Review

Novel concepts of neuropeptide-based drug therapy:
Vasoactive intestinal polypeptide and its receptors

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Abstract

Chronic inflammatory airway diseases such as bronchial asthma or chronic obstructive pulmonary disease (COPD) are major contributors to the global burden of disease. Although inflammatory cells play the central role in the pathogenesis of the diseases, recent observations indicate that also resident respiratory cells represent important targets for pulmonary drug development. Especially targeting airway neuromediators offers a possible mechanism by which respiratory diseases may be treated in the future. Among numerous peptide mediators such as tachykinins, calcitonin gene-related peptide, neurotrophins or opioids, vasoactive intestinal polypeptide (VIP) is one of the most abundant molecules found in the respiratory tract. In human airways, it influences many respiratory functions via the receptors VPAC1, VPAC2 and PAC1. VIP-expressing nerve fibers are present in the tracheobronchial smooth muscle layer, submucosal glands and in the walls of pulmonary and bronchial arteries and veins. Next to its strong bronchodilator effects, VIP potently relaxes pulmonary vessels, and plays a pivotal role in the mediation of immune mechanisms. A therapy utilizing the respiratory effects of VIP would offer potential benefits in the treatment of obstructive and inflammatory diseases and long acting VIP-based synthetic non-peptide compounds may represent a novel target for drug development.

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1. Introduction

Bronchial asthma and chronic obstructive pulmonary disease (COPD) are leading causes of chronic morbidity and mortality (Van Ganse et al., 2002; Anto et al., 2001). Despite considerable advances in the understanding of the pathogenesis of these airway diseases (Barnes et al., 2003; Chung, 2001), and recently identified potent transport pathways for aerosolically administered drugs (Groneberg et al., 2003d, 2004b), effective treatment options of these diseases remain essentially unchanged compared to the last decades with few exceptions such as leukotriene receptor antagonists. A large variety of new compounds that aimed to manipulate immune mechanisms including antibodies against interleukin 5 (IL-5) or recombinant IL-12 (Kips et al., 2001; Bryan et al., 2000) failed to be clinically superior to existing drugs. Considering the enormous research efforts directed to single immune mechanisms and the relatively disappointing history of drug development, current approaches to develop new strategies should be carefully reappraised.

So far, most approaches aimed to identify single mediators and to target their effects. Specifically, molecular and cellular mechanisms related to T lymphocytes and eosinophils were carefully studied and revealed their importance for airway inflammation (Baldacci et al., 2001; Kingham et al., 2003). The hypothesis that eosinophils are the central inflammatory cell in allergic airway inflammation (Kingham et al., 2003) propagated the development of an anti-IL-5 (Kips et al., 2001; Bryan et al., 2000) to fail to be clinically superior to existing drugs. Considering the enormous research efforts directed to single immune mechanisms and the relatively disappointing history of drug development, current approaches to develop new strategies should be carefully reappraised.

The respiratory innervation offers potent intervention options when targeting airflow obstruction, mucus hypersecretion, coughing or hyperemia since all of these symptoms are mediated by neurotransmitters (Belvisi, 2003; Widdicombe, 2003). Within the different subtypes of neurons innervating the airways, both potentially pro- and anti-inflammatory neuropeptides are expressed and released upon stimulation. In contrast to pro-inflammatory sensory neuropeptides such as tachykinins (Joos et al., 2001, 2004) or calcitonin gene-related peptide (CGRP) (Springer et al., 2004, 2003), the 28-amino acid peptide vaso-active intestinal polypeptide (VIP) is a potentially anti-inflammation mediator that may be used for the development of new compounds. VIP was originally isolated from the small intestine and the lung as a peptide with potent vasodilator effects (Said and Mutt, 1970a,b) and has recently been implicated to play a major regulatory role in a large number of acute and chronic diseases (Delgado et al., 2001; Delgado and Ganea, 2003). It belongs to the most abundantly expressed neuropeptides found in the upper and lower respiratory tract (Baraniuk et al., 1990; Filippatos et al., 2001) and influences many aspects of airway function (Said, 1982). Since VIP has recently been attributed to function not only as a vasodilator and bronchodilator but also as a potent immunomodulator, the present review aims to integrate both old and new findings to present a comprehensive and critical review of the potential use of VIP for a neuropeptide-based drug therapy.

2. VIP synthesis and expression in the respiratory tract

VIP is structurally related to a number of other peptidergic mediators such as pituitary adenylate cyclase activating polypeptide (PACAP), secretin, glucagon, peptide having carboxy terminal methionine/isoleucine (PHM/PHI), peptide histidine valine (PHV), helodermin, or growth hormone-releasing factor (GRF). VIP is synthesized from a precursor molecule (Pro-VIP), which also contains the sequence for peptide having carboxy terminal methionine-27. The human gene encoding for VIP is located on the chromosome region 6q24. It contains seven exons, each encoding a functional domain on the protein or the precursor mRNA (Delgado et al., 2001). Since VIP has recently been attributed to function not only as a vasodilator and bronchodilator but also as a potent immunomodulator, the present review aims to integrate both old and new findings to present a comprehensive and critical review of the potential use of VIP for a neuropeptide-based drug therapy.

In the human respiratory tract, VIP-expressing nerve fibers are found as branching networks (Ghatei et al., 1987). The frequency of these VIPergic fibers decreases as the airways become smaller, and only a few VIPergic fibers are present in bronchioles and alveolar space (Lundberg et al., 1984). The pattern of VIPergic nerve fiber distribution largely follows that of cholinergic nerves, which is consistent with the colocalization of VIP with acetylcholine (Laitinen et al., 1985). However, VIP has also been identified in some sensory nerves, including subepithelial airway nerves (Laitinen et al., 1985; Luts and Sundler, 1989). In pulmonary ganglia, VIP has been found in microganglia cells in the posterior trachea and around intrapulmonary bronchi. VIP-expressing nerve fibers
project to the tracheobronchial smooth muscle layer, to the walls of pulmonary and bronchial vessels, to submucosal glands and to the lamina propria (Dey et al., 1981; Lundberg et al., 1984). VIP has also been found in immune cells such as mast cells (Cutz et al., 1978; Goetzl et al., 1988), eosinophils (Aliakbari et al., 1987) and in different mononuclear and polymorphonuclear cells (O’Dorisio et al., 1981; Murphy et al., 1981).

3. PACAP and other related peptides

PACAP is a neuropeptide closely related to VIP and expressed by both neurons and lymphoid cells (Delgado et al., 2003). It also exerts a broad spectrum of functions and may participate in controlling immune homeostasis in the respiratory tract. Next to VIP, PACAP has been clearly identified as a potent anti-inflammatory factor in the past decade, exerting its effects by regulating the expression and release of both pro- and anti-inflammatory mediators. In this respect, PACAP has been shown to mitigate symptoms in septic shock. On the molecular level, PACAP is suggested to lead to a shift from a Th1-like to a Th2-like differentiation of T cells and to prevent the deleterious effects underlying experimental autoimmune arthritis along with VIP (Delgado et al., 2001). Therefore, PACAP and its primary receptor PAC1 also remain to be promising candidates in the development of future therapeutic strategies to treat numerous acute and chronic inflammatory diseases (Delgado et al., 2003).

4. Cleavage and metabolism of VIP

VIP is hydrolyzed by several enzymes including neutral endopeptidase (NEP, E. C. 3.4.24.11) (Fig. 1), mast cell chymase and mast cell tryptase (Goetzl et al., 1989; Di Maria et al., 1998). The fragments may then be rescavenged for the pulmonary amino nitrogen pool by peptide transporters which are expressed at high levels in the airways (Gronenberg et al., 2001a,b, 2002a,b). NEP hydrolysis fragments of VIP are physiologically inactive (Lilly et al., 1993) (Fig. 1) and also tryptase or chymase VIP fragments fail to relax vascular or tracheal smooth muscle (Caughey et al., 1988). Under physiological conditions, VIP is mainly cleaved by NEP, whereas in states of airway inflammation, mast cell enzymes dominate the degradation of VIP (Lilly et al., 1994). In this respect, VIP-induced pulmonary relaxation can be reversed by exogenous mast cell chymase or tryptase in the ferret (Franconi et al., 1989), or by the addition of exogenous chymotrypsin or papain in the guinea pig (Thompson et al., 1990).

Decreasing NEP activity by removing the airway epithelium leads to an enhancement of VIP-induced relaxation. This enhancement is equivalent to that reported upon treatment of intact epithelial airways with NEP inhibitors but not angiotensin converting enzyme inhibitors in guinea pig tracheal rings (Farmer and Togo, 1990). Protease inhibitors increasing VIP-induced relaxation include phosphoramidon or thiorphan and can be effectively combined with aprotinin and soybean trypsin inhibitors (Lilly et al., 1993, 1994; Tam et al., 1990).

5. VIP receptors

In the “pre-cloning” era, VIP binding sites have been identified in the respiratory tract of several species using binding techniques with $[^{125}I]$VIP (Carstairs and Barnes, 1986). Other studies assessed cyclic AMP formation which is induced by binding of VIP to its receptors (Frandsen et al., 1978). High densities of VIP binding sites were found in the pulmonary vascular smooth muscle layer and in airway smooth muscle of large, but not smaller airways. VIP binding sites are also present in submucosal glands, airway epithelium and in alveolar walls (Carstairs and Barnes, 1986). Immuno-histochemistry for cyclic AMP after stimulation by VIP confirmed the autoradiographic findings (Lazarus et al., 1986) in different species. In the human upper respiratory tract, VIP receptors were found on

Fig. 1. VIP-proteolysis: Neutral endopeptidase (NEP) activity and VIP cleavage sites. VIP-degrading NEP activity is abundantly present in the airways as demonstrated using a NEP activity assay in guinea pig airways (A). VIP is subject to proteolysis by NEP and other enzymes. The main cleavage sites (B) are Thr-Asp at position 7 and 8 and Ser-Ile at position 25 and 26 leading to the major cleavage products VIP (1–25) + VIP (26–28) and the minor products VIP (1–7) + VIP (8–28) that are all inactive. Original magnification ×200.
submucosal glands, epithelial cells, and arterial but not sinusoidal vessels (Baraniuk et al., 1990). In the past decade, molecular studies identified two major VIP receptors in the respiratory tract which are termed VPAC1 and VPAC2. A further receptor binding to VIP is the PAC1 receptor. This receptor may also play an important functional role in the respiratory tract by regulation of immune effects of VIP and PACAP in allergic diseases such as allergic bronchial asthma.

5.1. VPAC1

Until 1998, the nomenclature of VIP receptors was largely confusing: the VPAC1 receptor was originally termed the VIP receptor (Ishihara et al., 1992) and subsequently designated VIP1 receptor (Lutz et al., 1993), or VIP/PACAP type II receptor (Ciccarelli et al., 1994) or PVR 2 (Rawlings et al., 1995). In the current nomenclature proposed by the international union of pharmacology the receptor has been termed VPAC1 receptor (Harmar et al., 1998). It was first isolated and identified from the rat lung (Ishihara et al., 1992) and later identified in human tissues (Couvineau et al., 1994). There are no receptor splice variants known (Sreedharan et al., 1993). With regard to the potential use of VPAC1 for the development of new compounds, selective agonists and antagonists need to be identified. The VIP/GRF hybrid [Lys15, Arg16, Leu27] VIP (1–7)GRF(8–27)-NH2 (IC50, 1 nM) is a selective VPAC1 receptor agonist that does not activate GRF receptors (Gourlet et al., 1997a). [Arg16] chicken secretin (IC50, 2 nM) acts as an agonist of the VPAC1 receptor but also binds to the secretin receptor. This may limit its use in respiratory tissues in which a secretin receptor expression has not been excluded so far. However, in the central nervous system and other tissues that do not express the secretin receptor, [Arg16] chicken secretin may serve as a highly selective VPAC1 receptor agonist. [Acetyl-His3, D-Phe5, Lys15, Arg16]TVIP (3–7)GRF(8–27)-NH2 is known to be a selective antagonist of the VPAC1 receptor (IC50, 1 to 10 nM). Messenger RNA encoding the VPAC1 receptor was identified in the lung, CNS and other tissues (Ishihara et al., 1992; Sreedharan et al., 1995; Usdin et al., 1994) and in T lymphocytes (Delgado et al., 1996). Using immunohistochemistry, VPAC1-like immunoreactivity was identified in smooth muscle cells in the wall of blood vessels and in white blood cells (Busto et al., 2000).

5.2. VPAC2

The second receptor expressed in the human respiratory tract is VPAC2 (Groneberg et al., 2001c; Busto et al., 2000) (Fig. 2). As for VPAC1, it is a G protein-coupled receptor with seven transmembrane domains that is structurally related to receptors for many other biologically active peptides (Hanze et al., 2002). It was previously termed VIP2 receptor (Lutz et al., 1993), PVR3 (Rawlings et al., 1995), or PACAPR-3 (Inagaki et al., 1994). VPAC2 was cloned from human and other tissues and there are no splice variants described to date (Svoboda et al., 1994; Wei and Mojsov, 1996; Lutz et al., 1993; Usdin et al., 1994; Inagaki et al., 1994). The recombinant human and rat VPAC2 receptors recognize VIP (IC50, 3 to 4 nM), PACAP-38 (IC50, 2 nM), PACAP-27 (IC50, 10 nM), peptide histidine valine, and peptide having carboxy terminal isoleucine (IC50, 10 to 30 nM) and with a much lower affinity growth hormone-releasing factor and secretin (IC50, 5000 to 30,000 nM). Two highly selective agonists of the VPAC2 receptor have been described which are cyclic peptides: Ro 25-1553 (Gourlet et al., 1997b) first developed as an anti-inflammatory bronchodilator agent (O’Donnell et al., 1994a,b) and Ro 25-1392 (Xia et al., 1997).

Fig. 2. VPAC2 structure and distribution in human airways. VPAC2 is localized on the transcriptional level in airway epithelial cells as shown by non-radioactive mRNA in situ hybridization in human airways (A, negative control in the figure inlet). VPAC2 is proposed to be a 7-helix G protein-coupled receptor with the carboxy terminal end in the intracellular and the amino terminal end in the extracellular space (B). Original magnification × 100.
weak and restricted to a minority of smooth muscle cells. The specificity of the reaction was not confirmed by pre-absorption techniques and therefore not proven as specific for the VPAC2 protein (Busto et al., 2000).

6. Signal transduction

The predominant mode of VIP action in airway smooth muscle and vascular relaxation encompasses the stimulation of adenyl cyclase and cAMP production (Fig. 3) (Ganz et al., 1986; Sata et al., 1988). The increase of cAMP activates cAMP- and cGMP-dependent protein kinases (Francis et al., 1988) and leads to smooth muscle relaxation (Lincoln et al., 1990). VIP has also been reported to activate cAMP-mediated chloride channels (Martin and Shuttleworth, 1994). A number of respiratory effects of VIP may also be mediated via nitric oxide (Fischer et al., 2002a; Eynott et al., 2002, 2003), inducing the activation of NO synthetases and the subsequent stimulation of cytosolic guanylyl cyclase (Grider et al., 1992). It was reported that a combination of VIP and NO donors displays a synergistic effect on histamine-induced bronchoconstriction in guinea pigs (Kanazawa et al., 1997). Recently it was indicated that NO is probably responsible for mediating approximately 50% of the relaxant effect of VIP (Hasanean et al., 2003).

A VIP-binding protein (Stallwood et al., 1992) similar to porcine brain calmodulin (Andersson et al., 1993) has also been described, but its interactions with VIP are still poorly understood (Said, 1993). In other organs, VIP was also reported to induce catecholamine secretion (Malhotra et al., 1988), to increase intracellular Ca^{2+} levels (Tatsuno et al., 1992; Gressens et al., 1993; Sand et al., 1989) and to increase the breakdown of phosphoinositides to inositol phosphates (Audigier et al., 1986). Further intracellular interactions with other signaling cascades relevant for respiratory diseases such as mitogen-activated protein kinases (MAPKs), JUN proteins, or SMADs (Cano and Mahadevan, 1995; Groneberg et al., 2004a) have not been investigated in detail so far and need to be examined in future.

7. Effects on airway smooth muscle tone

VIP is a strong relaxant of airway smooth muscle (Groneberg et al., 2001e). Its bronchodilatory effects are almost 100 times more potent than adrenergic dilatation by isoproterenol in human bronchi and therefore, VIP belongs to the most potent endogenous bronchodilators so far described (Palmer et al., 1986a). VIP relaxes tracheal or bronchial segments in humans and significantly attenuates the constrictory effects of histamine, leukotriene D_{4}, prostaglandin F_{2α}, kallikrein, endothelin, NKA and NKB (Hamasaki et al., 1983; Groneberg et al., 2001e). This bronchodilation is independent of adrenergic and cholinergic receptors or cyclooxygenase (Altiere and Diamond, 1984; Saga and Said, 1984). The predominant site of VIP-induced bronchodilation is the central airways with a size-dependent mode of action (Altiere and Diamond, 1984). Therefore, its bronchodilatory effects may not be used effectively in diseases such as COPD which are characterized by a major airflow obstruction due to small airways alterations. The effects of VIP are more pronounced on lung resistance than on dynamic lung compliance (Diamond et al., 1983).

Although inhalation of VIP protects against histamine- and prostaglandin F_{2α}-bronchoconstriction (Cox et al., 1983) and intravenous VIP causes marked bronchodilation (Diamond et al., 1983) in animals, inhalation of VIP has no effect against exercise-induced asthma in human subjects (Bundgaard et al., 1983) and only minor protective effects against histamine-induced bronchoconstriction (Palmer et al., 1986b; Altiere et al., 1984). These unexpected weak effects of inhaled VIP are most likely based on the lack of penetration through the airway epithelium and protease activity (Morice et al., 1983; Altiere et al., 1984). In this respect it was shown that the VIP-induced relaxation of tracheal segments is increased after the removal of airway epithelium, probably due to removing hydrolyzing enzymes (Sharaf and Said, 1993). Protease-resistant endogenous and synthetic VIP analogs have also been shown to have more potent effects (Bolin et al., 1993; Ito and Tachibana, 1991).

Systemic administration of VIP has also not been reported to lead to significant bronchodilation in normal subjects (Palmer et al., 1986b). Since relaxant effects on the vascular smooth muscle tone are more potent than on the airway smooth muscle tone, systemic administration leads to hypotension, reflex tachycardia, and flushing, limiting higher systemic doses. Although VIP itself possesses potent bronchodilator effects in vitro, no significant therapeutical effects were reported after aerosolic or systemic delivery for the peptide. However, a recent study using the synthetic VPAC2 agonist Ro 25-1553 demonstrated rapid bronchodilatory effects (Linden et al., 2003).
8. Effects on vascular tone

The principal effect of VIP is vasodilation of pulmonary and systemic vessels and it potently relaxes the vessels supplying the upper airways (Lung and Widdicombe, 1987), trachea and bronchi (Laitinen et al., 1987) and pulmonary vessels (Obara et al., 1989; Nandiwada et al., 1985; Hamasaki et al., 1983). VIP-related vasorelaxation is more potent in the tracheal than in the bronchial circulation (Matran et al., 1989). The vasodilatory effect of VIP is 50 times more potent than prostacyclin (Saga and Said, 1984) and independent of the endothelium (Barnes et al., 1986; Greenberg et al., 1987). Due to its potent vasodilatory effects, VIP has recently been implicated in the treatment of primary pulmonary hypertension (PPH) (Petkov et al., 2003). In patients with primary pulmonary hypertension, VIP levels were decreased in the serum and lung tissues accompanied by an upregulation of VIP receptors. The substitution of VIP resulted in a substantial improvement of hemodynamic and prognostic parameters without side effects (Petkov et al., 2003). The mean pulmonary artery pressure was significantly decreased while the cardiac output and mixed venous oxygen saturation were increased indicating a potential future use of VIP or long lasting synthetic agonists in the treatment of primary pulmonary hypertension.

9. Effects on airway secretions

Mucus hypersecretion displays a serious problem in chronic inflammatory airway diseases of the upper and lower respiratory tract (Groneberg et al., 2002e, 2003c). Airway submucosal glands are densely innervated by VIPergic nerve fibers (Dey et al., 1981), and VPAC2 mRNA has been identified in human submucosal glands (Groneberg et al., 2001c), indicating a modulatory role for VIP in mucus secretion. However, the effects of VIP on mucus secretion seem to be very complex with different studies reporting controversial findings. Whereas cholinergic secretion was demonstrated to be inhibited by VIP in ferret trachea, it was stimulated in the cat trachea (Webber and Widdicombe, 1987; Shimura et al., 1988). In ferret and rat tracheal glands, VIP stimulated secretion (Peatfield et al., 1983; Wagner et al., 1998). Also, VIP stimulated the active secretion of chloride ions in the dog trachea (Nathanson et al., 1983) and a potent VIP-induced increase of the secretory response to phenylephrine was reported (Richardson and Webber, 1987).

In the human trachea, VIP inhibited metacholine-stimulated release of glycoproteins and lysozyme (Coles et al., 1981). Since VIP increases cyclic AMP formation in submucosal gland cells (Frandsen et al., 1978), it was expected that it stimulates secretion. In the upper airways, VIP was shown to stimulate lactoferrin secretion from human nasal mucosal cells, but had little effects on mucous glycoprotein release (Baraniuk et al., 1990).

With regard to these controversial findings, future studies using human tissues and cell lines need to be performed in order to identify the exact pathways of VIP signal transduction leading to either stimulation or inhibition of airway secretion. Especially in hypersecretory diseases such as COPD or asthma (Groneberg et al., 2002d, 2004c), neuropeptide-based compounds may be used to target hypersecretion since other major substance classes with an impact on secretion have not been identified in the past decades.

10. Effects on airway innervation

There is a vast body of evidence that VIP is a neurotransmitter of the autonomous airway innervation (Berisha et al., 2002) since it fulfills numerous relevant criteria of a neurotransmitter: It is present in the vagus nerve and airway nerves (Dey et al., 1988; Lundberg et al., 1979) and is colocalized with acetylcholine (Lundberg, 1981) and several other transmitters including nitric oxide (NO), peptide having carboxy terminal methionine, calcitonin gene-related peptide (CGRP), peptide having carboxy terminal isoleucine (PHI), neuropeptide tyrosine (NPY), galanin and opioids (Widdicombe, 1998). The airway smooth muscle relaxation induced by VIP is not affected by adrenergic blockade and mimics the electrophysiologic profile produced by stimulation of non-adrenergic non-cholinergic nerve populations (Cameron et al., 1983; Ito and Takeda, 1982). It is also released by electrical field nerve stimulation of tracheobronchial preparations in quantitative relation to the magnitude of nerve stimulation (Cameron et al., 1983; Matsuzaki et al., 1980).

Several further findings speak for a role of VIP as a neurotransmitter. Recently, the transport and effects of a VIPase monoclonal antibody capable of binding and cleaving VIP were studied. VIPase exhibited a concentration-dependent inhibitory effect on electrical field stimulation which was evident both in the absence and presence of blockade of beta-adrenergic and cholinergic receptors. It can therefore be concluded that circulating VIP binding and cleaving antibodies can reach the airways and attenuate smooth muscle relaxation by neutralizing endogenously released VIP indicating a major role of VIP as neuromediator in airway relaxation (Berisha et al., 2002).

Also, the relation between VIP and NO as potential co-transmitters of neurogenic airway relaxation was recently studied (Hasanean et al., 2003). In neuronal NO synthase (NOS-1/nNOS) gene-depleted mice it was shown that NO accounts for approximately 50% of the relaxant effect of VIP implying that NO is a major mediator of airway relaxation, whether due to neurogenic stimulation or to the neuropeptide VIP (Hasanean et al., 2003).

11. Effect on immune mechanisms

Recent experiments have proposed that besides neurally derived VIP, also lymphocyte-derived VIP may account for a large proportion of its biological effects although it is difficult to dissect the relative importance of both cellular sources. Early experiments indicated that VIP is associated with down-regulating T cell proliferation (Nio et al., 1993) and inhibition of mast cell mediator release (Undem et al., 1983). Recently, VIP was proposed to function as an important T helper-differentiating factor that promotes Th2-like and inhibits Th1-like immune responses via several cellular and molecular
mechanisms (Delgado, 2003). On the cellular level it positively influences the preferential survival of Th2 effector cells and the generation of memory Th2 cells (Voice et al., 2002; Gomariz et al., 2001). A complex picture of VIP-related immune effects has evolved over the past years. First studies demonstrated that in vitro treatment of macrophages with VIP leads to the induction of IL-4 and IL-5 and the inhibition of IFN-gamma and IL-2 in antigen-primed CD4 T cells (Delgado et al., 1998, 1999).

Recent studies employing VPAC2 gene-depleted and over-expressing murine strains also pointed to an important modulatory role of VIP and its receptor VPAC2 in mediating immune mechanisms (Voice et al., 2001, 2003; Goetzl et al., 2001). VPAC2 gene-depletion led to increased Th1-type responses which were characterized by an enhanced delayed-type hypersensitivity and a diminished immediate-type hypersensitivity (Voice et al., 2001). By contrast, T cell over-expression of VPAC2 led to a deviation from the normal CD4 T cell cytokine expression profile toward a Th2-like profile with elevated blood IgE and IgG1 levels and increased eosinophil numbers. The animals also had increased cutaneous allergic reactions and a decreased delayed-type hypersensitivity (Voice et al., 2001).

VPAC1 and VPAC2 have been reported to be expressed and differentially regulated in T cells in vitro (Gomariz et al., 2001). It was proposed that VPAC1 is constitutively expressed in T cells and downregulated during Th-cell stimulation while VPAC2 is upregulated during stimulation (Gomariz et al., 2001). However, these suggestions are based on in vitro data and have to be extrapolated for human in vivo situations with caution. Therefore, human T cells purified from patients with T cell-related diseases such as allergic asthma have to be assessed for VIP receptor expression and regulation. Following these necessary steps it has to be evaluated if novel VIP receptor-targeting therapeutic approaches should be developed.

On the molecular level, a positive feedback loop has been proposed for the relation between VIP and Th2 cells since it was reported that Th2 cells can express and release VIP after antigenic stimulation (Delgado and Ganea, 2001). Although the contribution of neurally released and inflammatory cell-derived VIP during inflammatory processes is difficult to assess, it seems likely that both sources play complementary roles and VIP and its receptors display useful targets for future drug development.

12. Role in inflammatory airway diseases

VIP is a potent dilator of airways and pulmonary vessels and impaired inhibitory VIPergic non-adrenergic non-cholinergic reflexes have been hypothesized to be involved in impaired bronchodilation. Also, VIP may participate in the regulation of mucus secretion and modulate local immune mechanisms in airways.

12.1. Upper respiratory tract diseases

A variety of inflammatory upper respiratory tract diseases exist in which VIP along with other neuromediators may contribute to pathogenesis and pathophysiology. Recently, different subtypes of chronic upper airway diseases including hyperreflectoritic rhinitis (Heptt et al., 2002), toxic rhinitis (Groneberg et al., 2003a) and aspirin-sensitive rhinitis (Groneberg et al., 2003b) were assessed for changes in nerve fiber profiles. Expression profiling for VIP, substance P, calcitonin gene-related peptide (CGRP), and neuropeptide tyrosine (NPY) in hyperreflectoritic rhinitis revealed significant increases for VIP and substance P (Heptt et al., 2002), indicating a role of both molecules in this chronic upper airway disease that is related to an unspecified hyperreactivity. To distinguish whether these alterations in nerve profiles are disease-specific or epiphenomena of airway inflammation, further subtypes including irritative toxic rhinitis and aspirin-sensitive rhinitis were examined (Groneberg et al., 2003a). Irritative toxic rhinitis is a nasal disorder induced by chemical compounds such as tobacco smoke, ozone, solvents, or formaldehyde that are also related to development and progression of bronchial asthma and COPD. In contrast to hyperreflectoritic rhinitis, VIP, substance P, calcitonin gene-related peptide (CGRP), and neuropeptide tyrosine (NPY) expression profiling showed significant increases only for VIP and neuropeptide tyrosine (NPY) (Groneberg et al., 2003a). These results demonstrated a differential regulation of subclasses of mucosal nerve fibers in the pathophysiology of toxic rhinitis. They also indicated that the alterations in nerve profiles found in toxic rhinitis and hyperreflectoritic rhinitis are disease-specific and not an epiphenomenon of airway inflammation.

Using nerve profiling, also aspirin-sensitive rhinitis was assessed for potential changes. This disease represents the manifestation of aspirin intolerance in the upper airways (Groneberg et al., 2003b) and is proposed to be a pseudoallergy against aspirin and related anti-inflammatory drugs. Nerve fiber profiling demonstrated that in aspirin-sensitive rhinitis, only VIPergic nerve fiber numbers are increased (Groneberg et al., 2003b) being paralleled by highly significant increases in eosinophil numbers. Further studies need to distinguish the role of VIP and other neuromediators in allergic nasal disorders.

12.2. Role in asthma

The precise role of VIP and related peptides in the development and progression of bronchial asthma has still not been fully revealed. In contrast to early proposals of a selective absence of VIPergic nerves fibers in asthmatic airways (Ollerenshaw et al., 1989), later studies did not support the hypothesis of an impaired VIP gene expression and indeed normal patterns of VIP expression for patients with asthma were shown in various other studies (Howarth et al., 1991; Lilly et al., 1995).

Studying the response of VIP plasma levels in relation to metacholine and exercise loading in children and adolescents with asthma indicated that VIP plasma VIP may rise in asthma attacks. Low VIP levels were found in patients with the largest fall of the respiratory function after exercise (Ohzeki et al., 1993). A further study addressing VIP levels during severe asthma exacerbations reported low VIP plasma concentrations
VIP-neutralizing antibodies were found in asthmatic patients but also in the plasma of normal subjects, so that they may not contribute significantly to lower levels of VIP (Paul et al., 1989).

In contrast to cystic fibrosis, VIP binding sites in lung tissue obtained from fatal asthma were similar to normal tissues (Sharma et al., 1995). However, these binding studies were obtained from fatal asthma were similar to normal tissues in inflammation (Kaltreider et al., 1997). An increase of VIP in bronchoalveolar lavage fluids (BALF) and increases of VPAC1 and VPAC2 receptor mRNA in BALF lymphocytes were found. Also, VPAC1 but not VPAC2 receptor mRNA was increased in alveolar macrophages following allergen challenge (Kaltreider et al., 1997).

12.3. Potential clinical use

The potent bronchodilatory properties of VIP gave rise to studies addressing the effectiveness of VIP-based therapy in bronchial asthma. Contrary to early studies (Morice et al., 1983) reporting a significant bronchodilation and protection against histamine-induced bronchoconstriction in asthmatic subjects, later studies failed to show protective effects of the peptide. In this respect, systemic administration had no effect on the airway tone in normal subjects (Palmer et al., 1986b). At higher doses, VIP even caused blood pressure decreases and tachycardia. Aerosol administration of VIP was shown to reduce the bronchial reactivity to histamine in mild asthma but did not significantly increase airway conductance (Barnes and Dixon, 1984). Also, a further study using inhaled VIP as pretreatment did not significantly prevent exercise-induced asthma (Diamond et al., 1983). The explanation for the inability of VIP to cause protective effects is the rapid inactivation of the peptide by airway peptidases.

To bypass the limited clinical effectiveness of the native peptide which is subject to degradation by many enzymes, two approaches where suggested: 1) a combination of VIP and peptidase inhibitors or 2) the use of peptidase-resistant VIP analogs. The later approach recently proved to be effective. After the characterization of amino acid residues required for VIP receptor binding and activation (O’Donnell et al., 1991), various compounds were synthesized and examined (O’Donnell et al., 1991; Bolin et al., 1996). The identification of major cleavage sites led to the synthesis of metabolically more stable analogs which are cyclic peptides incorporating disulfide and lactam ring structures (Bolin et al., 1995). The most potent synthetic VIP analogs that proved to have the best metabolic stability and the longest duration of activity are the cyclic peptides Ro 25-1553 that was developed as an anti-inflammatory bronchodilator and Ro 25-1392 (Xia et al., 1997). Both compounds have been characterized to be highly selective agonists of the VPAC2 receptor (Gourlet et al., 1997b; Xia et al., 1997; Schmidt et al., 2001).

Due to the extremely promising profiles of VIP’s respiratory effects, recently a double blind, randomized, placebo controlled, crossover study was performed to study the effects of Ro 25-1553. The compound was administered by inhalation to patients with asthma compared with the long acting beta(2) adrenoceptor agonist formoterol. Twenty four patients with moderate stable asthma were included in a crossover protocol with the primary variable being the bronchodilatory effect assessed as increase in forced expiratory volume in 1 s, FEV₁ after inhalation of Ro 25-1553 (100 or 600 μg) or formoterol (4.5 μg). Side effects were identified by assessing blood pressure, serum potassium, electrocardiography and echocardiography. In contrast to earlier unsuccessful studies with the native peptide VIP it was shown that the inhalation of 600 μg Ro 25-1553 caused a rapid bronchodilatory effect (geometric mean increase in FEV₁ compared with placebo) within 3 min of 6% (95% CI 4 to 9). The corresponding maximum bronchodilatory effect during 24 h was similar for 600 μg Ro 25-1553 (7% (95% CI 4 to 10)) and the reference bronchodilator formoterol (10% (95% CI 17 to 12)). However, for both doses of the synthetic VIP agonist the bronchodilatory effect was attenuated 5 h after inhalation whereas formoterol had a bronchodilatory effect 12 h after inhalation. Safety profiling of Ro 25-1553 and formoterol did not reveal any clinically relevant adverse reactions and side effects. Given the advantages of the strong topical effects of VIP, newly developed VIP-agonists with a longer duration of bronchodilatory effects will be very attractive for a potential clinical use and further research is required.

13. Conclusions

The past decade of respiratory research has been challenged by a revolutionary series of new insights into immune mechanisms of various diseases with numerous newly identified mediators. However, this scientific advance which based on new tools of molecular biology such as loss-of-function or gain-of-function models (Rubio-Aliaga et al., 2003; Kerzel et al., 2003) did not lead to the establishment of promising new immunomodulatory drug classes for asthma and COPD since most novel compounds failed to reach clinical practice. It may therefore be questioned if the vast research efforts aimed to identify new single immune targets might have displayed a too reductionistic approach. The current rate of introduction of new compounds to the pharmaceutical market is lower than at any time in the past 50 years (Horrobin, 2001) although the number of relevant new discoveries in immune mechanisms raises. It should therefore be reappraised if within complex diseases such as bronchial asthma or COPD, not only inflammatory or immune cells but also resident cells such as epithelial cells, smooth muscle myocytes, fibroblasts or nerves are potential targets to control disease symptoms. In view of the multitude of mediators expressed in these cells, single mediator approaches may be outlined in the future by dual or multiple-mediator/receptor targeting approaches. In the case of VIP and related peptides, further research has to be conducted to extrapolate recently gained knowledge from animal and in vitro studies to human disease conditions.
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