Guanylyl cyclases, nitric oxide, natriuretic peptides, and airway smooth muscle function

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Hamad, Ahmed M., Andrew Clayton, Baharul Islam, and Alan J. Knox. Guanylyl cyclases, nitric oxide, natriuretic peptides, and airway smooth muscle function. Am J Physiol Lung Cell Mol Physiol 285: L973–L983, 2003; 10.1152/ajplung.00033.2003.—Airway smooth muscle (ASM) plays an important role in asthma pathophysiology through its contractile and proliferative functions. The cyclic nucleotides adenosine 3’,5’-cyclic monophosphate (cAMP) and guanosine 3’,5’-cyclic monophosphate (cGMP) are second messengers capable of mediating the effects of a variety of drugs and hormones. There is a large body of evidence to support the hypothesis that cAMP is a mediator of the ASM’s relaxant effects of drugs, such as β2-adrenoceptor agonists, in human airways. Although most attention has been paid to this second messenger and the signal transduction pathways it activates, recent evidence suggests that cGMP is also an important second messenger in ASM with important relaxant and antiproliferative effects. Here, we review the regulation and function of cGMP in ASM and discuss the implications for asthma pathophysiology and therapeutics. Recent studies suggest that activators of soluble and particulate guanylyl cyclases, such as nitric oxide donors and natriuretic peptides, have both relaxant and antiproliferative effects that are mediated through cGMP-dependent and cGMP-independent pathways. Abnormalities in these pathways may contribute to asthma pathophysiology, and therapeutic manipulation may complement the effects of β2-adrenoceptor agonists.

atrial natriuretic peptide; guanosine 3’,5’-cyclic monophosphate; asthma

RECENT STUDIES HAVE SHOWN that both nitric oxide (NO) and atrial natriuretic peptide (ANP) have a significant bronchodilator effect in asthmatic subjects. Because NO and ANP share a common intracellular second messenger mechanism (i.e., cGMP), this suggests that cGMP may have important regulatory functions in human airway smooth muscle (ASM). Guanylyl cyclases (GC) are the enzymes that catalyze the conversion of GTP to guanosine cGMP and exist as soluble and particulate membrane-associated enzymes. NO is the natural activator of soluble GC, and a large part of this review will focus on its functions. Recent studies showed that carbon monoxide and pituitary adenylate cyclase-activating peptide can also activate soluble GC and relax guinea pig airways in vitro (15, 16, 110), but these are not discussed in detail here. Particulate GC act as plasma membrane receptors for natriuretic peptides and related peptides. Several membrane forms of the enzyme have been identified up to now. Some of them serve as receptors for the natriuretic peptides, a family of peptides that includes ANP, brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP), three peptides known to play important roles in renal and cardiovascular physiology. The type of GC present varies from tissue to tissue. In some tissues, the concentration of the soluble GC to particulate GC is nearly equal, whereas in others, such as the small intestine, the particulate form predominates (23). In some organs, such as the kidney, the relative abundance of both forms varies throughout the organ (22). However, the relative expression of different isoforms of GC in the lung have not been studied directly. This review will discuss the importance of cGMP as a second messenger in ASM, focusing on recent studies of soluble and particulate GC activators in intact and cultured ASM in vitro and in asthmatic subjects in vivo.

NO AND SOLUBLE GC

NO synthesis from the semiessential amino acid L-arginine is catalyzed by a family of enzymes called nitric oxide synthases (NOS). There are at least three isoforms of NOS that have been cloned and sequenced (37, 72, 83). There are two constitutive isoforms (constitutive NOS (cNOS)): the endothelial isoform (endothelial NOS) or type III NOS, normally present in endothelial cells, and the neuronal isoform (neuronal
NO donors or type I NOS, present in neuronal cells of the brain and the peripheral nerves. The constitutive isoforms are calcium dependent and induce transient production of picomolar concentrations of NO in response to various physiological stimuli. In contrast, the inducible isoform [inducible NOS (iNOS)] is upregulated in a number of cells, including airway epithelium, by endotoxins or cytokines (8, 102). This isoform generates larger (nanomolar) concentrations of NO for a more sustained period of time than cNOS (40). It is generally thought that iNOS is calcium independent. This has, however, been questioned by a recent report showing that iNOS contains calmodulin that is tightly bound and only requires very low levels of calcium for activation, merely giving the impression that it is calcium independent (18). In contrast, cNOS binds calmodulin loosely and requires much higher cytosolic levels of calcium to produce closer association with calmodulin, a step necessary for NO production. In the lung, vascular endothelial cells, macrophages, airway nerves [inhibitory nonadrenergic noncholinergic (iNANC)], and airway epithelium are thought to be the main sources of NO under basal conditions (40).

**Soluble GC**

Soluble GC is a heterodimeric heme-containing cytosolic enzyme comprising large (α-) and small (β-) subunits; three isoforms for the α- (α1-α3) and β- (β1-β3) subunits have already been cloned and sequenced (81). The α1/β1 form, which is the universal form with greatest activity, has been cloned from the rat and bovine lung (70, 82). Each subunit is divided into three functional domains: a heme-binding domain (confers NO sensitivity), a catalytic domain (identical between the subunits and also to the catalytic domain of particulate GC), and a dimerization domain (mediating the subunits’ association to form a heterodimer that is obligatory for enzyme activation) (48). Thiols are required for soluble GC activation by NO by forming more stable S-nitrosothiols with NO, and this may explain the inhibition of soluble GC by oxidizing agents such as methylene blue (39). The main effector cells for the effect of NO in the lung are vascular smooth muscle and ASM. Soluble GC, the primary receptor for NO, has been localized in the bronchial and vascular smooth muscle in the lung from various species (12, 70, 82, 97). Our own studies show that human airway smooth muscle cells (HASMC) express active soluble GC and accumulate cGMP after treatment with NO donors such as S-nitroso-N-acetyl penicillamine (SNAP) and sodium nitroprusside (SNP) (45).

**Desensitization of Soluble GC**

There is much literature on the use of NO donors as beneficial drugs for the treatment of acute forms of coronary artery disease. The major limitation of their use in vascular diseases is the rapid development of tolerance. This might also be a limitation in their effect on airways, although this has not been studied to the same extent. Several mechanisms for tolerance to the effects of nitroglycerin to NO due to depletion of intracellular thiols (80), 2 increased cGMP breakdown due to increased phosphodiesterase (PDE) activity (79), or 3 impaired cGMP formation due to desensitization of soluble GC (112, 122). Studies from our group have looked at tolerance to the effect of NO donors in cultured human airway smooth muscle (Fig. 1). We showed that soluble GC is desensitized in HASMC after pretreatment with NO donors, leading to a decrease in cGMP accumulation in response to subsequent treatment with NO donors in a dose- and time-dependent manner (46). The same phenomenon was seen in cell-free preparations, suggesting a GC desensitization instead of changes in NO release from the NO donors used.

**WHAT ARE THE BIOLOGICAL EFFECTS OF SOLUBLE GC ACTIVATORS ON ASM FUNCTION?**

**Contraction.** A number of studies have looked at exogenously applied NO both in vitro and in vivo (Tables 1 and 2). There is a general agreement among in vitro studies that NO relaxes airway smooth muscle with a potency intermediate between the β-adrenoceptor agonist isoprenaline and the PDE inhibitor theophylline (19, 25, 59, 78, 106, 107). Endogenous NO from several sources in the airway is capable of regulating bronchomotor tone. Epithelium-derived relaxing factor (EpDRF), a NO or NO-like substance, is a relaxant of ASM from a number of species. Exogenous NO is a weak bronchodilator in vivo (Table 2). NOS inhibition enhances agonist-induced increase in airway resistance in guinea pig, supporting a role for EpDRF in control of bronchomotor tone (87, 101, 128). Consistent
with this, NOS inhibition enhances agonist responses in guinea pig trachea with intact mucosa but not in epithelially denuded preparations (34, 87). Moreover, bradykinin increased cGMP and relaxed guinea pig trachea with intact epithelium but contracted epithelium-denuded trachea, suggesting that bradykinin stimulated the release of NO from the tracheal epithelium that in turn elevated cGMP levels and caused relaxation (34, 128). Studies in bovine trachea showed that NOS inhibition increased the basal tone, abolished histamine-induced increase in NO release from the epithelial layer, and enhanced histamine-induced contraction (107). More recent studies showed that repeated antigen exposure led to bronchial hyperresponsiveness in guinea pigs, probably due to a lack of EpDRF-mediated bronchodilatation (74). Indeed, clinical studies with NOS inhibitors in asthmatic subjects support this notion (99, 100). Furthermore, a recent study using human bronchial strips showed that the antiasthmatic affect of ginsenoside, an extract of ginseng, was enhanced by NOS inhibition, suggesting a potential role for NOS in the pathophysiology of asthma.

Table 1. Effects of soluble/particulate GC activators on ASM in vitro in different species

<table>
<thead>
<tr>
<th>Activator</th>
<th>Species</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANP, atriopeptins, SNP</td>
<td>Guinea pigs</td>
<td>Relaxation of both basal and agonist-induced tone</td>
<td>(14, 38, 47, 96, 125)</td>
</tr>
<tr>
<td>BNP, CNP</td>
<td>Guinea pigs</td>
<td>Relaxation</td>
<td>(117, 118)</td>
</tr>
<tr>
<td>S-nitrosothiols</td>
<td>Guinea pigs</td>
<td>Relaxation</td>
<td>(59)</td>
</tr>
<tr>
<td>ANP</td>
<td>Bovine</td>
<td>Reverse and protect against methacholine-induced tone</td>
<td>(6, 19, 85)</td>
</tr>
<tr>
<td>ANP, atriopeptins, SNP</td>
<td>Bovine</td>
<td>Relaxation of agonist-induced tone (ANP &gt; SNP); no effect on basal tone</td>
<td>(57, 58)</td>
</tr>
<tr>
<td>SIN-1, SNP, SNAP</td>
<td>Bovine and guinea pigs</td>
<td>Relaxation</td>
<td>(19, 78, 106, 107)</td>
</tr>
<tr>
<td>Nitrovasodilators</td>
<td>Canine</td>
<td>Relaxation and increased cGMP</td>
<td>(93, 129)</td>
</tr>
<tr>
<td>SNP, NO</td>
<td>Porcine</td>
<td>Relaxation and increased cGMP</td>
<td>(118)</td>
</tr>
<tr>
<td>ANP, SNP</td>
<td>Human</td>
<td>Reverse and protect against methacholine-induced tone ANP &gt; SNP</td>
<td>(6, 20, 84)</td>
</tr>
<tr>
<td>S-nitrosothiols, SNP, NO, SIN-1</td>
<td>Human</td>
<td>Relaxation and increased cGMP</td>
<td>(30, 36, 123, 124)</td>
</tr>
<tr>
<td>ANP, BNP, CNP, SNP, SNAP</td>
<td>Cultured human ASM</td>
<td>Increased cGMP (peptides &gt; NO donors)</td>
<td>(45)</td>
</tr>
<tr>
<td>ANP, SNP, SNAP</td>
<td>Cultured human ASM</td>
<td>Homologous desensitization of GC</td>
<td>(46)</td>
</tr>
<tr>
<td>SNP, SNAP</td>
<td>Cultured human ASM</td>
<td>Inhibition of proliferation</td>
<td>(42)</td>
</tr>
<tr>
<td>SNP, SNAP</td>
<td>Cultured rat ASM</td>
<td>Increased cGMP formation</td>
<td>(29)</td>
</tr>
</tbody>
</table>

GC, guanylyl cyclases; ASM, airway smooth muscle; ANP, atrial natriuretic peptide; SNP, sodium nitroprusside; BNP, brain natriuretic peptide; CNP, C-type natriuretic peptide; SIN-1, 3-morpholinosydnonimine; NO, nitric oxide; SNAP, S-nitroso-N-acetyl penicilamine.

Table 2. Effects of soluble/particulate GC activators on ASM function in vivo in different species

<table>
<thead>
<tr>
<th>Activator</th>
<th>Species</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNAP, NO</td>
<td>Guinea pigs</td>
<td>Reversing methacholine-induced bronchoconstriction &gt; basal tone</td>
<td>(29)</td>
</tr>
<tr>
<td>Infused ANP</td>
<td>Guinea pigs</td>
<td>Reverse and protect against agonist-induced bronchoconstriction but not compliance</td>
<td>(31)</td>
</tr>
<tr>
<td>Infused ANP, BNP, CNP</td>
<td>Guinea pigs</td>
<td>Reverse antigen-induced changes in lung resistance</td>
<td>(88)</td>
</tr>
<tr>
<td>Infused ANP</td>
<td>Sheep</td>
<td>Reverse methacholine-induced changes in lung resistance but not compliance; no effect on baseline lung function</td>
<td>(9)</td>
</tr>
<tr>
<td>Inhaled NO</td>
<td>Rabbits</td>
<td>Reverse methacholine-induced increase in lung resistance but not compliance</td>
<td>(49)</td>
</tr>
<tr>
<td>Inhaled NO, 80 ppm</td>
<td>Human (normal, COPD, asthma)</td>
<td>Increased sGaw in asthmatic subjects but not in normal and COPD patients</td>
<td>(50)</td>
</tr>
<tr>
<td>Inhaled NO, 80 ppm</td>
<td>Human (healthy men)</td>
<td>Reverse methacholine-induced changes in sGaw in healthy men; no effect on basal sGaw</td>
<td>(109)</td>
</tr>
<tr>
<td>Inhaled NO, 100 ppm</td>
<td>Human (asthma)</td>
<td>Small increase in FEV1 and FVC (but not FEF25) after methacholine-induced bronchoconstriction</td>
<td>(63)</td>
</tr>
<tr>
<td>Inhaled nitroglycerine</td>
<td>Human (asthma)</td>
<td>Bronchodilation</td>
<td>(104)</td>
</tr>
<tr>
<td>Inhaled isosorbide dinitrate</td>
<td>Human (asthma)</td>
<td>Reverse exercise-induced bronchoconstriction; no effect on baseline lung function</td>
<td>(121)</td>
</tr>
<tr>
<td>Infused nitroglycerine</td>
<td>Human (asthma)</td>
<td>↓tracheal cuff pressure in cardiac bypass surgery patients</td>
<td>(13)</td>
</tr>
<tr>
<td>Sublingual nitrates</td>
<td>Human (asthma)</td>
<td>Mild improvement in FEV1, FVC in mild asthma but no change in FEV1, FVC in acute severe asthma</td>
<td>(38, 66, 76, 90)</td>
</tr>
<tr>
<td>Infused ANP</td>
<td>Human (normal and asthma)</td>
<td>Bronchodilatation, increased plasma C50 levels in moderately severe asthmatics, but higher doses were required to reduce airway resistance in normal and mild asthmatics</td>
<td>(2, 17, 52, 53)</td>
</tr>
<tr>
<td>Infused ANP</td>
<td>Human (asthma)</td>
<td>Protection against direct and indirect bronchial challenges</td>
<td>(51, 73)</td>
</tr>
<tr>
<td>Inhaled ANP</td>
<td>Human (asthma)</td>
<td>Protect against subsequent bronchial challenge. Higher doses (5 mg) produced significant bronchodilatation</td>
<td>(3, 55, 56)</td>
</tr>
<tr>
<td>Inhaled ANP</td>
<td>Human (asthma)</td>
<td>Bronchodilator and bronchoprotective effects are enhanced by thiorphan (neutral endopeptidase inhibitor)</td>
<td>(4, 5)</td>
</tr>
</tbody>
</table>

COPD, chronic obstructive pulmonary disease; ppm, parts per million; sGaw, specific conductance; FEV1, forced expiratory volume; FVC, forced vital capacity; FEF25, forced expiratory flow.
Panax ginseng, is via stimulation of NO generation from airway epithelium and cGMP synthesis (119).

NO may also be produced endogenously by iNANC nerves and act locally on ASM. The iNANC mechanism is the only known neural bronchodilator pathway in humans, and NO is the only known neurotransmitter of iNANC nerves in humans (10, 11, 30, 124). In vitro studies in human airway preparations showing that the iNANC response is associated with a selective increase in cGMP and that selective inhibition of cGMP-specific PDE enzyme enhances the iNANC response are consistent with it being mediated by NO-induced increases in cGMP (32, 124).

In vivo studies in different species, including humans, showed a bronchodilator effect for inhaled NO. Hogman and colleagues (50) showed that inhalation of 80 parts per million of NO increased the specific airway conductance in asthmatic subjects, although to a lesser extent than after β₂-agonists. These findings were confirmed in subsequent studies (63, 109). Clinical trials with nitrovasodilators generally showed a weak bronchodilator effect, with the inhaled route confirmed in subsequent studies (63, 109). The relaxant effect of NO in different species correlates well with cGMP elevation, suggesting a causal role for cGMP in mediating its relaxation (36, 57, 129). More recently, the proliferative effects of endothelin-1 (ET-1), both alone and in combination with epidermal growth factor, and the effect of NO on the cell proliferation were investigated in cultured guinea pig bronchial smooth muscle (67). A NO donor, SIN-1, reduced the cell-proliferative effect of ET-1 in a concentration-dependent manner. A soluble GC inhibitor partly, but significantly, reversed the effect of SIN-1. Studies using NOS inhibitors have shown that HASMC express type I NOS and inhibition of NOS enhances DNA synthesis and cell proliferation (92).

**NATRIURETIC PEPTIDES AND PARTICULATE GC**

The natriuretic peptide family of hormones has an important role in salt and water homeostasis. The human natriuretic peptides include ANP, BNP, and CNP. At first, the sole source of ANP was thought to be the heart. It has been known for a long time that ANP is secreted from the atrial myocytes into the blood stream in response to distension or stretch of the atrial wall (105). Several more recent studies, however, show that the lung is another source of ANP in different species, including humans (7, 71, 108, 114). Studies in hamsters showed that the ANP gene is expressed mainly in the airway epithelium and smooth muscle, and, to a lesser extent, in the alveolar wall, muscular media of the pulmonary arteries, and extrapulmonary pulmonary veins (86). ANP and the other natriuretic peptides act on different particulate GC receptors. These are transmembrane proteins composed of a single transmembrane domain, a variable extracellular natriuretic peptide-binding domain, and a more conserved intracellular kinase homology domain (KHD) and catalytic domain. GC-A, the receptor for ANP and BNP, also named natriuretic peptide receptor-A or -1, has been studied widely. Its mode of activation by peptide ligands and mechanisms of regulation serve as prototypes for understanding the function of other particulate forms of GC. Activation of this enzyme by its ligand is a complex process requiring oligomerization, ligand binding, KHD phosphorylation, and ATP binding. Gene knockout and genetic segregation studies have provided strong evidence for the importance of GC-A in the regulation of blood pressure and heart and renal functions (68). Immunohistochemical studies have localized GC-A to the ASM and alveoli in bovine lung (65). However, specific receptors for ANP have not been sought directly in human lung. We have used pharmacological tools to characterize the presence of these receptors in cultured HASMC (45). In this study, we showed that treatment of HASMC with ANP, BNP, and CNP led to a time- and concentration-dependent increase of cGMP levels in these cells, suggesting that particulate GC is expressed in these cells. The order of potency seen in our experiments was: ANP ≥ BNP > CNP, consistent with type A and B of...
particulate GC being present in these cells (GC-A > GC-B). Although heat-stable enterotoxin (GC-C ligand) did not affect cGMP over the time course of our experiments, suggesting that GC-C is not expressed in HASMCs, a recent study showed that treatment of guinea pigs with uroguanylin (a ligand of GC-C receptor in gastrointestinal tissue) significantly inhibited leukotriene C4-induced pulmonary changes in a dose-dependent manner (89). The disparity between this study and ours may reflect species differences.

DESENSITIZATION OF PARTICULATE GC

Similar to soluble GC, particulate GC may undergo homologous desensitization after prolonged exposure to ANP through a cGMP-independent mechanism (46). We further studied the mechanism of particulate GC desensitization in HASMC (43); pretreatment of HASMC with phorbol 12-myristate 13-acetate (PMA), a protein kinase C (PKC) activator, led to time- and concentration-dependent desensitization of ANP-stimulated cGMP accumulation. GF-109203X, a selective PKC inhibitor, blocked the PMA-induced desensitization but did not block ANP-induced desensitization. In addition, desensitization by PMA and ANP showed an additive effect.

WHAT ARE THE BIOLOGICAL EFFECTS OF PARTICULATE GC ACTIVATORS ON ASM FUNCTION?

Contraction. ANP has a direct relaxant effect associated with cGMP accumulation in guinea pig airway in vitro (24, 75, 125). BNP and CNP have similar effects in guinea pig airway preparations (117, 118). ANP is a potent relaxant of the intrinsic tone as well as tone induced by various agonists in guinea pig trachea

Fig. 2. Role of NO/soluble GC/cGMP (A) and natriuretic peptides/particulate GC/cGMP (B) in regulation of ASM functions. GC stimulation by activators leads to increased formation of cGMP in ASM cells. cGMP exerts its effects through activation of cGMP-dependent protein kinase, with subsequent phosphorylation of different proteins. NO-induced relaxation could be cGMP independent (direct activation of maxi-K+ channels, oxidation of intracellular contractile proteins, or decreased sensitivity to intracellular Ca2+). NO also inhibits proliferation by cGMP-independent inhibition of ribonucleotide reductase. Atrial natriuretic peptide (ANP) clearance receptors mediate cGMP-independent antiproliferative effect of ANP. BNP, brain natriuretic peptide; CNP, C-type natriuretic peptide.
(95, 125). However, it may be more effective in relaxing methacholine- than in leukotriene D4-induced tone (46). In rat tracheal tissue, atriopeptins cause a weak relaxation of intrinsic tone as well as carbachol-induced tone (33). In bovine tracheal smooth muscle, ANP and atriopeptins have a direct relaxant effect on the tone induced by various agonists (57, 58). The potency of ANP was intermediate between isoprenaline and SNP. Infused ANP has been shown to reverse and protect against agonist-induced bronchoconstriction in guinea pigs in vitro (31, 88). Similar results were shown in sheep (9). More recently, BNP and CNP were reported to have a similar effect as ANP on antigen-induced changes in lung resistance in sensitized guinea pigs; the rank of order of inhibitory potency was BNP = ANP > CNP (88).

ANP also relaxes human airways in vitro. ANP was reported to reverse and protect against methacholine-induced contraction of human bronchi (6, 20, 84). ANP was more potent than SNP and salbutamol (20). Data from human and guinea pig airways suggest that the ANP relaxant effect may be due to cGMP-dependent activation of large conductance Ca2+-activated K+ channel (24, 75). A number of studies have looked at the effect of ANP on lung function in both normal and asthmatic subjects in vivo. Infused ANP at a concentration producing plasma levels in the pathophysiological range had a significant bronchodilator effect in asthmatics (52). The effect of infused ANP was similar to that of nebulized β2-agonists, although it was shorter lived. High dose-inhaled ANP produced less bronchodilator effect compared with intravenous ANP (3, 55, 56). This can be explained by the rapid degradation of ANP by the neutral endopeptidases (NEP) within the airways (6). This was confirmed in a subsequent study in which NEP inhibition greatly enhanced the bronchodilator effect of ANP in asthmatics (4, 5). However, it was observed that short-term exercise and acute asthma were associated with a rise in the plasma level of ANP (54, 103), suggesting that ANP could have a physiological role in the regulation of the bronchomotor tone. This increase in ANP may be due to hypoxia-induced pulmonary vasoconstriction, leading to increased right atrial pressure (127).

**Proliferation.** As with NO, ANP inhibits proliferation in several cell lines, but until recently, its role in regulation of HASMC proliferation was not known. We have shown that human ANP1-28 (a GC activator), rat ANP (rANP)104–126 (which binds selectively to the ANP clearance receptors without elevating cGMP), and a cGMP analog had an antiproliferative effect in HASMC, suggesting that both cGMP-dependent and cGMP-independent mechanisms are involved in ANP’s antiproliferative effect (42). Although we found that the human ANP1-28, like SNAP, had antiproliferative effects, it produced a smaller maximum effect. This difference in efficacy contrasts with relative abilities of ANP and SNAP to elevate cGMP (ANP > SNAP). This paradox suggests that compartmentalization of cGMP pools may enable cGMP generated by soluble GC to have a greater effect than that generated by particulate GC.

The lung is capable of synthesizing ANP (35, 41, 86), and both types of ANP receptors (GC-linked and clearance receptors) have been characterized and localized throughout the lung. In the heart, where more is known about the function of ANP, ANP release is stretch stimulated (28). Similarly, Springall et al. (115) suggested that stretch of rat pulmonary vein stimulates ANP release. Preliminary data support a similar stretch-dependent mechanism for ANP release in tracheal muscle of anesthetized sheep (93). Stretch-dependent release of ANP from ASM, occurring with deep inspiration, could lead to cGMP elevation with subsequent inhibition of ASM tone and proliferation.

**CROSS TALK BETWEEN CGMP AND CAMP PATHWAYS**

The other main cyclic nucleotide involved in ASM relaxation is cAMP, which is activated mainly by β2-adrenoceptor agonists (69). In the classic pathway, β2-adrenoceptor agonists bind to β2-receptors, which are coupled to adenylyl cyclase, leading to production of cAMP. cAMP then activates protein kinase A (PKA), and PKA phosphorylates a number of substrates to bring about its intracellular effects (94, 113). In parallel pathways, NO and ANP activate soluble and particulate GC, respectively, to produce cGMP, and cGMP activates protein kinase G (PKG), which then phosphorylates its own set of substrates (94, 113, 120). It has become clear, however, that the situation is much more complex, and although it has not been studied in detail in ASM, cross talk between cGMP and cAMP pathways is well recognized in many other biological systems (94, 113). This cross talk can occur at a number of levels.

First, cyclic nucleotides can repress the degradation of their counterparts through their actions on PDEs (120). For example, the cGMP-stimulated PDE-2 and the cGMP-inhibited PDE-3 preferentially hydrolyze cAMP (94, 113, 120). Both of these PDE isozymes are present in human airway smooth muscle (120). Second, cGMP and cAMP are both capable of cross-activating their respective kinases. For example, at physiological concentrations, both cGMP and cAMP can activate PKG in vascular smooth muscle (21). In contrast, in the same experiments, PKA was only activated by cAMP. Other investigations have, however, shown that cGMP can inhibit proliferation of cultured vascular smooth muscle by activating PKA (26). In contrast, cAMP relaxes pig coronary arteries via PKG (62). Third, both PKG and PKA have a number of common substrates. Sites of phosphorylation in ASM for PKA include phospholipase C, maxi-K+ channels, Na+-K+-ATPase, myosin light chain kinase, and sarcoplasmic reticulum Ca2+ pumps. PKG can phosphorylate maxi-K+ channels and Ca2+ uptake pumps in ASM, and in non-ASM cells, contractile proteins and phospholipase C can also be phosphorylated (reviewed in Ref. 69). There is also evidence that PKA and PKG may cooperatively phosphorylate some substrates such that phosphorylation...
by one kinase changes the conformation of the target protein, making serine threonine sites more accessible to the other kinase (94). Alternatively, PKG or PKA may regulate the activity of protein phosphatases, which then modifies the effect of the other kinase (94). Finally, cross talk in some systems occurs in the regulation of cGMP/cAMP synthesis (77).

Although cross talk can be complex, compartmentalization within the cell of the enzymes catalyzing cyclic nucleotide synthesis and degradation, the enzymes responsible for cyclic nucleotide-mediated phosphorylation and the protein targets of these kinases exert a degree of constraint and allows cell specificity in the interactions and functional responses (94).

Collectively, these studies suggest there is considerable potential for pathways activated by NO donors or natriuretic peptides to enhance the effects of β2-agonist agonists either on relaxation or proliferation of ASM. Further studies addressing possible interactions in vitro may be of great interest. The demonstration of an additive effect of NO and NO donors on β2-agonist-induced bronchodilation in asthmatic subjects (50, 104) and that a combination of ANP and salbutamol evokes a greater effect than either alone in reversing and protecting against methacholine-evoked contraction in isolated human bronchi (84) suggest that such combinations could be of benefit in the treatment of asthma, allowing lower doses of each individual drug to be used.

RELEVANCE OF GC TO ASThma

The evidence reviewed thus far suggests that both soluble and particulate GC may have important roles in asthma pathophysiology but that NO donors and natriuretic peptides act through a combination of cGMP-dependent and cGMP-independent effects. NO donors and natriuretic peptides have ASM relaxant properties that may be important under different physiological circumstances. NO produced by NO synthase may have a physiological role in reducing ASM proliferation. The epithelial shedding that occurs in the asthmatic airways could, therefore, lead to the removal of a paracrine braking mechanism acting to inhibit ASM proliferation.

The desensitization seen with NO may also have pathophysiological significance. Numerous studies have shown that NO is produced in large quantities in asthmatic airways, possibly as a result of iNOS induction (126). This excess NO could potentially desensitize soluble GC in ASM, thereby impairing NO-mediated bronchodilatation (Fig. 1). This may be particularly important for the iNANC nervous system in which NO is the major neurotransmitter. Consistent with this hypothesis, there is some evidence that iNANC is dysfunctional in asthmatic airways (78). In this study, guinea pigs were sensitized with ovalbumin and then challenged with ovalbumin for 3 consecutive days. On the day after the final challenge, iNANC responses elicited by electrical field stimulation or relaxation responses to SIN-1 were obtained in the tracheal strips precontracted by histamine. The iNANC responses and SIN-1-induced relaxation of the ovalbumin group were significantly attenuated, suggesting that allergic airway inflammation impairs neural NO-induced relaxation, presumably by inhibiting the access of neural NO to the ASM (Fig. 1).

Our studies with ANP suggest that PKC activation can desensitize particulate GC but that the desensitization induced by ANP itself is PKC independent. The pathophysiological relevance of this desensitization is not clear, but it is possible that PKC activation by proinflammatory cytokines in asthma may downregulate the bronchoprotective effect of ANP.

CONCLUSION

Agents acting through cGMP could prove to be adjuvance to therapy to the bronchodilators in current use. They utilize a complementary pathway to β-agonists, which signal through cAMP and anticholinergics that act specifically to block muscarinic cholinergic receptors. The demonstration of an additive effect of NO donors and β2-agonists, in some studies, suggests that such combinations could be of benefit in the treatment of asthma, allowing lower doses of each individual drug to be used. An alternative strategy might be to utilize S-nitrosylated derivatives of existing bronchodilator molecules. Interestingly, it was shown that S-nitrosylated derivatives of vasoactive intestinal peptide preserve the intrinsic function of vasoactive intestinal peptide but acquire NO-like vasoactivity when tested on aortic rings (61). Similar studies in the airways would be interesting. Currently, there is a great deal of interest in developing PDE inhibitors with a more favorable pharmacological profile than existing agents. The combined use of PDE inhibitors and GC activators could allow the use of smaller doses of both (32). It is also possible that the transient effects of ANP could be prolonged either by the concomitant use of NEP inhibitors or pharmacological modification of the ANP molecule.

Besides its relaxant effect, NO may also protect against airway remodeling by inhibiting ASM proliferation (an important component of airway thickening in asthma). Dysfunction of GC activation by endogenous stimuli may contribute to the bronchial hyperresponsiveness characteristic of asthma as asthmatic inflammation results in excess production of NO (Fig. 1). This excess NO would be expected to cause desensitization of soluble GC in ASM. Furthermore, activation of PKC as a result of asthmatic inflammation could desensitize particulate GC and impair cGMP production in response to endogenous natriuretic peptides (Fig. 1).

In conclusion, the GC/cGMP second messenger system has a parallel role to the adenyl cyclase/cAMP system in ASM, regulating its contractile and proliferative functions. Drugs activating this pathway have the potential to be new antiasthma therapies that could be used in conjunction with existing drugs.
REFERENCES


