Distribution of Receptor Targets in the Lung

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Most of the drugs that are currently used to treat airway diseases interact with receptors expressed by cells in the airways. This makes inhaled delivery efficient because it reaches the key target cells and has a low risk of systemic side effects. Both β_2-agonists and anticholinergics target autonomic receptors on airway smooth muscle of large and small airways. Inhaled β_2-agonists also interact with β_2-receptors expressed on other important target cells, including mast cells and postcapillary venules. Anticholinergic bronchodilators target M_3 muscarinic receptors on airway smooth muscle, which in small airways may be activated by extraneuronal acetylcholine. Corticosteroids target glucocorticoid receptors (GR), which are widely distributed so that they are best given by inhalation to interact with intracellular GR in the respiratory tract and to avoid side effects from activation of GR in extrapulmonary tissues such as bone. By contrast, cysteinyl-leukotriene 1 receptors are mainly expresses in airway smooth muscle so that antileukotrienes are less effective clinically than β_2-agonists and corticosteroids, but oral delivery is possible as there are minimal side effects. There are many other receptor targets in lung and for several of these receptors, such as receptors for chemotactic agonists, selective antagonists are in clinical development. For drugs that inhibit chemotactic receptors, systemic delivery is more appropriate to prevent the inflammatory cells that bear these receptors from being recruited into the airways by locally released chemotactic factors. Many novel receptors, including orphan receptors, have now been identified as these may be future targets for developing novel therapies for asthma and chronic obstructive pulmonary disease.

Keywords: β_2-adrenoceptor; chemokine receptor; cysteinyl-leukotriene receptor; glucocorticoid receptor; muscarinic receptor

Most of the drugs that are currently used to treat common respiratory diseases interact with cell receptors in the respiratory tract. These include β_2-adrenoceptor agonists, muscarinic receptor antagonists (anticholinergics), corticosteroids, and cysteinyl-leukotriene-1 (cys-LT_1) receptor antagonists (antileukotrienes). The distribution of receptors for these agonists or antagonists is an important determinant of their mechanism of action, as it defines which cells can be targeted by these drugs, but it also determines side effects, because most of these receptors are widely distributed in the body. This has led to the development of inhaled delivery for these drugs (β_2-agonists, anticholinergics, and corticosteroids) in order to maximize effects in the airways and minimize systemic side effects. For antileukotrienes the relevant receptors are largely confined to the respiratory tract and therefore systemic administration does not produce significant extrapulmonary (adverse) effects, so that inhaled delivery has no advantage. The distribution of receptors is obviously an important determinant of the clinical effect of the drug and this is highly relevant to new drugs in development that might target either novel receptors or specific receptor subtypes of known receptors.

TECHNIQUES FOR STUDYING RECEPTOR DISTRIBUTION IN LUNG

There are several complimentary approaches to determining the anatomical distribution of receptors in lungs.

Receptor Autoradiography

Receptor autoradiography depends on the technique of radioligand binding, in which a radiolabeled receptor agonist or antagonist binds to specific receptors in tissue sections of lung, airways, or isolated cells and the distribution of receptors is then mapped by autoradiography. As a control for nonspecific (nonreceptor) binding, the same concentration of radioligand is incubated with an excess of unlabeled agonist (preferably) or antagonist to displace the radioligand bound to receptors. For example, [^{125}I] iodocyanopindolol is a high-affinity radiolabeled β-agonist that has been used for localization of β-receptors in lung. Nonspecific binding is usually measured by a high concentration of isoproterenol, a nonselective β_2-agonist that occupies most of the β-receptor binding sites. It is important to demonstrate that the characteristics of specific binding are those expected of the particular receptor of interest in careful preliminary studies. Receptor subtypes may also be mapped using receptor subtype-selective ligands, for example a β_1-selective antagonist such as betaxolol to displace β_1-receptor binding but leaving the label bound to β_2-receptors which are then visualized. This technique has been useful for localizing many autonomic, neuropeptide and mediator G protein–coupled receptors (GPCR) in lung, although for some ligands there is problem of high nonspecific binding, which makes discrimination of specific receptor distribution difficult. The amount of binding may be quantified by image analysis. A relative disadvantage of autoradiography is that frozen sections are needed to preserve the binding sites of most receptors, and this has limited resolution of the imaging.

Receptor Immunohistochemistry

The coning of many receptors has now made it possible to develop high-affinity antibodies which may be lapelled in various ways, including fluorescence probes, to allow histochemical localization of receptors in tissue sections. Controls include sections incubated with the antibody with an excess of purified receptor. This technique can provide good resolution and may be possible with paraffin section and electron microscopy, giving a high level of resolution, but is difficult to quantify.

In Situ Hybridization

Sense oligonucleotides or riboprobes to receptor gene encoded mRNA are used to localize specific receptor mRNA by *in situ* hybridization. The probe is labeled radioactively or nonradioactively by digitonin. Nonspecific binding is measured using the complementary sense probe that does not hybridize with receptor mRNA. This technique gives information that is complementary to the techniques described above that measure receptor protein, but is particularly useful for receptors where no specific ligands

(*Received in original form and accepted in final form September 24, 2004*)

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Proc Am Thorac Soc Vol 1. pp 345–351, 2004
DOI: 10.1513/pats.200409-045MS
Internet address: www.atsjournals.org

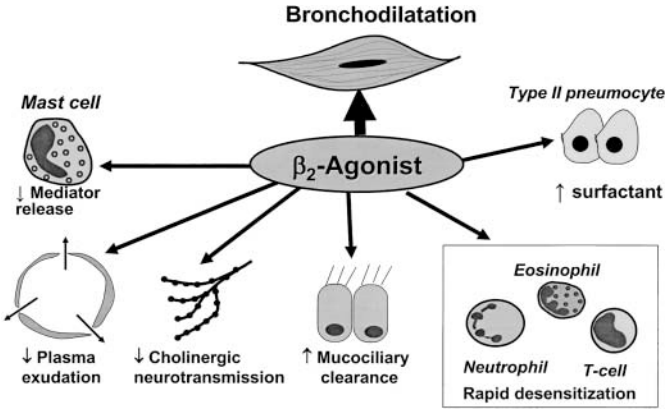

Figure 1. Effects of inhaled β_2-agonists on airways. Their major action is in directly relaxing airway smooth muscle of large and small airways, but they may also act on β_2-receptors of other airway cells. Infiltrating inflammatory cells also have β_2-receptors but are rapidly desensitized by β_2-agonists so that there are no persistent antiinflammatory effects.

are developed, or for receptor subtypes for which there are no very selective ligands available.

Functional Studies

Receptors may also be localized by functional studies that measure responses to specific agonists in tissues or specific cell types purified from lung. For example, β_2-agonists relax precontracted human bronchi and parenchymal lung strips *in vitro*, indicating the presence of β_2-receptors on airway smooth muscle of large and small airways. β_2-Agonists also inhibit the release of bronchoconstrictor mediators from isolated human lung mast cells, indicating that these cells have β_2-receptors and may be stimulated by inhaled β_2-agonists.

β-ADRENOCEPTORS

β-Adrenoceptors are present in high concentration in lung tissue, and autoradiographic mapping and *in situ* hybridization studies show that they are localized to several cell types (1) (Figure 1). Binding studies show that approximately 70% of pulmonary β-receptors are of the β_2-receptor subtype (2, 3). These receptors are localized to airway smooth muscle, epithelium, vascular smooth muscle, and submucosal glands (4), whereas β_1-receptors are localized to submucosal glands. There is a uniform distribution of β-receptors on the alveolar wall with a ratio of β_1:β_2 receptors of 2:1. This is confirmed by *in situ* hybridization studies that show a similar distribution of β_1- and β_2-receptor mRNA in human lung (5, 6).

Airway Smooth Muscle

The density of β_2-receptors in airway smooth muscle does not change at different airway levels, so that bronchioles have a similar density to large airways. This is confirmed by functional studies showing that β_2-agonists relax precontracted human bronchi *in vitro*, and also relax lung parenchymal strips. This suggests that β_2-agonists may bronchodilate all airways, and this is particularly important in asthma and chronic obstructive pulmonary disease (COPD), where small airways are involved.

Mast Cells

β_2-Agonists also have many other effects in the airways, as β_2-receptors are widely distributed (1). They inhibit the release of histamine and cysteinyl-leukotrienes from chopped human lung and purified human lung mast cells (7). Inhaled β_2-agonists have a greater protective effect against adenosine-induced bronchoconstriction, which is mediated by mast cell degranulation, than against histamine- and methacholine-induced bronchoconstriction, which are direct constrictor effects of airway smooth muscle. This indicates the additional inhibitory effect of inhaled β_2-agonists on mast cells (8, 9). This may be important in the use of β_2-agonists in preventing allergen- and exercise-induced asthma as well as in severe asthma and acute exacerbations, all of which involve mast cell activation. Interestingly, inhaled β_2-agonists are more effective than oral β_2-agonists that give equivalent bronchodilatation in protecting against exercise-induced asthma (10). β_2-Agonists also inhibit the release of acetylcholine from cholinergic nerves, thus reducing cholinergic neural (reflex) bronchoconstriction.

Plasma Exudation

Exudation of plasma from postcapillary venules is an important component of acute inflammation. β_2-Receptors are present on postcapillary venular endothelial cells, and β_2-agonists inhibit plasma exudation by preventing separation of endothelial cells in postcapillary venules (11). This effect is seen with all β_2-agonists (12, 13). In this way β_2-agonists may exert acute antiinflammatory and antiedema effects in the airways. While intravenously administered β-agonists are ineffective in inhibiting plasma exudation in guinea pigs (14), they are effective in inhibiting the leakage induced by inhaled mediators when given via the aerosol route, indicating that high local concentrations may be useful in inhibiting exudation of plasma (12, 15). Whether these effects of inhaled β-agonists are relevant to their antiasthma actions is not yet certain, as plasma exudation in the lower airways is difficult to quantify in human airways. Inhaled formoterol reduces the increase in plasma proteins induced by inhaled histamine in sputum of normal subjects, indicating that therapeutic doses of inhaled β_2-agonists can inhibit plasma exudation (16).

Sensory Nerves

β-Agonists may also have effects on activation of airway sensory nerves. β-Agonists inhibit excitatory nonadrenergic noncholinergic (NANC) bronchoconstrictor responses in guinea pig bronchi *in vitro* at concentrations that do not block equivalent tachykinin-induced responses (17). This modulatory effect is mediated via β_2-receptors on capsaicin-sensitive sensory nerves in the airways. Whether β-receptors modulate sensory nerves in human airways is less certain. Some evidence that suggests that β_2-receptors may be modulatory is provided by the inhibitory action of albuterol on cough responses (18).

Inflammatory Cells

Inflammatory cells that are involved in asthma and COPD, including eosinophils, neutrophils, T lymphocytes, and macrophages, all express a low number of β_2-receptors. *In vitro* β_2-agonists have been shown to inhibit the release of inflammatory mediators from these cells (19). However, these effects rapidly become tolerant due to downregulation of β_2-receptors (20). This means that β_2-agonists do not have chronic antiinflammatory actions in airway diseases.

Future Developments

β_2-Agonists are by far the most effective bronchodilators in asthma because they act as functional antagonists and therefore reverse and prevent bronchoconstriction from all the many bronchoconstrictor mechanisms that operate in asthmatic airways. The major advance has been the introduction of long-acting β_2-agonists (LABA) that have duration of action over 12 hours.

LABA are now commonly used in combination with inhaled corticosteroids to control asthma (*see below*). In the future β_2-agonists of even longer duration will be introduced and at least four once-daily β_2-agonists are now in clinical development. These drugs have been developed to have a slow dissociation from β_2-receptors and prolonged retention in the lung, thus enhancing their therapeutic ratio.

ANTICHOLINERGICS

Inhaled anticholinergics, which antagonize muscarinic receptors in the airways, are the most effective class of bronchodilator in patients with COPD in whom airway cholinergic (vagal) tone is the only reversible component of the disease. In asthma, anticholinergics are considerably less effective as bronchodilators than β_2-agonists, because they block only the cholinergic component of bronchoconstriction, which is usually minimal compared to the direct constrictor effects of mediators, such as cys-LTs. The major targets for anticholinergic drugs are muscarinic receptors in the respiratory tract, and these receptors are expressed on several cell types. The respiratory tract is innervated by the vagus nerve, which carries preganglionic cholinergic fibers that relay in local ganglia in the airway wall. Postganglionic fibers then innervate airway smooth muscle and submucosal glands. Vagal innervation of the airways is predominantly in large airway and diminishes peripherally with no motor innervation of small airway and lung parenchyma (21).

Muscarinic Receptors

The distribution of muscarinic receptors has been mapped in animal and human airways by receptor autoradiography. In ferret and guinea pig airways there is a high density of muscarinic receptors in smooth muscle of large airways with a progressive diminution of receptors as airways diminish in size, consistent with the distribution of cholinergic nerves (22, 23). In human airways, however, muscarinic receptors are localized to smooth muscle of all airways, although the density in larger airways is higher (23). Muscarinic receptors are also localized to airway epithelium and to submucosal glands, consistent with the stimulatory effect of acetylcholine (ACh) on mucus secretion.

Muscarinic Receptor Subtypes

Four subtypes of muscarinic receptor have now been identified in lung by binding studies and pharmacologically (24). The muscarinic receptors that mediate bronchoconstriction in human and animal airways belong to the M_3-receptor subtype, whereas mucus secretion appears to be mediated by M_1- and M_3-receptors. Muscarinic receptor stimulation results in vasodilatation via activation of M_3-receptors on endothelial cells which release NO. M_1-receptors are also localized to parasympathetic ganglia, where they facilitate the neurotransmission mediated via nicotinic receptors.

Inhibitory muscarinic receptors (autoreceptors) have been demonstrated on cholinergic nerves of airways in animals *in vivo*, and in human bronchi *in vitro* (24). These prejunctional receptors inhibit ACh release and may serve to limit vagal bronchoconstriction. Autoreceptors in human airways belong to the M_2-receptor subtype, whereas those on airway smooth muscle and glands belong to the M_3-receptor subtype. Drugs such as atropine and ipratropium bromide, which block both prejunctional M_2-receptors and postjunctional M_3-receptors on smooth muscle with equal efficacy, therefore increase ACh release, which may then overcome the postjunctional blockade. This means that such drugs will not be as effective against vagal bronchoconstriction as against cholinergic agonists, and it may be necessary to reevaluate the contribution of cholinergic nerves when drugs that are selective for the M_3-receptors are in clinical use. The presence of muscarinic autoreceptors has been demonstrated in human subjects *in vivo*. A cholinergic agonist, pilocarpine, which selectively activates M_2-receptors, inhibits cholinergic reflex bronchoconstriction induced by sulfur dioxide in normal subjects (25). Autoradiographic mapping using [^{3}H]N-methyl scopolamine and selective muscarinic antagonists has demonstrated the presence of M_2 and M_3 receptors in airway smooth muscle of human airways and M_1 and M_3 receptors in submucosal glands (23). There are also M_1 receptors in the lung parenchyma. A similar distribution of binding sites is seen with radiolabeled tiotropium bromide, the long-acting muscarinic antagonist (26). This autoradiographic distribution of muscarinic receptors in lung has been confirmed by in situ hybridization using riboprobes for *m1*, *m2*, and *m3* receptor genes (27). There is a high level expression of the *m3* receptor gene in airway smooth muscle of all airways, with clear evidence of expression in peripheral airways as well as submucosal glands. There is no evidence for expression of *m4* or *m5* receptors in human lung, although there is expression of m4 receptors in rabbit lung, indicating important species differences, not only in the distribution of receptors, but also in the subtypes expressed (28).

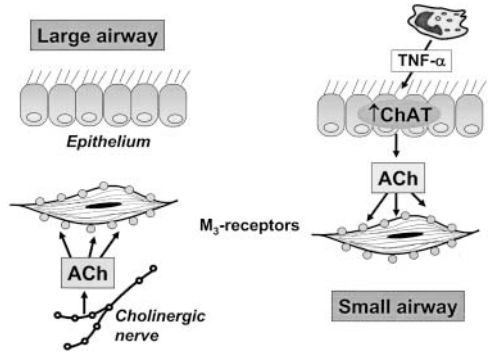

Figure 2. In proximal airways acetylcholine (ACh) is released from vagal parasympathetic nerves to activate M_3-receptors on airway smooth muscle cells. In peripheral airways M_3-receptors are expressed, but there is no cholinergic innervation; these receptors may be activated by ACh released from epithelial cells that may express choline acetyltransferase (ChAT) in response to inflammatory stimuli, such as tumor necrosis factor-α (TNF-α).

Extraneuronal ACh

Recent evidence suggests that ACh may also be released from cells in the airways other than nerves, including epithelial cells, but the role of extraneuronal ACh in human airways is currently uncertain (29, 30). The synthesis of ACh in epithelial cells is increased by inflammatory stimuli which increase the expression of choline acetyltransferase (ChAT), the enzyme responsible for synthesis of ACh. This could therefore theoretically contribute to cholinergic effects in airway diseases. Because muscarinic receptors are expressed in airway smooth muscle of small airways which do not appear to be innervated by cholinergic nerves (23, 27), this might be an important as a mechanism of cholinergic narrowing in peripheral airways that could be relevant in COPD (Figure 2). ChAT is also expressed in inflammatory cells, including macrophages and T-lymphocytes, indicating another source of ACh in inflammatory airway diseases (30). Human T-lymphocytes express ChAT and release ACh on immune activation, but also express muscarinic receptors, so have the ability to respond to ACh (31, 32).

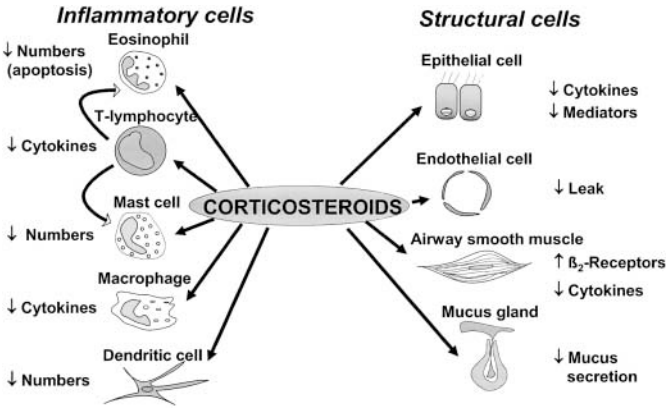

Figure 3. Glucocorticoid receptors are widely distributed in the airways and are expressed on inflammatory and structural cells.

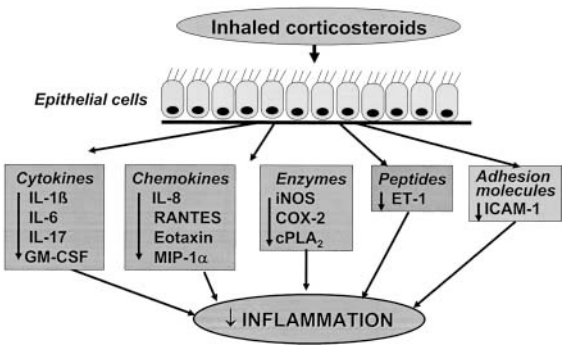

Figure 4. Airway epithelial cells are likely to be the major target for inhaled corticosteroids, which switch off multiple inflammatory genes. ET-1 = endothelin-1; GM-CSF = granulocyte-macrophage colony stimulating factor; ICAM-1 = intercellular adhesion molecule-1; IL = interleukin; iNOS = inducible nitric oxide synthase; MIP = macrophage inflammatory protein.

Cholinergic Mechanisms in Inflammation

Not only do inflammatory cells have the capacity to synthesize ACh, but they may also respond to ACh through the activation of muscarinic receptors. This suggests the possibility that anticholinergic drugs might have inhibitory effects on inflammatory cells that are activated by neuronal and extraneuronal release of ACh. A monocyte/macrophage line has been shown to express *m3* and *m5* receptor mRNA after treatment with interferon-γ, although it is not certain whether alveolar macrophages have the capacity to respond to ACh (33). Muscarinic receptors are also expressed on T-lymphocytes but not on neutrophils (34). T-lymphocytes are activated by ACh via M_1-receptors to release interleukin-2 and thus proliferate (35). ACh stimulates human bronchial epithelial cells to release monocyte and neutrophil chemotactic factors via M_1-receptors (36). ACh also releases neutrophil chemotactic factors, particularly leukotriene B_4 from bovine alveolar macrophages via an M_3-receptor (37). Whether anticholinergic drugs have any antiinflammatory effects is not yet established, but should be further investigated, particularly in cells from patients with COPD. It is possible that an antiinflammatory effect of muscarinic antagonists may account for the reduction (by approximately 25%) in exacerbations of COPD seen in long-term studies (38, 39).

CORTICOSTEROIDS

Inhaled corticosteroids are the mainstay of asthma management (40). Their effects are mediated by activation of glucocorticoid receptors (GR) in target cells in the lung (41). Inhaled corticosteroids cross the cell membrane and bind to cytoplasmic GR, which then translocate to the nucleus, where they may bind to recognition elements in the promoter region of antiinflammatory genes, or bind to coactivator molecules and recruit histone deacetylase-2 to reverse histone acetylation and thus switch off multiple activated inflammatory genes (42).

Distribution of GR

Almost every cell type expresses GR, but the number of GR per cell appears to vary and may be one of the determinants of steroid responsiveness. Using an antibody to GR we have examined the distribution of GR in airways and demonstrated that GR is expressed in all cell types, but with the highest density in endothelial and epithelial cells. It is likely that there are multiple cellular targets for inhaled corticosteroids, including inflammatory cells such as eosinophils and T lymphocytes, dendritic cells, and macrophages (Figure 3). In asthma structural cells including epithelial cells, smooth muscle, endothelial cells, and fibroblasts also express multiple inflammatory genes and may be the major cellular source of mediators. It is likely that airway epithelial cells are of particular importance as cellular targets for inhaled corticosteroids, as these cells express multiple inflammatory proteins that orchestrate the complex inflammation of asthma (43)(Figure 4).

GR are very widely distributed outside the lungs, so systemic side effects on bone, growth, skin, skeletal muscles, and blood vessels are common; this provides the rationale for the use of inhaled corticosteroids to reduce systemic exposure. The side effects and antiinflammatory effects may be dissociated to some extent, so it might be possible to develop safer "dissociated" steroids in the future (44).

A second type of glucocorticoid receptor GR-β is a splice variant of the GR gene and binds to the same DNA binding sites as GR-α. However, GR-β does not bind corticosteroids, so may theoretically function as a dominant-negative inhibitor of GR signaling. Increased expression of GR-β has been described in the airways of patients with steroid-resistant asthma (45), but is very unlikely to account for corticosteroid insensitivity in these patients as the amount of GR-β is much less than GR-α (46).

MEDIATOR RECEPTORS

Many different inflammatory mediators are involved in the pathophysiology of airway diseases and all produce their effects through specific surface receptors on target cells (47). The effects of these mediators vary because of the differential distribution of receptors in the lung and differences between the intracellular pathways activated by receptors. The distribution of several mediator receptors has been mapped in lungs by autoradiography, including receptors for bradykinin (48), tachykinins (49, 50), platelet-activating factor (51), and cys-LTs (52). Because so many mediators are involved in asthma and there is such redundancy of effects, specific antagonists of mediators have had little or no effects. The exception is cys-LTs, which mediate their effects predominantly through cys-LT_1 receptors, because cys-LT_1 antagonists have clinical effects in asthma (53).

Leukotriene Receptors

Cys-LTs are potent bronchoconstrictors and activate cys-LT_1 receptors on airway smooth muscle. Using labeled LTC4 and

LTD4, it was possible to detect specific cys-LT_1 receptor binding in lung, but the high nonspecific binding makes autoradiography difficult (52). The cloning of the cys-LT_1 receptor made it possible to express the receptor and generate specific antibodies that could be used in localization by immunohistochemistry (54). Using antibodies and *in situ* hybridization the distribution of cys-LT_1 receptors in lung was shown to be predominantly on airway smooth muscle, with some receptors on macrophages (55). This is consistent with the predominant functional action of cys-LTs and the fact that antileukotrienes, such as montelukast and zafirlukast, act predominantly to prevent leukotriene-induced bronchoconstriction. In this respect they are less effective as bronchodilators than β_2-agonists, which is not surprising as β_2-agonists counteract all bronchoconstrictors through functional antagonism. Antileukotrienes are weak antiinflammatory drugs however, and are poorly effective compared to inhaled corticosteroids (56). This may reflect that there are few cys-LT_1 receptors in the airways apart from airway smooth muscle. However cys-LT_1 receptors are expressed on peripheral blood eosinophils, so this may account for some reduction in eosinophilic inflammation seen with antileukotrienes (55). It also explains why antileukotriene therapy is safe, because cys-LT_1 receptors are strongly expressed in lungs and relatively little in other tissues (54, 57). This means that cys-LT_1 antagonists are suitable for oral administration and that there is no advantage to giving these drugs by the inhaled route. A second type of cys-LT receptor called cys-LT_2 has been identified in lung, but the function of this receptor and its distribution are currently unknown (58).

FUTURE TARGETS IN THE LUNGS

Many drugs are now in development for the treatment of airway diseases and several of these drugs target specific receptors (59, 60).

New Receptor Targets

Many new receptors have now been identified that are targeted for new therapies for asthma and COPD. Chemokine receptors are an attractive drug target in asthma and COPD, because these have a typical GPCR structure that is suited to the discovery of small molecule inhibitors (61). Small molecule inhibitors for CCR2 (monocytes), CCR3 (on eosinophils, mast cells, and Th2 cells), CCR4 and CCR8 (Th2 cells) are in development for asthma, and CCR2, CXCR2 (neutrophils, monocytes), and CXCR3 ($CD8^+$ cells) are being explored for COPD. These chemokine receptors are expressed on inflammatory cells in the circulation that respond to chemokines released locally in the airways, and therefore chemokine inhibitors are more likely to be useful as oral, rather than inhaled, drugs. By contrast, receptors for other cytokines have proved very difficult to block, as their structure is complex, often involving different subunits. There are no small molecule inhibitors for these cytokines, and inhibition of cytokines has been achieved by specific blocking antibodies or by soluble receptors (62).

Other receptors that are potential targets for therapy are LTB_4 receptors (BLT_1-receptors) in COPD, because these are expressed predominantly on neutrophils and LTB_4 is increased in the airways of patients with COPD. Because BLT_1-receptors are chemotactic LTB_4 antagonists would probably act most effectively in the circulation to prevent neutrophils trafficking into the lungs. Other receptors that are targeted are adenosine A_{2B} receptors, which are expressed on mast cells and inhibitory A_{2A} receptors on neutrophils and eosinophils (63). Prostaglandin D_2 activates a chemotactic receptor on Th2 cells, eosinophils called CRTh2, for which small molecule inhibitors are in development to inhibit inflammatory cell influx in asthma (64).

Orphan Receptors

Orphan receptors are gene products with receptor structure for which now ligands are known. Many novel receptors, including GPCRs and nuclear receptors, have now been identified by molecular cloning and these may form the basis for new drugs when ligands for these receptors are identified. It is estimated that there are ~200-500 orphan GPCRs in the human genome, providing many opportunities for drug discovery as half of our current therapies work through interacting with GPCRs (65). Similarly there are ~30 nuclear orphan receptors, which are now leading to the discovery of new classes of drug (66, 67). An example is agonists for the peroxisome proliferator receptor PPAR-γ (glitazones), which have an inhibitory effect on macrophages and therefore may have relevant in the treatment of COPD (68). Several steroid-like receptors have been identified, including Nur77 and ROR-γ which are expressed in T-lymphocytes and may have immunomodulatory actions (69).

Enzyme Targets

Many of the future targets for drugs relevant to airway diseases are inhibitors of enzymes. This means that intracellular delivery is required and drugs that cross the cell membrane are also likely to be absorbed systemically from the lungs so that side effects are more likely than with polar drugs that bind to surface receptors. This may necessitate the development of new delivery systems that target these drugs to particular cell types in the lungs, for example macrophages.

The most advanced enzyme inhibitors for treating airway diseases are inhibitors of phosphodiesterase-4 (PDE4), which is expressed in key inflammatory cells including eosinophils, neutrophils, macrophages, T cells, airway smooth muscle, and epithelial cells. PDE4 inhibitors therefore have a broad spectrum of antiinflammatory effects and are suitable for treating both asthma and COPD (70). However, a major limitation to their clinical development has been a relatively high frequency of side effects, such as nausea, gastrointestinal symptoms, and headaches when given orally, and this limits the dose that can be tolerated, resulting in relatively weak antiinflammatory actions. One solution is to give the PDE4 inhibitor by inhalation, but the inhaled drug needs to be retained in the lung and to have a low oral bioavailability to reduce systemic exposure. Inhaled PDE4 inhibitors with these characteristics are now in development.

There are many signaling enzymes that are targets for drugs in the lungs, particularly kinases. Selective inhibitors of kinases are now in clinical development (71) and these include p38 MAP kinase, I-κB kinase, and phosphoinositide kinase-γ inhibitors, all of which are in development for COPD and asthma (60). Because these kinase targets are widely distributed, it is likely that these inhibitors with have side effects, so inhaled delivery to reach these enzymes in the cells of the respiratory tract may be necessary to reduce the risk of systemic adverse effects. A major issue in the development of kinase inhibitors is their specificity, because all kinases recognize ATP at their active site, so it is more difficult to achieve selectivity than with receptor binding sites. Because there are over 700 kinases known, this may be a problem. Some selectivity may be achieved by inhaled delivery, however.

CONCLUSIONS

Most of the drugs that are currently used to treat airway diseases interact with receptors expressed by cells in the respiratory tract. This makes inhalation the most efficient way to treat airway diseases, because it delivers the drug to key target cells and has a low risk of systemic side effects. Both β_2-agonists and anticholinergics target autonomic receptors on airway smooth

muscle of large and small airways. β_2-Agonists may also interact with β_2-receptors expressed on other important target cells, including mast cells and postcapillary venules. Anticholinergic bronchodilators may also target M_3 receptors on inflammatory cells, but little is known about the distribution and function of these receptors. Extraneuronal acetylcholine may be important in activating these muscarinic receptors on inflammatory cells and small airways which are not innervated. Corticosteroids target GR, which are widely distributed. This means that corticosteroids are best given by inhalation to interact with intracellular GR in the respiratory tract but also largely avoid side effects from activation of GR in extrapulmonary tissues such as bone. By contrast, cys-LT_1 receptors have a limited distribution and are mainly expressed in airway smooth muscle, so that antileukotrienes are less effective clinically than the broad spectrum β_2-agonists and corticosteroids. However, this means that oral delivery is possible and there are minimal side effects. There are many other receptor targets in lung and for several of these receptors, such as receptors for chemotactic agonists such as chemokines, selective antagonists are in clinical development. For drugs that inhibit chemotactic receptors systemic delivery is more appropriate to prevent the inflammatory cells that bear these receptors from being recruited into the airways by locally released chemotactic factors. Many novel receptors, including orphan receptors, have now been identified, as these may be future targets for developing novel therapies for asthma and COPD; the distribution of these receptors is an important predictor of their likely use as drugs. In the future the major targets for treating airway disease are likely to be enzymes, particularly kinases and phosphodiesterases. These enzymes are widely distributed, so it is unlikely that inhaled delivery will be needed to target inflammation the lung without unacceptable side effects. New strategies to prolong the action of inhaled drugs are needed by designing agonists or antagonists that have only very slow dissipation from the receptor target, or drugs that are retained in the lung for long periods without systemic bioavailability.

Conflict of Interest Statement: P.J.B. has previously served as a consultant to GlaxoSmithKline (GSK) and is a member of Scientific Advisory Boards for GSK, Boehringer Ingelheim, Altana, and Pfizer and has received lecture fees from GSK, AstraZeneca, Boehringer Ingelheim and unrestricted grants from GSK, AstraZeneca, Boehringer Ingelheim, Novartis, Millenium and Scios.

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