

**ALLERGIC rhinitis is a condition which affects over 15% of the population in the United Kingdom. The pathological process involves two stages: nasal inflammation, and the development of nasal airway hyperresponsiveness (AHR) to allergen and a number of other stimuli. This results in the amplification of any subsequent allergic reaction, contributing to the chronic allergic state. A number of different hypotheses have been proposed to explain the underlying mechanism of AHR, including a role for eosinophil-derived proteins, free radicals and neuropeptides. While there may be a number of independent pathways which can result in AHR, evidence obtained from both animal models and *in vivo* experiments in humans indicate that some mediators may interact with one another, resulting in AHR. Further research into these interactions may open new avenues for the pharmacological treatment of chronic allergic rhinitis, and possibly other allergic airway diseases.**

**Key words:** Human nasal airway, Hyperresponsiveness, Eosinophils, Bradykinin, Neuropeptides, Nitric oxide

## Hyperresponsiveness in the human nasal airway: new targets for the treatment of allergic airway disease

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### Introduction

Allergic rhinitis and asthma are two of the most common immunological disorders producing chronic disease in man. For instance, allergic rhinitis affects over 15% of the general population.<sup>1</sup> In both diseases, a local allergic reaction results in an inflammatory response, causing wheezing and mucus secretion in asthma, and nasal congestion, rhinorrhoea ('runny nose'), pruritis (itching) of the nose and sneezing in allergic rhinitis. Continued exposure to allergen induces a state of chronic allergic inflammation. Contributing to this is the development of airway hyperresponsiveness (AHR), which results in the amplification of any subsequent allergic reaction.

While a variety of pharmacological agents can be used to treat the initial inflammatory response, only steroids are effective in preventing AHR. The mechanism by which AHR occurs remains unclear. If the mechanism of AHR can be described, then this may provide new pharmacological targets for the treatment of chronic allergic airway disease. While allergic rhinitis is not life-threatening, the same is not true for chronic asthma, and the problem is compounded by the adverse effects associated with long-term steroid use.

Unfortunately, animal models and *in vitro* experiments are of limited value, since they are not representative of the situation in man.<sup>2,3</sup> However, the human nasal airway is reasonably accessible for *in vivo* research into the development of nasal AHR, and may also yield some insight into the mechanism of

AHR in asthma. We review here current hypotheses for the development of AHR in the human nose, and indicate potential new pharmacological targets for the treatment of allergic rhinitis and possibly other allergic airway diseases.

### Pathogenesis of allergic rhinitis

There are two distinct forms of allergic rhinitis, depending on the allergen to which the subject is sensitive. In perennial allergic rhinitis (PAR), the subject is exposed to allergen throughout the year, while in subjects with seasonal allergic rhinitis (SAR), allergen exposure is limited to certain times during the year. Subjects with PAR are sensitive to allergens including those from the house-dust mite (*Dermatophagoides pteronyssinus*), other mites, animal danders and certain foods. In contrast, the allergens involved in SAR are tree and grass pollens, moulds and certain fungal spores.

### Nasal inflammation

When particles of allergen are inhaled into the nasal cavity, they cross the nasal mucosa and are exposed to the immune system; in susceptible individuals, this results in sensitisation, a process where immunoglobulin E (IgE) is synthesised and becomes fixed to cells, such as mast cells. If these cells subsequently encounter the same allergen, crosslinking occurs between IgE molecules on the cell surface, causing the release of inflammatory mediators including

histamine, kinins and eicosanoids, such as PGD<sub>2</sub>,<sup>4</sup> as shown in Table 1. These mediators are responsible for the symptoms of allergic rhinitis. Nasal blockage results from a combination of increased blood flow to the nose (causing swelling of the nasal tissue), plasma extravasation, and oedema. Rhinorrhoea is a consequence of the stimulation of nasal secretory glands, which produce a mixture of watery or serous secretion and viscous mucus secretion, mixed together with extravasated plasma. Stimulation of sensory nerves in the nasal cavity causes sneezing and pruritis.

One method used to study the inflammatory response in the human nose is nasal lavage, where the nasal cavity is rinsed with a solution (such as saline), and the lavage fluid subsequently collected and analysed. Using this technique, one can measure a number of inflammatory mediators using appropriate assays. Furthermore, by collecting and counting cells present in the lavage fluid, it is possible to correlate the release of inflammatory mediators with the different cells observed.

The response of subjects with allergic rhinitis to intranasal challenge can often be divided into an immediate phase, occurring during the first 2 h following exposure to antigen, and, in about 30–40% of subjects, a late phase occurring 6–12 h later. The late phase is associated with an infiltration of a number of different inflammatory cells (including eosinophils, neutrophils and T-lymphocytes) to the site of inflammation, where they release a variety of mediators including leukotrienes, cationic proteins such as eosinophil cationic protein (ECP), and cytokines (Table 1).

Interestingly, there may be differences in the precise mediators involved in PAR and SAR. While histamine is strongly associated with the early phase of SAR, histamine H<sub>1</sub> antagonists are not very effective in reducing the early allergic response of PAR.

**Table 1.** Mediators found in nasal lavage fluid collected following both the immediate and late phase response in atopic individuals challenged intranasally with allergen<sup>4</sup>

Mediator	Immediate phase	Late phase
Histamine	✓✓	✓✓
TAME-esterase	✓✓	✓✓
Prostaglandin D <sub>2</sub>	✓✓	×
Leukotrienes LTB <sub>4</sub> /C <sub>4</sub> /D <sub>4</sub>	✓	✓
Kinins	✓✓	✓
Platelet-activating factor (PAF)	✓	×
Albumin	✓✓	✓✓
Neuropeptides, e.g. CGRP and VIP	✓	×
Substance P	✓	✓
Eosinophil-derived mediators, e.g. ECP, MBP	×	✓✓
Interleukin 1 (IL-1)	✓	✓
IL-3, IL-4, IL-5, IL-6, TNF $\alpha$	×	✓

Instead, the kinins appear to be the major mediator causing symptoms in PAR.<sup>5</sup> There is also evidence that the allergen (house dust mite) in PAR can generate kinin independent of the inflammatory response, either by the direct activation of kallikrein, the enzyme which generates kinins,<sup>6</sup> or by possessing such biochemical activity itself.<sup>7</sup>

### Nasal airway hyperresponsiveness

Nasal hyperresponsiveness is a hallmark of allergic rhinitis.<sup>8,9</sup> Subjects with allergic rhinitis show an increased response to nasal challenge with a variety of stimuli, including histamine and bradykinin, both of which are released following allergen challenge. The nasal airway effectively becomes more sensitive to allergen, contributing to the chronic allergic state. AHR is usually associated with the late phase reaction, but can continue well beyond this stage. In fact, it is induced irrespective of whether a late phase of inflammation occurs.<sup>10</sup>

One important consideration is the type of response involved in AHR. In asthma, AHR is often defined as an increase in the contractile response of the airway smooth muscle. However, there is little smooth muscle present in the human nasal airway. Nasal blockage results from increased blood flow to the nasal mucosa, and not by an action on smooth muscle. This highlights the difficulty of extrapolating data from the nasal airway to the lower airways, and vice versa. AHR is also associated with increased mucus production and oedema following allergen challenge, in both the upper and lower airways. Therefore, AHR would appear to result from a direct or indirect potentiation of the overall receptor activation, and not simply from an action on a particular cell type alone. With this in mind, there are a number of potential mechanisms by which AHR might occur (Fig. 1).

#### (1) *The inflammatory cascade:*

Initial exposure to allergen might trigger a cascade reaction, causing increased mediator release and, therefore, greater receptor activation. For instance, subjects with SAR experience increasingly severe symptoms as the pollen season continues. However, a number of inflammatory mediators can induce AHR in the absence of any other mediator. Furthermore, the development of AHR can occur independently of a chronic inflammatory response.<sup>10</sup> It is likely, therefore, that the AHR potentiates the effect of the inflammatory cascade seen in chronic allergic rhinitis, rather than being caused by it.

#### (2) *Increased exposure of receptors on the nasal mucosa to the stimulus:*

A common histological finding in chronic airway allergy is the damage and shedding of epithelial cells

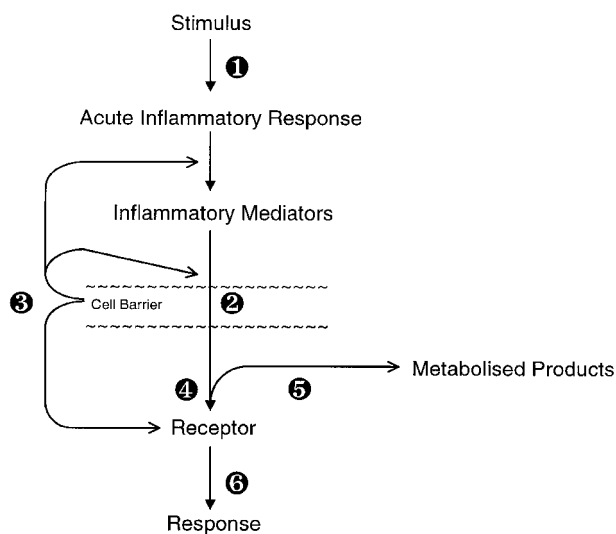


FIG. 1. Potential mechanisms in the development of AHR. ① Inflammatory Cascade; ② altered exposure of the receptor to the stimulus; ③ release of modulating agent by other cells; ④ change in the receptor properties; ⑤ altered metabolism of the mediator; ⑥ altered regulation of the intracellular signalling resulting in the response.

lining the airway. The airway epithelium can act as a physical barrier between the airway lumen and the underlying mucosa. Therefore, removal of these cells would increase the exposure of the receptors on the mucosa to any stimulus present. For example, in the lower airways, removal of the airway epithelium increases the response of smooth muscle in both animal models and human bronchi *ex vivo*.<sup>11</sup> While there may be a link between epithelial shedding and the presence of AHR in allergic individuals,<sup>12</sup> mediators which induce AHR on their own (e.g. platelet-activating factor) do not cause epithelial shedding in the time taken for AHR to develop, though the doses required are toxic to airway epithelial cells.<sup>13</sup> Alternatively, the loss of airway epithelial cell function, but not necessarily barrier function, may be sufficient to induce AHR.<sup>14</sup> In the human nasal airway, AHR to bradykinin is unlikely to be due to a reduction in epithelial barrier function,<sup>15</sup> and there is no evidence for increased epithelial permeability in subjects with allergic rhinitis.<sup>16</sup> Mediators such as PAF may also damage the mucociliary clearance system linked to the airway epithelium, contributing to the AHR.<sup>13</sup>

### (3) Modulating agents:

The ability of acetylcholine to cause vasodilatation in the vascular system is dependent upon the release of nitric oxide from the endothelium (and possibly other mediators, collectively known as endothelium-derived relaxing factors). It has been proposed that airway epithelial cells may generate mediators, such as arachidonic acid metabolites, nitric oxide and the putative epithelium-derived relaxing factor (EpDRF) (also known as EDHF (epithelium-derived hyperpolarising factor)) which modulate the responsiveness

of the airways. However, the evidence for such mediator release from airway epithelial cells is equivocal.<sup>11,17-19</sup> The epithelium may have an important role in the metabolism of various stimuli, particularly adenosine,<sup>20</sup> tachykinins<sup>18</sup> and acetylcholine.<sup>21</sup> Loss of epithelial cell function might reduce the metabolism of these mediators, enhancing their ability to activate receptors and effect a response. However, epithelial damage can occur without the presence of AHR, and vice versa.<sup>22</sup> For example, cationic proteins can cause AHR without any apparent damage to the epithelium.<sup>23</sup>

### (4) Receptors:

AHR may result from a change in the characteristics of receptors located on the nasal mucosa. For example, cholinergic agonists such as methacholine cause more secretion in allergic subjects than in non-allergic controls, and this could be explained by an increase in the density of cholinergic receptors.<sup>24</sup> However, the density of muscarinic receptors on nasal tissue from allergic subjects may actually decrease slightly, possibly as a result of adaptation due to overstimulation, although the remaining receptors exhibit an increased affinity, which might contribute to the hypersecretion.<sup>25</sup> No significant differences have been found for  $\alpha$ - or  $\beta$ -adrenoceptors.<sup>26</sup> The minor changes observed are probably a result, rather than the cause, of AHR.

### (5) Metabolism:

Alternatively, AHR may be a consequence of prolonged activity of a variety of mediators, due to an effect on their metabolism. For example, neuropeptides are degraded by neutral endopeptidase (NEP), and the activity of NEP is significantly lower in subjects with nasal AHR.<sup>27</sup> A second protein, lactoferrin, may prevent antigen-induced AHR in the lower airways of sheep by inhibiting tryptase.<sup>28</sup> Though the precise role of tryptase in airway allergy is not fully known, it can induce AHR in the lower airways of sheep<sup>29</sup> and in *ex vivo* tissue from human bronchi.<sup>30</sup>

### (6) Altered intracellular signalling:

Finally, the intracellular pathways which are activated by the receptor/agonist complex may be altered in AHR. Of particular interest is phosphodiesterase IV (PDE4), an enzyme which catalyses the metabolism of a number of intracellular messengers, such as cyclic GMP (cGMP). While PDE4 inhibitors abolish AHR in animal models,<sup>31</sup> the data in human asthmatic subjects are equivocal,<sup>32</sup> and there are no data with respect to the human nasal airway. Interestingly, inhibitors of nitric oxide synthase can also cause AHR, possibly by reducing the amount of cGMP.<sup>33</sup> AHR could result from a loss of a compensatory mechanism, involving cGMP; such a mechanism may be free radical dependent.<sup>34</sup> In the chronic allergic state, AHR

may result, in part, from a change in gene transcription within cells (e.g. the NF- $\kappa$ B transcription system), though the timescale of onset of hyperresponsiveness makes this process unlikely in the induction of AHR.

## Mediators implicated in the development of AHR

In 1933, Sir Henry Dale described a number of criteria which should be used to identify potential inflammatory mediators. Applying these to the development of AHR:

- administration of the potential mediator should cause AHR *in vitro* and *in vivo*;
- the mediator should be present in appropriate concentrations in allergic individuals with AHR;
- the mechanisms involved in the generation of the mediator should be present and increased in AHR;
- a mechanism should exist to terminate the action of the mediator (important, because AHR is a reversible phenomenon);
- antagonists of the mediator reduce the induction of AHR;
- receptors/signalling pathways should be present in the nasal mucosa, and be activated by the mediator.

The process by which AHR occurs remains unclear, no doubt due, in part, to the complexity of the mechanism and the difficulty in regulating the process under controlled experimental conditions. Nonetheless, because it is associated with the influx of cells to the site of inflammation, a number of hypotheses have been proposed involving mediators released from these cells. One particular cell type, the eosinophil, has been implicated in AHR. In both SAR and PAR, antigen challenge results in an increase of eosinophils in the nasal mucosa and the release of various eosinophil-derived mediators.<sup>35-39</sup> Consequently a major role has been proposed for eosinophils and their proteins in this process.

## Eosinophil granule-derived proteins

Eosinophils contain granules composed of four basic proteins. The core of these granules is major basic protein (MBP), while the matrix surrounding the core is composed of eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN) and eosinophil peroxidase (EPO).<sup>40</sup> The possible roles of these proteins in allergic airway disease are described in Table 2.

The levels of ECP, EPO and MBP are raised following antigen challenge in allergic rhinitis,<sup>37-39,41</sup> and these increases often coincide with the presence of AHR. MBP and other cationic proteins, including

**Table 2.** Cationic proteins found in eosinophil granules<sup>40</sup>

Protein	Cell content ( $\mu\text{g}/10^6$ cells)	Role in allergic airway disease
MBP	9	<ul style="list-style-type: none"> <li>● causes histamine release from basophils and mast cells</li> <li>● cytotoxic for human epithelial cells</li> <li>● causes bronchoconstriction and induces hyperresponsiveness in animals</li> <li>● activates neutrophils</li> </ul>
ECP	5	<ul style="list-style-type: none"> <li>● causes histamine release from mast cells</li> <li>● cytotoxic to a variety of cells</li> </ul>
EDN	3	<ul style="list-style-type: none"> <li>● undefined</li> </ul>
EPO	12	<ul style="list-style-type: none"> <li>● cytotoxic to airway epithelium</li> <li>● provokes bronchoconstriction in animals</li> <li>● in the presence of <math>\text{H}_2\text{O}_2</math> and halide, causes mast cell degranulation and histamine release and inactivates leukotrienes</li> </ul>

the synthetic protein poly-L-lysine, cause AHR in the lower airways of rats,<sup>23</sup> which appears to be dependent on their cationic charge. However, no study has yet been conducted to investigate whether these cationic proteins can themselves induce AHR in the human nasal airway.

The mechanism of MBP-induced AHR is unknown, though it may be dependent on causing epithelial damage, since MBP only induces AHR in guinea pig tracheal preparations with an intact epithelium.<sup>42</sup> However, as stated above, cationic proteins may induce AHR without causing epithelial damage.<sup>23</sup> Furthermore, although all the eosinophilic cationic proteins are cytotoxic to the airway epithelium,<sup>43</sup> only MBP caused AHR in a study on primates.<sup>44</sup> In the lower airway of the rat, AHR induced by MBP or poly-L-lysine is abolished by both neurokinin NK-1<sup>45</sup> and bradykinin B2<sup>46</sup> antagonists, indicating a role for substance P and the kinins in the development of AHR. It is also possible that eosinophil cationic proteins act on other inflammatory cells to generate the conditions required for AHR.<sup>22</sup> While there is evidence for a relationship between ECP levels in nasal lavage and nasal AHR,<sup>47</sup> this has not always been reproduced in other studies,<sup>48,49</sup> so ECP is unlikely to be solely responsible for the induction of nasal AHR.

## Platelet activating factor (PAF)

PAF is a naturally occurring phospholipid, and is the only endogenous compound known to induce AHR in both animals and man.<sup>50</sup> In normal, non-atopic subjects, it induces a nasal AHR to histamine and bradykinin<sup>51,52</sup> and causes an increased response to pollen in subjects with SAR.<sup>51</sup> The resulting AHR is

similar, in many ways, to that observed in allergic rhinitis, as PAF also causes a significant nasal neutrophilia and eosinophilia, together with raised ECP levels in nasal lavage fluid.<sup>52-54</sup>

Although PAF can induce AHR in the human nasal airway, its role in allergen-induced AHR is less defined. Antigen challenge with grass pollen in atopic individuals with SAR causes the release of lyso-PAF and PAF<sup>41,55</sup> but it is unclear whether PAF or lyso-PAF are released in PAR.<sup>56</sup> One possibility is that any PAF generated is converted to lyso-PAF by acetylhydrolase present in the lavage fluid. The activity of this enzyme in lavage fluid is significantly raised following grass pollen challenge in sensitive subjects.<sup>41,57</sup>

PAF may act directly on the nasal mucosa, and radioligand binding studies indicate the presence of specific binding sites for PAF in human lung tissue,<sup>58</sup> but no such studies have yet been performed using tissue obtained from the human nose. One of the main features of PAF is its ability to attract and activate a variety of inflammatory cells, including eosinophils, neutrophils, monocytes, macrophage and platelets.<sup>50</sup> PAF may also release from airway epithelial cells a range of chemotactic factors for neutrophils and eosinophils, including the cytokine GM-CSF.<sup>59</sup> The subsequent activation of these cells will release a range of mediators which can act on other cells in the airway. Administration of PAF into the nasal airway also causes ECP release, which could then contribute to the AHR.<sup>52,53</sup>

If PAF is an important mediator of nasal AHR, one would expect PAF-antagonists to reduce leukocyte infiltration and inhibit antigen-induced AHR. While this effect has been observed in some studies using animal models,<sup>60-62</sup> the PAF antagonists WEB 2086<sup>41</sup> and UK 74,505<sup>63</sup> did not alter AHR in the human nasal airway. However, both these antagonists exhibit a low potency for PAF receptors, and may cause only a weak inhibitory action in man.

PAF causes mucosal dysfunction and damage, inhibiting ciliary action and increasing exfoliation of the epithelial lining of the airway both *in vivo* in the lower airways of the rabbit<sup>13</sup> and *in vitro* using explants from human nasal mucosa.<sup>64</sup> Interestingly, the latter study found that PAF is itself cytotoxic to epithelial cells, without requiring the production of other cytotoxic mediators. PAF-induced AHR in the human nasal airway was almost abolished by pretreatment with the antioxidant vitamin E,<sup>52</sup> implying a mechanism which is dependent on the generation of free radicals. This supports the hypothesis that PAF causes tissue damage which may be independent of the release of other mediators, perhaps via the generation of free radicals. The source of the free radicals could be the epithelial cells, so in effect, the epithelium may cause its own destruction.<sup>65</sup>

It is unclear, however, whether sufficient quantities of PAF are produced in the antigen-induced allergic

response to cause AHR, and there are probably differences in the underlying mechanism of AHR induced by PAF and antigen.

## Leukotrienes

The leukotrienes are generated by the action of 5-lipoxygenase on arachidonic acid. They are released in both the early and late phases following antigen challenge in subjects with SAR<sup>66</sup> and during the early phase in PAR.<sup>67</sup> There are two classes of leukotrienes:  $LTB_4$  and the peptidyl-cysteinyl leukotrienes ( $LTC_4$ ,  $LTD_4$  and  $LTE_4$ ). The latter group are synthesised by eosinophils (among other cells) and cause a long-lasting eosinophilic infiltration, and have been associated with AHR in the lower airways in rats<sup>68</sup> and in man;<sup>69</sup> both these actions appear to be dependent on eosinophil activation. Although inhibitors of leukotriene synthesis reduce the nasal blockage experienced following challenge with grass pollen in allergic subjects,<sup>70,71</sup> no studies have investigated the effect of such drugs on AHR in the human nasal airway, though zileuton, a lipoxygenase inhibitor, reduced antigen-induced AHR in the lower airways of asthmatics.<sup>72</sup> Pharmacological intervention can also be achieved at the level of the receptor, and a number of leukotriene receptor antagonists have been developed which inhibit AHR in animal models.<sup>73</sup> Many of these are currently under study in man, including pranlukast, which may reduce AHR in asthmatics,<sup>74</sup> and montelukast, which has recently been approved for use in the USA. The role of  $LTB_4$  in AHR is not clear, but  $LTB_4$  antagonists have been shown to inhibit antigen-induced AHR in the lower airways of primates<sup>75</sup> and the guinea pig.<sup>76</sup> Although human eosinophils cannot produce  $LTB_4$  because they lack the enzyme needed for its synthesis, it is the main lipoxygenase product in neutrophils, and acts as a potent stimulus for leukocyte infiltration and subsequent degranulation.<sup>77</sup>

## Prostaglandins and thromboxanes

The prostaglandins  $PGD_2$  and  $PGE_2$  are detected at increased levels in nasal lavage fluid following allergen challenge in subjects with SAR<sup>78,79</sup> and PAR,<sup>80</sup> but only in the early response and not the late phase of inflammation.<sup>66</sup> Inhibitors of cyclooxygenase, the enzyme required for the synthesis of prostaglandins, do not affect the response to antigen in the human nasal airway.<sup>66</sup>  $PGE_2$  is synthesised by the airway epithelium and has been proposed as a possible EpDRE. In the lower airways, AHR may result from epithelial damage, reducing  $PGE_2$  generation by epithelial cells and, therefore, decreasing its relaxant effect on airway smooth muscle.<sup>81</sup> However, in the human nose, any action of  $PGE_2$  would presumably have to be on blood vessels (since there is little airway

smooth muscle in the human nose), and one would also expect a decrease in PGE<sub>2</sub> release if it was involved in AHR, yet the opposite is true. Thromboxane A<sub>2</sub> (TxA<sub>2</sub>) does appear to mediate AHR in animal models<sup>82,83</sup> and may do likewise in man.<sup>84</sup> Inhibition of thromboxane synthesis reduces AHR and also inhibits airway eosinophilia after allergen challenge.<sup>85</sup> The thromboxanes may, therefore, have an important role in upregulating the eosinophil-associated response.<sup>77</sup> The contribution of thromboxanes to nasal allergy remains undefined, and more research is needed to investigate their potential role in the development of AHR.

## Neuropeptides

Sensory nerve fibres contain a number of different peptides, including calcitonin gene-related peptide (CGRP) and the tachykinins substance P (sub P) and neurokinin A (NK-A). These neuropeptides, metabolised *inter alia* by the enzyme neutral endopeptidase (NEP), are released from sensory nerves which form part of the non-adrenergic non-cholinergic (NANC) nervous system, and are capable of generating a local axon reflex which causes an increase in vascular permeability, plasma leakage and subsequent tissue oedema. This response is known as neurogenic inflammation, and is mediated by the tachykinin NK-1 and NK-2 receptors. In addition, eosinophils are capable of producing vasoactive intestinal peptide (VIP) and sub P.<sup>86</sup> All these neuropeptides are found in nasal secretions following nasal challenge with grass pollen,<sup>87</sup> but their role in AHR remains unclear. However, there is a correlation between the presence of AHR and the activity of NEP in the human nose.<sup>27</sup> Furthermore, in both PAR and SAR, AHR to bradykinin is mediated by neural reflexes,<sup>15</sup> which could conceivably include a role for neuropeptides.

Neuropeptides appear to be important in AHR in a variety of animal models. In the lower airway of the rat, AHR induced by eosinophil-derived cationic proteins is inhibited by neurokinin NK-1 receptor antagonists and capsaicin.<sup>45</sup> In the guinea pig, application of capsaicin to the lower airway (an action which depletes sensory nerves of neuropeptides) also abolishes antigen-induced AHR.<sup>88,89</sup> The tachykinins, particularly sub P, may enhance eosinophil recruitment<sup>90,91</sup> and, therefore, cause AHR via an eosinophil-dependent mechanism. However, the inhibition of AHR by capsaicin does not affect lipoxygenase activity or eosinophil infiltration, suggesting that neuropeptides cause AHR independently of, or after, eosinophil activation and leukotriene synthesis.<sup>89</sup>

Neurogenic inflammation may be a phenomenon only found in animals, since one study found no evidence of capsaicin-induced neurogenic inflammation in the human nasal airway.<sup>92</sup> However, other

studies, some utilising higher doses of capsaicin, confirm that neurogenic inflammation does occur in allergic rhinitis.<sup>93,94</sup> Furthermore, application of capsaicin reduces the symptoms caused by antigen challenge in SAR<sup>95</sup> and PAR<sup>96</sup>, though neither of these studies investigated AHR. It is, therefore, possible that AHR in the human nasal airway may result, at least in part, from an upregulation of neurogenic inflammation, possibly due to epithelial damage increasing the exposure of sensory nerves. Bradykinin causes neuropeptide release *in vivo* in the human nasal mucosa,<sup>97</sup> while histamine has a similar effect on human lung tissue *ex vivo*.<sup>98</sup> Damage to the airway epithelium may increase exposure of sensory nerves to stimuli, effectively causing an upregulation of neurogenic inflammation. This would result in increased neuropeptide release, which might further potentiate AHR, perhaps by direct effects on inflammatory cells (e.g. eosinophils) or by stimulating cytokine release from cells in the nasal mucosa, a process which occurs in the human nasal airway.<sup>99</sup>

Alternatively, neuropeptides may not just upregulate the inflammation resulting in AHR, but also directly cause the increased response observed in AHR. A number of stimuli, including antigen, cause neuropeptide release in the human nasal airway. If tachykinin activity is potentiated, due to more neuropeptide release or inhibition of metabolism, this would cause an increase in neuropeptide-mediated symptoms such as nasal obstruction and rhinorrhoea: in effect the 'hyper'-response observed in AHR. Phosphoramidon, a NEP inhibitor, can cause AHR in the lower airways of guinea pigs<sup>100</sup> and man,<sup>101</sup> and potentiates the activity of neuropeptides in the human nasal airway.<sup>102</sup> NEP is present in the human nasal mucosa,<sup>103</sup> and correlates with the presence of nasal AHR.<sup>27</sup> Epithelial damage may cause AHR by reducing the activity of NEP associated with airway epithelial cells. However, in the human nasal airway, PAF-induced AHR is not associated with the release of sub P (Fig. 2). PAF may cause AHR by a different mechanism to antigen, the former being independent of neuropeptide release. Alternatively, PAF may potentiate subsequent neuropeptide release from sensory nerves, and not cause neuropeptide release *per se*, so an increase in sub P would only occur in the presence of a further stimulus, such as histamine.

It is unknown which particular neuropeptides may be involved in the development of AHR. Sub P is the most potent endogenous mediator at the NK-1 receptor, but it does not induce AHR in the lower airways of sheep, while NK-A does.<sup>104</sup> In the human nasal airway, NK-1 receptors are localised to the epithelium, glands and blood vessels, while NK-2 receptors are limited to arterial vessels.<sup>105</sup> This is in agreement with the findings that nasal obstruction is

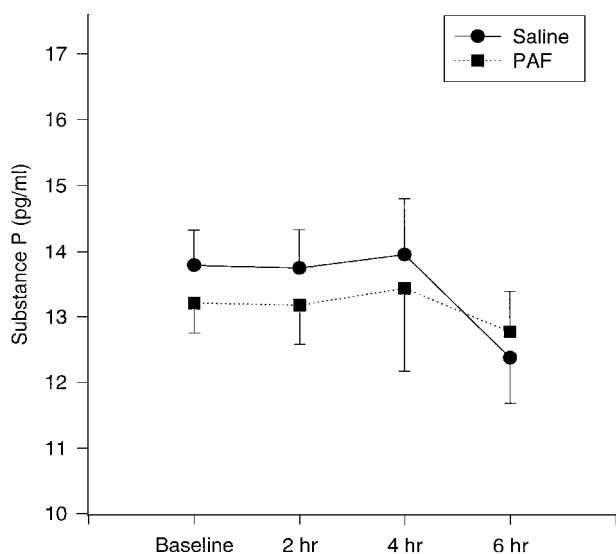


FIG. 2. The effect of PAF on the concentration of substance P in nasal lavage fluid. The nasal airway was challenged with either saline or PAF, 60  $\mu$ g, and the airway was lavaged 2, 4 and 6 h after challenge. Following lavage, a peptidase inhibitor was added to prevent degradation of substance P prior to assay. Data are means  $\pm$  SEM from nine subjects.

mediated through the NK-1 receptor, while plasma extravasation probably involves both NK-1 and NK-2 receptor activation,<sup>106</sup> and would explain the inconsistency that NK-1 or NK-2 receptor antagonists prevent AHR in some studies but not in others. In chronic asthma, both receptor subtypes are upregulated,<sup>107</sup> and this may happen in chronic allergic rhinitis as well. There are no reports investigating the role of CGRP in AHR.

## Cytokines

Cytokines are intercellular messenger peptides which are released by a variety of cells to influence the activity of other cells. Three cytokines are of vital importance in the development and regulation of eosinophil function: the interleukins IL-3 and IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF). All three prevent apoptosis and prolong the survival of eosinophils *in vitro*, and in particular, IL-5 is essential for the differentiation of progenitor cells into eosinophils.<sup>108</sup>

Both IL-4 and IL-5 have been implicated in the development of AHR.<sup>109</sup> In animals, IL-5 causes a marked eosinophilia, eosinophil activation and AHR.<sup>91</sup> Monoclonal antibodies to IL-5 abolish antigen-induced eosinophilia and AHR in the lower airway of the guinea pig.<sup>110</sup> IL-4 regulates the activity of CD4+ T-lymphocytes, which release a range of cytokines capable of priming and activating eosinophils,<sup>71</sup> and can also activate neutrophils.<sup>111</sup> Furthermore, memory T-cells in the nasal mucosa of patients with nasal allergy can produce IL-4 during allergen exposure, which may upregulate the inflammatory response.<sup>112</sup>

Patients with SAR or PAR have a raised number of CD4+ T-cells.<sup>113</sup>

Following nasal allergen challenge, the levels of IL-1 $\alpha$ , IL-1 $\beta$ , IL-5, IL-6, IL-8 and GM-CSF are raised in nasal secretions,<sup>114–116</sup> and human eosinophils are potential sources of these cytokines.<sup>117</sup> Epithelial cells, isolated from allergic rhinitics, showed increased immunostaining for GM-CSF, IL-8, the receptors for IL-1 and TNF- $\alpha$ ,<sup>118</sup> and also they release more IL-1 $\beta$ , IL-8, GM-CSF and TNF- $\alpha$  compared to epithelial cells from non-allergic subjects.<sup>119</sup> Similar increases in IL-4-, IL-5- and GM-CSF-positive cells are observed in biopsies from the nasal mucosa of atopics.<sup>120</sup> Both interferon- $\gamma$  and TNF- $\alpha$  (and possibly other cytokines) cause an upregulation of ICAM-1 on human nasal epithelial cells,<sup>121</sup> while IL-4 upregulates the expression of VCAM-1.<sup>113</sup> Both these adhesion molecules are upregulated in allergic rhinitis.<sup>113</sup>

Cytokines may promote AHR by upregulating the recruitment and activation of eosinophils (Fig. 3) and neutrophils. For example, there are close correlations between the number of eosinophils and GM-CSF levels in bronchoalveolar fluid (BALF) from subjects with asthma,<sup>122</sup> while the survival of eosinophils in BALF from subjects with allergic rhinitis correlates the concentrations of IL-5 and GM-CSF present.<sup>123</sup> Neurokinin NK-2 receptor antagonists inhibit AHR induced by IL-5 in the guinea pig, but not the associated eosinophilia,<sup>124</sup> indicating that cytokines are involved in cell recruitment, following which various mediators are released, such as neuropeptides which may cause the development of AHR. IL-1 may also be involved in the development of AHR,<sup>125</sup> but it has a wide range of cellular actions, so it is difficult to suggest a precise role for it in the pathogenesis of AHR. Interestingly, it may inhibit the activity of NEP,<sup>126</sup> or induce the generation of oxygen-based free radicals from macrophages and neutrophils.<sup>127</sup>

Finally, glucocorticoids downregulate the production of IL-3 and IL-5 following allergen challenge; this effect may contribute to the action of steroids in abolishing AHR,<sup>116</sup> although glucocorticoids have other actions too.

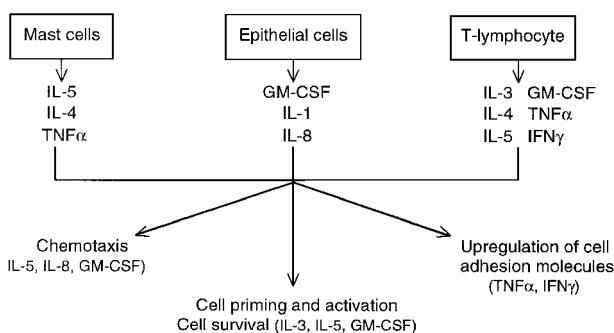


FIG. 3. Role of cytokines in the modulation of eosinophil function in allergy.<sup>71</sup>

## Chemokines

Chemokines are cytokines which possess chemotactic activity, and are divided into groups depending on their structure. The two main groups are CC chemokines, where two cysteine residues are adjacent to each other (e.g. RANTES, MIP-1 $\alpha$ , eotaxin) and CXC chemokines, in which the two cysteine residues are separated by a third amino acid (e.g. IL-8). Some chemokines (RANTES, eotaxin) appear to be selective for eosinophils, while IL-8 has a chemotactic activity only for neutrophils (though there are reports that it also has an action on primed human eosinophils).<sup>128</sup>

The levels of RANTES,<sup>115,129</sup> MIP-1 $\alpha$ ,<sup>115</sup> eotaxin<sup>130</sup> and IL-8<sup>115,131</sup> detected in nasal lavage are raised following nasal allergen challenge in man. Treatment with glucocorticoids, which inhibits inflammatory cell recruitment, abrogates these increases.<sup>115,132</sup> Interestingly, mucosal cells obtained from the noses of subjects with allergic rhinitis show increased expression of mRNA for RANTES<sup>129</sup> and eotaxin.<sup>130</sup> It is now generally accepted that RANTES and eotaxin are important in IL-5-mediated eosinophilia, where the latter causes the mobilisation of eosinophils into the circulation while the local release of chemokines provides a 'homing' mechanism for the migration of eosinophils into the tissues.<sup>128</sup> However, no study has yet investigated the specific roles of chemokines in nasal hyperresponsiveness. Notwithstanding, administration of RANTES into the nasal airway of subjects with allergic rhinitis causes an eosinophilia but not an influx of other inflammatory cells.<sup>133</sup> However, the same study also found that, after allergen challenge, administration of RANTES also caused an influx of basophils, neutrophils, lymphocytes and monocytes, as well as causing epithelial shedding, a response similar to that observed in nasal hyperresponsiveness. It is therefore likely that chemokines have an important role in the recruitment of inflammatory cells that is observed during the development of nasal hyperresponsiveness.

## Nitric oxide

Recently, a large amount of research has been carried out on the role of nitric oxide in airway disease. There is evidence that nitric oxide synthase (NOS) activity is increased in PAR<sup>134</sup> and in SAR.<sup>135,136</sup> This would increase the amount of nitric oxide available to react with superoxide, generating free radicals which may contribute to the development of AHR.<sup>33</sup> Nitric oxide may also have a role in the production of cytokines necessary for eosinophil survival, such as IL-4 and IL-5.<sup>137</sup> Therefore, one might expect inhibitors of NOS to prevent AHR, either by reducing free radical production or via the induction of eosinophil apoptosis. Paradoxically, NOS inhibitors actually induce AHR to histamine and bradykinin in both the human nasal airway<sup>138</sup> (Fig. 4) and lower airways of asthmatics,<sup>139</sup>

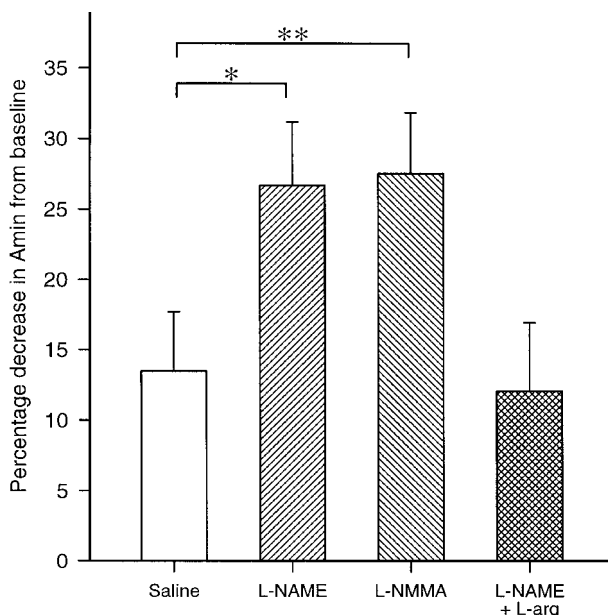


FIG. 4. The percentage decrease in the minimal cross-sectional area of the nasal airway ( $A_{\min}$ ) from baseline 10 min after challenge with histamine, 200  $\mu$ g. The nasal cavity was pretreated every 30 min with saline, a NOS inhibitor (L-NAME or L-NMMA, 1  $\mu$ mol), or L-NAME together with L-arginine, 30  $\mu$ mol, for 6 h prior to the histamine challenge. Data are means  $\pm$  SEM from 10 subjects. \*\*, \*Significant increase in the histamine-induced percentage change in  $A_{\min}$  following pretreatment with a NOS inhibitor compared to saline pretreatment (\*\*, \*\* $P$ <0.05 and  $P$ <0.01, respectively, Wilcoxon sign-rank test).

and also in animal models.<sup>140</sup> It is possible that in the normal, non-inflamed airway, nitric oxide is protective, so inhibitors of NOS would cause AHR, but in chronic airway inflammation, an inappropriate degree of nitric oxide production may be harmful and potentiate the allergic response.

Interestingly, NO is thought to be the central mediator of inhibitory NANC transmission. Therefore, inhibition of NOS may cause a reduction in the activity of inhibitory NANC nerves, which could cause AHR by potentiating neurogenic inflammation mediated by excitatory NANC nerves. In chronic allergy, excessive NO production could cause AHR by the formation of the peroxynitrite free radical, which causes AHR in the guinea pig airway, possibly by inhibiting cGMP production.<sup>33</sup> Furthermore, other NO metabolites, such as nitryl chloride, can be synthesised by neutrophils, inactivating endothelial cell angiotensin-converting enzyme.<sup>141</sup> This enzyme is involved in the degradation of kinins and possibly tachykinins in allergic rhinitis,<sup>102,142</sup> so inhibition of this enzyme may influence AHR by potentiating the action of these mediators.

## Kinins

A number of studies have identified a role for kinins in the development of AHR in the lower airways of both



the guinea pig<sup>143</sup> and sheep.<sup>144</sup> Furthermore, the ability of MBP and synthetic cationic proteins to induce AHR in the lower airways of rats is dependent on the generation of kinins.<sup>46</sup> In all three studies, administration of a bradykinin B2 receptor antagonist inhibited the development of AHR, and, where investigated, also appeared to have an effect on the recruitment of eosinophils into the airway. ECP can stimulate kallikrein activity *in vitro*,<sup>145</sup> resulting in kinin production, so eosinophil-derived cationic proteins may generate kinins which lead to AHR.

Recent investigations have found that AHR in the human nasal airway may also be kinin dependent. Icatibant, a highly potent antagonist at the bradykinin B2 receptor, prevents PAF-induced AHR (Fig. 5), while PAF causes an increase in the level of kinins in nasal lavage fluid (Fig. 6). Kinins are produced in both PAR and SAR, and could therefore contribute to AHR in allergic rhinitis.

One important consideration is the source of the kinins. In SAR, antigen challenge causes an increase in plasma extravasation, so the kinins detected may be a product of plasma kallikrein activity. However, antigen does not induce plasma extravasation in PAR,<sup>5</sup> and the role of plasma kallikrein has not been assessed in either allergic rhinitis or asthma.<sup>146</sup> The levels of tissue kallikrein in nasal secretions are increased in allergic rhinitis,<sup>147</sup> but it is not thought that tissue kallikrein can be activated by cationic proteins.<sup>46</sup> A novel solution to this anomaly is as follows. Neutrophils

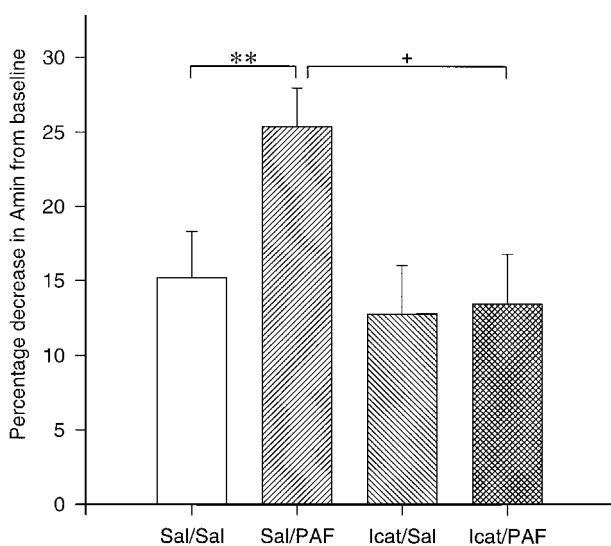


FIG. 5. The percentage decrease in the minimal cross-sectional area of the nasal airway ( $A_{min}$ ) from baseline 10 min after challenge with histamine, 200  $\mu$ g. The nasal cavity was pretreated with saline (Sal), icatibant (Icat), 200  $\mu$ g, and/or PAF, 60  $\mu$ g, 6 h prior to the histamine challenge. Data are means  $\pm$  SEM from 15 subjects. \*\*, \*Significant increase in the histamine-induced percentage change in  $A_{min}$  following saline/PAF pretreatment compared to pretreatment with saline only (\*\* $P$ <0.01, Wilcoxon sign-rank test) or icatibant/PAF (\* $P$ <0.05, Wilcoxon sign-rank test).

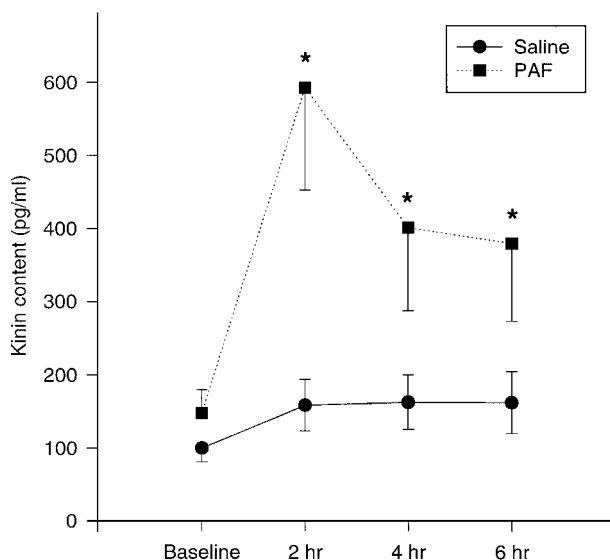


FIG. 6. The effect of PAF on the concentration of kinins in nasal lavage fluid. The nasal airway was challenged with either saline or PAF, 60  $\mu$ g, and the airway was lavaged 2, 4 and 6 h after challenge. Data are means  $\pm$  SEM from 13 subjects. \*Significant increase ( $P$ <0.05) in levels of kinin following challenge with PAF compared to saline (Wilcoxon sign-rank test).

contain tissue kallikrein and bind plasma kallikrein, together with high- and low-molecular weight kininogen, on the cell surface.<sup>148,149</sup> Antigen and PAF challenge cause a neutrophilia in addition to eosinophil recruitment,<sup>54</sup> therefore neutrophils may provide the components needed for the generation of kinins.

The mechanism by which kinins induce AHR remains unknown. Exogenous kinins applied to the airways do not cause AHR in animal models<sup>46</sup> nor in the human nasal airways (Turner & Foreman, unpublished data), though one study found that bradykinin can produce AHR to acetylcholine in the guinea pig airway.<sup>150</sup> Bradykinin causes sensitisation of C-fibres in the guinea pig trachea,<sup>151</sup> and there is evidence that in the human nose enhanced responsiveness to bradykinin is mediated by neural reflexes.<sup>15</sup> Bradykinin can also release sub P and other neuropeptides from sensory nerve endings,<sup>98,152</sup> so it may induce AHR by a neuropeptide-dependent mechanism. However, although administration of PAF into the human nose causes kinin generation, there is no increase in sub P release. Alternatively, bradykinin can initiate the production of the cytokines IL-1, IL-6 and IL-8 *in vivo*<sup>153</sup> and stimulate the release of TNF $\alpha$ / $\beta$  and IL-1 from macrophages.<sup>154</sup> These cytokines may contribute to AHR as previously mentioned. Interestingly, the eosinophilia induced by antigen challenge in the lower airway of the guinea pig is reduced by bradykinin receptor antagonists<sup>143</sup> or an inhibitor of tissue kallikrein,<sup>155</sup> thus providing evidence for the involvement of kinins in the recruitment of eosinophils following exposure to allergen.

## The role of inflammatory cells in hyperresponsiveness

From the evidence presented, it might appear that eosinophils have a vital role in the development of AHR. However, the relationship between eosinophil activation and AHR remains controversial. Certainly, eosinophils are involved in the late response, but the development of AHR may not be dependent on the presence of eosinophils. Studies conducted in animal models indicate that AHR can occur without a detectable eosinophilia, and vice versa.<sup>156</sup> Data obtained from studies in the human nose similarly imply that AHR does not necessarily occur together with eosinophil activation,<sup>48,49</sup> while antigen can induce an eosinophilia without causing AHR.<sup>157</sup> There are a range of airway inflammatory conditions which feature an eosinophilic infiltration, but no associated AHR.<sup>22</sup> Nonetheless, the close association between eosinophil recruitment, activation and AHR in many studies<sup>35,52,67</sup> implies, at the very least, that eosinophils may contribute to the development of AHR.

Although eosinophils are the main cells which have been implicated in the mechanism underlying AHR, it is likely that other cells are involved, including neutrophils, T-lymphocytes, macrophages, B-cells and basophils.<sup>71</sup> Basophils, which may be involved in the late allergic response, are capable of generating histamine, bradykinin, MBP, LTC<sub>4</sub>, IL-4 and IL-8,<sup>71,158</sup> which may contribute to AHR. Macrophages may also be involved in the development of nasal AHR, possibly by generating cytokines and free radicals.<sup>159</sup>

The involvement of the neutrophil in AHR is often overlooked, yet there is a wealth of evidence for an active role. Not only may they contribute to the generation of kinins and cause tissue damage via the superoxide burst, but they also generate PAF, LTB<sub>4</sub> and a variety of cytokines. It is interesting to note that ECP has now been detected in neutrophils.<sup>160</sup> Neutrophils isolated from atopic subjects have an increased capacity for myeloperoxidase release, and this enzyme can generate nitric oxide-derived oxidants, which can induce AHR and inhibit kininase II, an enzyme responsible for the metabolism of

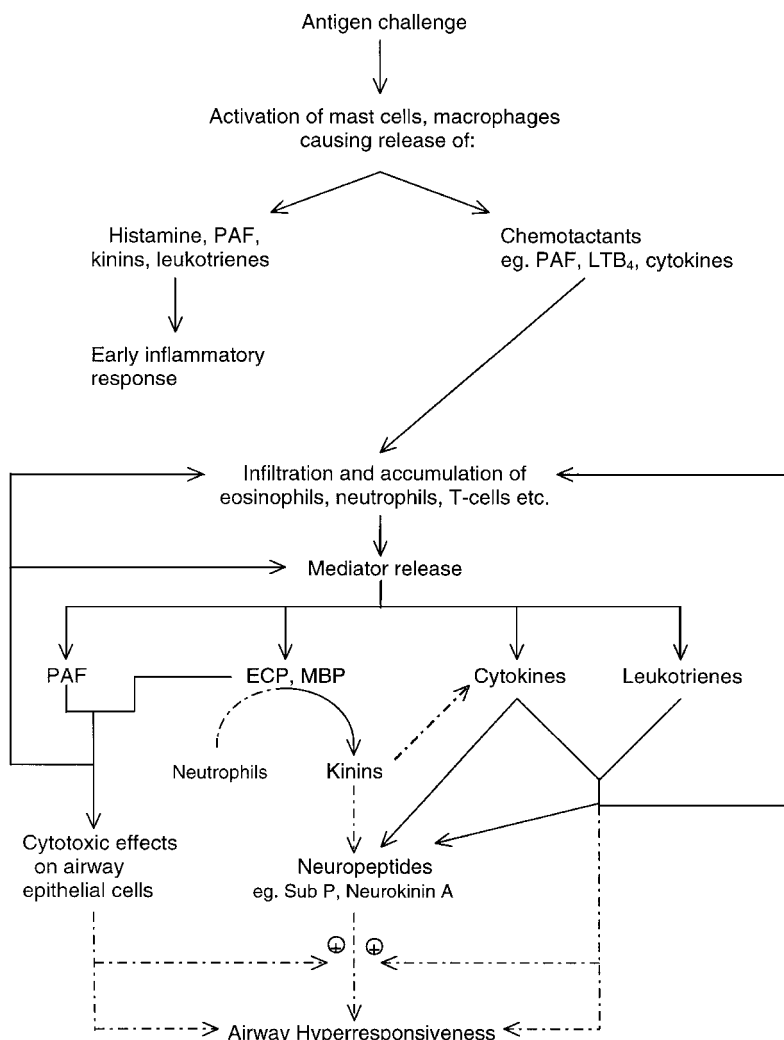


FIG. 7. Putative mechanism for the development of airway hyperresponsiveness.

kinins.<sup>141</sup> The hypothesis that both neutrophils and eosinophils act together in causing AHR is one which warrants further investigation.

The upregulation of ICAM-1 and VCAM-1 by cytokines in allergic rhinitis has already been discussed. ICAM-1 and VCAM-1, present on endothelial cells, bind respectively to integrins LFA-1 ( $\alpha$ L $\beta$ 2 integrin) and VLA-4 ( $\alpha$ 4 $\beta$ 1 integrin) on leukocytes, allowing leukocytes to adhere to the endothelium prior to migration. The interaction between VCAM-1 and VLA-4 appears to be particularly important in eosinophil migration, as neutrophils do not have the VLA-4 receptor on their surface.<sup>161</sup> Interfering with the VCAM-1/VLA-4 pathway may, therefore, prevent eosinophil-dependent hyperresponsiveness. Monoclonal antibodies (mAbs) to the integrin sub-unit  $\alpha$ 4 have been developed which abolish antigen-induced hyperresponsiveness in the lower airways of a number of animal models of airway allergy.<sup>162-164</sup> Interestingly, this effect was not always associated with an decrease in the airway eosinophilia,<sup>163,165</sup> possibly because other mechanisms exist (e.g. via ICAM-1) whereby eosinophil migration may occur. Further development of these mAbs may provide a useful therapeutic intervention for airway allergy in man.

## Summary

It would appear that a number of mediators are capable of inducing AHR in the human nasal airway, perhaps acting via different mechanisms, which would help explain the conflicting evidence regarding a role for eosinophils or airway epithelial damage in this process. However, the fact that inhibitors of leukotrienes, PAF, kinins and tachykinins can all inhibit antigen-induced AHR implies that there may be a central common pathway in the pathogenesis of AHR. Since neurogenic inflammation can potentially be modulated by all of these mediators, neuropeptides may be involved in the final stage of AHR induction, perhaps as shown in Fig. 7. Antagonists of these mediators appear to inhibit the development of hyperresponsiveness in animals, and the development of similar antagonists for use in man may provide new pharmacological treatments for allergic rhinitis, and perhaps for other allergic airway diseases as well.

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