Nonadrenergic, noncholinergic airway inhibitory nerves

J-W.J. Lammers*, P.J. Barnes**, K.F. Chung**


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 vagal intestinal 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.

Eur Respir J., 1992, 5, 239-246.

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-adrenoceptor blockers [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-adrenoceptor 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

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.
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.

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) 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.

![Graphs](image-url)
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 F2α (PGF2α)-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, quindine, 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, 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 demonstrable 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^3-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 post-junctional) 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 cotransmitter in cholinergic nerves, may act as a brake in inhibiting the bronchoconstrictor effects of acetylcholine release at the same effector 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 may be 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.

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 trypsin 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 trypsin isolated from canine mastocytoma cells markedly increases their contractile response to spasmodogens, 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

Neurogenic inflammation in the Cholinergic-VIP Coexistence


