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REVIEW

Nonadrenergic, noncholinergic airway inhibitory nerves

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ABSTRACT: Nonadrenergic, noncholinergic (NANC) nerves, which cause relaxation of airway smooth muscle, have been described in several species including man. Stimulation of efferent vagus nerves during cholinergic and adrenergic blockade induces a pronounced bronchodilation in the cat. In more recent studies in man, capsaicin inhalation or mechanical irritation of the larynx, under conditions of cholinergic and adrenergic blockade, have been shown to cause a transient bronchodilator response.

There is some evidence that neuropeptides such as vasointestinal peptide (VIP) or peptide histidine methionine (PHM) may be the neurotransmitter of NANC nerves, but this is not conclusive. Nitric oxide may be another neurotransmitter. In mild asthma, the NANC bronchodilator response is similar to that observed in normal subjects; on the other hand, a reduction in VIP immunoreactivity has been reported in more severe patients. The contribution of NANC dilator nerves in pathophysiological situations is not known, but their effect may be modulated during allergic responses.

Use of antagonists or inhibitors of putative neurotransmitters, and molecular biological techniques will be useful in defining both the physiological and pathophysiological roles of NANC inhibitory nerves in the airways.

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The autonomic nervous system of the airways is more complex than originally believed. In addition to parasympathetic or cholinergic and sympathetic or adrenergic neural pathways, there are other neural mechanisms which are neither adrenergic nor cholinergic. At the end of the 19th century, LANGLEY first described "atropine resistant" neural effects in the bladder and stomach [1, 2], but it was not until the 1960s that the existence of nonadrenergic, noncholinergic (NANC) nerves was demonstrated [3]. NANC innervation was initially demonstrated in the gastrointestinal tract and later in the urogenital and cardiovascular systems [4]. Because the airways embryologically develop from the foregut, it was not surprising that NANC nerves were also found in the respiratory tract [5, 6]. Stimulation of NANC nerves may result in bronchodilatation or bronchoconstriction, as well as mucus secretion, microvascular leakage, changes in airway blood flow and activation of inflammatory cells. Bronchoconstriction and inflammatory effects may be produced by the release of peptides such as tachykinins and calcitonin gene-related peptide (CGRP) from sensory nerves; this topic has been extensively reviewed [7–9].

In this review, we will discuss NANC bronchodilator nerves and their possible role in the pathophysiology of asthma, with particular emphasis on recent studies which have been performed in human subjects.

Evidence for inhibitory NANC nerves

In vitro *studies*

NANC nerves which cause relaxation of airway smooth muscle have been described in several species, including man, using *in vitro* techniques [6, 10–13]. In isolated human airway smooth muscle, electrical field stimulation (EFS) induces a contractile response that is blocked by pretreatment with atropine and the nerve toxin, tetrodotoxin, indicating that cholinergic nerves are involved. This is followed by a relaxant response which is unaffected by β-adrenoreceptor blockade [6, 10, 12], indicating that there is no functional sympathetic innervation of human airway smooth muscles. Histochemical studies confirm that adrenergic fibres do not innervate human airway smooth muscle directly [14], although there are adrenergic receptors on airway smooth muscle which are stimulated by circulating adrenaline [15, 16]. In guinea-pig trachealis muscle and canine bronchial smooth muscle the relaxant responses to EFS are partially inhibited by β-adrenoreceptor blockers [10, 17]. Thus, in these animals the relaxation of the airway smooth muscle is composed of an adrenergic and a nonadrenergic component. In all species, the inhibitory NANC response is blocked by tetrodotoxin, indicating that a neural mechanism is responsible for this phenomenon [6, 10, 12]. In some studies, a

component of the relaxant response to EFS is resistant to tetrodotoxin and is presumably non-neural in origin [12]. The nature of this non-neural relaxant effect is not understood but may be related to the presence of airway epithelium, since it is absent in guinea-pig tracheal rings denuded of epithelium [18], implying that an epithelial-derived relaxant factor is probably responsible.

In vivo *studies in animals*

Support for the presence of NANC inhibitory nerves has also been demonstrated *in vivo* in animals by electrical stimulation of the vagus nerves during cholinergic and adrenergic blockade [19–22]. Under these conditions stimulation of efferent vagal nerves induces a pronounced and long-lasting bronchodilatation in cats. This response is inhibited by the ganglion blocker, hexamethonium, indicating that NANC nerves are preganglionic as well as postganglionic [21]. Inhalation of capsaicin or mechanical stimulation of the larynx induces a similar bronchodilator response in cats after pretreatment with adrenergic and cholinergic blocking agents [23, 24], indicating that reflex activation of these pathways is possible.

relatively unchanged [19]. At present no such data are available on the distribution of NANC responses along the airways in man *in vivo*, although *in vitro* studies have demonstrated that peripheral airways (bronchioles) lack an inhibitory NANC response [25].

Although RICHARDSON and BELAND [5] first described inhibitory NANC nervous responses of human airway smooth muscle preparations *in vitro* about 15 yrs ago, it was not until recently that inhibitory NANC responses of the airways have been demonstrated in human subjects *in vivo* [26–28].

Studies in normal, non-asthmatic subjects

Several groups have recently demonstrated bronchodilator NANC effects in human subjects *in vivo*. Essentially, these studies, similar in design to those previously described in cats, stimulate the laryngeal afferent pathways with capsaicin, or mechanical irritation [26–28]. Capsaicin inhalation induces a transient bronchoconstrictor response in normal subjects [29] but, following cholinergic inhibition with ipratropium bromide and β-blockade with propranolol, capsaicin causes a bronchodilator response in the presence of increased bronchomotor tone induced by

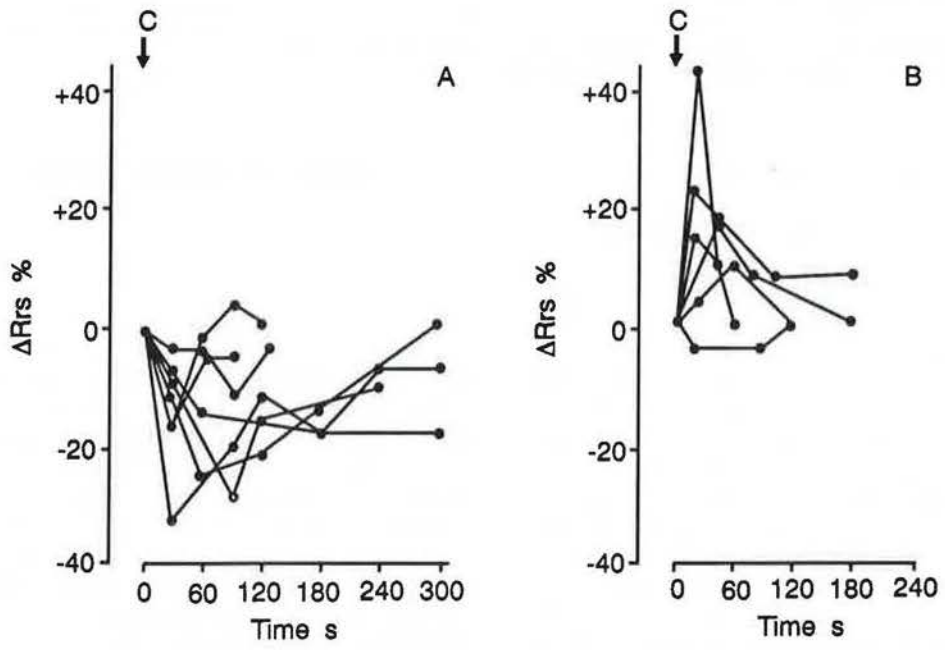

Fig. 1. – Individual time courses of changes in total respiratory resistance (Rrs) for normal volunteers induced by inhalation of capsaicin (C). These volunteers have been constricted with leukotriene D_4 aerosol and pretreated with ipratropium bromide and propranolol. Changes in Rrs (ΔRrs) have been calculated from the baseline bronchoconstricted state. Panel A: In the absence of lignocaine. Panel B: After inhalation of 2% lignocaine. Time on horizontal axis is taken from moment of inhalation of C. Lignocaine inhibited the bronchoconstrictor effect of capsaicin and uncovered a constrictor component of the capsaicin response. Reproduced by permission from LAMMERS *et al.* [27].

Although NANC-induced airway responses can be demonstrated *in vitro* both in trachea and bronchi, tantalum bronchography has revealed that NANC bronchodilatation occurs *in vivo* predominantly in medium sized airways in cats leaving the trachea and peripheral bronchi (less than 1 mm in diameter)

leukotriene D_4 (LTD_4) (fig. 1). This response is transient, lasting for less than two minutes, and does not totally reverse the bronchoconstrictor effect of LTD_4. This is in contrast to studies in cats [21, 22], where the bronchodilator effect lasted for several minutes, probably because of the greater nasal stimulation and

release of a greater amount of neurotransmitters by direct stimulation of the vagus nerves. After pretreatment with the local anaesthetic, lignocaine, there was only a further transient increase in bronchoconstriction, and the bronchodilator response was blocked (fig. 1). Apparently, the afferent nerve fibres, which mediate the capsaicin-induced bronchodilatation, were blocked by anaesthesia of the airway mucosa leaving the bronchoconstrictor response unchanged [27]. In a similar study design, ICHINOSE *et al.* [26] used capsaicin inhalation in normal subjects after cholinergic and β-adrenergic blockade and after prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced bronchoconstriction. A significant transient bronchodilator response was observed, an effect which was abolished by pretreatment with the ganglionic blocking agent, hexamethonium.

In another study [28], mechanical stimulation of the larynx induced transient decrease in airway resistance induced by histamine under cholinergic and beta-adrenergic blockade. These studies establish the presence of a nonadrenergic, noncholinergic bronchodilator system, which can only be demonstrated under conditions of increased bronchomotor tone. The determinants of the degree and duration of relaxation induced either by mechanical stimulation or by inhalation of capsaicin have not yet been investigated.

The neurotransmitter of inhibitory NANC responses

The precise identity of the inhibitory NANC neurotransmitter in airways is not yet established and no specific blockers have been identified, making detailed studies of the physiology of this mechanism difficult. In the gastrointestinal and genitourinary tract some evidence has implicated purines as neurotransmitters, it was therefore suggested that the NANC nervous system of the airways may also be purinergic [30–32].

Purines

Experimental evidence, however, argues against this possibility. Adenosine does not mimic inhibitory NANC effects and they are not blocked by the adenosine antagonist, theophylline [33]. Exogenously administered adenosine triphosphate (ATP) relaxes airway smooth muscle, but the ATP antagonist, quinidine, does not prevent NANC inhibitory responses either *in vitro* or *in vivo*. Moreover, the purine uptake inhibitor, dipyridamole, fails to enhance nonadrenergic bronchodilatation [22, 34].

Vasoactive intestinal peptide

Many NANC effects are mediated by peptide release from classical cholinergic nerves, but few peptides have bronchodilator effects. VIP is abundant in animal and human airways and is a potent bronchodilator. VIP and the related peptides, peptide histidine methionine (PHM) or isoleucine and peptide histidine valine (PHV-42), are candidates for the neurotransmitter [25, 35–38]. VIP is a 28 amino acid peptide which is localized to neurones and nerve terminals in human airways around submucosal glands, beneath airway epithelial cells, and in bronchial and pulmonary vessels, in addition to airway ganglia [39–41]. It is likely that VIP is present in cholinergic nerves and may be a co-transmitter of acetylcholine. VIP is a potent relaxant of airway smooth muscle *in vitro*, an effect which is unaffected by propranolol or indomethacin [25, 34, 42]. PHM is also a potent bronchodilator peptide which is co-transcribed and co-expressed with VIP in airway motor neurones [25, 43, 44]. PHV-42 is an N-terminal extended form of PHM, which has potent bronchodilator effects *in vitro* [45]. Heliodermin is a VIP-like peptide described in a species of lizards, and a heliodermin-like peptide has now been described in mammalian airways, which has potent bronchodilator effects [46]. *In vivo,* inhaled VIP is a bronchodilator and attenuates histamine-induced bronchoconstriction in animals [38]. In humans, inhaled VIP has no bronchodilator effect and provides only minor protection against histamine-induced bronchoconstriction, possibly because it is degraded by epithelial enzymes [47]. Infused VIP, similarly, has no demonstratable bronchodilator effect at maximally tolerated doses [48]. PHV-42 is similarly ineffective after infusion [49]. Electrical field stimulation of tracheobronchial strips releases VIP into the organ bath and this effect is blocked by tetrodotoxin [37] indicating a neurogenic release of this peptide.

Although no effective antagonists of airway VIP are available [50, 51], the responses of nonadrenergic, noncholinergic nerve stimulation can be reduced by an antibody against VIP [37], desensitization of VIP receptors [34] and peptidases such as chymotrypsin, aprotinin, leupeptin, phosphoramidon and soybean trypsin inhibitors, which are known to degrade VIP [52, 53].

VIP produces relaxation of airway smooth muscle by activating adenylyl cyclase and increasing cyclic adenosine monophosphate (AMP) in a similar manner to β-adrenoceptor induced responses. Cyclic AMP breakdown by phosphodiesterase (PDE) is inhibited by the PDE-Type III inhibitor SK&F 94120, which potentiates the bronchodilator response to VIP. The same inhibitor also enhances relaxant NANC responses [54]. By contrast, an inhibitor of cyclic guanylic acid (GMP) PDE, zaprinast, fails to enhance either VIP or inhibitory NANC responses, providing further evidence that VIP may be a transmitter. Additional circumstantial evidence is provided by the observation that while proximal airways have an inhibitory NANC response and relax to VIP *in vitro,* peripheral airways which do not have an inhibitory NANC response also fail to respond to VIP [25] and have no receptors for VIP [55]. These observations, while supporting the

hypothesis that this neuropeptide and/or other related peptides such as PHM may be the neurotransmitter of inhibitory NANC in the airways, are not conclusive.

Nitric oxide

Peptides such as VIP and PHM may not be the sole mediators of inhibitory NANC responses in airways. In a recent study, TUCKER *et al.* [56] demonstrated that the peptidase-resistant component of the NANC inhibitory response in guinea-pig tracheal smooth muscle is attenuated in a dose-related fashion by L-N^G-nitroarginine, which is an inhibitor of nitric oxide formation. This effect of nitroarginine is stereospecific and reversed by L-arginine. Thus an endogenous nitrate may partially mediate NANC relaxation, although its exact site of action (pre- or postjunctional) in tracheal smooth muscle is not yet clear. Preliminary studies also indicate that inhibition of nitric oxide synthesis diminishes inhibitory NANC responses in human airways at low frequencies of electric field stimulation, suggesting that the inhibitory NANC response may be modulated by nitric oxide release at low frequencies and by VIP release at higher frequencies [57]. Further studies are necessary in this area, particularly in human airway smooth muscle.

NANC inhibitory pathways in disease

A pathophysiological role for the inhibitory NANC nerves in human airways has long been proposed. It has been suggested that a defective function of the NANC nerves may contribute to bronchoconstriction and bronchial hyperresponsiveness in asthma [6, 58]. The NANC neurotransmitter which may be a co-transmitter in cholinergic nerves, may act as a brake in inhibiting the bronchoconstrictor effects of acetylcholine release at the same efferent motor endings. A reduction in the release of the NANC neurotransmitter may, therefore, lead to excessive bronchoconstrictor drive.

Asthma

An important question, which remains to be answered, is whether the inhibitory NANC system is dysfunctional in asthma or other airway diseases. In the absence of a specific inhibitor this question may be difficult to resolve. Inhibitory NANC responses *in vitro* do not appear to be impaired in airways of patients with chronic airflow limitation [59]. Airways from asthmatic patients at lung transplantation have been found to have a normal inhibitory NANC response [60]. Also airways from patients dying during severe asthmatic attacks showed similar NANC inhibitory responses to control airways from non-asthmatic subjects [61].

Two recent groups have investigated the NANC bronchodilator system in asthmatic subjects *in vivo* [62, 63]. They both demonstrated the presence of a NANC bronchodilator system in asthmatic patients with very mild asthma, who only required bronchodilator therapy. The protocol previously described for normal subjects was used, except that the use of β-blockade with propranolol was avoided, as β-blockade may provoke severe bronchoconstriction in asthma [64]. MICHOUD *et al.* [63] similarly extended their study to asthmatic subjects without β-blockade (fig. 2), but they also measured circulating plasma catecholamines in order to exclude a possible contribution of these circulating hormones. No significant major increases in catecholamines occurred during their experiments involving mechanical stimulation of the larynx. Both studies [62, 63] demonstrated that the degree of bronchodilator response observed was of similar duration and magnitude as that seen in normal subjects, suggesting that the NANC bronchodilator system was functioning in mild asthmatic subjects. These *in vivo* studies question the hypothesis that asthma may be the result of a defective NANC bronchodilatation, although a defect of this neural bronchodilator pathway in more severe asthmatic patients may still be present.

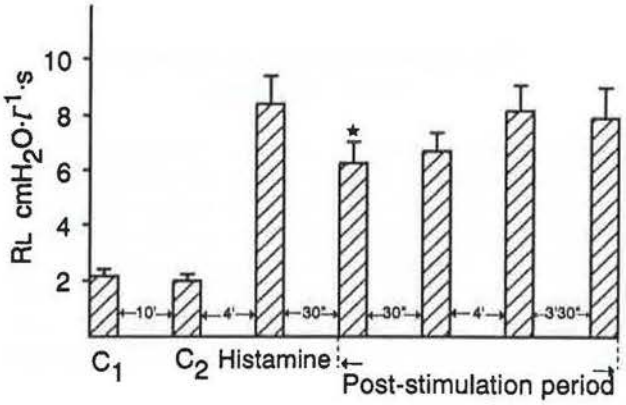

Fig. 2. – Effect of laryngeal stimulation on histamine-induced bronchoconstriction in six mild asthmatic subjects. Within 30 s of laryngeal stimulation there was a significant bronchodilator response. C_1 and C_2: control values. Reproduced by permission from MICHOUD *et al.* [63]. RL: pulmonary resistance.

A reduction in VIP immunoreactivity has recently been reported in the airways of asthmatic patients with severe disease, most of which were obtained at postmortem; this observation was also surprisingly described in pulmonary vessels [65]. Human tryptase obtained from lung extracts can rapidly hydrolyse VIP and, since tryptase is secreted by mast cells, it has been suggested that an increased release of this protease by mast cells may explain the loss of VIP from airway nerves [66]. However, a more recent preliminary study of mucosal biopsies obtained from a much milder group of asthma patients reported no difference between normal and asthmatic patients in terms of VIP immunoreactivity [67]. If VIP is the neurotransmitter for the NANC dilator system, these

observations would suggest that the reduction in VIP immunoreactivity is dependent on the severity and perhaps the chronicity of asthma.

Allergic responses

Little is known about the state of the NANC response during episodes of allergic bronchoconstriction in asthma. Recent studies during the allergic response in the airways have been performed in cats by MIURA and co-workers [68, 69], who showed that inhibitory NANC nerve stimulation potently inhibited antigen-induced bronchoconstriction and the increase in arterial plasma histamine [68], suggesting that the NANC bronchodilator prevents release of mediators such as histamine from activated sensitized mast cells as has been demonstrated *in vitro* [70]. It has also been demonstrated that the bronchodilator effects of VIP and of the NANC bronchodilator system were reduced after allergen exposure and that a protease inhibitor, leupeptin, abolished the allergen-induced impairment of NANC effects in sensitized cats [69]. These results would suggest that NANC dilatation is less effective in this situation because of degradation of the putative NANC neurotransmitter, such as VIP, by proteases released during the allergic response. Both VIP and PHM are degraded by mast cell proteases, particularly tryptase which is the major secretory protease of human lung mast cells [71]. Thus, during allergic airway responses, this may contribute to bronchial hyperresponsiveness and perhaps to the decrease in immunoreactive VIP in airway nerves that has been reported in asthma [65], as mast cells are often found in close proximity to airway nerves [72]. Recently, *in vitro* studies have shown that tryptase isolated from canine mastocytoma cells markedly increases their contractile response to spasmogens, possibly by degrading endogenously released VIP [73]. It may be of interest to investigate the role of the NANC dilator system following allergen challenge, after the early response, and during the late phase response in asthmatic patients. Indeed, other airway challenges such as ozone and viral infections, which induce a transient state of bronchial hyperresponsiveness [74, 75] may also be associated with reduced inhibitory NANC responses.

Other effects of NANC nervous system

The NANC inhibitory nervous system also seems to be important in the regulation of pulmonary artery blood flow as has been demonstrated in guinea-pigs and cats [76–77]. The identity of the neurotransmitter is not yet certain as several neuropeptides (VIP, tachykinins) and CGRP can induce pulmonary vasodilation. It may well be that VIP is one of the neurotransmitters of NANC mediated relaxation of pulmonary arteries, since these vessels are richly supplied with VIP-containing nerves [78, 79]. In addition, VIP is a potent vasodilator of tracheal vessels *in vivo* [80] and may be the mediator of NANC vasodilation in tracheal vessels in pig airways [66].

Histochemical studies have shown that VIP-immunoreactive nerves are closely related to submucosal glands in mammalian airways [40]. Electrical stimulation of the vagus nerves in ferrets and cats increases the secretion of mucus glycoproteins into the trachea, an effect that is partially cholinergic but mainly nonadrenergic, noncholinergic [81, 82]. In contrast to airways of normal human subjects VIP does not seem to inhibit the release of mucus glycoconjugates from submucosal glands of patients with chronic bronchitis [83]. VIP can also stimulate ion and water transport across the epithelium in canine airways [84] and, together with the effect on mucin secretion, VIP-ergic nerves may influence mucociliary transport in the airways [85]. Although few studies have investigated VIP effects on airway secretions, autoradiographic mapping studies have demonstrated a high density of VIP receptor over submucosal glands and epithelium of human airways [55].

Conclusion

NANC neural bronchodilation has been demonstrated in several species *in vivo* and *in vitro*, including man. The precise physiological or pathophysiological role of this system remains to be defined and more experimental work is needed particularly in asthmatic patients. The greatest insight will be provided by work which uses pharmacological tools such as antagonists or inhibitors of putative neurotransmitters, or of inhibitors of breakdown of these peptides. In combination with *in vivo* studies, molecular biological approaches to investigate regulation of VIP and nitric oxide synthesis at a transcriptional level in normal and diseased airway biopsies will be potentially very valuable.

References

1. Langley JN, Anderson HK. – The innervation of the pelvic and adjoining viscera. IV. The internal organs. *J Physiol,* 1985; 19: 122–130.
2. Langley JN. – On inhibitory fibres in the vagus for the end of the oesophagus and the stomach. *J Physiol,* 1988; 23: 407–414.
3. Burnstock G, Campbell G, Bennett M, Holman ME. – Inhibition of the smooth muscle of the taenia coli. *Nature,* 1963; 200: 581–582.
4. Burnstock G. – Evolution of the autonomic innervation of visceral and cardiovascular systems in vertebrates. *Pharmacol Rev,* 1969; 21: 247–324.
5. Richardson J, Beland J. – Non-adrenergic inhibitory nervous system in human airways. *J Appl Physiol,* 1976; 41: 764–771.
6. Editorial. – NANC nerves in asthma. *Lancet,* 1986; ii: 1253–1254.
7. Barnes PJ. – Neurogenic inflammation in airway and its modulation. *Arch Intern Pharmacodyn,* 1990; 303: 67–82.

8. McDonald DM. – Neurogenic inflammation in the respiratory tract: actions of sensory nerve mediators on blood vessels and epithelium of the airway mucosa. *Am Rev Respir Dis,* 1987; 136: S65 –S71.
9. Barnes PJ, Lundberg JM. – Airway neuropeptides and asthma. *In*: Asthma and airway hyperresponsiveness: pathophysiology and treatment. C. Persson, P.J. Barnes, M. Kaliner eds, Marcel Dekker, New York, 1991; pp. 385–407.
10. Taylor SM, Pare PD, Schellenberg RR. – Cholinergic and nonadrenergic mechanisms in human and guinea-pig airways. *J Apply Physiol: Respirat Environ Exercise Physiol,* 1984; 56: 958–965.
11. Davis C, Kannan MS, Jones TR, Daniel EE. – Control of human airway smooth muscle. *in vitro* studies. *J Appl Physiol: Respirat Environ Exercise Physiol,* 1982; 53: 1080–1087.
12. De Jongste JC, Mons H, Bonta IL, Kerrebijn KF. – Non-neural components in the response of fresh human airways to electric field stimulation. *J Appl Physiol,* 1987; 63: 1558–1566.
13. Altiere RJ, Szarek JL, Diamond L. – Neurally mediated nonadrenergic relaxation in cat airway occurs independent of cholinergic mechanisms. *J Pharmacol Exp Ther,* 1985; 234: 590–597.
14. Partanen M, Laitinen A, Hervonen A, Toivanen M, Laitinen LA. – Catecholamine and acetylcholinesterase containing nerves in human lower respiratory tract. *Histochemistry,* 1982; 76: 175–188.
15. Barnes PJ. – Neural control of human airways in health and disease. *Am Rev Respir Dis,* 1986; 136: 1289–1314.
16. Richardson JB. – Nerve supply to the lung. *Am Rev Respir Dis,* 1979; 119: 785–802.
17. Coburn RF, Tomita T. – Evidence for nonadrenergic inhibitory nerves in guinea-pig trachealis muscle. *Am J Physiol,* 1973; 224: 1072–1080.
18. Rhoden KJ, Barnes PJ. – Epithelial modulation of NANC and VIP-induced responses: role of neutral endopeptidase. *Eur J Pharmacol,* 1989; 171: 247–250.
19. Matsumoto N, Inoue H, Ichinose M, Ishii M, Inoue C, Sasaki H, Takishima T. – Effective sites by sympathetic beta-adrenergic and vagal nonadrenergic inhibitory stimulation in constricted airways. *Am Rev Respir Dis,* 1985; 132: 1113–1117.
20. Irvin CG, Boileau R, Tremblay J, Martin RR, Macklem PT. – Bronchodilatation: noncholinergic, nonadrenergic mediation demonstrated *in vivo* in the cat. *Science,* 1980; 207: 791–792.
21. Diamond L, O'Donnell M. – A nonadrenergic vagal inhibitory pathway to feline airway. *Science,* 1980; 208: 185–188.
22. Irvin CG, Martin RR, Macklem PT. – Nonpurinergic nature and efficacy of nonadrenergic bronchodilatation. *J Appl Physiol: Respirat Environ Exercise Physiol,* 1982; 52: 562–569.
23. Inoue H, Ichinose M, Miura M, Katsumata U, Takishima T. – Sensory receptors and reflex pathways of non-adrenergic inhibitory nervous system in feline airways. *Am Rev Respir Dis,* 1989; 139: 1175–1178.
24. Szarek JL, Gillespie MN, Altiere RJ, Diamond L. – Reflex activation of the nonadrenergic, noncholinergic inhibitory nervous system in feline airway. *Am Rev Respir Dis,* 1986; 133: 1159–1162.
25. Palmer JBD, Cuss FMC, Barnes PJ. – VIP and PHM and their role in nonadrenergic inhibitory responses in isolated human airway. *J Appl Physiol,* 1986; 61: 1322–1328.
26. Ichinose M, Inoue H, Miura M, Takishima T. – Nonadrenergic bronchodilation in normal subjects. *Am Rev Respir Dis,* 1988; 138: 31–34.
27. Lammers J-WJ, Minette P, McCusker M, Chung KF, Barnes PJ. – Nonadrenergic bronchodilator mechanism in normal human subjects *in vivo. J Appl Physiol,* 1988; 64: 1817–1822.
28. Michoud M-C, Amyot R, Jeanneret-Grosjean A, Couture J. – Reflex decrease of histamine-induced bronchoconstriction after laryngeal stimulation in humans. *Am Rev Respir Dis,* 1987; 136: 616–622.
29. Fuller RW, Dixon CMS, Barnes PJ. – The bronchoconstrictor response to inhaled capsaicin in humans. *J Appl Physiol,* 1985; 85: 1080–1084.
30. Burnstock G. – Purinergic nerves. *Pharmacol Rev,* 1972; 24: 509–581.
31. Souhrada JF, Melzer E, Grantham P. – Some characteristics of the purinergic nervous system in normal and sensitized airway smooth muscle. *Respir Physiol,* 1980; 40: 199–209.
32. Satchell D. – Nonadrenergic, noncholinergic nerves in mammalian airway: their function and the role of purines. *Comp Biochem Physiol,* 1982; 72: 189–196.
33. Karlsson JA, Persson CGA. – Neither vasoactive intestinal peptide (VP) nor purine derivatives may mediate nonadrenergic tracheal inhibition. *Acta Physiol Scand,* 1984; 122: 589–598.
34. Ito Y, Takeda K. – Nonadrenergic inhibitory nerves and putative transmitters in the smooth muscle of cat trachea. *J Physiol (Lond),* 1982; 330: 497–511.
35. Joos GFP, Pauwels RAR, van der Straeten MERP. – The role of neuropeptides as neurotransmitters of nonadrenergic, noncholinergic nerves in bronchial asthma. *Bull Eur Physiopathol Respir,* 1988; 23: 619–637.
36. Venugopalan CS. – Vasoactive intestinal peptide (VIP), a putative neurotransmitter of nonadrenergic, noncholinergic (NANC) inhibitory innervation and its relevance to therapy. *J Vet Pharmacol Ther,* 1989; 12: 113–123.
37. Matsuzaki Y, Hamasaki Y, Said SI. – Vasoactive intestinal peptide: a possible transmitter of nonadrenergic relaxation of guinea-pig airways. *Science,* 1980; 210: 1252–1253.
38. Said SI. – Vasoactive peptides in the lung, with special reference to vasoactive intestinal peptide. *Exp Lung Res,* 1982; 3: 343–348.
39. Uddman R, Sundler F. – Vasoactive intestinal polypeptide nerves in human upper respiratory tract. *Otorhinolaryngology,* 1979; 41: 221–226.
40. Dey RD, Shannon WA, Said SI. – Localization of VIP-immunoreactive nerves in airway and pulmonary vessels of dogs, cats and human subjects. *Cell Tissue Res,* 1981; 220: 231–238.
41. Laitinen A, Partanen M, Hervonen A, Pelto-Huikko M, Laitinen LA. – VIP-like immunoreactive nerves in human respiratory tract. Light and electron microscopic study. *Histochemistry,* 1985; 82: 313–319.
42. Altiere RJ, Diamond L. – Comparison of vasoactive intestinal peptide and isoproterenol relaxant effects in isolated cat airway. *J Appl Physiol: Respirat Environ Exercise Physiol,* 1984; 56: 986–992.
43. Lundberg JM, Fahrenberg J, Hokfelt T. – Coexistence of peptide HI (PHI) and VIP in nerves regulating blood flow and bronchial smooth muscle in various animals, including man. *Peptides,* 1984; 5: 593–606.
44. Linder S, Barkhem T, Norberg A. – Structure and expression of the gene encoding the vasoactive intestinal peptide precursor. *Proc Natl Acad Sci USA,* 1987; 84: 605–609.

45. Yiangou Y, Di Marzo V, Spokes RA, Panico M, Morris HR, Bloom JR. – Isolation, characterization and pharmocological actions of peptide histidine valine 42, a novel prepro-vasoactive intestinal peptide-derived peptide. *J Biol Chem,* 1987; 262: 14010–14013.
46. Foda HD, Higuchi J, Said SI. – Heliodermin, a VIP-like peptide, is a potent and long-acting pulmonary vasodilator. *Am Rev Respir Dis,* 1990; 141: A486.
47. Barnes PJ, Dixon CMS. – The effect of inhaled vasoactive intestinal peptide on bronchial hyperreactivity in man. *Am Rev Respir Dis,* 1984; 130: 162–166.
48. Palmer JBD, Cuss FMC, Warren JB, Blank M, Bloom SR, Barnes PJ. – Effect of infused vasoactive intestinal peptide on airway function in normal subjects. *Thorax,* 1986; 41: 663–666.
49. Chilvers ER, Dixon CMS, Yiangou Y, Bloom SR, Ind PW. – Effect of peptide histidine valine on cardiovascular and respiratory function in normal subjects. *Thorax,* 1988; 43: 750–755.
50. Thompson DC, Altiere RJ, Diamond L. – The effects of antagonists of vasoactive intestinal peptide on nonadrenergic, noncholinergic inhibitory resposes in feline airways. *Peptides,* 1988; 9: 443–447.
51. Ellis JL, Farmer SG. – The effects of vasoactive intestinal peptide (VIP) antagonists, and VIP and peptide histidine isoleucine antisera on nonadrenergic, noncholinergic relaxations of tracheal smooth muscle. *Br J Pharmacol,* 1989; 96: 513–520.
52. Ellis JL, Framer SG. – Effects of peptidases on nonadrenergic, noncholinergic inhibitory responses of tracheal smooth muscle; a comparison with effects on VIP- and PHI-induced relaxation. *Br J Pharmacol,* 1989; 96: 521–526.
53. Tam EK, Franconi GM, Nadel JA, Caughey GH. – Protease inhibitors potentiate smooth muscle relaxation induced by vasoactive intestinal peptide in isolated human bronchi. *Am J Respir Cell Mol Biol,* 1990; 2: 449–452.
54. Rhoden K, Barnes PJ. – Potentiation of nonadrenergic neural relaxation in guinea-pig airway by a cAMP phosphodiesterase inhibitor. *J Pharmacol Exp Ther,* 1990; 282: 396–402.
55. Carstairs JR, Barnes PJ. – Visualization of vasoactive intestinal peptide receptors in human and guinea-pig lung. *J Pharmacol Exp Ther,* 1986; 239: 249–255.
56. Tucker JF, Brave SR, Charalambous L, Hobbs AJ, Gibson A. L-N^{G}-nitroarginine inhibits nonadrenergic, noncholinergic relaxations of guinea-pig isolated tracheal smooth muscle. *Br J Pharmacol,* 1990; 100: 663–664.
57. Belvisi MG, Stretton D, Verleden GM, Yacoub MH, Barnes PJ. – Inhibitory NANC nerves in human tracheal smooth muscle: involvement of VIP and NO. *Am Rev Respir Dis,* 1991; 143: A355.
58. Barnes PJ. – The third nervous system in the lung: physiology and clinical perspectives. *Thorax,* 1984; 39: 561–567.
59. Taylor SM, Pare PD, Armour CL, Hogg JC, Schellenberg RR. – Airway reactivity in chronic obstructive pulmonary disease. Failure of *in vivo* methacholine responsiveness to correlate with cholinergic, adrenergic, or nonadrenergic responses *in vitro. Am Rev Respir Dis,* 1985; 132: 30–35.
60. Adcock JM, Belvisi MG, Stretton D, Yacoub M, Barnes PJ. – Inhibitory NANC responses and VIP mRNA levels in asthmatic and denervated human lung. *Am Rev Respir Dis,* 1991; 143: A355.
61. Bai TR. – Abnormalities in airway smooth muscle in fatal asthma. *Am Rev Respir Dis,* 1990; 141: 552–557.
62. Lammers J-WJ, Minette P, McCusker M, Chung KF, Barnes PJ. – Capsaicin-induced bronchodilatation in mild asthmatic subjects: possible role of nonadrenergic inhibitory system. *J Appl Physiol,* 1989; 66: 856–861.
63. Michoud M-C, Jeanneret-Grosjean A, Cohen A, Amyot R. – Reflex decrease of histamine-induced bronchoconstriction after laryngeal stimulation in asthmatic patients. *Am Rev Respir Dis,* 1988; 138: 1548–1552.
64. McNeill RS. – Effect of a β-adrenergic blocking agent, propranolol, on asthmatics. *Lancet,* 1964; ii: 1101–1102.
65. Ollerenshaw S, Jarvis D, Woolcock A, Sullivan C, Scheibner T. – Absence of immunoreactive vasoactive intestinal polypeptide in tissue from the lungs of patients with asthma. *N Engl J Med,* 1989; 320: 1244–1248.
66. Matran R, Alving K, Martling CR, Lacroix JS, Lundberg JM. – Vagally mediated vasodilation by motor and sensory nerves in the tracheal and bronchial circulation of the pig. *Acta Physiol Scand,* 1989; 135: 29–37.
67. Howarth PH, Britten KM, Djukanovic RJ, Wilson JW, Holgate ST, Springall DR, Polak JM. – Neuropeptide containing nerves in human airways *in vivo*: a comparative study of atopic asthma, atopic non-asthma, and non-atopic non-asthma. *Thorax,* 1990; 45: 786–787, (Abstract).
68. Miura M, Inoue H, Ichinose M, Kimura K, Katsumata U, Takishima T. – Effect of nonadrenergic, noncholinergic inhibitory nerve stimulation on the allergic reaction in cat airways. *Am Rev Respir Dis,* 1990; 141: 29–32.
69. Miura M, Kimura K, Takahashi T, Inoue H, Takishima T. – Possible mechanisms of the antigen-induced dysfunction of nonadrenergic, noncholinergic inhibitory nervous system. *Am Rev Respir Dis,* 1990; 141: A387.
70. Unden BJ, Dick EC, Buckner CK. – Inhibition by vasoactive intestinal peptide of antigen-induced histamine release from guinea-pig minced lung. *Eur J Pharmacol,* 1983; 88: 247–250.
71. Tam EK, Caughey GH. – Degradation of airway neuropeptides by human lung tryptase. *Am J Respir Cell Mol Biol,* 1990; 3: 27–32.
72. Kakuta Y, Stead RH, Perdue MH, Marshall JS, Bienenstock J. – Micro-anatomical relationship of mast cells and nerves in rat lung and trachea. *Am Rev Respir Dis,* 1989; 139: 118A, (Abstract).
73. Sekizawa K, Caughey GH, Lazarus SC, Gold WM, Nadel JA. – Mast cell tryptase causes airway smooth muscle hyperresponsiveness in dogs. *J Clin Invest,* 1989; 83: 175–179.
74. Laitinen LA, Elkins RB, Empey DW, Jacobs L, Mills J, Nadel JA. – Bronchial hyperresponsivenesses in normal subjects during attenuated influenza virus infection. *Am Rev Respir Dis,* 1991; 143: 358–361.
75. Seltzer J, Bigby BG, Stulbarg M, Holtzman MJ, Nadel JA, Ueki IF, Leikauf GD, Goetzl EJ, Boushey HA. – O_3-induced changes in bronchial reactivity to methacholine and airway inflammation in humans. *J Appl Physiol,* 1986; 60: 1321–1326.
76. Martling C-R, Angaard P, Lundberg JM. – Noncholinergic vasodilatation in the tracheobronchial tree of the cat induced by vagal nerve stimulation. *Acta Physiol Scand,* 1985; 125: 343–346.
77. Kubota E, Hamasaki Y, Sata T, Said SI. – Autonomic innervation of pulmonary artery: evidence for a nonadrenergic, noncholinergic inhibitory system. *Exp Lung Res,* 1988; 14: 349–358.
78. Altiere RJ, Diamond L. – Relaxant effects of vasoactive intestinal peptide and betanechol in cat intrapulmonary artery. *Eur J Pharmacol,* 1983; 93: 121–124.

79. Greenberg B, Rhoden K, Barnes PJ. – Relaxant effects of vasoactive intestinal peptide and peptide histidine isoleucine in human and bovine pulmonary arteries. *Blood Vessels,* 1987; 24: 45–50.
80. Laitinen LA, Laitinen A, Salonen RO, Widdicombe JG. – Vascular actions of airway neuropeptides. *Am Rev Respir Dis,* 1987; 136: 559–564.
81. Peatfield AC, Barnes PJ, Bratcher C, Nadel JA, Davis B. – Vasoactive intestinal peptide stimulates tracheal submucosal gland secretion in ferret. *Am Rev Respir Dis,* 1983; 128: 89–93.
82. Peatfield AC, Richardson PS. – Evidence for noncholinergic, nonadrenergic nervous control of mucus secretion into the cat trachea. *J Physiol Lond,* 1983; 342: 335–345.
83. Coles SJ, Said SI, Reid LM. – Inhibition by vasoactive intestinal peptide of glycoconjugate and lysozyme secretion by human airways *in vitro. Am Rev Respir Dis,* 1981; 124: 531–536.
84. Nathanson I, Widdicombe JH, Barnes PJ. – Effect of vasoactive intestinal peptide on ion transport across dog tracheal epithelium. *J Appl Physiol: Respirat Environ Exercise Physiol,* 1983; 55: 1844–1848.
85. Barnes PJ. – Nonadrenergic, noncholinergic neural control of human airways. *Arch Intern Pharmacodyn,* 1986; 280: 208–228.