play a role in pulmonary development. In addition to serotonin, these cells also contain the regulatory peptides bombesin, calcitonin, katacalcin and calcitonin gene-related peptide[27,66]. Recent studies suggest that, in animals and humans, these cells are closely associated with afferent nerves, so perhaps these cells have a sensory role and their peptides function as 'neurotransmitters'[48,72]. The stimuli which may activate these cells are uncertain, but there is some evidence that they may sense hypoxia[66].

Bombesin, which may be released from neuroendocrine cells is a bronchoconstrictor in guinea pig airways[39], but has no effect on human airways in vitro (unpublished observation). In pancreas, bombesin has a trophic role, and it is possible that it may play a role in the development of the respiratory tract, since neuroendocrine cells and bombesin immunoreactivity decrease with maturation[27,37]. A marked reduction in bombesin immunoreactivity has been observed in lungs of human infants who died of acute respiratory distress syndrome[37].

Non-adrenergic non-cholinergic innervation

The nerve supply to the lungs is more complex than previously recognised, since, in addition to classical cholinergic and adrenergic innervation[6,63], there are nerves which are non-adrenergic and non-cholinergic (NANC)[3,4,77]. NANC nerves were first described in the gut and therefore their existence in lung is to be expected.

Non-adrenergic inhibitory nerves, which relax airway smooth muscle, have been demonstrated in vitro by electrical field stimulation after adrenergic and cholinergic blockade in several species, including humans[4,77]. In human airway smooth muscle the NANC inhibitory system is the only bronchodilator pathway, since there is no functional sympathetic innervation, and because NANC innervation is the sole inhibitory pathway from trachea to the smallest bronchi that has been of considerable interest in the neurotransmitter. NANC inhibitory nerves have also been demonstrated in vivo in animals by electrical stimulation of the vagus after adrenergic and cholinergic blockade[29,40]. Stimulation of this pathway produces pronounced and long-lasting bronchodilatation, which may be inhibited by ganglion blockers. This pathway may be activated reflexly by mechanical or chemical stimulation of the larynx[81]. Although it is difficult to study this pathway in humans in vivo, studies of laryngeal stimulation indicate that reflex non-adrenergic bronchodilation may occur[61].

Evidence argues against purines as neurotransmitters of NANC inhibitory nerves in airways. Although exogenous ATP relaxes airway smooth muscle[41], an antagonist quinidine fails to block NANC relaxation in vitro or in vivo and the purine uptake inhibitor, dipyridamole, does not enhance non-adrenergic bronchodilatation[40,41]. Similarly, adenosine fails to mimic NANC relaxation and antagonists, such as theophylline, have no blocking effect[43]. Evidence is now more in favour of a neuropeptide as a neurotransmitter of NANC inhibitory nerves and, of the several neuropeptides identified in airways, only vasoactive intestinal peptide (VIP) and the related peptide histidine isoleucine (PHI) relax airway smooth muscle and are, therefore, the only known peptide candidates[9,79].

Electrical stimulation of guinea pig bronchi, and occasionally trachea in vitro, and vagal nerve in vivo produces a component of bronchoconstriction which is not inhibited by atropine[2]. There is now convincing evidence that substance P and related neuropeptides may be neurotransmitters of these non-cholinergic excitatory nerves.

Other NANC responses have been described in lung. NANC secretion of mucus has been demonstrated in cats in vivo using vagal nerve stimulation[75], and in ferret airways in vitro using electrical field stimulation[17].

Vasoactive intestinal peptide

VIP was originally extracted from porcine lung as a vasodilator peptide, and is localised to motor nerves in lung of several species, including humans. VIP has potent effects on airway and pulmonary vascular tone and on airway secretion, which suggest that it may have an important regulatory role[9,79].

Localisation. VIP has been isolated from lung extracts of several species, including humans[36]. VIP-immunoreactivity is localised to nerves and ganglia supplying airways and pulmonary vessels. VIP-immunoreactivity is present in ganglion cells in the posterior trachea and around intrapulmonary bronchi, diminishing in frequency as airways become smaller. VIP-immunoreactive nerves are widely distributed throughout the respiratory tract and pulmonary vasculature. There as a rich VIP-ergic innervation of the nasal mucosa and upper respiratory tract, but the density of innervation diminishes peripherally so that few VIP-ergic fibres are found in bronchioles. VIP-ergic nerves are also found in airway smooth muscle, around bronchial vessels and surrounding submucosal glands[28,47,76]. VIP-ergic fibres are also found in the adventitia of pulmonary vessels, particularly medium sized arteries.

VIP-receptors. VIP-receptors have been identified in the lung of several species by receptor binding techniques using [^{125}I]-VIP[78]. Binding of VIP to its receptor activates adenylate cyclase, and VIP stimulates cyclic AMP formation in lung fragments. The actions of VIP are, therefore, very similar to those of β-adrenoceptor agonists and any differences in response of different tissues to VIP or β-agonists depends on the relative densities of their respective receptors. The distribution of VIP-receptors in lung has recently been investigated, using an autoradiographic method to map out specific VIP binding sites[21,51]. VIP-receptors are found in high density in pulmonary vascular smooth muscle and in airway smooth muscle of large, but not small, airways. VIP-receptors are also found in high density in airway epithelium and submucosal glands. While the distribution of receptors is consistent with known functions of VIP, a high density of receptors is also found in the alveolar walls. The physiological function of these receptors is obscure, since there is no VIP-ergic innervation of peripheral lung. It is possible that these VIP-binding sites represent sites of uptake of VIP since VIP is taken up from the circulation and metabolised by pulmonary capillary endothelial cells. The distribution of VIP-receptors has also been studied by an immunocytochemical method using an antibody to cyclic AMP. After stimulation by VIP, cyclic AMP increases in those cells with specific receptors, and this technique confirms the autoradiographic studies in demonstrating VIP receptors in airway smooth muscle, epithelium and submucosal glands of several species[49].

Airway smooth muscle. VIP is a potent relaxant of airway smooth muscle in vitro and this relaxation is independent of adrenergic receptors[67,79]. VIP is 50–100 times more potent than isoprenaline in relaxing human bronchi, making it the most potent endogenous bronchodilator so far described. Since there is a VIP-ergic innervation of human bronchi, this suggests that VIP may be an important regulator of bronchial tone and may be involved in counteracting the bronchoconstriction of asthma.

There appear to be differences in response to VIP, depending on the size of airway. In bovine airways the responsiveness to VIP diminishes with decreasing size of airway, whilst the relaxant response to isoprenaline is unchanged[71]. Similarly, in human airways, whilst bronchi are potently relaxed by VIP, bronchioles are unaffected, although they relax to an equal degree with isoprenaline[67]. This response of human airways is consistent with the distribution of VIP-receptors, since receptors are to be seen in bronchial smooth muscle, but not in bronchiolar smooth muscle[21]. This peripheral fad-

ing of VIP-receptors is also consistent with the distribution of VIP-immunoreactive nerves which diminish markedly as airways become smaller. These studies suggest that VIP, while regulating the calibre of large airways, is unlikely to influence small airways.

VIP also causes bronchodilatation in vivo. VIP given i.v. causes potent bronchodilatation in cat airways[30], and inhaled VIP protects against the bronchoconstrictor effects of histamine and prostaglandin $F_2\alpha$[79]. In humans, however, inhaled VIP has no bronchodilator effect, although a β-agonist in the same subjects is markedly effective[16]. Inhaled VIP has only a small protective effect against the bronchoconstrictor effect of histamine[16] and has no effect against exercise-induced bronchoconstriction[19]. This lack of potency of inhaled VIP may be explained by problems of diffusion of VIP across the airway epithelium to reach receptors in airway smooth muscle, or by enzymatic degradation of the peptide. Infused VIP similarly has no bronchodilator effect in normal subjects who bronchodilate with isoprenaline[70]. Infused VIP has a marked effect on the systemic cardiovascular system, with flushing, hypotension and tachycardia. These effects limit the dose which can be given by infusion and, as VIP is more potent on vessels than on airway smooth muscle, this prevents giving a dose which will affect airways. Infused VIP causes bronchodilation in asthmatic subjects, but the effect is small[62], and might be explained by sympathoadrenal activation secondary to the cardiovascular effects of VIP[10]. Thus, although VIP has a potent bronchodilator effect in vitro, it has no significant action in vivo, and therefore has no therapeutic potential, although it is possible that more stable analogs or novel compounds which activate VIP-receptors might be developed in the future as bronchodilators.

Airway secretion. VIP-immunoreactive nerves are closely associated with airway submucosal glands and form a dense network around the gland acini. VIP is a potent stimulant of mucus secretion[74], being significantly more potent than isoprenaline. VIP increases cyclic AMP formation in submucosal gland cells, and there is some suggestion that, as with β-agonists, there may be preferential effects on mucous rather than serous cells of these glands[49], indicating that VIP may stimulate mucus secretion rich in glycoprotein. VIP-receptors have also been localised to human submucosal glands, suggesting that VIP-ergic nerves may also regulate mucus secretion in human airways[21]. VIP has been found to have an inhibitory effect on glycoprotein secretion from human tracheal explants[26], which is surprising since agonists which stimulate cyclic AMP formation would be expected to stimulate secretion.

VIP is a potent stimulant of chloride ion transport and therefore water secretion in the gut, and a similar effect has also been found in dog tracheal epithelium[64], suggesting that VIP may be a regulator of airway water secretion and therefore mucociliary clearance. High densities of VIP-receptors have been localised to epithelial cells of human airways, so a similar effect might be expected[21].

VIP also inhibits antigen-induced histamine release from guinea pig lung, suggesting that VIP-receptors are present on pulmonary mast cells[86], but whether human lung mast cells have VIP-receptors is uncertain.

Blood vessels. VIP is a potent dilator of systemic vessels and pulmonary vessels. In vitro, VIP potently relaxes pulmonary vessels in may species, including man[12,38], and the relaxation is independent of endothelial cells, indicating that VIP acts directly on vascular smooth muscle cells, rather than releasing a relaxant factor from endothelial cells[38]. This is confirmed by autoradiographic studies showing the high density of receptors in smooth muscle with no labelling of endothelial cells[12,21]. In bovine pulmonary arteries the receptor density is greatest at the adventitial surface of the medial and diminishes towards the lumen[12]. The density of VIP-receptors is significantly greater in human pulmonary vessels than on bronchial smooth muscle, which may explain why VIP is about 10 times more potent as a vasodilator than a bronchodilator in vitro. VIP may have a role in regulating pulmonary blood flow, although the precise physiological role as a vasodilator mechanism is not yet certain.

Regulatory peptides in lung

Nerves	Neuroendocrine cells
Vasoactive intestinal peptide	Bombesin
Peptide histidine isoleucine	Leu-enkephalin
Substance P	Katacalcin
Neurokinins A and B	Calcitonin gene-related peptide
Calcitonin gene-related peptide	
Neuropeptide Y	
Galanin	
Gastrin releasing peptide	
Cholecystokinin	
Somatostatin	

VIP also relaxes bronchial vessels[46] and may regulate airway blood flow. Since VIP is likely to have a greater effect on bronchial vessels than on airway smooth muscle, it may provide a mechanism for increasing blood flow to contracted smooth muscle. Thus, if VIP is released from cholinergic nerves (see later), this may result in improved perfusion during cholinergic contraction. Perhaps the apparent protective effect of inhaled VIP against histamine-induced bronchoconstriction in human subjects[16], despite a lack of effect on bronchomotor tone, may be explained by an increase in bronchial blood flow which would more rapidly remove inhaled histamine from sites of deposition in the airways.

Neuromodulation. VIP is localised to nerves which surround airway ganglia, suggesting a possible neuromodulatory effect on cholinergic neurotransmission. In bovine airways VIP reduces cholinergic nerve effects, although this is seen only at high frequencies of stimulation, suggesting that the neuromodulatory effect is frequency-dependent[71].

VIP as NANC transmitter. Several lines of evidence implicate VIP as a neurotransmitter of NANC inhibitary nerves in airways. VIP produces prolonged relaxation of airway smooth muscle which is unaffected by adrenergic or neural blockade, and mimics the time-course of NANC inhibitory responses both in vitro and in vivo. VIP mimics the electrophysiological changes in airway smooth muscle produced by NANC nerve stimulation[20,41]. Electrical field stimulation of tracheobronchial preparations releases VIP into the bathing medium and this release is blocked by tetrodotoxin, proving that it is derived from nerve stimulation[20,60]. Furthermore, the amount of VIP released is related to the magnitude of nerve stimulation. Although there are no specific blockers of VIP-receptors, incubation of cat and guinea pig trachea with high concentrations of VIP induces tachyphylaxis and also reduces the magnitude of NANC nerve relaxation, while responses to sympathetic nerve stimulation and isoproterenol are unaffected[41]. Furthermore, preincubation of guinea pig trachea with a specific antibody to VIP reduces responses to exogenous VIP and to NANC stimulation[60]. The close association between responses to VIP and NANC relaxation in different sizes of human and bovine airways[71] also points to the role of VIP as a neurotransmitter. Although evidence in favour of VIP is persuasive, until the development of a specific receptor antagonist, its neurotransmitter role cannot be proved. Indeed, there is some evidence against it as a neurotransmitter in airways. After pretreatment of guinea pig trachea with maximally effective concentration of VIP, there is no diminution of NANC relaxation, Which would be expected if all VIP-receptors were occupied[43]. However, it is likely that other peptides, such as PHI, will also be released from these nerves and VIP may not have ready access to the VIP-receptors related to VIP-ergic nerves.

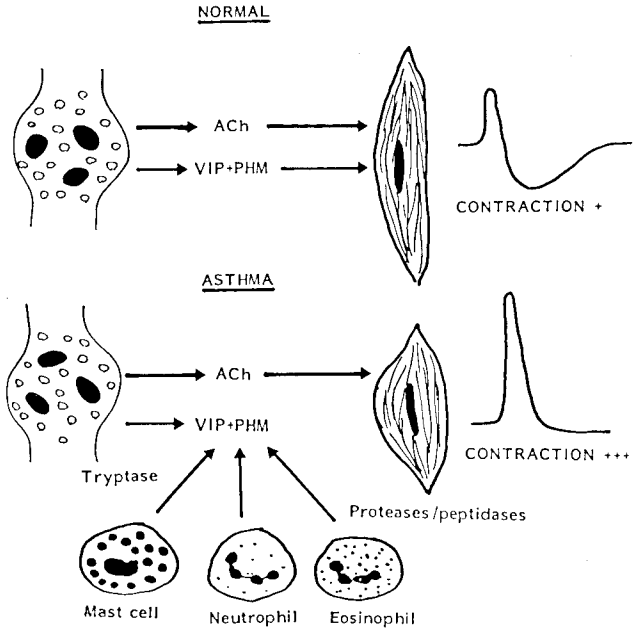

Figure 1. Schematic representation of increased degradation of VIP and PHM in asthmatic airways as a result of enzymes released from inflammatory cells. This may result in increased cholinergic bronchoconstriction. (From Barnes[4]).

Co-transmission with acetylcholine. VIP coexists with acetylcholine (ACh) in some cholinergic nerves, supplying exocrine glands and potentiates the salivary secretory response to ACh[52]. VIP may be released from cholinergic nerves only with high frequency firing and may serve to increase the blood flow to exocrine glands under conditions of excessive stimulation. VIP also appears to coexist with ACh in airways, and it seems likely that there is a functional relationship between VIP and cholinergic neural control. It is possible that excessive stimulation of cholinergic nerves on certain patterns of firing result in VIP release. In bovine tracheal smooth muscle VIP has an inhibitory effect on cholinergic nerve-induced contraction, only with high frequency firing, and also reduces the contractile effect of exogenous ACh[71]. This does not involve any change in muscarinic receptor density or affinity, and may be due to functional antagonism. This indicates that VIP counteracts the bronchoconstrictor effect of acetylcholine and thus may function as a 'braking' mechanism for airway cholinergic nerves[8]. If this mechanism were to be deficient, this might result in exaggerated bronchoconstrictor responses.

Abnormalities in asthma. Whether dysfunction of VIP-ergic innervation contributes to any abnormality in pulmonary diseases is uncertain. It seems unlikely that there would be any primary abnormality in VIP content, neurotransmission or receptors, but it is possible that a secondary defect might develop as a result of the disease process[3,8].

Bronchial hyperresponsiveness in asthma may be related to an inflammatory response in the airway. The inflammatory cells present in the asthmatic airway (mast cells, macrophages, basophils, neutrophils) may release a variety of peptidases which break down VIP. Little is known of the enzymatic pathways involved in inactivation of these peptides, although several peptidases may inactivate them. If these peptidases are released into the airway in asthma, with increased degradation of VIP and PHI, this would result in a loss of the braking effect on cholinergic bronchoconstrictor responses (fig. 1). This might then contribute to the bronchial hyperresponsivess of asthma.

Peptide histidine isoleucine

PHI and its human equivalent peptide histidine methionine (PHM) have a marked structural similarity to VIP, with 50% amino acid sequence homology. PHI and PHM are encoded by the same gene as VIP and both peptides are synthesised in the same prohormone. It is therefore not surprising to find that PHI has a similar immunocytochemical distribution in lung to VIP, and thus PHI-immunoreactive nerves supply airway smooth muscle (especially larger airways), bronchial and pulmonary vessels, submucosal glands and airway ganglia [55]. The levels of PHI-immunoreactivity are very similar to values obtained or VIP-immunoreactivity in respiratory tract[24].

Like VIP, PHI stimulates adenylates cyclase and may activate the same receptor as VIP. However, this is unlikely as PHI has different relative potencies compared with VIP in different tissues, being less potent as a vasodilator and more potent as a stimulant of secretion. In human bronchi in vitro PHM is a potent relaxant, and is equipotent to VIP[67]. It is therefore likely that PHI/PHM is released with VIP from airway nerves and may also be a neurotransmitter of NANC relaxation. Although VIP and PHI may activate separate receptors, no synergy has been demonstrated between these peptides in airway smooth muscle (Palmer, J. B., and Barnes, P. J., unpublished). Because PHM has equal potency in relaxing airway smooth muscle, but is less potent as a vasodilator than VIP, it is possible to infuse higher concentrations before cardiovascular effects are limiting. In a preliminary study it has not proved possible to demonstrate bronchodilatation in 3 mild asthmatics with the highest infused concentration of PHM (Palmer, J. B., Cuss, F. M., and Barnes, P. J., unpublished).

PHI is significantly less potent as a pulmonary vasodilator than VIP, and, like VIP, produces its effects independently of endothelial cells[38]. The effects of PHI on airway mucus secretion and ion transport have not yet been studied, but PHI has potent effects on secretion in the gastrointestinal tract.

Substance P and tachykinins

While substance P (SP) was isolated over 50 years ago, structurally related peptides (tachykinins) called neurokinin A and B have recently been identified[65]: NKA is coded by the same gene as SP, whereas NKB is coded by a different gene.

Localisation. SP is localised to nerves in the airways of several species, including humans[56], although there has been debate about whether SP can be demonstrated in human airways[45]. However, rapid degradation of SP in airways, and the fact that SP concentrations may decrease with age, and possibly smoking, could explain the difficulty in demonstrating this peptide in some studies. SP-immunoreactive nerves in the airway are found beneath and within the airway epithelium, around blood vessels and, to a lesser extent, within airway smooth muscle. SP appears to be localised to afferent nerves in the airways and SP is synthesised in the nodose ganglion of the vagus nerve and then transported down the vagus to peripheral branches in the lung. Treatment of animals with capsaicin releases SP from sensory nerves acutely and chronic administration depletes the lung of SP[54]. Neurokinin-like immunoreactivity has also been found in lung and may also play a regulatory role[83].

Tachykinin receptors. SP exerts its effects on target cells via specific receptors, which have now been identified by direct binding techniques. Recent autoradiographic studies have demonstrated the distribution of SP-receptors in guinea pig and human lung using Bolton-Hunter [^{125}I]-SP[22]. SP-receptors are found in high density in airway smooth muscle from trachea down to small bronchioles, whereas pulmonary vascular smooth muscle and epithelial cells are less densely la-

belled. Submucosal glands in human airways are also labelled.

There are at least three types of tachykinin receptor which may be differentiated by different responses to a series of tachykinins[50]. In some tissues SP is more potent than neurokinins (SP-P or NK-1 receptor), whereas in others the order of potency is either NKA > NKB > SP (SP-E or NK-2 receptor), or NKB > NKA > SP (SP-N or NK-3 receptor).

Airway smooth muscle. In vivo SP infusion causes bronchoconstriction in animals[1], which may be partially blocked with atropine, suggesting that SP release of acetylcholine may be responsible for some of the bronchoconstrictor action in vivo[82]. In human subjects infusion of SP has profound cardiovascular effects, but little effect on airway function; a small bronchoconstrictor response is followed by bronchodilation at higher infusion doses[35]. The cardiovascular actions may limit the dose that can be given and result in reflex bronchodilatation (by a reduction in vagal tone), which counteracts the bronchoconstriction. Even when given by inhalation SP has no significant effect on airway function in mild asthmatic subjects who are hyperresponsive to histamine given in the same way[35]. This may be due to enzymatic degradation of SP in the airway and its inability to cross the epithelium. Inhalation of capsaicin in human subjects, which should release SP, induces marked coughing but only transient bronchoconstriction, seen in both normal and asthmatic subjects to an equal extent. This bronchoconstrictor response is inhibited by cholinergic antagonists, suggesting that it may be due to reflex stimulation. The intense irritation of capsaicin may preclude giving a dose sufficiently large to release SP from airway nerves.

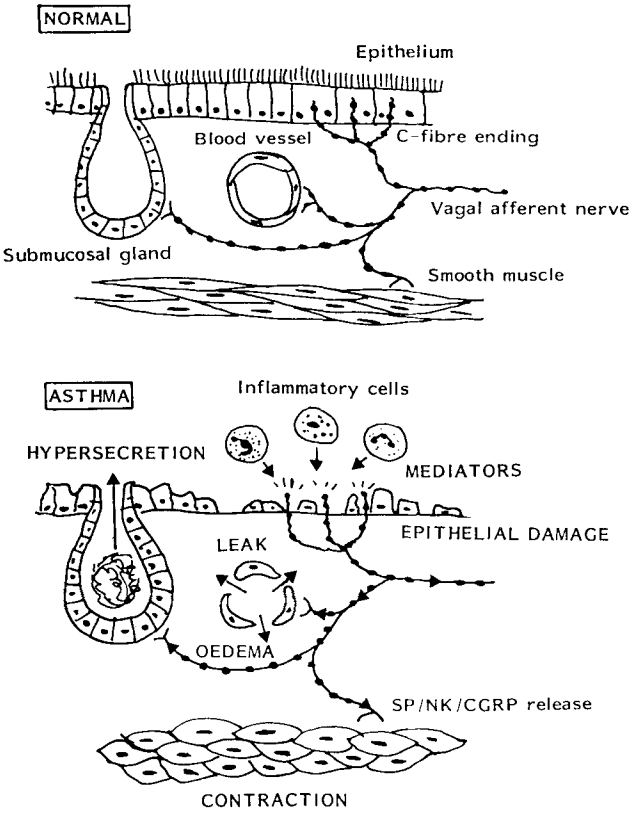

Figure 2. Axon reflex mechanisms in asthma. Damage to airway epithelium in asthma exposes unmyelinated nerve endings which may be triggered by mediators (e.g. bradykinin) resulting in release of sensory neuropeptides such as substance P (SP), neurokinins (NK) and calcitonin gene-related peptide (CGRP), which together contribute to the pathology of asthma. (From Barnes[5]).

In vitro SP contracts airway smooth muscle of several species, including man[42,57]. Moreover, capsaicin is capable of inducing a similar contraction, indicating release of SP from intrinsic nerves within airway smooth muscle. The contracile effect of SP on airway smooth muscle in vitro may be inhibited by SP antagonists, suggesting a direct effect on smooth muscle cells, although the specificity of these antagonists has been questioned.

In guinea pig and human airways neurokinin A is more potent than SP in causing contraction, suggesting that bronchial smooth muscle has NK-2 receptors, and that neurokinins, rather than SP, regulate bronchomotor tone[42,68].

Microvascular effects. In rats and guinea pigs, both SP and capsaicin induce edema of the airway wall by increasing microvascular permeability. Depletion of SP nerves by neonatal capsaicin pretreatment prevents irritants, such as cigarette smoke and mechanical stimulation, from causing mucosal edema, suggesting that SP nerves mediate this effect[58]. In human skin SP causes a wheal and flare response[32,11], but NKA and NKB are much less potent, suggesting this is mediated by NK-1 receptors[13].

Secretion. SP is a potent stimulant of airway mucus secretion in isolated canine and human airways[15,25].

Mediator release. The wheal and flare response to intradermal SP is blocked by antihistamines[32] and increases the release of histamine into draining veins[11]. This suggests that SP might be capable of causing histamine release in the airways, although there is no direct evidence for this. The flare induced by intradermal SP and neurokinins is also reduced by aspirin, suggesting that cyclo-oxygenase products are also released[14].

It has also been suggested that SP might amplify neutrophil and eosinophil responses to chemotactic agents, and therefore magnify the inflammatory response in the airways[73].

Calcitonin gene-related peptide (CGRP)

CGRP is localised to afferent nerve terminals in airways and may be co-localised with SP[53]. Human CGRP is a potent vasodilator, and produces a wheal and flare in the skin when injected locally[18], and potentiates the effect of SP[11]. CGRP has been extracted from human airways and is localised to nerves[69]. In vitro human CGRP contracts isolated human airways, being much more potent than SP, and producing equivalent contraction to carbachol, whereas SP causes only partial contraction[69]. CGRP-induced contraction is unaffected by cholinergic, histamine or leukotriene antagonists and probably acts via specific receptors.

Axon reflexes and asthma

Sensory neuropeptides produce many of the pathological features of asthma, including contraction of airway smooth muscle, edema and plasma extravasation, mucus hypersecretion and possibly increased mediator release, it is possible that they may contribute to the pathology of asthma. Damage to airway epithelium may occur even in relatively well-controlled asthmatics, probably as a result of eosinophil products[42], exposing afferent nerve endings which are stimulated by inflammatory mediators. C-fibre endings may be selectively stimulated by bradykinin and other mediators produced in the inflammatory reaction, which could result in a reflex cholinergic bronchoconstriction. Bradykinin is a potent bronchoconstrictor in asthmatic subjects, which may selectively stimulate C-fibre afferents[34]. However, anticholinergic drugs are not very effective in clinical asthma and it is possible that stimulation of C-fibre endings may result in an axon reflex, with release of sensory neuropeptides from sensory collaterals in the airway[5], or a local ganglionic reflex,

since SP and CGRP are found in airway ganglia, suggesting an afferent input. The release of SP, neurokinins and CGRP may then result in bronchoconstriction, mucus hypersecretion and microvascular leakage of plasma to produce edema of the airway wall and extravasation of plasma into the airway lumen (fig. 2). The axon reflex may, therefore, contribute to the pathology of asthma, and may help to explain how patchy epithelial damage leads to widespread pathophysiological changes. Drugs which interfere with the axon reflex mechanisms may, therefore, be useful in asthma, and there is some evidence to suggest that sodium cromoglycate may have such an effect.

Other neuropeptides

Neuropeptide Y. NPY has been found in human lung and is localised primarily to innervation of blood vessels[80]. The distribution of NPY is similar to that of sympathetic nerves and therefore few NPY-immunoreactive nerve fibres are localised to airway smooth muscle. NPY is probably a co-transmitter of noradrenaline and is a potent constrictor of vascular smooth muscle[59]; it may play an important role in regulating pulmonary and bronchial vessels but is likely to be less important in influencing airway tone.

Galanin. Galanin has recently been isolated and localised to motor nerves in the respiratory tract, possibly co-localised with VIP and acetylcholine[23]. The function of this peptide is uncertain and, while it acts as a neuromodulator reducing cholinergic nerve effects in gut, it has no effect on guinea pig trachea[31].

Gastrin-releasing peptide. Gastrin-releasing peptide (GRP) is probably the mammalian form of bombesin and has been localised to nerves in the airway wall of several species, but again its function is uncertain[84].

Conclusions

Many neuropeptides have now been localised to the lung, and almost certainly more will be discovered. These peptides often have potent actions on airway and vascular tone and on lung secretions, but the presence of so many peptides raises questions about their physiological role. It seems most likely that they may act as subtle regulators under physiological conditions, but in inflammatory diseases such as asthma they may have a pathogenetic role. Until specific antagonists have been developed it will be difficult to evaluate the precise role of these neuropeptides in disease. It is certainly possible that pharmacological agents which interact with neuropeptides by affecting their release, metabolism or receptors may be developed in the future and may have therapeutic potential.

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Endocrine cells producing regulatory peptides

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Summary. Recent data on the immunolocalization of regulatory peptides and related propeptide sequences in endocrine cells and tumours of the gastrointestinal tract, pancreas, lung, thyroid, pituitary (ACTH and opioids), adrenals and paraganglia have been revised and discussed. Gastrin, xenopsin, cholecystokinin (CCK), somatostatin, motilin, secretin, GIP (gastric inhibitory polypeptide), neurotensin, glicentin/glucagon-37 and PYY (peptide tyrosine tyrosine) are the main products of gastrointestinal endocrine cells; glucagon, CRF (corticotropin releasing factor), somatostatin, PP (pancreatic polypeptide) and GRF (growth hormone releasing factor), in addition to insulin, are produced in pancreatic islet cells; bombesin-related peptides are the main markers of pulmonary endocrine cells; calcitonin and CGRP (calcitonin gene-related peptide) occur in thyroid and extrathyroid C cells; ACTH and endorphins in anterior and intermediate lobe pituitary cells, α-MSH and CLIP (corticotropin-like intermediate lobe peptide) in intermediate lobe cells; met- and leu-enkephalins and related peptides in adrenal medullary and paraganglionic cells as well as in some gut (enterochromaffin) cells; NPY (neuropeptide Y) in adrenalin-type adrenal medullary cells, etc.. Both tissue-appropriate and tissue-inappropriate regulatory peptides are produced by endocrine tumours, with inappropriate peptides mostly produced by malignant tumours.
Key words. Bombesin; substance P; CRF; ACTH; opioids; calcitonin; somatostatin; PP; glucagon; GRF; secretin; GIP; gastrin; CCK; motilin; neurotensin; endocrine cells; endocrine tumours.

Introduction

Endocrine cells producing regulatory peptides are specialized epithelial cells characterized by their secretory granules of variable size, shape, density and inner structure enveloped by a unit membrane. The granules are formed at the trans side of the Golgi complex, from condensing vacuoles whose contents, simultaneously with the process of controlled proteolysis of prohormones, undergo progressive densification to form clathrin-coated 'immature' progranules and then 'mature' secretory granules storing the active hormones[107]. Besides hormonal peptides and related prohormone fragments, secretory granules store hormone-unrelated proteins like chromogranins, monoamines, such as catecholamines and serotonin, polyamines and metal cations[122,178].

In addition to secretory granules, like those storing peptides and chromogranins, and the large, dense-cored vesicles of nerves, a population of small clear vesicles, closely resembling the small synaptic vesicles which store classic neurotransmitters, has been described in some endocrine cells, such as paraganglionic, adrenal medullary and pulmonary endocrine cells[28,78,98]. Recently these small vesicles of nerves and endocrine cells (including adrenal medullary and pituitary cells) have been found to be selectively marked by a Ca^{2+}-binding membrane glycoprotein, the synaptophysin or P38 protein[102]. P38 protein immunoreactivity has been detected in adrenal medullary and paraganglionic cells, pancreatic islets, adenohypophysis and thyroid C cells as well as in pulmonary and gastric endocrine cells and related growths, while no reactivity has been observed in intestinal endocrine cells, cardiac atrial cells producing natriuretic hormone and parathyroids[24,102,177]. Cholinergic[33,173], aminergic[110] and GABAergic[62] mechanisms have been found to operate in at least some of the P38-positive cells. Two other vesicle membrane proteins[21,97], neuron specific enolase[10], three distinct chromogranin proteins[122,125,143,178] and a number of regulatory peptides and amines are now known to be common markers of nerves and endocrine cells.

Thus, although the proposed neural crest origin of endocrine cells[110] has been confirmed only for adrenal medulla, carotid body and thyroid C cells[89], the ability of many (not all) endocrine cells, independently from their neural crest origin, to express morphologic and functional patterns characteristic of nerve cells is widely documented and may justify the designation of such cells as 'neuroendocrine' cells[111]. It seems interesting that during phylogenesis nerve cells first develop as peptidergic elements scattered in both the ectoderm and endoderm of coelenterates, partly as elongated 'sensory' cells contacting the epithelial surface, with processes at their basal part[56], a pattern resembling some paracrine cells of mammalian endodermal derivatives[137,141].

As a rule, in different endocrine cell types distinct genes are expressed coding for different propeptides. However, alternative processing of the same m-RNA may result in two distinct propeptides leading to different regulatory peptides, as in the case of calcitonin and calcitonin gene-related peptide (CGRP), coded by the same gene through different propeptides showing tissue specific, though partly overlapping, distributions[126,158]. More often, two or more active peptides, showing the same cellular distribution, may be the products of the same propeptide, coded by a single gene, as in the case