Mechanisms of Disease

Airway receptors

Peter J. Barnes

Department of Thoracic Medicine, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY, UK.

Introduction

Airway smooth muscle tone is influenced by many hormones, neurotransmitters, drugs and mediators, which produce their effects by binding to specific surface receptors on airway smooth muscle cells. Bronchoconstriction and bronchodilation may therefore be viewed in terms of receptor activation or blockade and the contractile state of airway smooth muscle is probably the resultant effect of interacting excitatory and inhibitory receptors.

It is important to recognize that airway calibre is not only the result of airway smooth muscle tone, but in asthma it is likely that airway narrowing may also be explained by oedema of the bronchial wall (resulting from microvascular leakage) and to luminal plugging by viscous mucus secretions and extravasated plasma proteins, which may be produced by a 'soup' of mediators released from inflammatory cells, including mast cells, macrophages and eosinophils. Activation of receptors on other target cells, such as submucosal glands, airway epithelium, post capillary venules, mast cells and other inflammatory cells may, therefore, also influence airway calibre.

In this article I will concentrate on some of the receptors present on airway smooth muscle which may be relevant to airway disease.

Indirect regulation of airway smooth muscle

There is a complex interaction between different cells in the airway and, while many stimuli may act directly on airway smooth muscle cells, others may affect smooth muscle tone indirectly, either via neural control mechanisms, via release of mediators from inflammatory cells, or possibly via release of epithelial factors. Thus, bradykinin is a potent bronchoconstrictor when given by inhalation in man, but has little effect on human airway muscle in vitro, suggesting an indirect action which, in part, is due to activation of a cholinergic reflex, since the bronchoconstriction may be reduced by a cholinergic antagonist. Other mediators may have a bronchoconstrictor effect which, in the case of adenosine, is due to mast cell mediator release, or in the case of platelet-activating factor due to platelet products.

Epithelial-derived relaxant factor

Recently there has been considerable interest in the possibility of a relaxant factor released from airway epithelial cells, which may be analogous to endothelial-derived relaxant factor. The presence of airway epithelium in bovine airways in vitro reduces the sensitivity to and maximum contractile effect of spasmogens, such as histamine, acetylcholine or serotonin, although not potassium which depolarises airway smooth muscle directly. Similar results have been obtained in dog, guinea pig and human airways. One possibility is that these spasmogens release factor from epithelium, rather like endothelium-derived relaxant factor, which directly relaxes airway smooth muscle. The nature of this putative factor is uncertain, but it does not appear to be influenced by either cyclo-oxygenase or lipoxygenase blockade. Another possibility is that enzymes present in epithelial cells normally degrade mediators so that epithelial removal enhances their effect.

Airway receptors and disease

Since surface receptors may determine tissue responsiveness it is possible that alterations in receptors on airway smooth muscle might account for increased airway responsiveness seen in asthma, and, to a lesser extent, in chronic obstructive airways disease. Many different factors are known to alter receptor expression and could change either receptor density, affinity, or coupling. Thus, inflammatory mediators which are formed in the airway wall may have effects on various receptors which could lead to an increased responsiveness. Since in asthma the increased responsiveness is

Correspondence: Professor P.J. Barnes, M.A., D.Sc., D.M., F.R.C.P.
Received: 27 December 1988
Supported by Medical Research Council and Asthma Research Council

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found with many different bronchoconstrictor stimuli, it is unlikely that there is an effect on a single type of receptor (e.g. muscarinic or histamine receptors). It is more probable that there is enhanced coupling of all receptors, perhaps via phosphoinositide hydrolysis, or that there is a defect in inhibitory receptors (e.g. beta-adrenoceptors).

**Receptor coupling and second messengers**

There have recently been considerable advances in molecular pharmacology of receptors. Several receptors have now been cloned and expressed and this has given important insights into the mechanisms of receptor activation. Many receptors are linked to adenylyl cyclase by a coupling protein (G protein), which either stimulates (Gs) or inhibits the enzyme (Gi). Thus, beta-adrenoceptors and vasoactive-intestinal peptide (VIP) stimulate adenylyl cyclase in airway smooth muscle, resulting in increased intracellular cyclic AMP and bronchodilatation. Conversely, acetylcholine inhibits adenylyl cyclase, resulting in reduced cyclic-AMP and bronchoconstriction. It is now apparent that several receptors which interact with G proteins have C-terminal homology and thus a common sequence of amino acids may be involved in the interaction with these coupling proteins. Indeed, there are remarkable similarities in structure between these receptor proteins with severe hydrophobic membrane spanning sections, hydrophilic loops on the external surface which contain the ligand recognition sites, and the hydrophilic intracellular loops which link with the G protein.

It is now apparent that several receptors are coupled in a different fashion and activation leads to the hydrolysis of membrane phosphoinositides (PI) with the formation of inositol triphosphate which releases calcium from intracellular stores. This stimulation of PI turnover is initiated by activation of phospholipase C via a distinct G protein. In airway smooth muscle many spasmodens stimulate PI hydrolysis. For acetylcholine there is a close relationship between muscarinic receptor occupancy and the stimulation of PI turnover and, in general, there is a close relationship between receptor density and the magnitude of PI hydrolysis. The discovery of this transduction mechanism clarifies the mechanisms of bronchoconstriction and may, in the future, lead to novel bronchodilator drugs.

Activation of PI hydrolysis also leads to the formation of diacyl glycerol which stimulates protein kinase C, a key enzyme involved in phosphorylation of several regulatory proteins, including receptors and G proteins. Thus, activation of one receptor may influence quite different receptors, and this may be pertinent in diseases such as asthma.

**Autonomic receptors in airways**

Autonomic innervation of the airways is complex. In addition to classical cholinergic pathways which cause bronchoconstriction and adrenergic mechanisms which are usually bronchodilator, there is a more recently recognized component of autonomic control which is neither cholinergic nor adrenergic. Autonomic nerves influence airway tone by activating specific receptors on airway smooth muscle. In the case of cholinergic pathways acetylcholine released from postganglionic nerve endings stimulates muscarinic cholinergic receptors. Adrenergic mechanisms include sympathetic nerves which release noradrenaline, and circulating adrenaline secreted from the adrenal medulla; these catecholamines activate alpha- or beta-receptors. The neurotransmitters of the non-adrenergic non-cholinergic (NANC) nervous system are not certain, but the most likely candidate for non-adrenergic inhibitory nerves is VIP, whereas that of non-cholinergic excitatory nerves is probably substance P (SP) or a related peptide. These neuropeptides interact with specific receptors on target cells.

The different components of the autonomic nervous system interact with each other in a complex way, both by affecting release of neurotransmitter (via prejunctional receptors), at ganglia in the airways, and by interaction at postjunctional receptors. Thus, airway tone may be determined by a complex interplay between different components of the autonomic nervous system.

**Beta-adrenoceptors**

Both histochemical and functional studies indicate that there are few, if any, adrenergic nerve fibres directly supplying airway smooth muscle in human airways, although in other species, such as cat and dog, adrenergic bronchodilator nerves have been described. This suggests that beta-receptors in airway smooth muscle are under the control of circulating adrenaline.

Direct receptor binding studies indicate that beta-receptors are present in high density in lung of many species, including humans. Autoradiographic studies have revealed that beta-receptors are found on many different cell types within lung, including airway smooth muscle from trachea down to terminal bronchioles. This is consistent with functional studies indicating that beta-agonists are potent relaxants of bronchi, bronchioles and peripheral lung strips. While a direct relaxant effect of beta-agonists on airway smooth muscle is undoubtedly their major mode of action as bronchodilators, they may also lead to bronchodilatation indirectly, either by inhibiting release of bronchoconstrictor mediators from airway.
mast cells,\textsuperscript{14} by inhibiting acetylcholine release from cholinergic nerves,\textsuperscript{19,20} or by stimulating release of an epithelial relaxant factor.\textsuperscript{4} Indeed, a very high density of beta-receptors is seen in airway epithelium, which far exceeds the density of receptors in smooth muscle itself.\textsuperscript{16}

**Beta-receptor subtypes**

Although originally beta-receptors of airway smooth muscle were classified as beta\textsubscript{2}-receptors, later studies showed that, in several species, relaxation of tracheal smooth muscle was intermediate between a beta\textsubscript{1} and beta\textsubscript{2} mediated response, suggesting the presence of beta\textsubscript{1} in addition to beta\textsubscript{2}-receptors. Using direct receptor binding techniques and selective beta-antagonists the coexistence of beta\textsubscript{1} and beta\textsubscript{2}-receptors was confirmed in animal and human lung.\textsuperscript{21} In dog tracheal smooth muscle, while beta\textsubscript{2}-receptors predominate, 20\% of receptors are of the beta\textsubscript{1} subtype.\textsuperscript{22} Functional studies of the same tissue \textit{in vitro} show that relaxation to exogenous beta-agonists is mediated by beta\textsubscript{2}-receptors, but relaxation to sympathetic nerve stimulation is mediated by beta\textsubscript{1}-receptors. These findings are consistent with the hypothesis that beta\textsubscript{1}-receptors are regulated by sympathetic nerves (‘neuronal’ beta-receptors), whereas beta\textsubscript{2}-receptors are regulated by circulating adrenaline (‘hormonal’ beta-receptors).

Further support for this idea is provided by studies of airway beta-receptor function in other species. Thus, in cat trachea, which has a dense sympathetic nerve supply, relaxation to beta-agonists is mediated predominantly by beta\textsubscript{1}-receptors, whereas in lung strips, which contain bronchioles devoid of sympathetic nerves, responses are mediated by beta\textsubscript{2}-receptors.\textsuperscript{23} In human airway smooth muscle, with its absence of significant innervation, no beta\textsubscript{1}-receptor mediated effects would be expected. This has been confirmed in functional studies \textit{in vitro}, in which relaxation of central and peripheral airways is mediated by beta\textsubscript{2}-receptors.\textsuperscript{17} Similarly, \textit{in vivo} pranethol, a beta\textsubscript{2}-selective agonist, has no bronchodilator effect in asthmatic subjects, despite significant cardiac effects.\textsuperscript{24} Autoradiographic studies of human lung have confirmed that the beta-receptors of human airway smooth muscle from bronchi to terminal bronchioles are entirely of the beta\textsubscript{2} subtype.\textsuperscript{25}

**Beta-receptor dysfunction in asthma**

The suggestion that there may be a defect in beta-receptor function in asthma\textsuperscript{26} provided a great impetus to research and the question is still unresolved. While the defects in peripheral beta-receptor function described in asthmatic subjects can largely be ascribed to the effects of prior adrenergic therapy,\textsuperscript{14} it is still not certain whether airway smooth muscle beta-receptor function is impaired, largely because of the difficulties in obtaining asthmatic airways to study \textit{in vitro}. Asthmatic subjects are apparently less responsive to inhaled beta-agonists than normal subjects,\textsuperscript{27} but this could be explained by reduced aerosol penetration or by functional antagonism (a larger dose of beta-agonist is required to reverse a greater initial degree of bronchoconstriction), rather than a defect in airway beta-receptors. Recent observations in asthmatic airways \textit{in vitro}, however, suggest that there is a reduced relaxant response to isoprenaline with no evidence of an increase in responsiveness to spasmogens.\textsuperscript{28,29}

A reduction in lung beta-receptor density is found in a guinea pig model of asthma,\textsuperscript{30} and this reduction is found in several cell types, including airway smooth muscle.\textsuperscript{31} In bovine tracheal smooth muscle cholinergic stimulation reduces beta-receptor density and uncouples beta-receptors.\textsuperscript{32} This is probably mediated via activation of protein kinase C, since phorbol esters, which also activate this enzyme, have a similar effect. Several inflammatory mediators stimulate PI turnover in airway muscle, which may result in a reduction in beta-receptor function. In guinea pigs, exposure to the inflammatory mediator, platelet-activating factor, increases bronchial responsiveness and reduces the bronchial responsiveness to isoprenaline \textit{in vivo}, although tracheal smooth muscle responds normally to isoprenaline \textit{in vitro} and the density or affinity of beta-receptors is not altered.\textsuperscript{33} Beta-adrenoceptor function in asthmatic airways has recently been reviewed.\textsuperscript{33a}

**Alpha-adrenoceptors**

Alpha-receptors which mediate contraction of airway smooth muscle have been demonstrated in many species, including human,\textsuperscript{34} although it may only be possible to demonstrate their presence under certain conditions. Human peripheral lung strips contract with alpha-agonists, although it is likely that contractile elements other than airway smooth muscle are responsible.\textsuperscript{35} Autoradiographic studies confirm a very low density of alpha\textsubscript{2}-receptors in smooth muscle of large airways, but have revealed a surprisingly high density in small airways.\textsuperscript{36}

**Alpha-receptor subtypes**

The classical alpha-receptor which mediates contractile effects is the alpha\textsubscript{1}-receptor, which is selectively blocked by prazosin, whereas prejunctional alpha-receptors, mediating negative feedback of noradrenaline release, are alpha\textsubscript{2}-receptors and selectively blocked by yohimbine. More recently, alpha-
receptors have also been found postjunctionally. In dog tracheal smooth muscle the contractile response to both sympathetic nerve stimulation and to exogenous alpha-agonists are mediated almost entirely by alpha2-receptors, and the majority of alpha-receptors measured by radioligand binding in the same tissue are of this subtype. The role of alpha2-receptors in human airways is not yet certain.

**Alpha-receptors in asthma**

There is some evidence that alpha-adrenergic responses may be increased in asthma and may, therefore, contribute to bronchial hyperresponsiveness. The alpha-agonist methoxamine causes bronchoconstriction in asthmatic but not in normal subjects, even in the absence of beta-blockade. This suggests that alpha-adrenergic responses may be increased in the airways of asthmatics.

No alpha-adrenergic response can be demonstrated in normal canine or human smooth muscle in vitro, even after beta-blockade, but in diseased human airways or after pretreatment of normal canine airways with histamine or serotonin a marked alpha-adrenergic contractile response is seen, suggesting an activation of alpha-adrenergic responses by mediators or disease. Similar activation of alpha-adrenergic responses can also be demonstrated in vivo. This suggests that inflammatory mediators may 'turn on' alpha-adrenergic responses in asthma. The mechanism for this activation does not involve any change in density or affinity of alpha-receptors in airway smooth muscle and is likely to be a post-receptor mechanism, possibly involving voltage-dependent calcium channels. In a guinea pig model of asthma there is an increase in lung alpha,-receptors, although no increased alpha-adrenergic responsiveness has been found in airway smooth muscle.

If exaggerated alpha-adrenergic responsiveness of airway smooth muscle were an important factor in bronchial hyperresponsiveness, then alpha-blockers should be beneficial in asthma. Alpha-antagonists, such as phentolamine and thymoxamine, have been shown to inhibit bronchoconstriction induced by histamine, allergen and exercise, but such drugs lack specificity and their protective effects may be explained by their pharmacological actions such as antihistamine activity. The specific alpha,-blocker prazosin given by inhalation has no bronchodilator effect in asthmatics, who readily bronchodilate with a beta-agonist, suggesting that an alpha-hyperresponsiveness does not contribute to resting bronchomotor tone in asthma. Similarly, prazosin has no effect on histamine-induced bronchoconstriction, but has a weak protective effect against exercise-induced bronchospasm, which may be explained by an effect on bronchial blood flow rather than on airway smooth muscle. It is possible that stimulation of alpha-receptors may, if anything, be beneficial in asthma since adrenaline prevents microvascular leakage in guinea pig airways via alpha,-receptor stimulation, and alpha2-agonists inhibit both cholinergic and NANC constrictor nerve effects prejunctionally.

**Cholinergic receptors**

Acetylcholine released from cholinergic nerves causes contraction of airway smooth muscle by activation of muscarinic receptors which are blocked by atropine. There is a close association between receptor occupation and stimulation of PI hydrolysis but the contraction curve is well to the left and maximum contraction is obtained when only about 20% of receptors are occupied, indicating the existence of 'spare' receptors. This is confirmed by the use of phenoxycbenzamine which irreversibly alkylates muscarinic receptors and with progressive loss of receptors, although there is a rightward shift in the concentration response curve, the maximum response is only reduced when receptor density falls below 20%.

Direct receptor binding studies have demonstrated a high density of muscarinic receptors in smooth muscle of large airways, and this has been confirmed autoradiographically. The density of muscarinic receptors decreases as airways become smaller, so that terminal bronchioles are almost devoid of muscarinic receptors. This is consistent with physiological studies in dogs using tantalum bronchography, showing that vagal stimulation has a marked effect on large airways but little effect on bronchioles. In humans, anticholinergic drugs have more effect on large than on small airways, as measured by helium-oxygen flow-volume curves, whereas beta-agonists relax all airways.

**Muscarinic receptor subtypes**

Recent evidence from binding and functional studies with selective antagonists suggests that muscarinic receptors may be subclassified into at least three types, and four distinct receptor proteins have now been cloned, although there is still considerable confusion about terminology. In gut, pirenzepine selectivity blocks muscarinic receptors on ganglia, which are termed M1-receptors, but not those on smooth muscle which are designated M2-receptors. In airway smooth muscle, as expected, there is no evidence for M1-receptors, since pirenzepine has low affinity in both inhibition of receptor binding and in blocking PI hydrolysis. Recently it has been possible to demonstrate M2-receptors in human cholinergic reflex pathway, probably localized to ganglia.
It has also been possible to demonstrate a different type of muscarinic receptor in postganglionic airway nerves, since in cat and guinea pig the non-competitive antagonist gallamine selectively inhibits a muscarinic receptor on cholinergic nerve terminals which enhances acetylcholine release and has little inhibitory effect on acetylcholine in airway smooth muscle.\cite{59,60} This suggests that acetylcholine inhibits its release from cholinergic nerves via an autoreceptor which differs from the receptor on airway smooth muscle. Similar observations have recently been made in human airway smooth muscle \textit{in vitro}\cite{54} and \textit{in vivo}.\cite{55} Since the gallamine-sensitive receptor in heart is classified as an $M_2$-receptor, it follows that the receptor on airway smooth muscle must be of a different subtype, and may be designated an $M_4$-receptor. This receptor is selectively inhibited by the drugs 4-DAMP and hexahydorsila-difenidol, whereas the prejunctional $M_2$-receptor is inhibited by gallamine, AF-DX 116 and the recently developed selective drug methoctramine. There is some evidence that the prejunctional $M_2$-receptor may be dysfunctional in asthma, which would lead to exaggerated cholinergic reflex effects, and this may also account for the profound bronchoconstriction which may occur after beta-blockade in asthma.

**Neuropeptide receptors**

Many different neuropeptides have now been localized to airway nerves in several species, including humans.\cite{56,57} There is increasing evidence that these peptides play a neurotransmitter or cotransmitter role, and may be the neurotransmitters of NANC nerves. These neuropeptides have effects on airway smooth muscle which are mediated by specific surface receptors. The functional significance of airway neuropeptides is still uncertain and will remain so until specific receptor antagonists become available. Both inhibitory and excitatory NANC nerves have been described; evidence is in favour of VIP as a neurotransmitter of the inhibitory nerves, and of substance P (SP) and related tachykinins as the neurotransmitters of excitatory nerves.

**VIP-receptors**

VIP is a potent relaxant of animal and human airways \textit{in vitro} and is 50–100 times more potent than isoprenaline as a bronchodilator.\cite{58} VIP is a potent bronchodilator in cats when given intravenously, but in human subjects the cardiovascular effects of the peptide prevent the infusion of a dose high enough to bronchodilate.\cite{59} Inhaled VIP also has no bronchodilator effect in man, probably because it is not able to reach receptors on smooth muscle.\cite{60} VIP is the most favoured candidate as neurotransmitter of non-adrenergic inhibitory nerves in airways since in many respects it mimics NANC inhibitory nerve effects. VIP produces its effects by activation of specific receptors on airway smooth muscle and it is unaffected by beta-blockers, indomethacin or tetrodotoxin. VIP, like beta-agonists, stimulates adenylylate cyclase in target cells and therefore increases cyclic AMP in lung tissue. Using an immunocytochemical method, it has been possible to demonstrate increases in cyclic AMP content of airway smooth muscle cells in several species.\cite{61} VIP-receptors have been identified by \textit{125I}-VIP binding in lung homogenates,\cite{62} and the distribution of these receptors in lung has been studied by autoradiography.\cite{63} VIP-receptors are found in several cell types, including smooth muscle of large airways, but not of small airways, which is consistent with the lack of effect of VIP in relaxing small airways, although isoprenaline relaxes both large and small airways to an equal extent.\cite{64} The lack of VIP-receptors in small airway smooth muscle is also in keeping with the paucity of VIP-immunoreactive nerves, and with the lack of NANC inhibitory nerves in small airways.

Peptide histidine isoleucine (PHI) which exists in humans, with a terminal methionine (PHM), is closely related in structure to VIP and is coded by the same gene. It has similar effects to VIP and is equipotent in relaxing airways smooth muscle. It may activate the same receptors, although evidence now suggests that it may have different receptors since it has a different potency from VIP on vascular smooth muscle.

**Tachykinin receptors**

Substance P is localized to sensory nerves in the airways and may be released as part of an axon reflex. SP constricts airway smooth muscle of animals and humans \textit{in vitro} by activating specific receptors. \textit{In vivo} SP infusion causes bronchoconstriction in animals which may be partially blocked with atropine, suggesting that SP release of acetylcholine may contribute to its bronchoconstrictor effect.\cite{64} Autoradiographic studies using labelled SP have demonstrated high densities of SP receptors on smooth muscle of guinea pig and human airways from large airways down to terminal bronchioles.\cite{65}

Recently, related peptides (tachykinins) have been isolated from the mammalian nervous system. Neurokinins A and B appear to activate distinct receptors which have been termed NK-2 and NK-3 receptors, respectively, whereas SP activates NK-1 receptors.\cite{66} In airway smooth muscle of several species, including humans, the order of potency is NKA $>$ NKB $>$ SP (NKA being about 100-fold more potent than SP), indicating an NK-2 receptor.\cite{67,68} This suggests that NKA is the endogenous bronchoconstrictor tachykinin. This has recently been
confirmed in human subjects in vivo, since infused and inhaled NKA causes bronchoconstriction with little cardiovascular effect, whereas SP has profound cardiovascular actions but no bronchoconstrictor effect. Tachykinins appear to cause airway smooth muscle contraction by stimulating PI hydrolysis and, as expected, NKA is more potent than NKB or SP. Mechanical removal of airway epithelium markedly enhances the contractile response to NKA in guinea pig trachea, and a similar effect is produced in the absence of phosphoramidon, an inhibitor of the major degrading enzyme (enkephalinase). Thus, loss of epithelium in asthma may enhance the effects of tachykinins released from sensory nerves, since degradation would be prevented by loss of the enzyme contained in epithelial cells.

Other neuropeptides

Calcitonin gene-related peptide (CGRP) is also localized to sensory nerves in airways and contracts on human bronchi in vitro. Autoradiographic mapping of CGRP receptors in human and guinea pig lung have shown a high density of receptors in airway blood vessels, suggesting that CGRP may regulate airway blood flow.

Neuropeptide Y (NPY) is a cotransmitter of noradrenaline but has no direct effect on airway smooth muscle, but modulates cholinergic nerve effects via a prejunctional receptor. This suggests that NPY receptors are localized to cholinergic nerve terminals rather than to airway smooth muscle.

Mediator receptors

Many inflammatory mediators have effects on airway smooth muscle and produce their effects by activation of specific receptors on airway smooth muscle cells. Several mediators produce their effects indirectly on airway smooth muscle, either by activating bronchoconstrictor nerves or by releasing bronchoconstrictor mediators from other inflammatory cells. Indeed, there is increasing emphasis on the concept that asthma is, to a considerable degree, an inflammatory disease.

Histamine receptors

Histamine produces its effects by activation of H₁- and H₂-receptors. Inhaled histamine causes bronchoconstriction in vivo and contraction of large and small human airways in vitro by activating H₁-receptors, which are antagonized by the classical antihistamines such as chlorpheniramine. H₁-receptors have been identified in guinea pig and human lung homogenates. H₁-receptors have been characterized in bovine tracheal smooth muscle by [³H]-pyrilamine binding; there is a close relationship between H₁-receptor occupancy and stimulation of PI turnover by histamine, indicating that H₁-receptors may lead to contraction by stimulating PI hydrolysis and release of intracellular calcium, as in other tissues. There is also a close association between the contractile response and H₁-receptor occupancy in this tissue, indicating that there are no ‘spare’ receptors.

The role of H₂-receptors in airways is less certain. In some species H₂-receptors mediate bronchodilatation. Human peripheral lung strips may also relax with histamine, although this is likely to reflect the presence of vascular smooth muscle in these preparations. In vivo H₂-receptor antagonists have no effect on the bronchoconstrictor effect of histamine, suggesting that H₂-receptors do not play a role in regulating human airway smooth muscle tone. H₂-receptors have been identified in guinea pig lung homogenates by receptor binding using [³H]-tiotidine, but their localization is uncertain.

Prostanoid receptors

Prostaglandin receptors have not been well characterized since few specific antagonists are available. Prostacyclin receptors have been identified in guinea pig lung by measuring activation of adenylate cyclase and by direct receptor binding. These receptors are probably localized to pulmonary vessels, rather than airways, since prostacyclin has little effect on airway smooth muscle. PGD₂ and PGF₂α are potent constrictors of human airways in vitro and in vivo, presumably by activation of specific receptors in airway smooth muscle. PGD₂ enhances histamine and methacholine responsiveness, possibly via participation of a thromboxane receptor. Thromboxane A₂ is also a potent bronchoconstrictor which activates specific receptors and specific thromboxane receptor antagonists have recently been developed.

Leukotriene receptors

There has been considerable interest in the role of leukotriene B₄ and the sulphidopeptide leukotrienes C₄, D₄ and E₄ (which comprise slow-reacting substance of anaphylaxis) in the pathogenesis of asthma. Leukotrienes produce their effects by activating specific, and probably distinct, receptors which have recently been identified using [³H]-labelled leukotrienes. LTB₄ has potent chemotactic activity, particularly for neutrophils, and has little direct effect on airway smooth muscle.

Functional studies with sulphidopeptide leukotrienes have indicated that there may be discrete receptors for LTC₄ and LTD₄ in guinea pig lung, and...
binding studies have confirmed that there are two distinct binding sites in lung homogenates which correspond with the functional LTC₄ and LTD₄ receptors. LTC₄ appears to bind to the D₂ receptor but, although it is less potent than LTD₄, it is more slowly metabolized and so may have a more significant functional effect and contribute to the prolonged duration of bronchoconstriction of leukotrienes. In human airways there may be only a single leukotriene receptor, however. The signal transduction system of the LTD₄ receptor has very recently been reviewed.

The biochemical mechanisms by which LT receptors lead to contraction of airway smooth muscle have recently been investigated. Both LTC₄ and LTD₄ stimulate PI hydrolysis in guinea pig treachea, but LTC₄ is significantly more potent and has a greater stimulatory effect. Functionally, the two LTs have a similar potency, however, and the greater effect of LTC₄ may be related to the much higher number of binding sites. Both LTs also inhibit adenylate cyclase but, again, LTC₄ is more potent than LTD₄. Autoradiographic studies have mapped the distribution of LTC₄ and D₂ receptors in guinea pig lung. There appears to be a differential distribution, with LTC₄ receptors being more widely distributed and present in higher density, particularly in airway smooth muscle, than LTD₄ receptors.

**Platelet activating factor receptors**

Platelet activating factor (PAF) is a phospholipid which is a potent bronchoconstrictor in several animal species, including humans. Bronchoconstriction is indirect, since PAF has no direct effect on airway smooth muscle in vitro and is presumed to interact with other cells, such as platelets, neutrophils, macrophages or eosinophils. PAF produces its effects by activation of specific receptors which have been identified by direct binding assays on human platelets, neutrophils and lung membranes. PAF receptors are presumably not present on human airway smooth muscle, since PAF has no direct constrictor effect, but may cause constriction in the presence of platelets.

PAF also causes microvascular leakage in airways, which appears to be due to a direct action of PAF on airway vascular endothelial cells.

**Adenosine receptors**

At least two cell surface receptors for adenosine have been recognized with the development of a series of adenosine analogues. A₁-receptors are usually excitatory and associated with a fall in intracellular cyclic-AMP, whereas A₂-receptors are usually inhibitory and associated with a rise in cyclic-AMP. Inhaled adenosine causes bronchoconstriction in asthmatic subjects. Adenosine relaxes guinea pig airways by an A₂-receptor and has little effect on isolated human airways in vitro, suggesting that the bronchoconstrictor effect may be indirect, possibly by potentiating inflammatory mediator release. Theophylline is a specific antagonist of both A₁- and A₂-receptors, but there is evidence against this as its major mechanism of bronchodilatation, since an analogue of theophylline, enprofylline, is a more potent bronchodilator, without significant adenosine antagonism.

**Bradykinin receptors**

Bradykinin is a potent bronchoconstrictor when given by inhalation to asthmatic subjects, being significantly more potent than methacholine, but has little effect on human airways in vitro, suggesting that its bronchoconstrictor action is indirect, and that human airway smooth muscle does not have a significant population of bradykinin receptors. Bradykinin releases prostaglandins in several species, suggesting that its effects might be mediated via bronchoconstrictor prostaglandins, but aspirin has no inhibitory effect. To some extent the bronchoconstrictor effect is diminished by anticholinergic drugs, implicating a vagal cholinergic reflex. There is evidence that bradykinin may activate C-fibre afferent nerve endings in bronchi, so that the effect of bradykinin may be due to a cholinergic reflex and also due to release of bronchoconstrictor sensory neuropeptides via a local (axon) reflex. Two receptor subtypes (designated B₁ and B₂) have been recognized using peptide analogues of bradykinin and there is some evidence that experimental inflammation may enhance the responsiveness of B₁ receptors.

**Conclusions**

Many different receptors have now been characterized in airway smooth muscle by functional studies and by direct receptor binding methods. Recently the mechanisms by which receptors are coupled to contraction of airway smooth muscle have been elucidated. The development of techniques to measure electrophysiological changes after receptor activation, and the study of isolated airway smooth muscle cells, should further increase our understanding of airway smooth muscle receptors. The demonstration that several agonists may have different effects on different sized airways is important, since the receptor population of smooth muscle in peripheral airways may be quite different from that in proximal airways. Since studies have usually been performed in larger airways, this may give misleading information about responsiveness of peripheral airways which may be involved in airway disease. The question of whether receptor
populations may be changed in airway disease is still not answered. Stimulation of one receptor may change the expression of another. Thus, stimulation of muscarinic receptors may reduce the number and coupling of beta-adrenoceptors, possibly via the enzyme protein kinase C which is stimulated by PI breakdown. This implies that other inflammatory mediators, which also lead to receptor-mediated PI hydrolysis, may produce marked changes in surface receptor populations, resulting in altered responsiveness in asthma. Advances in molecular biology have now made it possible to study gene expression of receptor proteins and this should give great insight into the regulation of receptor expression in disease.

Acknowledgement
I am very grateful to Madeleine Wray for the careful preparation of the manuscript.

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Airway receptors.

P. J. Barnes

*Postgrad Med J* 1989 65: 532-542
doi: 10.1136/pgmj.65.766.532

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