulation has demonstrated that this is, in fact, the case (Olsen et al. 1965). The contraction of smooth muscle in response to vagal stimulation has been shown to occur from the trachea to at least the level of the 0,5-1mm diameter bronchioles (Nadel et al. 1971).

Diverse stimuli originating in multiple sites in and remote from the airway result in reflex alterations in bronchomotor tone by modulating parasympathetic neural output. Thus:

i. Mechanical or chemical stimulation of the nose and epipharynx produces a modest fall in airway resistance

(Tomori and Widdicombe, 1969).

ii. Inflation of the lungs results in reflex relaxation of the tracheal muscle but large inflation volumes may also stimulate irritant receptors, resulting in the constriction of both lower and upper airway smooth muscle (Widdicombe, 1966).

iii. A marked increase in total airway resistance results from the application of various physical and chemical irritative stimuli to the larynx, trachea and intrapulmonary airways (Nadel and Widdicombe, 1962; Nadel and Widdicombe, 1963; Nadel et al. 1965; Tomori and Widdicombe, 1969; Boushey et al. 1972).

iv. Tracheal smooth muscle tone exhibits a small but significant inverse relationship to arterial pressure, which is mediated by reflexes involving carotid sinus baroreceptors (Nadel and Widdicombe, 1962).

v. Arterial hypoxaemia and hypercapnia increase both lower and upper airway resistance by an effect on the carotid body chemoreceptors (Nadel and Widdicombe, 1962).

The sensory fibres involved in those reflexes which originate in the airway appear to lie mainly within the vagal trunks (Sant'Ambrogio, 1982). Some afferent activity has been recorded in the sympathetic nerves of dogs, however, (Kostreva et al. 1975). These fibres may be partly involved in the mediation of the rapid, shallow respiration that is produced by irritant substances such as bradykinin (Coleridge et al. 1983). The sensory receptors involved are probably closely related to the

intra-epithelial nerves which have been shown to be present in sites both adjacent to the lumen and to the basement membrane of the larger airways in humans (Laitinen, 1985).

A number of functionally different receptors appear to be present in the airways. These are: (i) the "rapidly-adapting irritant receptors with myelinated afferent fibres", (ii) the "C-fibre receptors associated with non-myelinated afferent fibres", (iii) the "slowly-adapting receptors" (Widdicombe, 1985). The latter are associated with the Hering-Breuer inflation reflex, and with the reflex bronchodilatation and cardiac acceleration produced by airway distension, and do not seem to be stimulated by pathological processes in the respiratory tract. By contrast the "rapidly-adapting receptors" are strongly stimulated by a variety of pathological, mechanical chemical processes. The "C-fibre receptors" appear to be similarly "polymodal" being, however, somewhat less sensitive to mechanical stimuli (Widdicombe, 1985). Like the response associated with electrical vagal stimulation, the time-course of the reflex increase in airway resistance produced by irritative stimuli is rapid (Nadel et al. 1965; Tomori and Widdicombe, 1969), suggesting that it is due to smooth muscle contraction.

In the absence of any stimuli, there is normally a degree of resting airway smooth muscle tone, which has been shown in animals to be related to tonic parasympathetic activity (Nadel and Widdicombe, 1963; Widdicombe, 1966). The increase in airway calibre that occurs in normal human subjects following the administration of cholinergic antagonists (Nadel and Widdicombe,

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A number of functionally different receptors appear to be present in the airways. These are: (i) the "rapidly-adapting irritant receptors with myelinated afferent fibres", (ii) the "C-fibre receptors associated with non-myelinated afferent fibres", (iii) the "slowly-adapting receptors" (Widdicombe, 1985). The latter are associated with the Hering-Breuer inflation reflex, and with the reflex bronchodilatation and cardiac acceleration produced by airway distension, and do not seem to be stimulated by pathological processes in the respiratory tract. By contrast the "rapidly-adapting receptors" are strongly stimulated by a variety of pathological, mechanical and chemical processes. The "C-fibre receptors" appear to be similarly "polymodal" being, however, somewhat less sensitive to mechanical stimuli (Widdicombe, 1985). Like the response associated with electrical vagal stimulation, the time-course of the reflex increase in airway resistance produced by irritative stimuli is rapid (Nadel et al. 1965; Tomori and Widdicombe, 1969), suggesting that it is due to smooth muscle contraction.

In the absence of any stimuli, there is normally a degree of resting airway smooth muscle tone, which has been shown in animals to be related to tonic parasympathetic activity (Nadel and Widdicombe, 1963; Widdicombe, 1966). The increase in airway calibre that occurs in normal human subjects following the administration of cholinergic antagonists (Nadel and Widdicombe,

1963; De Troyer et al. 1979) confirms that this situation applies as well in man, the effect being mainly due to dilatation of the larger more central airways (Ingram et al. 1977; Hensley et al. 1978).

In addition to its modulatory effect on smooth muscle tone, the parasympathetic nervous system regulates the output of the secretions of the mucous glands of the airway epithelium. Different irritative stimuli applied to the nose, pharynx, larynx and lower airway produce a reflex increase in the output of mucus from the tracheobronchial glands (Phipps and Richardson, 1976; German et al. 1980), and this response is inhibited by atropine and by cooling of the vagal trunks (German et al. 1980). The sensory receptors for these reflexes also appear to be the "rapidly-adapting" and "C-fibre receptors" (Widdicombe, 1985), and the efferent fibres to lie within the vagal trunks. In vitro and in vivo electrical and pharmacological studies in a number of mammalian species, including man, have confirmed that cholinergic mechanisms are at least partly involved (Davis et al. 1976; Shelhamer et al. 1980a; Borson et al. 1980; Borson et al. 1984; Leikauf et al. 1984). In addition, it has been shown that acetylcholine stimulates the movement of sodium and chloride ions into the lumen of the human airway in vitro (Knowles et al. 1984).

2.2 MUSCARINIC RECEPTORS

There is ample evidence of the existence of muscarinic

receptors in the mammalian respiratory tract. Studies using the radioligand (3H)quinuclidinyl benzilate have demonstrated their presence in the canine trachea (Murlas et al. 1982) as well as in bovine trachea and peripheral lung (Cheng and Townley, 1982). The density of the receptors appears to be considerably greater in the upper airway than in peripheral lung, and the contractile responses of the preparations to methacholine correlate with receptor density (Cheng and Townley, 1982). There also appears to be a correlation between the ability of different muscarinic agonists to inhibit the binding of the radioligand to canine tracheal membranes, and their relative contractile potency (Murlas et al. 1982). Radioligand binding to muscarinic receptors has also been demonstrated in fresh human lung obtained from surgical specimens (Raaijmakers et al. 1983).

Recent light microscopic autoradiographic studies have given an indication of the differential distribution of muscarinic receptors among the numerous cell types of the mammalian respiratory tract. The density of muscarinic receptors in the respiratory tract of the ferret is greatest in the smooth muscle of the trachea and the intrapulmonary cartilaginous airways and in the submucosal glands, and considerably less in bronchiolar smooth muscle, airway epithelium and vascular smooth muscle. There appear to be no receptors in the alveoli (Barnes et al. 1983a; Barnes et al. 1983c).

CHAPTER 3

SYMPATHETIC MECHANISMS

3.1 THE SYMPATHETIC INNERVATION OF THE RESPIRATORY TRACT

The sympathetic nerve supply to the mammalian respiratory tract is generally less profuse than the parasympathetic supply but is characterised by a considerable variation between different species (Doidge and Satchell, 1982). As with the lower primates (El-Bermani, 1978), the adrenergic innervation of the airways and lungs of humans appears to be sparse (Doidge and Satchell, 1982).

The sympathetic outflow to the lungs arises from the upper four or five thoracic segments of the spinal cord (Widdicombe and Sterling, 1970). In contrast to parasympathetic fibres, sympathetic fibres tend to synapse in ganglia distant from the organ being innervated. In the case of the pulmonary sympathetic nerves, the preganglionic fibres relay mainly in the stellate and mid-cervical ganglia, from which postganglionic fibres pass to the lungs, mingling with the parasympathetic nerves, and forming part of the extra- and subchondral nerve plexuses of the airway (Mann, 1971) before undergoing distal distribution.

In humans as well as other mammals, postganglionic sympathetic fibres terminate in ganglia located within the airway wall (Richardson, 1979; Doidge and Satchell, 1982). While there is definite evidence in a number of mammalian species of direct postganglionic sympathetic nervous innervation of both airway wall glands and smooth muscle, some workers have been unable to demonstrate catecholamine-containing nerves in human bronchi or lungs despite these being readily demonstrable in control

sections of guinea-pig trachea (Richardson and Beland, 1976). This has led to the suggestion that no similar nerve supply exists in man (Richardson, 1979), and that the pulmonary sympathetic nerve supply is primarily involved with vascular structures (Widdicombe and Sterling, 1970; Doidge and Satchell, 1982; Widdicombe, 1985). However, both histochemical and electron microscopic studies have confirmed the presence of catecholamine-containing nerves in the human airway. They appear to be most abundant in relation to the bronchial glands where their amount and distribution is similar to that of the cholinergic fibres (Partanen et al. 1982), and are, in addition, present in relation to both blood vessels and airway smooth muscle (Partanen et al. 1982; Pack and Richardson, 1984; Laitinen A et al. 1985a). In addition, the presence of adrenergic fibres has been demonstrated by means of catecholamine antibodies in the upper respiratory tracts of various mammalian species, including man, where they are related to both smooth muscle and blood vessels, and to a lesser extent in the lungs, in relation to the vasculature (Sheppard MN et al. 1983).

The neurotransmitter of the sympathetic nervous system is norepinehprine, a potent alpha-adrenergic, but weak beta-adrenergic agonist, with no bronchodilator action in vivo in man (Berkin et al. 1986; Larsson et al. 1986). Although electrical field stimulation of in vitro preparations of canine bronchi results in the release of norepinephrine (Vermeire and Vanhoutte, 1979), studies of the effects of sympathetic nerve stimulation on airway smooth muscle tone in different mammalian species have

given markedly conflicting results, probably related to inter-species differences.

In an early study using an isolated guinea-pig tracheal preparation in which the parasympathetic response had been abolished by atropine, transmural electrical stimulation resulted in smooth muscle relaxation, the effect being blocked by a beta-adrenergic antagonist, suggesting its mediation by postganglionic catecholamine release (Foster, 1964). In addition, electrical sympathetic nerve stimulation in both cats and dogs was shown to diminish the increase in airway resistance resulting from simultaneous vagal stimulation (Olsen et al. 1965). In a later study in dogs, supramaximal electrical sympathetic stimulation produced some inhibition of vagally induced bronchoconstriction (Cabezas et al. 1971), but after vagotomy similar stimulation of the sympathetic trunks appeared to have no effect on airway smooth muscle tone (Cabezas et al. 1971). While an inhibitory response was produced in isolated strips of guinea pig tracheal muscle by electrical field stimulation in the presence of atropine, this was unaffected by either propranolol or practolol (Coburn and Tomita, 1973). Other workers, however, elicited a similar response in the isolated guinea-pig trachea to which there did appear to be an adrenergic component (Richardson and Bouchard, 1975). Likewise, stimulation of the sympathetic trunks in intact guinea-pigs appeared to elicit an adrenergic inhibitory response of the airway smooth muscle, which was clearly attenuated by propranolol (Chesrown et al. 1980). However, two in vitro studies of human respiratory tissue, obtained surgically

and from autopsies, have clearly demonstrated that the smooth muscle relaxation produced by electrical field stimulation in the presence of cholinergic blockade is unaffected by adrenergic antagonists (Richardson and Beland, 1976; Davis et al. 1982).

The role of the sympathetic nervous system as a primary bronchomotor inhibitory mechanism in the human airway is therefore questionable. Furthermore, while it may be a significant bronchodilator mechanism in other species (Foster, 1964; Olsen et al. 1965; Cabezas et al. 1971; Richardson and Beland, 1976; Chesrown et al. 1980), it has been suggested that the smooth muscle inhibitory responses obtained in these studies may relate to the release of norepinephrine from the perivascular nerves (Kirkpatrick, 1984). In any event, no similar results have been obtained with human tissue. It seems likely, therefore, that the physiological role of adrenergic fibres in the control of bronchomotor tone in man is insignificant despite their undoubted anatomical relation to airway smooth muscle. (Partanen et al. 1982; Sheppard MN et al. 1983; Pack and Richardson, 1984; Laitinen A et al. 1985a).

Although the precise role of sympathetic neurones in the control of airway glandular secretions is still undefined, recent studies in mammalian tracheal preparations have suggested the involvement in the secretory processes of adrenergic mechanisms (Borson et al. 1980; Borson et al. 1984; Leikauf et al. 1984).

3.2 ADRENERGIC RECEPTORS

Adrenergic receptors were divided into two functional classes - alpha and beta - on the basis of the relative potency with which they are stimulated by the various sympathomimetic amines (Ahlquist, 1948; Ahlquist, 1966). Subsequently, those adrenergic receptors with equipotent responses to epinephrine and norepinephrine, which are approximately 10-20% of that to isoproterenol, were designated beta-1 receptors. Those having responses to norepinephrine only 0.1-1% of the responses to isoproterenol were designated beta-2 receptors (Lands et al. 1967). Beta-1 and beta-2 receptors were further differentiated on the basis of the blocking potency displayed by selective beta-adrenergic antagonists such as practolol. By means of these principles it has been shown that the stimulation of beta-1 adrenergic receptors is associated with a positive effect on cardiac chronotropy and inotropy as well as a lipolytic effect and that of beta-2 receptors with the inhibition of the tone of smooth muscle in various sites, including the airway (Lands et al. 1967).

Alpha-adrenergic receptors are those most potently stimulated by epinephrine and somewhat less by norepinephrine, displaying negligible responses to isoproterenol. They are further characterised by the fact that various adrenergic antagonists such as phenoxybenzamine and phentolamine demonstrate a greater affinity for them than for the beta-receptors. On the

basis of these principles it has been recognised that alpha-receptors are associated with a stimulatory effect on the tone of smooth muscle in various sites, most notably the vasculature.

More recently, the existence of two different subtypes of alpha-adrenergic receptor - designated alpha-1 and alpha-2 - has been confirmed. The alpha-1 subtype includes the conventional post-synaptic receptors such as those which mediate the contraction of vascular smooth muscle. Alpha-2 receptors, on the other hand, are presynaptic autoregulatory sites on peripheral adrenergic nerve endings. The stimulation of alpha-2 receptors by the norepinephrine molecules released into the synaptic cleft reduces the degree to which continuing incoming neuronal impulses can stimulate further release of the neurotransmitter (Hoffman and Lefkowitz, 1980). There appear, in addition, to be alpha-adrenergic receptors in the central nervous system which, when stimulated, diminish peripheral sympathetic vascular tone resulting in a reduction in arterial blood pressure (van Zwieten, 1973; Scriabine et al. 1976). These central receptors have various characteristics in common with the peripheral alpha-2 receptors (Berthelsen and Pettinger, 1977; Miach et al. 1978).

Among the alpha-adrenergic agonists, it has been suggested that methoxamine and phenylephrine are alpha-1 selective, and clonidine alpha-2 selective, and that epinephrine and norepinephrine which stimulate both subtypes of alpha-receptor with equal potency, are relatively non-selective (Berthelsen and Pettinger, 1977; Hoffman and Lefkowitz, 1980). Among the alpha-adrenergic antagonists, prazosin and phenoxybenzamine are

considered to be alpha-1 selective, yohimbine alpha-2 selective, and phentolamine relatively non-selective (Cavero et al. 1977; Doxey et al. 1977; Hoffman and Lefkowitz, 1980). Radioligand binding data using clonidine, yohimbine and alphamethyl-norepinephrine, and studies of alpha-agonist mediated inhibition of adenyl cyclase and 3,5 cyclic adenosine monophosphate (3,5 cyclic AMP), have demonstrated that post-synaptic alpha-2 receptors exist in a wide variety of tissues (Wood et al. 1979; Exton, 1982).

3.2.1 Beta-Adrenergic Receptors

There is ample evidence of the existence of beta-receptors in the mammalian respiratory tract. Radioligand studies have demonstrated the presence of beta-adrenergic binding sites in respiratory tract tissue obtained from the rat, guinea-pig, rabbit and dog, as well as man (Furchgott et al. 1975; Rugg et al. 1978; Minneman et al. 1979; Barnes et al. 1980d; Engel, 1981; Goldie et al. 1982; Carswell and Nahorski, 1983; Barnes et al. 1983d; Brodde et al. 1983). The combination of this technique with studies utilising the known affinities of various adrenergic agonists and antagonists has permitted, in addition, the conclusion that the beta-1 and beta-2 receptor subtypes coexist in respiratory tract tissue (Furchgott et al. 1975; Rugg et al. 1978; Minneman et al. 1979; Engel, 1981; Goldie et al. 1982; Carswell and Nahorski, 1983; Barnes et al. 1983d; Brodde et al. 1983). Although beta-2 receptors appear to predominate, the proportions of the two subtypes appears to vary considerably

between different species, and even possibly between different animals of the same species (Furchgott et al. 1975). The beta-1:beta-2 receptor ratio in human lung appears to be approximately 30:70 (Engel, 1981; Brodde et al. 1983).

While clearly demonstrating that the mammalian respiratory tract contains a high density of beta-receptors, these studies permit no conclusions regarding their differential distribution among the numerous cell types and structures present in it. Canine tracheal muscle contains beta-receptor binding sites in a considerably lower density than a similar preparation of peripheral lung, suggesting the possible location of considerable numbers of beta-receptors in sites other than smooth muscle (Barnes et al. 1983d). The technique of light microscopic autoradiography has permitted the localisation of beta-receptors among the different cell types in the respiratory tract. In ferret lung, the highest density occurs in bronchiolar smooth muscle, with a somewhat lower density in the smooth muscle of large airways. Beta-receptors are also present in the airway epithelium, the submucosal glands, the vascular smooth muscle, and the alveolar walls (Barnes et al. 1982a).

Autoradiographic studies of human lungs obtained from surgical procedures have shown that beta-receptors are also widely distributed in man (Carstairs et al. 1984; Carstairs et al. 1985). The greatest density appears to occur in the airway epithelium, submucosal glands, and alveolar walls, with a lesser density in the smooth muscle of both large and small airways and in the vasculature. Combination with agonist and antagonist

competition data show that the beta-1:beta-2 receptor ratio is roughly 1:3, confirming the radioligand studies (Engel, 1981; Brodde et al. 1983). The beta-2 receptor is the exclusive subtype in the smooth muscle of both the large and the small airways, the vasculature, and the airway epithelium, while in both the submucosal glands and the alveolar walls, both receptor subtypes appear to co-exist (Carstairs et al. 1984, 1985).

These findings are consistent with the concept that beta-adrenergic receptors regulate numerous physiological processes in the respiratory tract other than airway smooth muscle tone. These include the inhibition of mast cell mediator release, the control of vascular smooth muscle tone, effects on the capillary endothelial cells and type II pneumocytes and the regulation of glandular mucus secretion (Barnes et al. 1984; Carstairs et al. 1985). Evidence for the involvement of both beta-receptor subtypes in the latter function has been provided by the demonstration that both dobutamine and salbutamol increase the glycoprotein secretion in in vitro feline and human large airway preparations (Phipps et al. 1982; Peatfield and Richardson, 1982). The significance of the high beta-receptor density in airway epithelium is uncertain. It is of considerable interest, however, that removal of the epithelium appears to increase the sensitivity of the smooth muscle to provoked constriction and to diminish the protective effect of bronchodilators in in vitro preparations (Flavahan et al. 1985; Barnes et al. 1985; Goldie et al. 1986). These findings suggest the possible existence of a beta-adrenergically mediated bronchodilator mechanism located

within the airway epithelium. Beta-adrenergic agonists are also known to inhibit the release of mediators from human mast cells (Butchers et al. 1980; Peters et al. 1982; Hughes et al. 1983). It is possible that mast cell beta-receptors which appear to be of the beta-2 subtype, (Butchers et al. 1980; Hughes et al. 1983) account, at least in part, for the high intra-epithelial density of these receptors.

3.2.2 Alpha-Adrenergic Receptors

The role of alpha-receptors in the regulation of normal airway smooth muscle tone is considerably less well defined. Taking into account its effects in other sites, alpha-receptor stimulation might be expected to result in airway smooth muscle contraction. There is little doubt, however, at least in health, that the predominant result of adrenergic stimulation of airway smooth muscle is the beta-receptor mediated inhibitory response. Thus, even such potent alpha-agonists as norepinephrine and phenylephrine have, if anything, a slight bronchodilator effect in vitro, despite being extremely weak beta-agonists (Mathe et al. 1971; Goldie et al. 1984).

In 1962 Castro de la Mata et al. showed that the administration of epinenephrine and norepinephrine, as well as sympathetic nerve stimulation, produced airway smooth muscle constriction in anaesthetised beta-adrenergically blocked dogs, the response being abolished by the alpha-adrenergic blocker tolazoline. Subsequently, similar results were obtained in airway preparations in different mammalian species (Everitt and

Cairncross, 1969; Fleisch et al. 1970; Adolphson et al. 1971; Mathe et al. 1971). The conclusion reached as a result of these studies was that alpha-adrenergic receptors were present in mammalian airways and could stimulate smooth muscle contraction. These findings were not, however, consistently demonstrable in vitro (Foster, 1966) or in vivo (Cabezas et al. 1971).

The results of in vitro studies of isolated human airway preparations likewise yielded conflicting results. Thus, the contraction of smooth muscle could be elicited with both epinephrine and norepinephrine in the presence of beta-blockade, the response being abolished by phenoxybenzamine (Adolphson et al. 1971), and being 10-20% of the maximal bronchoconstrictor responses elicited by cholinergic agonists or histamine (Mathe et al. 1971; Simonsson et al. 1972). However, in another study, no increase in carbachol-induced contractions was obtained with epinephrine in the presence of propranolol, suggesting that alpha-receptors were not present (Guirgis et al. 1969).

It has subsequently been demonstrated that the addition of alpha-adrenergic agonists to isolated tracheal and bronchial smooth muscle from human subjects with no evidence of respiratory disease, produces no contractile response unless the preparations have been pretreated with either potassium chloride or histamine (Kneussl and Richardson, 1978), and both in vitro and in vivo canine studies have produced similar results (Brown et al. 1983; Barnes et al. 1983e). However, in tissue from humans with evidence of significant respiratory disease, norepinephrine produces smooth muscle contraction without any pretreatment, the

response being abolished by alpha-blockade (Kneussl and Richardson, 1978). Similarly, beta-adrenergic blockade appears to potentiate the contractile response of canine tracheal smooth muscle to alpha-stimulation (Leff et al. 1986). It is possible that alpha-receptors are present in respiratory tract tissue and are in some way activated in the presence of an abnormality or disease process.

Radioligand studies using (3H)prazosin have confirmed the presence of alpha-receptor binding sites in homogenised human lung tissue (Barnes et al. 1980d). Although the distribution of these receptors in the human respiratory tract has not yet been clearly defined, autoradiographic distribution studies in ferret lung using the same radioligand have shown that alpha-1 receptors are relatively widely distributed, being present in a high density in the vascular smooth muscle and the smooth muscle of the peripheral airways. Lesser densities occur in the airway glands, epithelium and alveolar walls, and few alpha-1 receptors are present in the large airways (Barnes et al. 1983a; Barnes et al. 1983b). Radioligand studies with (3H)prazosin and (3H)yohimbine in canine tissue suggest that tracheal alpha-receptors are predominantly of the alpha-2 subtype (Barnes et al. 1983e; Barnes et al. 1983f), whereas the alpha-1 subtype predominates in the peripheral lung (Barnes et al. 1983f). The contractile response to electrical field stimulation of beta-adrenergically and cholinergically blocked tracheal smooth muscle is more potently inhibited by yohimbine than by prazosin. In addition, the alpha-2 agonist clonidine appears to be the most

potent alpha-agonist in contraction studies and in competition with the selective alpha-2 antagonist ligand (3H)yohimbine. These data confirm the presence of a significant number of alpha-2 receptors in the canine trachea (Barnes et al. 1983f).

The above data suggest that alpha-receptors are present in human airway smooth muscle, but precise knowledge of receptor subtypes and of their role in the regulation of smooth muscle tone, both in health and disease is, for the most part, lacking. Evidence for the involvement of alpha-receptors in the regulation of mucous glandular secretion has been provided by studies showing that phenylephrine and norepinephrine increase the rate of glycoprotein secretion in the feline and human large airway (Shelhamer et al. 1980a; Phipps et al. 1982, Peatfield and Richardson, 1982). Studies in the cat suggest, in addition, that alpha-adrenergically mediated bronchial glandular secretions may be more watery than those stimulated by beta-adrenergic agonists (Leikauf et al. 1984).

A study of canine mast cells has provided no evidence of the existence in them of alpha-adrenergic receptors (Phillips et al. 1985).

3.3 ENDOGENOUS CIRCULATING CATECHOLAMINES

3.3.1 Epinephrine

Epinephrine is a product of tyrosine metabolism being synthesised from norepinephrine in the chromaffin cells which contain the enzyme phenylethanolamine-N-methyl transferase. In

post-natal life the bulk of these cells are found in the adrenal glands where they constitute the adrenal medullae. Bilateral adrenalectomy in humans results in a fall in the plasma concentration of epinephrine to undetectable levels even in the presence of insulin-induced hypoglycaemia, normally a potent stimulus to its secretion (Cryer, 1976). The rate of synthesis of tyrosine to norepinephrine in the adrenal medullae appears to be mainly regulated by sympathetic neuronal discharges, and the rate of conversion of norepinephrine to epinephrine mainly by adrenocorticotropic hormone and corticosteroids (Axelrod and Weinshilboum, 1972). Epinephrine is stored in granules within the chromaffin cells and released by the process of exocytosis at a basal rate of about 0.2/ug/kg/min (Guyton, 1981b). Sudden surges in the rate of secretion occur as a result of increased neuronal discharges in the preganglionic sympathetic supply to the adrenal medullae, these being part of the generalised adrenergic response to such stimuli as fear, rage, exercise, hypoxaemia, hypoglycaemia and shock.

Epinephrine is more potent than norepinephrine in its effects on alpha-adrenergic receptors, but less potent than isoproterenol in its effects on beta-receptors (Ahlquist 1966). It is also more potent in its bronchodilator and vasodilator - beta-2 adrenergic - effects than in its lipolytic and cardiac stimulatory - beta-1 adrenergic - effects (Lands et al. 1967) .

The combined data from a number of studies suggest that mean basal levels of plasma epinephrine in normal supine human subjects are approximately 20-50pg/ml (conversion to nmol/l x

0.0055) (Cryer, 1976). Plasma epinephrine levels normally exhibit a circadian variation with peak levels occurring at about 16h00 and trough levels at about 04h00 (Barnes et al. 1980b).

Epinephrine functions as a circulating hormone mediating diverse adrenergic functions throughout the body, and its effects on the respiratory tract include the beta-adrenergically mediated inhibition of airway smooth muscle tone. Infusions achieving plasma levels close to the normal physiological range are associated with a rise in both heart rate and systolic blood pressure, a fall in diastolic blood pressure, a variety of characteristic metabolic changes, and a significant increase in specific airway conductance (Fitzgerald et al. 1980; Warren and Dalton, 1983). In both asthmatic patients and normal subjects epinephrine has been shown to reverse the bronchospasm provoked by various stimuli (Warren et al. 1984; Sands et al. 1985; Larsson et al. 1985).

3.3.2 Norepinephrine

Synthesis of norepinephrine from tyrosine via the compounds dihydroxyphenylalanine and dopamine takes place both in the central nervous system and in the axon terminals of peripheral postganglionic sympathetic neurones. In addition, a significant concentration gradient of norepinephrine exists from the adrenal veins to the inferior vena cava and norepinephrine is estimated to constitute as much as 20% of the total catecholamine production of the adrenal medullae, being secreted at a rate of 0.05 ug/kg/min (Guyton, 1981b). Despite this, bilateral

adrenalectomy appears to have little effect on basal plasma norepinephrine levels (Cryer, 1976). Norepinephrine is released from postganglionic sympathetic neurones as a result of neural activity. It is a potent alpha-adrenergic agonist being, however, less potent than epinephrine in its effect on alpha-receptors (Ahlquist, 1966). It is equipotent with epinephrine in its effect on beta-1 receptors, and has little effect on beta-2 receptors (Lands et al. 1967).

The combined data obtained from a number of studies suggest that the basal plasma norepinephrine levels of normal supine subjects are approximately 200-220pg/ml (conversion to nmol/l x 0.0059) (Cryer, 1976). The excess of plasma norepinephrine over epinephrine levels may be related to a greater rate of release combined with a slower rate of clearance from the circulation (Fitzgerald et al. 1980). Although an infusion of norepinephrine administered to normal subjects produces significant haemodynamic and metabolic effects, these are only evident with plasma levels considerably in excess of resting values (1800pg/ml), suggesting that in the absence of significant stress, such as that related to prolonged or maximal exercise or a major acute illness, norepinephrine functions as a neurotransmitter only (Silverberg et al. 1978). It has recently been demonstrated that neither norepinephrine nor phenylephrine cause adrenergic metabolic effects or significant broncnodilatation in either asthmatic or non-asthmatic subjects despite significant increments in blood pressure (Barkin et al. 1986; Larsson et al. 1986).

3.3.3 Circulating Catecholamines and The Human Airway

Barnes et al (1983d) have shown that whereas both beta-1 and beta-2 receptors are present in canine tracheal smooth muscle in a ratio of 1:4 respectively, the relaxation response to beta-agonists is mediated mainly by the beta-2 receptors with only a small contribution from the beta-1 subtype. The relaxation response to norepinephrine released by electrical sympathetic nerve stimulation is, on the other hand, predominantly mediated by the beta-1 receptors. While both beta-1 and beta-2 receptors undoubtedly co-exist in the human lung (Engel, 1981; Goldie et al. 1982; Brodde et al. 1983; Carstairs et al. 1985), the beta-receptors in human airway smooth muscle appear to be exclusively of the beta-2 subtype (Carstairs et al. 1985). The lack of a significant functional sympathetic nerve supply and the apparent lack of beta-1 receptors in human airway smooth muscle suggest, therefore, that the adrenergic inhibitory modulation of its tone is primarily mediated by the effect of circulating epinephrine on the high density of beta-2 receptors present in both large and small airways (Carstairs et al. 1985). However, while relatively small doses of propranolol have been shown to cause some increase in airway resistance in non-asthmatic individuals (MacDonald et al. 1967), the lack of a consistent effect of beta-blockade on the calibre of the normal human airway raises some doubts about the physiological importance of this mechanism in man, at least in health (Zaid and Beall, 1966; Richardson and Sterling, 1969; Mattersfield et al. 1973).

CHAPTER 4

NON-ADRENERGIC NON-CHOLINERGIC MECHANISMS

4.1 NON-ADRENERGIC NON-CHOLINERGIC NERVES

It has been known for some time that non-adrenergic non-cholinergic (NANC) fibres exist in the gastrointestinal tracts and urogenital systems of various mammalian species (Burnstock, 1972). NANC fibres inhibit gastrointestinal smooth muscle tone, but appear to be excitatory in the urogenital system. The presence in the mammalian respiratory tract of a NANC system has been confirmed in a number of animals, including the guinea-pig (Coburn and Tomita, 1973; Richardson and Bouchard, 1975), the cat (Irvin et al. 1980) and the cow (Palmer et al. 1985b). The existence of the NANC system in man has been convincingly confirmed in lungs obtained from surgical excisions and autopsies (Richardson and Beland, 1976; Davis et al. 1982; Palmer et al. 1986a).

The NANC inhibitory system in the gastrointestinal tract has been shown to migrate with cholinergic fibres in rodent foetuses and to be functional significantly earlier in foetal life than the adrenergic system (Gershon and Thompson, 1973). It is possible that this pattern applies as well during the development of the respiratory tract. The NANC inhibitory effects on airway smooth muscle tone elicited in intact cholinergically blocked animals by electrical vagal stimulation (Chesrown et al. 1980; Irvin et al. 1980; Diamond and O'Donnell, 1980) can be considerably attenuated by interruption of the recurrent laryngeal nerves and by the administration of hexamethonium (Diamond and O'Donnell, 1980). These findings suggest that the

anatomical location of the NANC preganglionic fibres is within the vagal trunks. It appears from electrical field studies that NANC fibres are present from the mid-tracheal level to that of the distal bronchi, suggesting that postganglionic NANC fibres are widely distributed throughout the airways (Richardson and Beland, 1976). However, tantalum bronchography in cats suggests that while the NANC system inhibits bronchomotor tone from the trachea to the small airways, it has the most effect on those with a diameter of 4mm (Matsumoto et al. 1985). Studies in human tissue have shown that in contrast to the more central airways, the bronchioles have little NANC innervation (Palmer et al. 1986a).

NANC mechanisms appear, in addition, to be involved in the control of the secretions of the airway glands. This has been suggested by the finding that electrical field stimulation increases the secretion of radiolabelled macromolecules by the submucosal glands of the isolated ferret trachea in the presence of cholinergic and adrenergic blockade (Borson et al. 1984).

4.2 NEUROTRANSMITTERS OF THE NON-ADRENERGIC NON-CHOLINERGIC NERVOUS SYSTEM

4.2.1 Vasoactive Intestinal Polypeptide

The neurotransmitters of the NANC system have not been identified with certainty. Burnstock (1972) thought that adenosine triphosphate was involved, and accordingly used the term "purinergic" to describe the system. This postulate could

not, however, be substantiated in a number of later studies (Ito and Takeda, 1982; Cameron et al. 1983).

A considerable amount of experimental evidence has now been accumulated suggesting that the inhibitory neurotransmitter of the NANC system is vasoactive intestinal polypeptide (VIP). The relaxation by VIP of isolated bovine (Cameron et al, 1983; Palmer et al. 1985b) and guinea-pig tracheal smooth muscle (Said et al. 1974) has been clearly demonstrated. In isolated precontracted feline tracheal smooth muscle, the amplitude of electrically-induced relaxation can be significantly diminished by prior partial desensitisation to VIP (Ito and Takeda, 1982). All these effects appear to be unrelated to either cholinergic or adrenergic mechanisms, suggesting therefore that the effect of VIP is similar to that of NANC nerve stimulation.

VIP has been shown to be released during the electrical stimulation of intrinsic airway nerves in both bovine and guinea-pig preparations (Matsuzaki et al. 1980; Cameron et al. 1983) in an amount correlating with the degree of smooth muscle relaxation induced, and prior incubation with VIP-antiserum significantly reduces this response (Matsuzaki et al. 1980). VIP is equipotent with isoproterenol in its inhibitory effect on the smooth muscle of the isolated feline large airway (Altiere and Diamond, 1984) and has a considerably greater effect than isoproterenol on human bronchi of 3-4mm diameter (Palmer et al. 1986a). It has little effect, however, on bronchioles (Palmer et al. 1986a).

The peptide has been shown by both radioimmunoassay and immunocytochemical techniques to exist within the autonomic

nerves of the airway walls in various mammalian species, including man (Dey et al. 1981; Polak and Bloom, 1982; Hakanson et al. 1983; Sheppard MN et al, 1983; Laitinen A et al. 1985b) and is detectable in the human foetus after 20 weeks of gestation (Sheppard et al. 1984a). It is closely associated not only with airway smooth muscle, but also with mucous glands and pulmonary and bronchial blood vessels, suggesting its probable involvement in the regulation of secretory processes and vascular and airway smooth muscle tone. Its involvement in the control of airway glandular secretions has been confirmed by the finding that it has a direct effect on the stimulation of the output of labelled macromolecules by ferret submucosal glands (Peatfield et al. 1983), and increases the transport of chloride ions across the canine tracheal epithelium (Nathanson et al. 1983). VIP has also been shown to have a far more potent relaxant effect than isoproterenol on the feline pulmonary artery (Hamasaki et al. 1983). In minced guinea-pig lung it inhibits antigen-induced histamine release suggesting a possible effect on mast cells (Undem et al. 1983).

The suggestion that VIP is involved in diverse aspects of pulmonary function has been strengthened by recent autoradiographic studies demonstrating the presence of VIP receptors in the epithelium, submucosal glands, vascular smooth muscle, alveoli and smooth muscle of large - but not small - airways in the guinea-pig lung (Carstairs and Barnes, 1986b).

The mode of action of VIP may be similar to that of beta-adrenergic agonists in that it has been shown to induce the

accumulation of 3,5 cyclic adenosine monophosphate (3,5 cyclic AMP) in the guinea-pig trachea (Frandsen et al. 1978) as well as in the tracheal submucosal glands of ferrets and the epithelial cells of the canine trachea (Lazarus et al. 1983). It has also been shown to stimulate the adenyl cyclase activity of rat lung more potently than either beta-adrenergic agonists or prostaglandins (Robberecht et al. 1981). It has been suggested, however, that VIP mediates the stimulation of salivary gland secretion in cats by enhancing the binding of muscarinic agonists to receptors (Lundberg et al. 1982) and that it may inhibit the contractile effect of acetylcholine on bovine tracheal smooth muscle by a post-receptor mechanism (Palmer et al. 1985b).

Despite all this evidence, however, and despite the potent bronchodilator effect of VIP in intact animals (Diamond et al. 1983) and isolated human airway smooth muscle (Palmer et al. 1985a; Palmer et al. 1986a), in vivo human studies have yielded somewhat less convincing results. Inhaled VIP has little effect on the specific airway conductance in asthmatic patients, and diminishes the bronchoconstrictor response to histamine far less than inhaled salbutamol (Barnes and Dixon, 1984). Intravenous VIP appears to diminish the contractile response to inhaled histamine (Morice et al. 1983), but has, at most, a small bronchodilator effect (Morice et al. 1983; Palmer et al. 1986b). It has been suggested that these somewhat disappointing results may relate either to the fact that the neurotransmitter has not been delivered to the target organ, namely smooth muscle, by the normal physiological route (Barnes and Dixon, 1984) or to its

rapid enzymatic breakdown (Palmer et al. 1986b).

In addition to VIP, another closely related peptide found in human airway nerves - peptide histidine methionine - appears to be a potent smooth muscle relaxant, at least in vitro (Palmer et al. 1986a).

4.2.2 Other Regulatory Polypeptides

A considerable number of other regulatory peptides have been shown to be present in mammalian airways. These include bombesin, cholecystokinin, somatostatin, calcitonin-gene-related-peptide, galanin, neuropeptide tyrosine and substance P (Polak and Bloom, 1982; Cutz, 1982; Hakanson et al. 1983; Pernow, 1983; Sheppard et al. 1984b; Palmer et al. 1985c; Cheung et al. 1985), the latter being of particular interest. It appears to be present in fibres originating from the vagal trunks (Pernow, 1983), and has been demonstrated, in addition, in the nerve fibres of human airways where it is related to smooth muscle, epithelium and blood vessels (Sheppard MN et al. 1983), although some workers have been unable to confirm its presence (Laitinen et al. 1983). Substance P produces a dose-dependent contraction of human bronchial smooth muscle in vitro, which is resistant to atropine and appears, therefore, not to be cholinergically mediated (Lundberg et al. 1983). In addition to its effect on airway smooth muscle, it appears to stimulate the secretion of macromolecules by canine tracheal glands (Baker et al. 1977; Coles et al. 1984) with a potency far in excess of that of methacholine (Coles et al. 1984), and also specifically to

increase the secretion of chloride ions (Al-Bazzaz et al. 1985).

Both substance P and calcitonin-gene-related-peptide produce a wheal-and-flare reaction following intradermal injection into humans which is associated with, but not dependent upon, dermal histamine release (Barnes et al. 1986a;Barnes et al. 1986b). It has been postulated that axon reflexes resulting from epithelial damage to the airway may cause the release of neuropeptides from sensory fibres (Barnes, 1986).

It appears likely, therefore, that substance P is a neurotransmitter of a NANC excitatory system, and substance P receptors have recently been identified by autoradiographic studies in the airways and lungs of both the guinea-pig and man (Carstairs and Barnes, 1986a). In addition, however, another peptide, eledoisin, has been shown to be even more potent in its in vitro contractile effect on human airway smooth muscle (Karlsson and Persson, 1985).

As with VIP, there is evidence suggesting possible interaction of substance P with other components of the autonomic nervous system. It has been suggested that the contractile effect of the peptide on rabbit tracheal smooth muscle in vitro, is mediated by an acceleration in the release of acetylcholine from parasympathetic nerve endings (Tanaka and Grunstein, 1984; Tanaka and Grunstein, 1985) . In addition, it has been demonstrated that NANC-mediated contraction of the guinea-pig trachea is inhibited by norepinephrine and antagonist studies have suggested that it may be associated with alpha-2-receptors (Grundstrom et al, 1984).

In conclusion, the NANC system appears to be an important component of the autonomic regulatory control of airway smooth muscle tone and airway glandular secretion. Apparent abnormalities of the NANC inhibitory system in the gasttrointestinal tract have been linked to Hirschprung's disease (Frigo et al. 1973), and it has also been suggested that NANC abnormalities are associated with disorders of the urinary bladder (Polak and Bloom, 1983). No abnormalities of the system have yet been described in association with bronchial asthma.

CHAPTER 5

MOLECULAR MECHANISMS INVOLVED IN RECEPTOR FUNCTION

The combination of specific molecules of either endogenous origin - for example hormones and neurotransmitters - or exogenous origin - for example pharmacological agents - with receptors on the surfaces of cells, initiates intracellular events such as the activation of enzyme systems or alterations in ion fluxes, which ultimately result in characteristic physiological effects. Beta-receptors of both subtypes are associated with a specific biochemical system involving the catalytic enzyme adenyl cyclase which converts the substrate adenosine triphosphate (ATP) to 3,5 cyclic adenosine monophosphate (3,5 cyclic AMP). As this system has been extensively studied and is involved as well in the function of both alpha-2 receptors and muscarinic receptors, it is convenient to deal with beta-receptor function first.

5.1 BETA-ADRENERGIC RECEPTORS

5.1.1 Activation of Beta-Adrenergic Receptors

Hormone sensitive adenyl cyclase activity is present in virtually all animal cells and can be either stimulated or inhibited by a large number of different hormones and drugs. The components of the system include:

(i) The receptor.

(ii) The catalytic moiety of adenyl cyclase.

(iii) A guanine nucleotide regulatory coupling protein (G-protein).

This exists in two structurally similar forms, namely: (a) a

stimulatory form which allows the coupling of adenyl cyclase to beta-1 and beta-2 receptors, (b) an inhibitory form which allows coupling to alpha-2 receptors. Both of these regulatory molecules are multi-subunit proteins sharing in common two subunits, namely the beta-subunit of molecular weight 35,000 Daltons, and the gamma-subunit of molecular weight 8,000 Daltons. The alpha-subunits of the two forms of G-protein differ, the molecular weight being 45,000 Daltons in the stimulatory protein, and 41,000 Daltons in the inhibitory protein (Gilman, 1984). Furthermore, the alpha-subunit of the stimulatory protein provides a substrate for adenosine diphosphate (ADP)-ribosylation by cholera enterotoxin (Moss and Vaughan, 1979), whereas the alpha-subunit of the inhibitory regulatory protein provides a substrate for ADP-ribosylation by B. pertussis toxin (Katada et al. 1984).

(iv) Guanine nucleotides such as guanosine triphosphate(GTP).

This entire system is found in the cellular plasma membrane and the combination of an agonist molecule with the beta-receptor results, after a number of coupling processes, in the stimulation of adenyl cyclase followed by an increase in the intracellular level of 3,5 cyclic AMP (Lefkowitz et al. 1983; Lefkowitz et al. 1984).

It has been shown that while antagonists bind to the beta-receptors on frog erythrocyte membranes with uniform affinity, agonists exhibit states of either high or low affinity (Kent et

al. 1980). The high affinity state appears to be converted to one of low affinity by guanine nucleotides (Kent et al. 1980). Agonist occupancy of the beta-receptor appears to result in a physical coupling of the receptor itself to the G-protein. This reaction does not occur, however, after receptor occupancy by an antagonist (Limbird et al. 1980a). The affinity of the receptor-G protein complex for agonists is greater than that of free receptors, and it has been suggested, therefore, that the complex represents the high affinity form of the beta-receptor and the free receptor the low affinity form. Furthermore, the ability of an agonist to activate adenyl cyclase correlates closely with the degree of high affinity agonist-beta-receptor binding (Kent et al. 1980), giving credence to the suggestion that the formation of a high affinity ternary complex is a necessary intermediate step in the activation of adenyl cyclase (De Lean et al. 1980).

GTP appears to be essential for hormone adenyl cyclase responsiveness (Ross et al. 1977) and it has been suggested that catecholamines may promote the release of guanosine diphosphate (GDP) from the G-protein, thereby vacating a binding site for GTP (Cassel and Selinger, 1978). It is currently believed that GTP binds to the ternary agonist-receptor-G protein complex, activating and dissociating the G-protein, with simultaneous formation of the low affinity free receptor (Lefkowitz et al. 1984). The activated G-protein-GTP complex then itself activates the catalytic moiety of adenyl cyclase.

It has been suggested that the activation of beta-receptors leads ultimately to a dissociation of the alpha-subunit from the

beta- and gamma-subunits of the stimulatory G-protein and that the alpha-subunit then provides the stimulus for adenyl cyclase activation (Gilman, 1984). The series of reactions is terminated by the eventual hydrolysis of GTP to GDP by a hydrolytic GTPase activity. This reaction appears to be dependent on receptor occupancy by agonist but is unrelated to a catalytic adenyl cyclase effect. The GTPase reaction appears to be slower than adenyl cyclase activation, and ultimately permits the return of the system to the inactive state (Cassel and Selinger, 1976). The latter reaction is inhibited by cholera enterotoxin thereby resulting in a marked enhancement of adenyl cyclase activity (Cassel and Selinger, 1977; Moss and Vaughan, 1979).

5.1.2 Th Mediation of Smooth Muscle Relaxation by Beta-Adrenergic Receptors

Protein kinases in diff ent types of muscle cells, including smooth muscle, are sensitive to the intracellular levels of 3,5 cyclic AMP. These enzymes are composed of regulatory and catalytic subunits with the former inhibiting the activity of the latter. 3,5 cyclic AMP binds to the regulatory subunits thereby effecting a functional dissociation between the two moieties such that catalytic activity is no longer inhibited. The activated enzyme can then initiate a series of phosphorylation reactions which ultimately result in typical beta-adrenergic effects in different cell types (Robison et al. 1971).

It is currently believed that smooth muscle relaxation may

be effected by one or both of two mechanisms, namely: (i) A limitation of the availability of free calcium ions to the contractile apparatus of muscle cells. (ii) Inhibition of the interaction between actin and myosin due to the phosphorylation of myosin light chain kinase.

(i) Limitation of the availability of calcium ions: A considerable body of evidence suggests that changes in myoplasmic free calcium ion levels result in the contraction and relaxation of smooth muscle as well as other types of muscle cells. When intracellular calcium levels reach 10^{-6} molar, calcium binds to a protein with a molecular weight of 16,500 Daltons, known as calmodulin. The calcium-calmodulin complex binds to and activates myosin light chain kinase, and the activated enzyme can then phosphorylate the light chain of myosin. This permits the interaction of myosin and actin which results in muscle contraction (Adelstein and Hathaway, 1979; Braunwald 1982). In the presence of intracellular calcium levels of 10^{-8} molar or less, calmodulin cannot bind to myosin light chain kinase. The enzyme therefore remains inactive and muscle contraction cannot occur (Adelstein and Hathaway, 1979).

The importance of calcium ions in the process of smooth muscle contraction is demonstrated by the fact that the ability of 3,5 cyclic AMP to relax guinea-pig bowel is inversely related to the concentration of both calcium ions and calmodulin (Meisheri and Rugg, 1983). A reduction in the myoplasmic free calcium ion activity has been shown to occur as a result of a number of different mechanisms mediated by beta-adrenergic

agonists or 3,5 cyclic AMP. These include the intracellular sequestration of calcium ions, cellular calcium ion extrusion and the inhibition of entry of calcium ions into cells. There is experimental evidence supporting each of these mechanisms (Tomiyama et al. 1973; Kroeger et al. 1975; Mueller and van Breemen, 1979; Bülbring and den Hertog, 1980; Meisheri and van Breemen, 1982; Cauvin et al. 1984).

While it appears that beta-adrenergic receptor activation may relax smooth muscle by a reduction in the myoplasmic calcium ion level, the effect being mediated by 3,5 cyclic AMP, the precise mechanisms involved remain undefined. It is possible that relaxation is related to the activation of protein kinases which can then phosphorylate proteins involved in the regulation of intracellular calcium ion levels.

(ii) The phosphorylation of myosin light chain kinase: A considerable amount of evidence suggests that beta-adrenergic receptor stimulation results in smooth muscle relaxation by an effect on the myosin light chain kinase system which mediates the interaction of actin and myosin thus permitting muscle contraction (Adelstein and Hathaway, 1979). The incubation of avian myosin light chain kinase with the catalytic unit of 3,5 cyclic AMP-dependent protein kinase results in the incorporation of a mole of phosphate per mole of myosin light chain kinase. This, in turn, causes a two-fold diminution in the rate at which the enzyme phosphorylates the light chain of smooth muscle myosin (Adelstein et al. 1978). It has been suggested that the diminished activity of phosphorylated myosin light chain kinase

results from its inability to bind tightly to calmodulin (Conti and Adelstein, unpublished observations, cited in Adelstein and Hathaway, 1979).

It has been demonstrated that calcium-dependent phosphorylation of the light chain of myosin is depressed by 3,5 cyclic AMP in the presence of protein kinases (Silver and DiSalvo, 1979), the reaction being attenuated by the addition of calmodulin (Silver et al. 1981), suggesting its relation to a shift in the calcium sensitivity of the system. In addition, the rate and extent of both tension development and the phosphorylation of the myosin light chain induced by carbachol in bovine tracheal muscle can be inhibited by prior incubation with isoproterenol (Silver and Stull, 1982). Similarly, calcium-ion-activated tension in avian smooth muscle has been shown to be inhibited by pretreatment with the catalytic unit of protein kinase. This apparent reduction in calcium sensitivity can be reversed by the addition of calmodulin, and the rate of inhibition of tension increased, moreover, by the addition of 3,5 cyclic AMP (Kerrick and Hoar, 1981).

As a result of these and other studies, it has been hypothesised that beta-receptor stimulation results in the activation of 3,5 cyclic AMP and, therefore, in turn, to the activation of 3,5 cyclic AMP-dependent protein kinases. These phosphorylate myosin light chain kinase lowering its affinity for the calcium ion-calmodulin complex. The final result is a reduction in myosin phosphorylation associated with diminished interaction with actin and the inhibition of muscle contraction.

It is possible that 3,5 cyclic AMP may act by an integrated mechanism both lowering the intracellular calcium ion concentration and inhibiting the phosphorylation of myosin light chain kinase, both of which mechanisms result in the relaxation of smooth muscle.

It must be emphasised that the data on which these hypotheses are based have been obtained from in vitro studies in anima. preparations of smooth muscle, often originating, moreover, from sites other than the airway. Their applicability, therefore, to the physiology of the airway smooth muscle in man remains largely unproved.

5.2 ALPHA-ADRENERGIC RECEPTORS

The intracellular molecular events which follow ligand binding to alpha-receptors are, in general, not as well understood as those associated with binding to beta-receptors. It is clear from both radioligand binding studies and physiological data, that alpha-1 and alpha-2 receptors are distinct entities. As discussed above (Chapter 3.2.2), alpha-1 receptors appear to be exclusively postsynaptic, whereas alpha-2 receptors exist in both presynaptic and postsynaptic sites.

5.2.1 Activation of Alpha-1 Adrenergic Receptors

The binding protein of alpha-1 receptors has recently been identified using a radio-iodinated structural analogue of prazosin (Leeb-Lundberg et al. 1984). Their activation appears to

be associated with effects on both cellular calcium ion fluxes and phospholipid metabolism, and alpha-1 receptors have been identified and specifically related to these effects in a wide variety of tissues (Michell, 1975; Exton, 1982).

(i) The role of calcium ions: Much of the current understanding of alpha-1 receptor function is based on studies of adrenergically mediated glycogen breakdown in rat liver cells. It has been shown that both alpha- and beta-adrenergic agonists mediate an increase in hepatic glucose output related to the activation of glycogen phosphorylase - the rate limiting step in hepatic glycogenolysis. However, whereas this glycogenolytic reaction is associated with an increase in intracellular 3,5 cyclic AMP levels when beta-adrenergically mediated, it appears to be 3,5 cyclic AMP-independent when stimulated by alpha-agonists (Sherline et al. 1972; Osborn, 1975). In addition, cyclic AMP-independent phosphorylase activation and glycogenolysis appear to be calcium ion-dependent, the process being impaired in the presence of intracellular calcium ion depletion (van de Werve et al. 1977; Assimacopoulos-Jeannet et al. 1977; Blackmore et al. 1978).

Studies of hepatocyte calcium homeostasis have shown that phenylephrine increases the cytosolic free calcium ion concentration, the time course and dose-response relationship being closely paralleled by an increase in the intracellular levels of phosphorylase. These effects appear, moreover, to be antagonised by alpha-adrenergic blockade (Murphy et al. 1980). Phenylephrine retains its calcium mobilising effect, but fails to

activate phosphorylase in a strain of rat with deficiency of the enzyme phosphorylase kinase (Blackmore and Exton, 1981). It is currently believed, therefore, that the alpha-adrenergically mediated rise in the cytosolic calcium ion level initiates a glycogenolytic reaction, with the most likely site of action being at the level of this enzyme (van de Werve et al. 1977; Assimacopoulos-Jeanet et al. 1977; Blackmore et al. 1978; Kunos, 1984). Phosphorylase kinase can, in addition, be shown to be stimulated by calcium ions (Shimazu and Amakawa, 1975).

The rise in the cytosolic free calcium ion level that occurs in response to alpha-adrenergic stimulation, may be the result of the mobilisation of calcium from intracellular pools, and/or of the influx of extracellular calcium. A norepinephrine-responsive calcium pool appears to be located in the mitochondria of hepatocytes (Babcock et al. 1979). However, more recently it has been suggested that it is a non-mitochondrial vesicular pool of calcium that is involved (Streb et al. 1983; Joseph et al. 1984), and the exact source of alpha-adrenergically responsive calcium ions remains undefined. Most of the calcium released into the cytosol appears to be rapidly extruded from the cells following the initial rise in intracellular levels, reaccumulating once the effect of the agonist is terminated (Kunos, 1984).

(ii) The role of phospholipids: It appears unlikely that intracellular organelles respond directly to alpha-agonists, since neither mitochondria nor microsomes possess catecholamine receptors (El-Refai et al. 1979). It has been shown that the incubation of rat hepatocytes with the glycogenolytic hormones

angiotensin, vasopressin and epinephrine, stimulates the breakdown of phosphatidylinositol and its labelling with P^{32} during resynthesis (Billah and Michell, 1979), and similar responses have been observed in association with alpha-1 adrenergic stimulation in various other tissues (Michell, 1975). It has been postulated, therefore, that a phospholipid mechanism couples the stimulation of alpha-1 receptors to calcium-mobilising receptors, and that the binding of alpha-agonists to receptors on the cell surface results in the breakdown of phosphatidylinositol to phospholipid "second messengers" which in turn mediate calcium mobilisation (Michell, 1975). The stimulation of insect salivary glands with 5-hydroxytryptamine has been shown to result in the rapid hydrolysis of phosphatidylinositol 4,5-biphosphate, with the subsequent intracellular accumulation of 1,2-diacylglycerol and myoinositol 1,4,5 triphosphate (Berridge, 1983). The latter compound has, in turn, been shown to stimulate the selective release of calcium ions from a non-mitochondrial store in rat pancreatic acinar cells (Streb et al. 1983) and hepatocytes (Joseph et al. 1984), as well as in a variety of other cell types (Joseph, 1984).

It is currently believed that the key substrate in the activation of alpha-1 receptors is phosphatidylinositol 4,5 biphosphate which is hydrolysed to the two "second messengers", myoinositol 1,4,5 triphosphate and diacylglycerol (Berridge 1984; Nishizuka, 1984; Bell, 1986), the former having an effect on calcium ion mobilisation, and the latter being involved in the activation of the enzyme protein kinase C, which together with

calcium ions appears to be responsible for the induction of typical alpha-1 adrenergic effects (Nishizuka, 1984; Bell, 1986). The demonstration, however, that vasopressin-stimulated hydrolysis of phosphatidylinositol 4,5-biphosphate in hepatocytes is calcium dependent, and requires a far greater dose of agonist than is necessary for either an increase in phosphorylase activity or calcium mobilisation has raised some doubts about the hypothesis (Rhodes et al. 1983). The role of inositol 1,4,5 triphosphate in the mobilisation of calcium ions has, moreover, recently been questioned (Kunos, 1984).

(iii) The role of cyclic guanosine triphosphate regulatory proteins: These compounds may also be involved in alpha-1 adrenergic responses (Cockcroft and Gomperts, 1985; Litosch et al. 1985; Homcy and Graham, 1985) although their precise role is still poorly understood. It has been suggested that the receptor-mediated turnover of inositol 4,5 biphosphate may be coupled through GTP-dependent activation of the enzyme phospholipase C (Bell, 1986).

5.2.2 The Mediation of Smooth Muscle Contraction by Alpha-1 Adrenergic Receptors

The smooth muscle from most tissues except the intestine contracts in response to alpha-1 adrenergic stimulation, and it is generally accepted that a rise in intracellular calcium ion levels is involved (Bolton, 1979). It is likely that subsequently a calcium-calmodulin complex activates myosin light chain kinase with phosphorylation of the light chain of myosin, thereby

allowing the interaction of this protein with actin and permitting smooth muscle contraction (Adelstein and Hathaway, 1979) (see Chapter 5.1.2).

5.2.3 Activation of Alpha-2 Adrenergic Receptors

While presynaptic alpha-2 receptors are known to mediate the inhibition of norepinephrine release from adrenergic nerve endings, the molecular mechanisms involved in their function remain uncertain (Exton, 1982; Homcy and Graham, 1985).

Postsynaptic alpha-2 receptors are involved in a number of tissue reactions, including platelet aggregation and the inhibition of insulin secretion. They appear to share with muscarinic receptors the property of lowering intracellular levels of 3,5 cyclic AMP by inhibiting adenyl cyclase (Jakobs et al. 1984), and have been identified by radioligand binding, and shown to be associated with this property in a variety of tissues (Exton, 1982). In a situation analagous to the beta-adrenergically mediated stimulation of adenyl cyclase, the alpha-2 mediated inhibition of the enzyme has been clearly shown to be guanine nucleotide dependent in both human platelets (Steer and Wood, 1979) and rat islets of Langerhans (Katada and Ui, 1981). Furthermore, the alpha-2 receptors of rabbit platelets exhibit either a high or a low affinity state for epinephrine, that of high affinity being converted into one of low affinity by guanine nucleotides (Michel et al. 1980).

The activating effect of GTP on the epinephrine-induced inhibition of adenyl cyclase in rat islets has been shown to be

reduced by the addition of "islet activating protein", a toxin from B. pertussis (Katada and Ui, 1981). This toxin catalyses the ADP-ribosylation of a 41,000 Dalton membrane-bound protein which appears to be one subunit of a protein with 41,000 Dalton and 35,000 Dalton subunits which can be purified from rabbit liver (Katada et al. 1984; Bokoch et al. 1984). The protein is capable of regulating adenyl cyclase activity in human platelets. It appears to be identical with the inhibitory guanine nucleotide regulatory protein (G-protein) which couples alpha-2 agonist receptor binding with guanosine triphosphate-associated adenyl cyclase inhibition (Katada et al. 1984). It has been suggested that the stimulation of alpha-2 receptors results in a dissociation of the beta- and alpha-subunits, and that as the concentration of the inhibitory G-protein is considerably in excess of its stimulatory analogue, the 35,000 Dalton beta-subunit binds to the alpha-subunit of the stimulatory protein, thereby preventing it from activating adenyl cyclase (Gilman, 1984) (see Chapter 5.1.1). The precise molecular mechanisms involved have not, however, been defined with certainty.

5.3 MUSCARINIC RECEPTORS

Although it has recently been suggested (Venter, 1983) that muscarinic receptors lack major structural and phylogenetic diversity, they appear to display heterogeneity in the affinity of their binding to the antagonist ligand pirenzepine on the basis of which they have been classified into M1- and M2-

subtypes. The former occur in sympathetic ganglia, and the latter in peripheral effector organs such as the heart (Hammer and Giachetti, 1982).

5.3.1 Muscarinic Receptor Activation

The binding of agonists to muscarinic receptors appears to stimulate two major biochemical responses which are identical to those associated with alpha-1 and alpha-2 receptor stimulation respectively, namely (i) an increase in the cytosolic free calcium ion concentration, probably mediated by a phospholipid coupling mechanism; (ii) the inhibition of adenyl cyclase with a fall in the intracellular level of 3,5 cyclic AMP.

(i) The role of calcium ions:

While the effects of agonist binding to muscarinic receptors have been known for some time to be mediated by a rise in the cytosolic free calcium ion concentration in a variety of tissues (Rasmussen and Goodman, 1977), doubts have existed until relatively recently about the coupling of these two events. As in the case of alpha-1 adrenergic receptors, it is possible that phospholipid compounds may act as "second messengers".

Hokin and Hokin reported in 1954 that muscarinic agonists stimulated an enhanced turnover of phospholipids in pancreatic tissue, both in vitro and in vivo, and that this was associated with an increased incorporation of P^{32}. An increased turnover of inositol lipids has subsequently been demonstrated to be associated with cholinergic activity in a variety of tissues (Michell, 1975), and it has been proposed, therefore, that, as

in the case of alpha-1 adrenergic receptors, phosphoinositide breakdown is a necessary antecedent to calcium ion mobilisation. This postulate has been supported by the finding that carbachol accelerates the synthesis and accumulation of phosphatidate in frog smooth muscle cells. The time course of the effect is similar to that of muscle contraction, which can also be stimulated directly by the phosphatidate itself (Salmon and Honeyman, 1980).

A number of studies have suggested that phosphatidylinositol 4,5 biphosphate is the key substrate in this mechanism, as well as in alpha-1 adrenergic responses and intracellular levels of the compound have been shown to decline in rat parotid acinar cells (Weiss et al. 1982) and pancreas (Putney et al. 1983) in response to muscarinic stimulation, the effects being rapid and independent of calcium ions (Weiss et al. 1982; Putney et al. 1983). In addition, whereas both carbachol and myoinositol 1,4,5 triphosphate stimulate the release of calcium from intracellular stores, prior treatment of the cells with the latter compound appears to abolish the response to the former. This clearly suggests that both agents are acting on the same pool of releasable calcium, and that myoinositol 1,4,5 triphosphate is the mediator of the response to carbachol (Streb et al. 1983). It appears, therefore, that, as with alpha-1 receptor activation, the key substrate is phosphatidylinositol 4,5 biphosphate which is hydrolysed to myoinositol 1,4,5 triphosphate and diacylglycerol (Berridge, 1984; Nishizuka, 1984; Bell, 1986), the former having an effect on calcium ion mobilisation and the

latter being involved in the activation of protein kinase C which, together with calcium ions, is responsible for the induction of typical muscarinic effects (Nishizuka, 1984; Bell, 1986). In both canine and bovine tracheal smooth muscle, the link between the stimulation of muscarinic receptors, the hydrolysis of phosphatidylinositol 4,5 biphosphate and calcium ion mobilisation has recently been co (Hashimoto et al. 1985; Grandordy et al. 1986).

Recent work has suggested that GTP regulatory proteins may also be involved in these responses (Cockroft and Gomperts, 1985; Litosch et al. 1985; Bell, 1986).

(ii) Inhibition of Adenyl Cyclase:

In 1962 Murad et al. demonstrated that cholinergic agonists diminished the level of 3,5 cyclic AMP in canine myocardial homogenates, and it is now known that the stimulation of muscarinic - as well as alpha-2 adrenergic - agonists results in the inhibition of adenyl cyclase (Jakobs et al. 1984). It has been suggested (see Chapter 5.2.3) that the beta- and gamma-subunits of the inhibitory guanine nucleotide regulatory protein dissociate from the alpha-subunit in response to muscarinic - as well as alpha-2 adrenergic - receptor stimulation, and that as the inhibitory guanine nucleotide regulatory protein exists in a greater concentration than its stimulatory analogue, a reassociation may occur between the beta-subunit of the inhibitory protein and the alpha-subunit of the stimulatory protein preventing the latter from activating adenyl cyclase (Gilman, 1984). An alternative mechanism that has been suggested

is that the alpha-subunit of the inhibitory regulatory protein directly inhibits adenyl cyclase in association with GTP (Hildebrandt et al. 1985).

A number of important questions relating to muscarinic receptor function remain unanswered. The question of the relative importance of the two major biochemical responses to muscarinic stimulation in different tissues requires clarification. It has recently been suggested that either one or the other of these occurs in different experimental cell lines (Harden et al. 1986). Studies of muscarinic receptors in embryonic chick heart cells, however, have suggested that both responses are present, and that the receptors associated with them can be differentiated on the basis of the affinity of their binding to different muscarinic agonists (Brown and Brown, 1984). In addition, the importance of the functional differentiation of muscarinic receptors into the M1- and M2-subtypes on the basis of the affinity of their binding to the antagonist ligand pirenzepine is uncertain. It has recently been suggested that the smooth muscle and epithelial receptors of the bovine airway are predominantly of the M2- (low pirenzepine affinity) subtype (Madison et al. 1985; Grandordy et al. 1986).

CHAPTER 6

FUNCTIONAL CHANGES IN AUTONOMIC RECEPTORS

A number of endogenous and exogenous factors may produce changes in the function of autonomic receptors. Because beta-adrenergic receptors are linked to the relatively well defined biological effector system involving adenyl cyclase, much of current knowledge regarding autonomic receptor regulation is based on studies involving them. Accordingly, virtually all the data reviewed in the present section is concerned with functional changes in beta-adrenergic receptors.

6.1 AGONIST-INDUCED RECEPTOR DESENSITISATION

Although it is well known that the stimulation of receptors by agonists in any tissue results, after a variable period, in a diminished level of responsiveness, the mechanisms underlying the phenomenon have only recently begun to be elucidated.

Kakiuchi and Rall reported in 1968 that, whereas, the incubation of rabbit cerebellum with norepinephrine resulted in an initial large increase in the tissue content of 3,5 cyclic adenosine monophosphate (3,5 cyclic AMP), this was followed by the progressive disappearance of the nucleotide. Prolonged exposure was associated, moreover, with a reduced 3,5 cyclic AMP response on the subsequent readdition of agonist. Later studies confirmed that this desensitisation process occurred following exposure to different adrenergic agonists in a variety of cell types (de Vellis and Brooker, 1974; Franklin et al. 1975; Browning et al. 1976).

The mechanism of this desensitisation remained uncertain. It

was suggested that it was related to the release from cells of an inhibitory substance (Ho and Sutherland, 1971; Fain and Shepherd, 1975), and the desensitising effect was shown to be attenuated by inhibitors of RNA and protein synthesis (de Vellis and Brooker, 1974; Browning et al. 1976). Studies attempting to relate the desensitisation process to the induction of phosphodiesterase yielded conflicting results (de Vellis and Brooker, 1974; Browning et al. 1976).

It appeared, moreover, that the process of desensitisation could remain limited to the agonist - or class of agonists - by which it had been induced or could, once established, involve differer classes of agonists as well. Thus, while both catecholamines and prostaglandin E_1 could induce a diminished 3,5 cyclic AMP response in cultured cells, desensitisation induced by incubation with either of these types of agonists for less than 30 minutes remained specific for the inducing agonist. Incubation for a longer period with either agonist, however, desensitised the cell, not only to the original inducing agonist, but to the other as well (Su et al. 1976). The latter form of non-specific desensitisation was designated "heterologous" and hormone-specific desensitisation was designated "homologous" (Su et al 1976). It was further observed that the incubation of the cells with dibutryl cyclic AMP, an analogue of 3,5 cyclic AMP induced diminished responsiveness to both norepinephrine and prostaglandin E_1, with a time course and extent similar to that associated with heterologous desensitisation, suggesting that the latter process was in some way mediated by the same mechanism

which activates adenyl cyclase. (Su et al. 1976). In a later study it was shown that whereas isoproterenol induced a markedly decreased 3,5 cyclic AMP response to catecholamines together with some diminution in beta-receptor numbers, prostaglandin E_1, while also inducing a diminished response to catecholamines, had no effect on the number of beta-receptors. It was concluded that agonist-specific or "homologous" desensitisation might be related to a reduction in the number of beta-receptors as well as to the cyclic AMP-mediated phenomenon observed in association with non-specific or "heterologous" desensitisation (Johnson et al. 1978).

6.1.1 "Downregulation" and "Uncoupling" of Receptors

It is now evident that a diminution or "downregulation" in the number of beta-receptors is associated with catecholamine-specific desensitisation in a wide variety of cell types. A marked reduction in catecholamine-stimulated adenyl cyclase activity occurs in the erythrocytes of frogs treated for 24 hours with isoproterenol and is associated with a decrease in the number of beta-adrenergic receptors (Mukherjee et al. 1976). Similar changes occur following the incubation of frog erythrocytes with catecholamines in vitro (Mickey et al. 1976). It has also been shown that short periods of therapy with beta-adrenergic agonists are associated with a marked diminution in the density of beta-receptors as well as the 3,5 cyclic AMP responses of human leukocytes (Conolly and Greenacre, 1976; Galant et al. 1978; Scarpace et al. 1982).

Author Kallenbach J m
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