Regulation of cGMP by soluble and particulate guanylyl cyclases in cultured human airway smooth muscle

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Hamad, Ahmed M., Simon Range, Elaine Holland, and Alan J. Knox. Regulation of cGMP by soluble and particulate guanylyl cyclases in cultured human airway smooth muscle. Am. J. Physiol. 273 (Lung Cell. Mol. Physiol. 17): L807–L813, 1997.—Although guanosine 3′,5′-cyclic monophosphate (cGMP) acts as a relaxant second messenger, the regulation of intracellular cGMP has not been comprehensively studied in human airway smooth muscle. We studied the production of cGMP by cultured human airway smooth muscle cells (HASMC) after stimulation with activators of soluble guanylyl cyclase [sodium nitroprusside (SNP) and S-nitroso-N-acetylpenicillamine (SNAP)] and particulate guanylyl cyclase [atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP), and Escherichia coli heat stable enterotoxin (STa)]. cGMP was measured by enzyme-linked immunosorbent assay. Both SNP (10⁻⁶ to 10⁻³ M) and SNAP (10⁻⁶ to 10⁻³ M) caused concentration-dependent elevation of cGMP in the presence of the nonselective phosphodiesterase (PDE) inhibitor 3-isobutyl-1-methylxanthine (10⁻³ M), with cGMP increasing 6- and 15-fold in response to SNP and SNAP, respectively, at the highest concentration tested (10⁻³ M). The increases in cGMP in response to SNP (5 x 10⁻⁵ M) and SNAP (10⁻⁵ M) were inhibited by hemoglobin (Hb; 5 x 10⁻³ M), a nitric oxide scavenger, and methylene blue (MB; 5 x 10⁻⁴ M), an inhibitor of guanylyl cyclase. cGMP accumulation after SNAP was abolished by both Hb and MB. The response to SNP was inhibited by 79% with Hb and was abolished with MB, ANP, BNP, and CNP (10⁻⁹ to 10⁻³ M) + phosphoramidon (10⁻⁶ M) caused a concentration-dependent elevation in cGMP with an order of potency ANP > BNP > CNP. cGMP formation in the presence of the highest concentration of the most potent natriuretic peptide (10⁻⁵ M ANP) was two- to threefold greater than with the highest concentration of SNAP. The increase in cGMP seen with natriuretic peptides was similar in the presence or absence of phosphoramidon, a neutral endopeptidase (NEP) inhibitor, suggesting that NEP is not playing a role in modulating the effect of natriuretic peptides in HASMC. STa (400 IU/ml) had no effect on cGMP levels. SNAP- and ANP-induced cGMP accumulation was increased by the selective type V PDE inhibitors SKF-96231 and zaprinast, suggesting that type V PDE is responsible for cGMP breakdown in HASMC. These results suggest that cultured HASMC contain both soluble and particulate guanylyl cyclases. The order of potency of the natriuretic peptides ANP > BNP > CNP suggests that type A particulate membrane-bound guanylate cyclase predominates.

Nitric oxide; natriuretic peptides; guanosine 3′,5′-cyclic monophosphate

AIRWAY SMOOTH MUSCLE relaxation is the dominant mechanism of action of bronchodilator drugs in the treatment of asthma and chronic obstructive pulmonary disease (30). The airway smooth muscle relaxant drugs in current use (β-adrenoceptor agonists, theophyllines) act to elevate the intracellular second messengers adenosine 3′,5′-cyclic monophosphate (cAMP; see Ref. 30). Guanosine 3′,5′-cyclic monophosphate (cGMP) is also a relaxant second messenger in airway smooth muscle cells (8, 11, 12, 14, 15, 29, 48). This may be important physiologically as nitric oxide (NO), which elevates cGMP, is the relaxant neurotransmitter of nonadrenergic noncholinergic nerves (5, 28, 32, 48). Elevation of cGMP is also a potential target for pharmacological intervention.

cGMP is produced from GTP by the action of guanylyl cyclases. Guanylyl cyclases exist in two forms, a soluble form (GCs) and a particulate membrane-bound form (GCm; see Refs. 45, 47, and 50). Each form is activated by distinct agonists. GCs is activated by NO and NO donors, whereas GCm is a plasma membrane receptor for the natriuretic peptides and related hormones (45, 50). cGMP produced by guanylyl cyclases activates a group of distinct cGMP-dependent protein kinases, which have several sites of action in airway smooth muscle to bring about relaxation (8, 9, 21, 30, 33, 36). cGMP is metabolized to 5′-GMP by phosphodiesterases (PDE; see Refs. 32 and 46).

Agents that activate guanylyl cyclase have been shown to relax airway smooth muscle in vitro and bronchodilate in vivo. Nitrobronchodilators relax guinea pig (26), bovine (18), and human airway smooth muscle (14, 38, 48), atrial natriuretic peptide (ANP) relaxes guinea pig (10, 35), rat (10), and bovine (25) airway smooth muscle in vitro, and brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) relax guinea pig trachealis (43, 44). Studies with ANP in human airway smooth muscle in vitro have shown conflicting results, with some studies showing relaxation (2) and others showing no relaxation (7, 31). Inhaled NO has a weak bronchodilator effect in vivo (22), whereas ANP is a bronchodilator in both normal (23) and asthmatic subjects (1, 24).

Despite the potential importance of cGMP pathways in regulating airway smooth muscle tone, no previous studies have comprehensively examined the regulation of cGMP concentrations in human airway smooth muscle. The purpose of this study was to use a number of pharmacological tools to characterize the mechanisms responsible for cGMP formation and breakdown in cultured human airway smooth muscle cells (HASMC).

MATERIALS AND METHODS

Materials. SKF-96231 was a gift from SmithKline Beecham Pharmaceuticals (Welwyn, UK). Unless otherwise stated, all other chemicals were obtained from Sigma-Aldrich (Poole, Dorset, UK). The peptides used were human 28-ANP and human 32-BNP and 22-CNP. All reagents were dissolved in Dulbecco’s modified Eagle’s medium (DMEM) with the excep-
tion of 3-isobutyl-1-methylxanthine (IBMX), zaprinast, and SKF-96231, which were first dissolved in ethanol to give a final concentration of 3% vol/vol ethanol in the bathing solution. The same concentration of ethanol was added to the bathing solutions of control experiments. All concentrations of reagents shown refer to the final concentration in the cell suspension.

**Cell culture.** Primary cultures of HASMC were prepared from explants of airway smooth muscle according to the method reported by Hall and co-workers (20) and Widdop et al. (49) with some modifications. Human trachea was obtained from postmortem individuals within 12 h of death. No patients had evidence of airway diseases as determined by history and pathological examination of the trachea and lungs. The tissue was transported to the laboratory in DMEM containing 10% fetal calf serum (FCS), penicillin G (50 U/ml), streptomycin (50 µg/ml), amphoterin B (2.5 µg/ml), and L-glutamine (4 mM). Tissue was then washed several times in the same medium. The tracheal smooth muscle was then dissected free of epithelium and connective tissue under sterile conditions. Small (2 × 2-mm) explants of airway smooth muscle were then excised, and about 10 explants were placed in small petri dishes in DMEM containing FCS, antibiotics, and amphotericin B, and L-glutamine. The explants were incubated in humidified 5% CO2-95% air at 37°C, and the medium was changed every 3 days. Smooth muscle cells were usually seen about 7 days later. When cells were near confluence, the explants were removed. Once confluent, cells were trypsinized with 0.25% trypsin and 0.02% EDTA in phosphate-buffered saline, centrifuged, and resuspended in the above medium. Next, the cells were counted and plated out in several 175-cm² flasks and were grown to confluence. Cells were then detached with trypsin-EDTA, resuspended in 90% FCS-10% dimethyl sulfoxide at a density of 10⁶ cells/ml, frozen in liquid nitrogen, and stored until required.

All experiments were performed in confluent HASMC that had been growth arrested for 24 h in serum-free DMEM containing insulin (6 µg/ml), transferrin (5 µg/ml), and ascorbic acid (35 µg/ml). The culture medium was changed immediately before each experiment, and cells were incubated with the pharmacological agents or vehicle controls at 37°C for 2 h (unless otherwise stated) in humidified 5% CO2-95% air. The pharmacological agents or vehicle controls at 37°C for 2 h (unless otherwise stated) in humidified 5% CO2-95% air. The cell suspensions were then detached with trypsin-EDTA, resuspended in 90% FCS-10% dimethyl sulfoxide at a density of 10⁶ cells/ml, frozen in liquid nitrogen, and stored until required. Cells were thawed before use and were plated at a density of 2 × 10⁴ cells/well in 12-well culture plates containing the above medium.

**Characterization of the HASMC.** We employed morphological and immunocytochemical staining to determine whether the cultured cells had the characteristics of airway smooth muscle cells. Under the light microscope, subconfluent HASMC were spindle shaped with central oval nuclei, whereas the confluent cells depicted the "hill-and-valley" appearance that is characteristic of smooth muscle cells in culture. For the identification of the markers of airway smooth muscle cell phenotype, the cells were plated in an eight-well chamber slide system, grown to confluence, growth arrested for 24 h, and fixed with methanol. The cells were then examined by standard immunocytochemical techniques using antibodies against smooth muscle cell-specific α-actin (Sigma) and mature muscle cell-specific desmin (Daco, High Wycombe, Bucks, UK). We demonstrated that >95% of the cells were positively labeled. In contrast, staining for the epithelial marker cytokeratin was negative.

**Cyclic GMP measurement.** cGMP was extracted by adding 1 ml of ice-cold 0.1 M hydrochloric acid. Cells were removed from culture wells mechanically. The resulting suspension was freeze-dried (SB9; Lab Plant, Huddersfield, Yorkshire, UK) before the measurement of cGMP content. cGMP was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (RPN 226; Amersham, Little Chalfont, Buckinghamshire, UK). The samples were first acetylated with a mixture of acetic anhydride and triethylamine to increase the sensitivity of the assay to 4 fmol/100 µl. The coefficient of variation of the assay was 11%. All samples were assayed in duplicate. The cells from six wells not used for cGMP assay were used to give a representative estimate of protein content in each experiment using the method of Bradford (6).

**RESULTS**

Effect of IBMX on basal and stimulated cGMP levels. Preliminary experiments were performed to determine if PDE inhibition was necessary to show effects of guanylyl cyclase activators in HASMC. S-nitroso-N-acetylpenicillamine (SNAP) was used as an NO donor and GCc activator, and IBMX was used as a nonspecific PDE inhibitor. After 1 h of incubation, cGMP levels in cells incubated with IBMX (10⁻³ M; 102 ± 24 fmol/mg protein) did not differ significantly from cells incubated with control (84 ± 12 fmol/mg protein; P = 0.3, n = 4). IBMX (10⁻³ M) increased cGMP production in response to SNAP (10⁻⁶ M), ANP, and 10⁻⁶ M SNAP was measured at 0, 1, 60, and 120 min with SNAP (10⁻³ M) plus IBMX (10⁻³ M) alanine (P < 0.01, n = 4; Fig. 1). Similar experiments using ANP as a GCc activator showed that ANP had no effect in the absence of IBMX. cGMP levels were 969 ± 201 fmol/mg protein in controls, 984 ± 118 in cells incubated with ANP (10⁻⁶ M) alone, and 2,179 ± 239 in cells treated with ANP (10⁻⁶ M) plus IBMX (10⁻³ M; P < 0.01; ANP + IBMX vs. ANP alone). In view of these results, all subsequent experiments were carried out in the presence of IBMX (10⁻³ M) unless otherwise stated.

**Time course of cGMP production.** cGMP production in response to 10⁻⁴ M SNAP was measured at 0, 1, 20, 40, 60, 120, and 180 min. There was a time-dependent increase in cGMP from 157 ± 12 fmol/mg protein at time 0 to 599 ± 191 fmol at 1 min (P < 0.01, n = 6), 1,054 ± 142 fmol/mg at 20 min (P < 0.001, n = 6), 1,146 ± 271 fmol/mg protein at 40 min (P < 0.01, n = 6), 1,882 ± 216 fmol/mg protein at 60 min (P < 0.001, n = 6), 3,493 ± 235 fmol/mg protein at 120 min (P < 0.001, n = 6), and 4,260 ± 451 fmol/mg protein at 180 min (P < 0.001, n = 6; Fig. 2).

**cGMP production in response to 10⁻⁵ M ANP, 10⁻⁵ M BNP, and 10⁻⁵ M CNP was measured at 1, 60, and 120
There was a time-dependent increase in cGMP for each peptide. ANP (10^{-5} M) increased cGMP levels from 170 ± 65 fmol/mg protein at time 0 to 1,747 ± 148 fmol/mg protein at 1 min (P < 0.001, n = 4), 3,920 ± 449 fmol/mg protein at 60 min (P < 0.01, n = 4), and 12,520 ± 618 fmol/mg protein at 120 min (P < 0.001, n = 4). BNP (10^{-5} M) increased cGMP levels from 170 ± 65 fmol/mg protein at time 0 to 729 ± 35 fmol/mg protein at 1 min (P < 0.01, n = 4), 3,589 ± 434 fmol/mg protein at 60 min (P < 0.01, n = 4), and 10,879 ± 652 fmol/mg protein at 120 min (P < 0.001, n = 4). CNP (10^{-5} M) increased cGMP levels from 170 ± 65 fmol/mg protein at time 0 to 1,784 ± 171 fmol/mg protein at 1 min (P < 0.001, n = 4), 3,337 ± 168 fmol/mg protein at 60 min (P < 0.001, n = 4), and 4,604 ± 466 fmol/mg protein at 120 min (P < 0.01, n = 4; Fig. 3).

In view of these results, in all subsequent experiments, HASMC were incubated with the pharmacological agents/vehicle control for 2 h.

Effect of activators of GCs on cGMP levels. The NO donors sodium nitroprusside (SNP; 10^{-6} to 10^{-3} M) and SNAP (10^{-6} to 10^{-3} M) were used to activate GCs. Both SNP and SNAP generated concentration-dependent elevations in cGMP levels. SNP increased cGMP levels from a control of 418 ± 42 fmol/mg protein to 2,539 ± 175 fmol/mg protein at the highest concentration tested (10^{-3} M; P < 0.001, n = 5; half-maximal effective concentration (EC_{50}) = 1.05 × 10^{-4} M). SNAP increased cGMP levels from a control level of 283 ± 34 fmol/mg protein to 4,369 ± 1,868 fmol/mg protein at the highest concentration tested (10^{-3} M; P < 0.05, n = 5; EC_{50} = 1.1 × 10^{-4} M; Fig. 4). SNAP was more potent than SNP, with a higher maximal response at 10^{-3} M (P < 0.01).
Effect of hemoglobin and methylene blue. The effect of hemoglobin (Hb; 5 × 10⁻⁵ M), which binds free NO (16), and methylene blue (MB; 5 × 10⁻⁴ M), a guanylyl cyclase inhibitor (19), were studied on SNP (5 × 10⁻⁵ M) and SNAP (10⁻⁵ M)-induced cGMP production. We used these relatively small concentrations of SNP and SNAP as Hb competitively binds NO and MB competitively inhibits guanylyl cyclase (51); therefore, small quantities of Hb and MB will not have a major effect on the response to large concentrations of SNP and SNAP. The effect of 1H[1,2,4]oxadiazolo[4,3-α]quinazolin-1-one (ODQ; 10⁻⁶ M), a new selective inhibitor of NO-sensitive guanylyl cyclase (13), was also studied on SNAP (10⁻⁵ M)-induced cGMP production. Hb, MB, and ODQ were preincubated with the cells for 1 h before the addition of SNP and SNAP.

cGMP production in response to SNP (5 × 10⁻⁵ M) was 832 ± 178 fmol/mg protein compared with 293 ± 21 fmol/mg protein in controls. Hb inhibited the SNP-induced increase by 79% to 404 ± 49 fmol/mg protein (P < 0.05, n = 4). MB abolished the SNP-induced increase, with the cGMP value in the presence of MB being 299 ± 63 fmol/mg protein (P < 0.05, n = 4). cGMP production in response to SNAP (10⁻⁵ M) was 2,632 ± 333 fmol/mg protein compared with 728 ± 52 in controls. SNAP-induced increases in cGMP were abolished by Hb or MB (both P < 0.001, n = 5), with cGMP values in the presence of Hb or MB being 411 ± 40 and 488 ± 78 fmol/mg protein, respectively (Fig. 5). ODQ (10⁻⁶ M) abolished the SNAP-induced cGMP response (P < 0.001, n = 5). cGMP production in response to SNAP (10⁻⁵ M) alone was 1,716 ± 144 compared with 1,173 ± 112 fmol/mg protein in controls and 996 ± 133 fmol/mg protein in the presence of SNAP plus ODQ.

Effect of activators of GCm on cGMP levels. ANP, BNP, CNP (all 10⁻⁹ to 10⁻⁵ M), and Escherichia coli heat stable toxin (Sta; 400 IU/ml) were used to activate GCm in the presence of phosphoramidon (10⁻⁶ M), a neutral endopeptidase inhibitor. All three natriuretic peptides produced significant elevations in cGMP levels (Fig. 6). cGMP levels increased from 78 ± 20 to 12,520 ± 618 fmol/mg protein with 10⁻⁵ M ANP (n = 4, P < 0.001, EC₅₀ = 1.0 × 10⁻⁷ M), from 278 ± 9 to 10,879 ± 652 fmol/mg protein with 10⁻⁵ M BNP (n = 4, P < 0.001, EC₅₀ = 1.8 × 10⁻⁷ M), and from 487 ± 126 to 4,604 ± 466 fmol/mg protein with 10⁻⁵ M CNP (n = 4, P < 0.001, EC₅₀ = 1.2 × 10⁻⁷ M; Fig. 6). To determine whether enkephalasenes were functionally active in HASMC, the effects of all three natriuretic peptides were studied in the presence and absence of phosphoramidon (10⁻⁶ M), a neutral endopeptidase inhibitor added concurrently. Phosphoramidon did not alter the response to any of the peptides (all P > 0.5, data not shown).

No significant change was observed in cGMP after incubation with Sta (400 IU/ml). cGMP levels were 156 ± 39 and 170 ± 65 fmol/mg protein in the presence and absence of Sta, respectively (n = 4, P = 0.3).

Effect of selective PDE inhibitors. Experiments were performed to compare the effect of selective type V PDE inhibitors SKF-96231 (10⁻⁴ M) and zaprinast (10⁻⁴ M) on cGMP levels after stimulation with SNP (10⁻³ M) or ANP (10⁻⁵ M). The concentrations of SKF-96231 and zaprinast used have been shown to be selective for PDE type V (46). In these experiments, the drugs or vehicle controls were added 1 h before the addition of SNAP (10⁻³ M) or ANP (10⁻⁵ M). cGMP levels increased significantly from 1,606 ± 34 fmol/mg protein with SNAP (10⁻³ M) alone to 2,515 ± 298 fmol/mg protein with SNP (10⁻³ M) plus SKF-96231 (10⁻⁴ M; P < 0.05, n = 4) and to 1,987 ± 166 fmol/mg protein with SNAP (10⁻³ M) plus zaprinast (10⁻⁴ M; P < 0.05, n = 4). cGMP levels increased significantly from 755 ± 34 fmol/mg protein with ANP (10⁻³ M) alone to 2,224 ± 154 fmol/mg protein with ANP (10⁻⁶ M) plus SKF-96231 (10⁻⁴ M; P < 0.001, n = 4) and to 1,250 ± 58 fmol/mg protein with ANP (10⁻⁵ M) plus zaprinast (10⁻⁴ M; P < 0.001, n = 4; Fig. 7).

**DISCUSSION**

Our results suggest that cultured HASMC contain both GC₅ and GCm. We performed experiments in growth-arrested cultured HASMC in their first and...
different from control at all values of 10⁻¹¹ M SKF (SKF; 10⁻¹¹ M BNP (●), and 10⁻⁸ to 10⁻³ M CNP (△), all in the presence of IBMX (10⁻² M) + phosphoramidon (10⁻⁶ M). Data represent means ± SE; n = 4. Error bars that are not visible lie within the data point. Values for ANP were significantly different from control at all concentrations of 10⁻⁹ M (P < 0.01) or greater, values for BNP were significantly different from control at all values of 10⁻⁸ M or greater (P < 0.01), and values for CNP were significantly different from control at all values of 10⁻⁷ M or greater (P < 0.05).

The time course of cGMP accumulation in response to both NO donors and natriuretic peptides showed a progressive increase over 2 h. We used a 2-h incubation in subsequent experiments to maximize the cGMP signal and to allow pharmacological characterization of the response with inhibitors. The time course of cGMP production in our experiments is similar to that seen in other cultured cells (15) and airway epithelial cells (39) in which measurements were made in the presence of PDE inhibitors. In experiments with bovine and human tracheal strips in the absence of PDE inhibitors, a more rapid response was seen, which waned with time (40, 48). The different time course is likely to be related to the use of PDE inhibitors.

In experiments using SNP and SNAP to activate GCₐ, we saw large increases in cGMP with both compounds, with SNAP being the more potent. The response to SNP and SNAP was inhibited by MB, an inhibitor of GCₐ (19), indicating involvement of GCₐ in the response. The response to SNP and SNAP was also inhibited by Hb, which acts as an NO scavenger (16), suggesting that these compounds act via NO release. Although no previous studies have looked at the effect of GCₐ activators on cGMP levels in cultured HASMC, there have been studies measuring cGMP production in whole airway tissue preparations from animal airways. The increases in cGMP seen in our experiments with NO donors are broadly similar to those seen in previous studies in bovine (40), canine (51), and human (48) tracheal strips and intact human bronchi (14). The functional correlates of these findings have been studied in canine (51), guinea pig (42), and bovine (29, 40) tracheal strips in which SNP and other nitrovasodilators produced concentration-dependent relaxation that correlated with the cGMP elevation. In HASMC, NO donors also cause relaxation (48). Until recently, no endogenous mechanism for the control of airway tone was known to use the cGMP pathway in airway smooth muscle. However, several reports have shown that NO synthase inhibitors can inhibit the relaxant response to electrical field stimulation (28, 32), suggesting that NO is the neurotransmitter responsible for nonadrenergic noncholinergic airway relaxation.

We used ANP, BNP, CNP, and STa to activate GCₐ (50). All three natriuretic peptides increased cGMP in a concentration-dependent manner, whereas STa was without effect. Four classes of GCₐ, that can be distinguished in part by their binding affinity for natriuretic peptides and related compounds have been described (50). Type A guanylyl cyclase preferentially binds ANP and BNP. Type B guanylyl cyclase preferentially binds CNP. Type C guanylyl cyclase is mainly found in the gastrointestinal tract and binds the STa as well as the endogenous peptide hormone guanylin. The specificity of the fourth member of this class, retinal guanylyl cyclase, has been less well characterized. The order of potency seen in our experiment (ANP > BNP > CNP) is consistent with the type A GCₐ subclass of GCₐ present in HASMC. The ANP that we used (human 28-ANP) is that which has previously been shown to have bronchodilator properties in humans (23, 24). The lack of response to STa suggests that C-type GCₐ is not expressed in these cells.
Early studies in human isolated bronchi were unable to show relaxation with ANP (7, 31). Candenas et al. (7) and Labat et al. (31) suggested that one possible explanation for this was that there are no ANP receptors on human airway smooth muscle and that the bronchodilator effect seen was an indirect one. Our result shows that this is clearly not the case. Subsequent studies by other authors have shown that ANP can relax HASMC and shift the concentration curve to methacholine to the right, suggesting that the increases in cGMP that we have seen are functionally important (2). None of these studies measured cGMP accumulation in response to natriuretic peptides.

ANP produces significant bronchodilatation when given intravenously to both normal (23) and asthmatic subjects (1) and by inhalation in asthmatics (24). The effect of ANP was greater and more prolonged by the intravenous route, suggesting that peptidases present in airway epithelium may degrade it. Experiments in intact human tracheal preparations in vitro are consistent with this and have shown that the response to ANP can be potentiated by phosphoramidon, an inhibitor of neutral endopeptidase (2). In our experiments, we found no effect of phosphoramidon on cGMP levels produced by the natriuretic peptides. The most likely explanation for this is that neutral endopeptidase is functionally more important in airway epithelial or submucosal tissue and is not present in large quantities in airway smooth muscle. This is consistent with immunocytochemical studies showing a predominantly epithelial distribution of neutral endopeptidase (27).

The greater rise in cGMP seen in our experiments with the natriuretic peptides suggest that GC_m is present in higher quantities than GC_s in cultured HASMC. This contrasts with bovine trachea in which SNP produced a greater rise in cGMP than ANP (40). The relative effects of activators of GC_s and GC_m in our studies mirror the relative effects of these agents on human bronchomotor tone in vivo in which ANP causes moderate bronchodilatation (1, 24), whereas the effects of NO donors have been less marked (22).

Cyclic nucleotides are broken down by PDE, of which there are at least seven subtypes that differ in their affinity for cAMP and cGMP (3, 34, 46). Anion exchange chromatography has revealed the presence of types II, III, IV, and V in human tracheal strips (17). Type V PDE is cGMP specific. Our results suggest that type V PDE is involved in cGMP degradation in HASMC, as SKF-96231 and zaprinast, which both selectively inhibit type V PDE (3, 46), increased SNAP- and ANP-induced cGMP accumulation. These results are consistent with functional studies in human tracheal strips showing that zaprinast relaxes spontaneous and methacholine-induced tone (4).

In conclusion, our study demonstrates that both GC_s and GC_m are present in HASMC. Pharmacological characterization shows that type A is the dominant form of GC_m expressed. Type V PDE is likely to be responsible for cGMP degradation in HASMC. Cultured HASMC provide a useful model to study the regulation of guanylyl cyclases.

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