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COMMENTARY

Neural Mechanisms and Axon Reflexes in Asthma

WHERE ARE WE?

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ABSTRACT. For many years, asthma has been classified as a "neural" disease, with an imbalance between constrictor and dilator nerves being responsible for the symptomatology. Although, nowadays, asthma is recognized as an inflammatory disorder of the airways, neural mechanisms remain very important; axon reflexes, in particular, have received a lot of attention in recent years. In this commentary, an overview is given on the innervation of the airways and its relevance in asthma, and potential new insights in airway innervation are discussed. In a second part, the role of axon reflexes is highlighted. Although neuropeptides such as substance P and neurokinin A are present in human airways, where they produce many of the features characteristic of asthma, and although there is an elevation of their content in induced sputum from asthmatics, there is still no clear direct evidence for the existence of operational axon reflexes in human airways. Most of the research focused on this subject is performed in guinea pigs, where such an axon reflex clearly operates in the airways. In these animals, different receptors have been identified on C-fiber endings, which, upon stimulation, cause inhibition of neuropeptide release. Some of these receptors have also been identified on human airway nerves. Therefore, it has been suggested that modulation of axon reflexes could be of potential benefit in asthma treatment. Indeed, some drugs (e.g. sodium cromoglycate, nedocromil sodium, and ketotifen), which have been demonstrated to partially inhibit neuropeptide release in guinea pig airways, have anti-inflammatory effects in asthma. Other drugs, however, such as β_2-mimetics, which have a much more pronounced inhibitory effect on neuropeptide release in guinea pig airways, do not seem to have any anti-inflammatory effects in human asthma. In conclusion, although there is a lot of indirect evidence for the existence of axon reflex mechanisms in human airways, most of the data now available are derived from animal studies. The key question of whether axon reflexes are operational in human airways remains unanswered. Hopefully, the near future will bring a solution to this enigma with the introduction of very potent tachykinin antagonists for the treatment of human asthma. BIOCHEM PHARMACOL 51;10:1247–1257, 1996.

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For a long time it has been thought that an imbalance between constrictor and dilator nerves could explain the symptomatology of asthma. However, nowadays it is accepted that asthma is pathologically characterized by a more or less intense inflammation of the airways, which mainly consists of activated eosinophils, T-lymphocytes and mast cells [1–3]. The intensity of this airway inflammation, as assessed with bronchoalveolar lavage and bronchial biopsies, is grossly correlated with the degree of airway hyperresponsiveness that is present in almost every asthmatic patient [4–7]. Furthermore, it is accepted now that chronic airway inflammation results in epithelial damage, airway smooth muscle hypertrophy and hyperplasia [8], and subepithelial fibrosis [9], resulting in airway wall remodelling [10]. All these structural changes probably represent reparative mechanisms in order to restore the epithelial damage in asthmatic airways [11, 12]. As a consequence, epithelial damage in asthmatic airways is one of the key pathological findings [13], although its exact cause is still speculative: it could be due to edema fluid [11], which is supported by the finding of vascular endothelial gaps [14]. Others have found a correlation between increased eosinophilic infiltration and increased intracellular spaces of bronchial epithelium [15], suggesting a causative role for the eosinophils, and, indeed, a high concentration of major basic protein, a cytotoxic protein released by eosinophils [16], has been detected in the sputum of asthmatics [17]; it has also been localized on damaged bronchial epithelium from patients dying of asthma [18]. In fact, major basic protein could be responsible, at least in part, for the epithelial damage that results in loss of various regulatory functions [19] and in intraluminal exposition of sensory nerve fibers [20], which may lead to activation of local axon reflexes to amplify and spread airway inflammation [21]. As a consequence, modulation of axon reflex mechanisms and,

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therefore, modulation of neurogenic inflammation in the airways is a potential target for asthma treatment [22].

AIRWAY INNERVATION

Guinea pigs are frequently employed as a model for the study of axon reflexes and neurogenic inflammation in the airways. Studies with this model have identified contractile cholinergic and noncholinergic innervation as well as adrenergic and nonadrenergic relaxant innervation [23].

Cholinergic Innervation

Mammalian airways receive a rich cholinergic innervation consisting of afferent and efferent nerve fibers [24]. Cholinergic innervation has been studied extensively in guinea pigs and others animals [23, 25]. However, as there exists considerable variation between different species, some caution must be made when extrapolating these data to humans [26]. The views on the innervation of the human lung tissue are built for the most part on studies based on nonspecific histological staining procedures, although recent studies have used newer techniques such as immunochemistry and electron microscopy [27, 28].

EFFERENT NERVE FIBERS. Cholinergic efferent nerves arise in the vagal nuclei of the brainstem and pass down the vagus nerve to synapse in ganglia that are situated in the airway wall. These fibers are called preganglionic fibers. From these ganglia, relatively short postganglionic fibers pass to target cells, such as smooth muscle, blood vessels, and glands. Using staining for AchE,† AchE-containing nerve fibers supplying the smooth muscle and bronchial glands were demonstrated in different mammalian species, including humans [29]. In humans, the AchE-positive fibers supplying the smooth muscle were found from secondary bronchi to terminal bronchioli. These data are supported further by electron microscopy studies. If small agranular vesicles contain acetylcholine, then the majority of motor nerves in the airways are cholinergic [28]. Stimulation of the parasympathetic nerves causes constriction from the trachea to the small airways, although bronchoconstriction is most prominent in airways with a resting diameter of 1–5 mm and is less significant in airways smaller than 0.5 mm in diameter [30].

EFS of isolated airway preparations from humans [31] or guinea pigs [23] causes a contraction that can be prevented by tetrodotoxin, indicating that nerves are responsible for this response, or by atropine, indicating release of acetylcholine from cholinergic nerve terminals. Acetylcholine released from postganglionic nerve terminals activates muscarinic cholinergic receptors on the target cells of the airways. Direct receptor binding studies have demonstrated a high density of muscarinic receptors in smooth muscle of large airways, with a decreasing density in smaller airways, so that terminal bronchioles are almost devoid of muscarinic receptors [32]. Muscarinic receptors are also found on submucosal glands and airway epithelium [33]. Muscarinic receptor subtypes have been differentiated recently by the development of selective muscarinic antagonists and by the use of specific complementary deoxyribonucleic acid probes [34]. Five distinct human muscarinic receptor genes have been identified thus far, although only three seem to be relevant in human airways [34]. Muscarinic M_1-receptors are usually found in neuronal tissue, and there is evidence that they are localized to parasympathetic ganglia, where they may have a facilitatory effect on neurotransmission. This has been demonstrated in rabbit airways [35] and has been suggested to be present in human airways [36]. M_2 muscarinic receptor density is rather low in the human lung, although these receptors may have a very important role in the regulation of the cholinergic neurotransmission [37]. In several species, including guinea pig and human, prejunctional M_2-receptors seem to be present on postganglionic airway cholinergic nerves. In human airways *in vitro*, stimulation of these M_2-receptors inhibits cholinergic nerve-induced contraction of the smooth muscle [38]. This has also been confirmed in human non-asthmatics *in vivo*. In asthmatic patients, however, this M_2-receptor seems to be dysfunctional [39, 40]. M_3-receptors are present in human airway smooth muscle of large and small airways. Activation of these receptors results in smooth muscle contraction. M_3-receptors are also present on submucosal glands in human airways, and their activation results in mucus secretion [41]. These receptors have also been localized on human airway epithelial cells, although their function is not quite clear at the moment [42].

AFFERENT NERVE FIBERS. Afferent or sensory nerve fibers from the airways terminate in the vagal nuclei, and the nerve cell bodies are localized in the nodose ganglia [26]. Three different types of afferent nerve fibers have been recognized from physiologic studies [43], their role being to send sensory information up the vagus nerve.

(1) Slowly adapting (stretch) receptors. These are myelinated nerve terminals localized to smooth muscle of conducting airways [44]. These nerves are responsible for the Hering–Breuer reflex, which inhibits sustained inspiratory activity. They also lead to a reflex bronchodilation by inhibiting vagal tone [45]. In humans, however, slowly adapting receptors are uncommon, which may be related to the fact that in humans the Hering–Breuer reflex is weak [28].

(2) Rapidly adapting (irritant) receptors. These are also myelinated nerve terminals, which are anatomically situated below the epithelium and between epithelial cells [45]. Rapidly adapting receptors in the larynx and the trachea

† *Abbreviations:* AchE, acetylcholinesterase; EFS, electrical field stimulation; SP, substance P; NK, neurokinin; CGRP, calcitonin gene-related peptide; NANC, nonadrenergic, noncholinergic; iNANC, nonadrenergic inhibitory; VIP, vasoactive intestinal peptide; NO, nitric oxide; eNANC, noncholinergic excitatory; BK, bradykinin; NEP, neutral endopeptidase; and ACE, angiotensin converting enzyme.

are very sensitive to particulate irritants (cigarette smoke, dust), rather than to chemicals and, therefore, have been termed cough receptors [46]. Intrapulmonary irritant receptors are stimulated by gases such as ammonia, sulfur dioxide, and ozone and also by histamine, serotonin, and prostaglandin $F_{2\alpha}$ [45, 47]. Stimulation of these irritant receptors causes bronchoconstriction by a reflex increase in vagal efferent activity [45] and rapid shallow breathing, which is often seen in patients with chronic obstructive pulmonary disease, suggesting that in these patients there is an increased activity of irritant receptors [48].

(3) C-fiber endings. These are non-myelinated nerve endings, usually found within airway epithelium, also in humans [20]. These nerve endings are stimulated by capsaicin (the hot extract of pepper), bradykinin, prostaglandins, and sulfur dioxide [49]. Stimulation of C-fiber endings produces reflex bronchoconstriction, increased microvascular permeability and edema formation, and an increase in mucus secretion [21]. All these effects are due presumably to the release of neuropeptides (SP, NK A, and CGRP) from C-fiber endings [50, 51]. The role of C-fiber endings in local axon reflexes will be discussed further under noncholinergic innervation.

Adrenergic Innervation and Catecholamines

Adrenergic control of airways consists of sympathetic nerves, which release norepinephrine, and circulating catecholamines (in humans predominantly epinephrine), released from the adrenal medulla.

SYMPATHETIC INNERVATION. Preganglionic nerve fibers originate from the upper six thoracic segments of the spinal cord and synapse in the middle and inferior cervical ganglia and the upper four thoracic prevertebral ganglia. Postganglionic fibers run from these ganglia, entering the lung at the hilum to intermingle with the cholinergic nerves that form a dense plexus around airways and vessels [45]. Although it is evident that adrenergic nerves are present in the smooth muscle layer of human airways, they are fewer in number than AchE-containing nerve fibers [29]. While sympathetic nerves supply pulmonary and bronchial blood vessels, submucosal glands, and ganglia, there are few adrenergic nerves in airway smooth muscle [24]. There is, however, considerable variation between different species: airways of cats have a more pronounced sympathetic innervation, for instance, than rabbits and rats [52]. EFS of isolated human trachea induces a contraction, abolished by atropine, followed by a relaxation, which is not prevented by propranolol, suggesting that in human airways this relaxation response is not due to adrenergic innervation [31]. In other species such as guinea pigs [23], dogs [53], and cats [54], a functional adrenergic innervation exists, since propranolol can partially block the EFS-induced relaxation of the airways. Although there appears to be no functional adrenergic innervation in human airway smooth muscle, it is possible that adrenergic nerves may influence bronchomotor tone indirectly by modulating the cholinergic and ganglionic neurotransmission [26] and by acting on bronchial vessels and mucus glands [55].

CIRCULATING CATECHOLAMINES. Although human airway smooth muscle lacks a functional sympathetic innervation, there are a large number of β-adrenergic receptors on the smooth muscle cells of large and small airways. β-Agonists are known as very potent airway smooth muscle relaxants [56]. This suggests that circulating catecholamines may be important in regulating human airway tone. Norepinephrine in plasma is derived almost entirely from overspill of sympathetic nerve activity [57], although it has no significant effect on airway function when infused at physiologic concentrations in humans [58]. Epinephrine, on the other hand, is capable of causing bronchodilation in both normal and asthmatic subjects and of antagonizing the effect of inhaled histamine [59].

NANC Airway Innervation

In addition to the classical cholinergic and adrenergic pathways, neural mechanisms that are neither cholinergic nor adrenergic have been described [24, 60]. This NANC neural mechanism has already been studied extensively in different systems: the gastrointestinal tract, the urogenital tract, the eye, the cardiovascular system, and the lung. The role of these nerves in controlling airway function is still under debate, although it was established recently that NANC responses can stabilize smooth muscle tone in guinea pig isolated airways [61, 62]. The exact neurotransmitters of this nervous system remain undefined, although the recent discovery of numerous peptides, found to be present in peripheral nerves and to have multiple pharmacologic effects that can mimic NANC nerve stimulation, points to neuropeptides as possible neurotransmitters of the NANC (peptidergic) nervous system [63]. Several different regulatory peptides have now been localized to nerves in the airways of different species, including humans [64].

iNANC NERVES. iNANC nerves produce airway relaxation and were first described in the guinea pig trachea [65]. From then on, nonadrenergic bronchodilatation was reported to be present in several species, including humans [31]. In the absence of a functional adrenergic innervation, this iNANC system is the only dilator neural pathway in human airways, which suggests that it might be of particular importance [66]. Although the stimulus for this nervous system is not quite clear, it has been demonstrated that mechanical irritation of the larynx produces a nonadrenergic bronchodilation in feline [67] and in human airways [68]. It was further demonstrated that capsaicin evokes a nonadrenergic inhibitory system reflex in feline [69] and in human airways [70], suggesting that C-fiber receptors may be involved in the reflex pathway for nonadrenergic bronchodilation [71]. iNANC nerves also regulate the secretion of airway mucus in animals [72]. There has been a lot of debate on the possible nature of the neurotransmitter for

the iNANC nervous system, with purines being the first candidates [73]. There was, however, a lot of evidence that argued against purines as neurotransmitters of the iNANC system. As a consequence, peptides, such as VIP and peptide histidine isoleucine have been forwarded as neurotransmitter candidates. Indeed, VIP has been localized in both animal and human lungs to neurones and nerve terminals in airway smooth muscle, around submucosal glands, and in bronchial and pulmonary vessels [74]. VIP is a very potent relaxant of human bronchial smooth muscle *in vitro;* however, *in vivo,* VIP has either a weak [75] or no bronchodilating effect in human subjects, and it has only a weak protective effect against histamine-induced bronchoconstriction [76]. VIP-immunoreactive nerves are often distributed with cholinergic nerves, and ultrastructural studies suggest that VIP may co-exist in the same nerve terminals as acetylcholine [45]. Therefore, it has been assumed that VIP is co-transmitted with acetylcholine, acting as a functional brake to cholinergic bronchoconstriction [45]. Recently, it was hypothesized that the guinea pig trachealis receives excitatory and inhibitory innervation from distinct vagal parasympathetic pathways, and that neurons mediating nonadrenergic relaxation are associated with the esophagus, prior to innervating the trachealis [77]; therefore, co-release from the same nerve terminals seems unlikely. This was further supported by a study from our laboratory in which it was demonstrated that opioids modulate the cholinergic contraction in guinea pig trachea, but not the iNANC relaxation [78]. On the other hand, VIP modulates cholinergic [79, 80] as well as noncholinergic contractions [79] in guinea pig airways *in vitro.*

In a lot of species, NO has now been implicated as a potential neurotransmitter in nonadrenergic relaxation. In feline trachealis muscle, NO is the primary mediator of iNANC relaxation [81]. Also, in human airways, NO seems to be the only mediator of the iNANC relaxation [82–84]. In rat anococcygeus muscle [85], in canine gut [86], and in guinea pig airways [87], NO has been demonstrated to be at least partially responsible for the iNANC relaxation. There is convincing evidence now that NO can be released from nerves themselves, since a particular form of NO-synthase (a constitutive, cytosolic calcium-dependent enzyme) has been localized to peripheral nerves [88]. Endogenous NO appears to modulate cholinergic neurotransmission in both guinea pig [80] and human [89] airways, but whether NO is released from cholinergic nerves in the airways is not yet certain [90].

eNANC NERVES. eNANC nerves have been demonstrated in guinea pig airways both *in vivo* and *in vitro.* In the presence of atropine, EFS of guinea pig bronchi [23] and lower trachea [91, 92] produces a long-lasting contraction. This contraction is due to the release of neuropeptides such as SP, NK A, and CGRP from airway sensory nerves, since pretreatment of the animal with capsaicin, a neurotoxin that damages unmyelinated C fibers, abolishes this contractile response [92, 93] and since an SP antagonist inhibits this contraction [94, 95]. Stimulation of C-fiber afferents also produces vasodilatation and local edema, due to an increase in vascular permeability to plasma proteins. This has been studied extensively in the respiratory tract of guinea pigs. This response is known as neurogenic inflammation and is assumed to be due to release of SP and related peptides from the peripheral endings of C-fibers [50, 51]. Therefore, SP and related tachykinins (NK A and CGRP) are considered nowadays as the neurotransmitters of the eNANC nervous system. SP is localized to sensory nerves in the airways of several species, including humans [96]. SP immunoreactive nerves in the airways are found beneath and within the airway epithelium, around blood vessels and in airway smooth muscle. Compared with rodents the nerve fibers containing SP are less dense in human airway tissue. Studies on human airways, however, suggested an increased amount of SP in asthmatic versus normal subjects [97]. SP-immunoreactive nerve fibers also innervate parasympathetic ganglia, and they modulate both cholinergic and iNANC neurotransmission in guinea pig airways [98, 99]. SP appears to be localized predominantly to capsaicin-sensitive unmyelinated nerves in the airways [100]. NK A immunoreactivity has also been demonstrated in human airways and appears to be co-localized with SP [101].

At the moment, three types of tachykinin receptors (i.e. NK-1, NK-2, and NK-3) are recognized, at which SP, NK A or NK B, respectively, are thought to be the most relevant natural agonist [102]. Recently, it was established that the bronchoconstrictor response to sensory nerve activation in guinea pig airways is influenced to only a small degree by CP96345 [103], a selective NK-1 receptor antagonist [104], although the same antagonist perfectly blocks the tracheal protein extravasation response to cigarette smoke inhalation, nerve stimulation, and capsaicin [105, 106]. FK 224, a dual antagonist at both NK-1 and NK-2 receptors [107], inhibits plasma extravasation as well as the noncholinergic contraction induced by vagus nerve stimulation [108], whereas FK 888, a highly selective antagonist at NK-1 receptors [109], only inhibits plasma extravasation in guinea pigs *in vivo* [108]. This suggests that in guinea pig airways, eNANC contraction is mediated predominantly by NK-2 receptors, whereas neurogenic inflammation is mediated by NK-1 receptors.

In human airways, such a noncholinergic response is not easily demonstrable [110]. Nevertheless, the existence of noncholinergic excitatory nerves in human airways has been argued by the findings that capsaicin induces contractions of isolated human bronchi [110] and that inhaled capsaicin causes cough and transient bronchoconstriction in both normal and asthmatic subjects [111, 112]. *In vitro,* SP contracts human airway smooth muscle, although NK A is more potent, indicating that an NK-2 receptor is likely to be involved [113–115]. *In vivo,* however, SP does not consistently cause bronchoconstriction in humans [116], whereas NK A causes bronchoconstriction after intravenous administration [117] and after inhalation in asthmatics [118–120] and in normal volunteers [121]. The bron-

choconstrictor effect of inhaled NK A in asthmatics is prevented by pretreatment with nedocromil sodium, suggesting that it is mediated indirectly [122]. Such an indirect effect of tachykinins has also been demonstrated in rabbit [115] and rat [123] airways. In the latter species, mast cells seem to be involved in the bronchoconstriction induced by tachykinins [124]. In humans, however, at the moment there is no valid explanation for this indirect effect, although nedocromil sodium has been demonstrated to modulate neuropeptide release from guinea pig sensory nerves by a prejunctional mechanism [125], probably by blocking sensory nerve activation through inhibition of a chloride channel [126]. An alternative explanation is that nedocromil sodium and sodium cromoglycate act as tachykinin antagonists [127], although a recent study could not validate this hypothesis in human airways [128].

SP also stimulates mucus secretion from submucosal glands of human airways, and it is more potent than NK A, indicating that NK-1 receptors are involved [129].

AXON REFLEXES

Tachykinins such as SP and NK A produce many of the features of asthma (bronchoconstriction, enhanced mucus secretion, microvascular leakage). Damage to the airway epithelium occurs in almost every asthmatic patient [11], exposing C-fiber endings that are readily stimulated by inflammatory mediators such as BK and prostaglandins. This stimulation of intraluminal C-fibers could result in a reflex bronchoconstriction, but also in a local axon reflex, with release of tachykinins from sensory nerve collaterals in the airways. The axon reflex might then further amplify the inflammatory response in the airways [21].

Evidence for the Existence of an Axon Reflex in Human Airways

SP and NK A are present in the human lung. SP nerve fibers have been demonstrated in human airways, but they are few in number compared with the nerve fibers containing VIP [130]. It has been demonstrated recently that there is an elevated SP content in induced sputum from patients with asthma, indicating that tachykinins may be involved in the airway inflammatory process in asthma [131]. Furthermore a significantly higher amount of SP was found in the bronchoalveolar lavage fluid of subjects with allergic asthma, compared with nonallergic patients [132]. This is in agreement with the study of Ollerenshaw *et al.* [97], in which it was demonstrated that both the number and the length of SP-immunoreactive nerve fibers are increased in the airways of asthmatics compared with normal subjects.

NEP, which is the major enzyme responsible for the degradation of airway tachykinins, is present in human airways. When NEP is inhibited pharmacologically in different species (with NEP inhibitors such as phosphoramidon or thiorphan) or by cigarette smoke, respiratory viral infections, or inhalation of the industrial pollutant toluene diisocyanate, neurogenic responses, which are believed to be due to the release of tachykinins from airway sensory nerves, are exaggerated [133]. Thiorphan also increases the airway response to inhaled NK A in normal subjects [121] as well as in asthmatics *in vivo* [119]. In humans, experimental virus infections *in vivo* produce an increase of airway responsiveness to various agents; however, the mechanism of this virus-induced airway hyperresponsiveness is not quite clear at the moment [134], although interference with the epithelium has been suggested [135], which could result in a decreased activity of NEP and an enhanced exposure of nerves, which are then readily stimulated by inflammatory mediators such as BK to sustain the inflammatory response. In guinea pig tracheal smooth muscle *in vitro*, BK-induced contractions were reduced significantly by tetrodotoxin, whereas atropine had no effect. On the other hand, after capsaicin pretreatment of the animals the BK-induced contraction of the trachealis was reduced significantly. This indicates that BK-induced contraction of the airway smooth muscle *in vitro* is mediated by tachykinins released from sensory nerves, presumably via an axon reflex [136]. It has been demonstrated previously that the BK-induced bronchoconstriction in guinea pigs *in vivo* is also mediated by neural mechanisms involving both cholinergic nerves and sensory neuropeptides [137]. In human airways *in vitro*, BK causes a contraction of intact and epithelium-denuded airways, although epithelium removal resulted in a 7-fold increased sensitivity to BK, whereas NEP inhibition only marginally increased the sensitivity to BK. Tetrodotoxin had no effect, suggesting that in human peripheral airways *in vitro* tachykinin release is not involved in the BK-induced contraction [138]. This was further confirmed in the guinea pig, where BK instillation into the airways significantly increased the leakage of dye in all airway segments, whereas FK888 (an NK-1 antagonist) partially inhibited this leakage but only in the trachea and in the main bronchi. In the more peripheral airways, tachykinins seemed not to be involved in the BK-induced increased leakage [139], which is in agreement with the studies on human airways [138]. In asthmatic patients, the bronchoconstrictor response to BK is reduced by pretreatment with anticholinergic drugs [140] and with sodium cromoglycate and nedocromil sodium, indicating that C-fiber activation is likely to be involved [141]. Indeed, BK stimulates bronchial C-fibers in dogs [142]. Sodium cromoglycate and nedocromil sodium also protect asthmatics against various challenges that are believed to involve sensory nerve activation, such as SO_2, metabisulphite and distilled water [143, 144].

If tachykinins are not involved in the inflammatory process in asthmatic airways, then an alternative hypothesis has been put forward, in which it is suggested that tachykinins are responsible for non-productive cough [145]. Indeed, it seems that SP, released from sensory nerves in the airways of guinea pigs, may be an endogenous substance causing cough, since intraperitoneal injection of phosphoramidon produces a cough response, while FK888 inhibits

this phosphoramidon-induced cough [146]. In humans, ACE inhibitors may produce cough, probably by accumulation of BK in the lung, since its breakdown is inhibited by ACE inhibitors [147]. Sodium cromoglycate was able to reduce ACE-inhibitor-induced cough [148], which is another indirect piece of evidence for a role of tachykinins in human airways.

It has been demonstrated recently that FK224 (an NK-1 and NK-2 receptor antagonist) is able to protect against BK-induced bronchoconstriction in asthmatics [149], which seemed to be the first proof of the possible existence of an axon reflex in human airways *in vivo*. On the other hand, FK224 does not protect against the NK A-induced bronchoconstriction in asthmatics, which casts some doubt on FK224 being an NK antagonist in human airways [150]. Since direct evidence of the existence of an axon reflex mechanism in human airways is still lacking, further studies with potent and selective tachykinin antagonists in asthmatic patients are awaited to define the exact role of tachykinins in asthma and to possibly establish the existence of an axon reflex in human airways.

Is Modulation of Axon Reflex Mechanisms Useful in the Treatment of Asthma?

Inhibition of tachykinin release from airway sensory nerves can be achieved via two different mechanisms: either inhibition of sensory nerve activation or activation of a prejunctional receptor, located on sensory nerves, that inhibits the release of tachykinins. In guinea pig airways, loop diuretics, such as furosemide and bumetanide, as well as nedocromil sodium and sodium cromoglycate have been demonstrated to inhibit tachykinin release from airway sensory nerves, probably through inhibition of sensory nerve activation [125, 151]. Loop diuretics also inhibit the EFS-induced cholinergic contraction [151] and the noncholinergic, nonadrenergic relaxation [152] in guinea pig airways. Furthermore, they also inhibit the EFS-induced cholinergic contraction in human epithelium denuded airways *in vitro* [153]. The exact molecular mechanism of this inhibitory effect is still unknown at the moment, although various hypotheses have been put forward [154].

Furosemide and nedocromil sodium also have an inhibitory effect against various indirect acting bronchoconstrictor challenges in humans [144, 154]. Nedocromil sodium, however, has a proven anti-inflammatory effect in human asthma [155], whereas loop diuretics have not [156].

Different prejunctional receptors have been localized to sensory nerve endings: BK B2 receptor, which enhances eNANC responses [157] and various receptors that inhibit tachykinin release: μ-opioid receptor [158], and α_2-adrenergic [159] and a β_2-adrenergic [160] receptor, a purinoceptor [161], a $GABA_B$ receptor [162], a histamine H_3 receptor [163], a 5-hydroxytryptamine$_1$-like receptor [92, 164], and so on. It was thought that all these receptors operated a common potassium channel, since cromakalim (a potassium channel activator) also inhibited this eNANC responses via a prejunctional mechanism [165]. Recently, further evidence in favor of a common inhibitory mechanism was published by Stretton *et al.* [166]. In this study, it was shown that charybdotoxin, a blocker of the large conductance Ca^{2+}-activated K^+-channels, completely reversed the inhibitory effects of a μ-opioid agonist of neuropeptide Y and of an α_2-adrenoceptor agonist on eNANC responses in guinea pig airways *in vitro*, although this was not confirmed in another study [167].

If the release of tachykinins from airway sensory nerves is important to sustain the inflammatory response in asthmatic airways [21], then inhibition of tachykinin release from these nerves may be an important mechanism to reduce this neurogenic compound of airway inflammation [22]. As a consequence, agents that interfere with sensory nerve neurotransmission may become anti-inflammatory agents in asthma treatment. This, however, seems not to be the case since only nedocromil sodium and sodium cromoglycate have a proven anti-inflammatory effect in asthma, while, for instance, β_2-adrenoceptor agonists, which also inhibit the release of tachykinins from airway sensory nerves [160], have no anti-inflammatory effect *in vivo*. On the other hand, ketotifen and epinastine, two antihistaminic drugs with an affinity for various other receptors, also inhibit tachykinin release in guinea pig airways [168, 169], while they both are of clinical benefit in human asthma [170, 171].

Therefore, one cannot assume that inhibition of tachykinin release *per se* is equivalent to therapeutic (i.e. anti-inflammatory) efficacy in asthma. This could be explained by an imbalance between pro-inflammatory and anti-inflammatory
tachykinins: if a drug inhibits tachykinin release from airway sensory nerves by 100% (for example, β_2-adrenoceptor agonists [160]), there seems to be no anti-inflammatory activity, although β_2-adrenoceptor agonists can inhibit neurally mediated airway microvascular leakage in guinea pigs *in vivo*, the mechanism of which is unexplained [172], although capsaicin-sensitive sensory nerves seem not to be involved [173]. On the other hand, if a drug only partially inhibits tachykinin release (for example nedocromil sodium, ketotifen, epinastine), then possibly there is some anti-inflammatory effect. There is some evidence in favor of this hypothesis. Sensory nerves release SP and NK A, but also CGRP. Topical application of capsaicin to the hamster cheek pouch causes *in vivo* release of CGRP, resulting in a marked vasodilation but no leakage of plasma [174]. Furthermore, topical pretreatment with low concentrations of CGRP markedly inhibits the plasma leakage evoked by histamine in the hamster cheek pouch [174]; local pretreatment with capsaicin also inhibits histamine-induced plasma leakage, indicating that endogenous CGRP may inhibit inflammatory plasma extravasation [174]. Although CGRP may also act pro-inflammatory by enhancing edema evoked by different inflammatory mediators in the rabbit skin [175], this contradicts with studies on human skin, where CGRP injected simultaneously with SP has no potentiating

effect on SP-induced edema [176]. As a consequence, it could be concluded that sensory nerve activation is clearly pro-inflammatory in some situations, whereas CGRP release from sensory nerves might also act as an endogenous anti-inflammatory mechanism. Therefore, inhibition of all tachykinins, including CGRP, released from airway sensory nerves, could fail to produce an anti-inflammatory effect. Additional studies, however, are necessary to elucidate the importance of a possible imbalance between pro-inflammatory and anti-inflammatory tachykinins.

CONCLUSION

Airway innervation in mammals remains a difficult entity, and it is widely accepted now that there is a very close interrelationship between the different neural mechanisms. One of the most important questions, however, remains to be answered: does an axon reflex mechanism operate in human asthma? At the moment, there is no convincing evidence that an axon reflex mechanism exists in human airways, because it is impossible to study this phenomenon directly in humans. Lots of indirect evidence is present: the bronchoconstrictor response to tachykinins and to capsaicin, which is exaggerated after pretreatment with NEP inhibitors, the increased amount of SP in the sputum of asthmatics, and the effectiveness of nedocromil sodium and sodium cromoglycate in inhibiting indirect acting bronchoconstrictor challenges, which are believed to operate via stimulation of C-fiber endings. Furthermore, it is not clear at the moment why some drugs that interfere with the neurotransmission of sensory nerves (e.g. nedocromil sodium and sodium cromoglycate) have potent anti-inflammatory effects, while other drugs that are much more potent at inhibiting tachykinin release (e.g. β_2-adrenoceptor agonists) have no proven anti-inflammatory effect *in vivo*. Hopefully, the solution to this interesting question will be provided in the near future, when potent and selective tachykinin antagonists will be available to treat human asthma.

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