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Differential effects of soluble and particulate guanylyl cyclase on Ca^{2+} sensitivity in airway smooth muscle

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Rho, Edwin H., William J. Perkins, Robert R. Lorenz, David O. Warner, and Keith A. Jones. Differential effects of soluble and particulate guanylyl cyclase on Ca^{2+} sensitivity in airway smooth muscle. *J Appl Physiol* 92: 257–263, 2002.—Maximal relaxation of airway smooth muscle (ASM) in response to atrial natriuretic peptide (ANP), which stimulates particulate guanylyl cyclase (pGC), is less than that produced by nitric oxide (NO) and other compounds that stimulate soluble guanylyl cyclase (sGC). We hypothesized that stimulation of pGC relaxes ASM only by decreasing intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), whereas stimulation of sGC decreases both $[Ca^{2+}]_i$ and the force developed for a given $[Ca^{2+}]_i$ (i.e., the Ca^{2+} sensitivity) during muscarinic stimulation. We measured the relationship between force and $[Ca^{2+}]_i$ (using fura 2) under control conditions (using diltiazem to change $[Ca^{2+}]_i$) and during exposure to ANP, diethylamine-NO (DEA-NO), sodium nitroprusside (SNP), and the *Sp* diastereoisomer of β-phenyl-1,N^2-etheno-8-bromoguanosine-3′,5′-cyclic monophosphorothionate (*Sp*-8-Br-PET-cGMPS), a cell-permeant analog of cGMP. Addition of DEA-NO, SNP, or *Sp*-8-Br-PET-cGMPS decreased both $[Ca^{2+}]_i$ and force, causing a significant rightward shift of the force-$[Ca^{2+}]_i$ relationship. In contrast, with ANP exposure, the force-$[Ca^{2+}]_i$ relationship was identical to control, such that ANP produced relaxation solely by decreasing $[Ca^{2+}]_i$. Thus, during muscarinic stimulation, stimulation of pGC relaxes ASM exclusively by decreasing $[Ca^{2+}]_i$, whereas stimulation of sGC decreases both $[Ca^{2+}]_i$ and Ca^{2+} sensitivity.

calcium sensitivity; bronchodilation; nitrovasodilators

INCREASES IN THE INTRACELLULAR concentration of cGMP ($[cGMP]_i$) relax smooth muscle. Sources of cGMP include both soluble (sGC) and particulate (pGC) forms of guanylyl cyclase. sGC is a soluble enzyme that is activated by the binding of nitric oxide (NO) and NO donors to a heme iron center (15). pGC is a membrane receptor for natriuretic peptides, such as atrial natriuretic peptide (ANP) and related hormones (21). Studies in a variety of smooth muscle types show that cGMP activates cGMP-dependent protein kinases (cGK), which phosphorylate substrates that subsequently reduce both the concentration of intracellular Ca^{2+} ($[Ca^{2+}]_i$) (13, 18, 24, 25) and the force developed for a given $[Ca^{2+}]_i$ (i.e., the Ca^{2+} sensitivity) (6, 24, 29, 31, 38, 41). cGMP from both sources is metabolized to 5′-GMP by phosphodiesterases (11).

Airway smooth muscle expresses both sGC and pGC (11, 12), and agents that stimulate sGC or pGC can relax airway smooth muscle in vitro (16, 17, 40) and produce bronchodilation in vivo (3, 4, 14). Presumably, similar increases in $[cGMP_i]$ from either sGC or pGC should have similar effect on the airways. However, prior studies have noted that the maximal relaxation produced by stimulation of pGC via ANP is considerably less than that produced by NO donors such as sodium nitroprusside (SNP) (16, 40). This occurs even though the increases in $[cGMP]_i$ produced by pGC stimulation were similar to those produced by stimulation of sGC. There are at least three possible explanations for this observation. First, our laboratory and others have suggested that some NO donors may have additional actions to relax smooth muscle that are not mediated via cGMP (9, 31, 32, 36, 37). Stimulation of membrane receptors by agents such as ANP would not be expected to produce such effects. Second, it has been suggested that inhomogeneities in intracellular cGMP distribution arising from different sources (sGC vs. pGC) may affect its mechanism of action (40). For example, local concentrations of cGMP may be high at sites immediately adjacent to the cell membrane when generated via pGC, whereas distribution may be more homogeneous with sGC stimulation. If this is so, then stimulation of pGC may produce effects primarily via affecting membrane targets such as ion channels that affect $[Ca^{2+}]_i$, rather than cytosolic targets such as smooth muscle protein phosphatases that affect Ca^{2+} sensitivity. Finally, pGC consists of at least four distinct receptors [natriuretic peptide receptor (NPR)-A, B, C, and D] (7), some of which have other actions. For example, NPR-C is devoid of guanylyl cyclase activity, and it activates guanine nucleotide-binding proteins (2). Thus it is possible that, in addition to causing increases in $[cGMP]_i$, ANP could activate other second-messenger systems that regulate force.

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The aim of this study was to explore the mechanisms responsible for the differences in response of airway smooth muscle to compounds that activate sGC and pGC. We tested the hypothesis that, unlike compounds that stimulate sGC, ANP relaxes airway smooth muscle primarily by reducing $[Ca^{2+}]_i$ rather than by reducing Ca^{2+} sensitivity. We measured the relationship between force and $[Ca^{2+}]_i$ under control conditions and during exposure to ANP, NO [provided by the NO-nucleophile adduct diethylammonium (*Z*)-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (DEA-NO)], the NO donor SNP, and the *Sp* diastereoisomer of β-phenyl-1,N^2-etheno-8-bromoguanosine-3′,5′-cyclic monophosphorothionate (*Sp*-8-Br-PET-cGMPS), a stable cell-permeant analog of cGMP.

MATERIALS AND METHODS

Experimental Techniques

Tissue preparation. All experiments were performed in accordance with the guidelines established by our Institutional Animal Care and Use Committee. Adult pigs (80–95 kg) of either gender were anesthetized with an intravenous injection of pentobarbital sodium (25 mg/kg) and exsanguinated or were obtained immediately after slaughter from a local abattoir. A 5- to 10-cm portion of extrathoracic trachea was excised and immersed in chilled physiological salt solution (PSS) of the following composition (mM): 110.5 NaCl, 25.7 $NaHCO_3$, 5.6 dextrose, 3.4 KCl, 2.4 $CaCl_2$, 1.2 KH_2PO_4, and 0.8 $MgSO_4$. For all experiments, the PSS was bubbled with 94% O_2-6% CO_2 ($pH \approx 7.4$, $P_{O_2} \approx 400$ Torr, $P_{CO_2} \approx 39$ Torr). Fat, cartilage, connective tissue, and epithelium were removed with tissue forceps and scissors. Thin strips of tracheal smooth muscle were dissected from the sheet of tissue under microscopic observation.

Isometric force and fura 2 fluorescence measurements. Muscle strips (width 0.2–0.4 mm and length 6–9 mm) were incubated with PSS (25°C) containing 5 μM fura 2-AM (fura 2) for 3–4 h (18). Fura 2 was dissolved in DMSO and 0.004% cremaphor. After fura-2 loading, the muscle strips were mounted in a 0.1 ml quartz cuvette and continuously superfused at 2 ml/min with PSS (37°C) for ~2–3 h to remove extracellular fura 2 and DMSO and to allow deesterification of any remaining cytosolic fura 2. One end of the strips was anchored via stainless steel microforceps to a stationary metal rod and the other end via stainless steel microforceps to a calibrated force transducer (model AE801, Aksjeselskapet Mikro Elektronikk). During the washout period, the length of the strips was increased after repeated isometric contractions (of 2- to 3-min duration) induced by 1 μM ACh until the optimal length was obtained (resting tension 0.02–0.04 mN). Each strip was maintained at this optimal length for the remainder of the experiment.

Fura 2 fluorescence intensity was measured by a photometric system (model ph2, Scientific Instruments, Heidelberg, Germany) that measures optical and mechanical parameters of isolated tissue simultaneously. This system has been described in detail previously (13). Light from a xenon high-pressure lamp was monochromatically filtered to restrict excitation light to 340-nm and 380-nm wavelengths. Excitation light at these two wavelengths was alternated every 2 ms and was focused onto the muscle strips by a high-aperture objective. Surface fluorescence emitted from the strips was filtered at 500 ± 5 nm and detected by a photomultiplier. The emission fluorescence intensities due to excitation at 340 nm (F_{340}) and 380 nm (F_{380}) wavelengths were measured and the F_{340}-to-F_{380} ratio was used as an index of $[Ca^{2+}]_i$ (18).

Cyclic nucleotide measurements. Frozen muscle strips (width 1–2 mm and length 1.5–2.0 cm) were homogenized in 4 ml of cold (2°C) 100% ethanol by using a ground-glass pestle and homogenizing tube. The precipitated pellet was separated from the soluble extract by centrifugation at 4,000 *g* for 10 min. The soluble extract was evaporated to dryness at ~55°C under a stream of nitrogen and suspended in 0.3 ml of 4 mM EDTA (pH 7.5). [^{3}H]cGMP (0.4 μCi) was added as a tracer for cGMP recovery determinations. Commercially available radioimmunoassay kits were used to determine the concentrations of cGMP in the soluble extract (5). The protein content of the precipitated pellet was determined by the method described by Lowry et al. (23), with bovine serum albumin dissolved in 1 N NaOH as the standard. $[cGMP]_i$ was expressed as picomoles per milligram of protein.

Experimental Protocols

Each experimental protocol was conducted with separate sets of muscle strips. For each protocol, all strips exposed to SNP, ANP, DEA-NO, or *Sp*-8-Br-PET-cGMPS were incubated with 10 μM indomethacin to prevent the formation of prostanoids, which might affect measurement of cyclic nucleotides (19, 43). Preliminary studies showed that indomethacin did not affect the force-Ca^{2+} relationship in response to diltiazem (data not shown).

Effect of SNP, ANP, (Sp-8-Br-PET-cGMPS), or DEA-NO on the relationship between isometric force and $[Ca^{2+}]_i$. In this protocol, the relationship between $[Ca^{2+}]_i$ and isometric force was determined in strips precontracted with the physiological agonist ACh. A control relationship was determined by exposing strips to increasing concentrations of diltiazem, which reduces Ca^{2+} influx and $[Ca^{2+}]_i$ without affecting Ca^{2+} sensitivity. The effect of SNP, ANP, or DEA-NO on the relationship was determined by comparing this control relationship with the relationship measured during exposure to each compound. In preliminary studies, we confirmed that diltiazem (10 μM) does not affect Ca^{2+} sensitivity when added to strips permeabilized with β-escin according to our laboratory's published techniques (20) and stimulated with Ca^{2+} and ACh (data not shown). Preliminary studies also showed that the response of both force and F_{340}/F_{380} to ACh was stable in the absence of these compounds over the time needed to complete the study (data not shown), a finding consistent with our laboratory's previous work (13, 18, 19).

Each muscle strip was contracted for ~15 min with a concentration of ACh sufficient to produce ~30% of maximal isometric force (0.04 ± 0.02 μM ACh) until both isometric force and F_{340}/F_{380} were stable. The response to diltiazem (0.3, 1, and 3 μM), SNP (0.1, 0.4, and 4 μM), ANP (200 nM), *Sp*-8-Br-PET-cGMPS (1, 3, and 10 μM), or DEA-NO (0.003, 0.01, and 0.1 μM) was then determined. If, after washout with PSS, force and F_{340}/F_{380} returned to baseline values, and if a subsequent response of isometric force and F_{340}/F_{380} to ACh was identical to the initial response, a second compound was studied in a single strip.

Effect of ANP and SNP on $[cGMP]_i$. Two sets of six muscle strips obtained from each animal were pinned in wells containing PSS and stimulated with 0.04 μM ACh for 15 min. One strip from each set was rapidly frozen by immersion in liquid N_2 for 0.5 min to obtain the baseline $[cGMP]_i$ measurements. Then, either 200 nM ANP or 0.4 μM SNP was added to the other five strips from each set for 0.5, 1, 1.5, 2, and 10 min, and the strips were frozen with liquid N_2. The strips were kept frozen at −70°C until $[cGMP]_i$ measurements were made.

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Materials

Radioimmunoassay kits for cGMP measurements were purchased from Amersham (Arlington Heights, IL). DEA-NO was purchased from Cayman Chemical (Ann Arbor, MI). SNP was purchased from Research Biochemicals International (Natick, MA). All other drugs and chemicals were purchased from Sigma Chemical (St. Louis, MO). Stock solutions of fura 2-AM were prepared in DMSO and cremaphor. All other drugs and chemicals were prepared in distilled water.

Statistical Analysis

Isometric force and F_{340}/F_{380} were normalized to the steady-state initial values measured immediately before the addition of the test compound to the superfusate. Values for both were measured after stable responses to each compound were achieved.

Comparisons of $[cGMP]_i$ were made by repeated measures analysis of variance and Dunnett's *t*-test for post hoc analysis. To determine whether the single dose of ANP studied altered Ca^{2+} sensitivity, a sigmoidal four-parameter regression of the control isometric force values measured during relaxation by diltiazem was generated. With use of this regression, the F_{340}/F_{380} value was calculated for the amount of isometric force measured during relaxation with ANP. Then this interpolated F_{340}/F_{380} value was compared with the F_{340}/F_{380} value measured during relaxation induced by ANP using an unpaired Student's *t*-test. To determine whether SNP, DEA-NO, or *Sp*-8-Br-PET-cGMPS altered Ca^{2+} sensitivity, the F_{340}/F_{380} at half-maximal relaxation induced by diltiazem (control), SNP, DEA-NO, or *Sp*-8-Br-PET-cGMPS was compared by using sigmoidal four-parameter regression to calculate interpolated values for each condition. These interpolated values were then compared by analysis of variance, and Student-Newman-Keuls test was used for post hoc analysis. A P value < 0.05 was considered statistically significant.

RESULTS

Effect of ANP, SNP, DEA-NO, or Sp-8-Br-PET-cGMPS on the Relationship Between Isometric Force and $[Ca^{2+}]_i$

Figures 1, 2, and 3 show representative tracings of the effect of diltiazem, SNP, and ANP, respectively, on isometric force and F_{340}/F_{380} in a tracheal smooth muscle strip contracted with 0.04 μM ACh. ACh caused sustained increases in both isometric force and F_{340}/F_{380} that reached steady-state levels within 15 min. Addition of diltiazem (Fig. 1) or SNP (Fig. 2) caused sustained, concentration-dependent decreases in both isometric force and F_{340}/F_{380}. The addition of ANP (Fig. 3) caused an initial rapid decline in both isometric force and F_{340}/F_{380}, which partially recovered over 5–10 min. Isometric force and F_{340}/F_{380} returned to baseline values in all strips after washout with PSS within 5 min.

The stable responses of force and F_{340}/F_{380} to increasing concentrations of diltiazem and to the single concentration of ANP were plotted (Fig. 4). The decrease in F_{340}/F_{380} produced by ANP for the observed decrease in force did not differ significantly from that predicted by the control F_{340}/F_{380}-force relationship measured by using diltiazem (32 ± 18 and 34 ± 16%, respectively.) That is, ANP did not change the relationship between force and $[Ca^{2+}]_i$, indicating that it relaxed the strips solely by decreasing $[Ca^{2+}]_i$, without affecting Ca^{2+} sensitivity.

Addition of DEA-NO, SNP, or *Sp*-8-Br-PET-cGMPS also caused concentration-dependent decreases in both F_{340}/F_{380} and force. Compared with the relationship measured during exposure to diltiazem, all of these

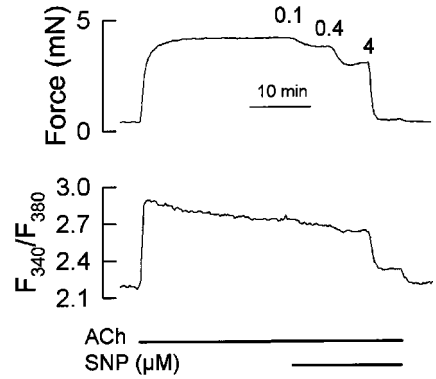

Fig. 2. Representative tracing of the effect of sodium nitroprusside (SNP; 0.1, 0.4, and 4 μM) on isometric force *(top)* and F_{340}/F_{380} *(bottom)* in a tracheal smooth muscle strip contracted with 0.04 μM ACh.

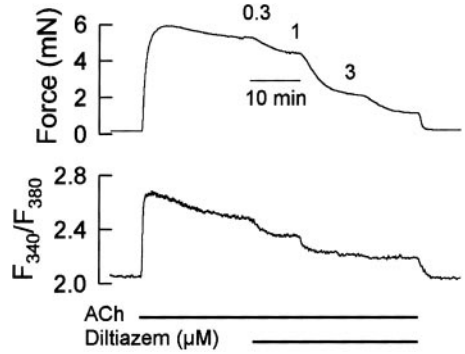

Fig. 1. Representative tracing of the effect of diltiazem on isometric force *(top)* and ratio of emission fluorescence intensities due to excitation at 340- and 380-nm wavelengths (F_{340}/F_{380}; *bottom*) in a tracheal smooth muscle strip contracted with 0.04 μM ACh.

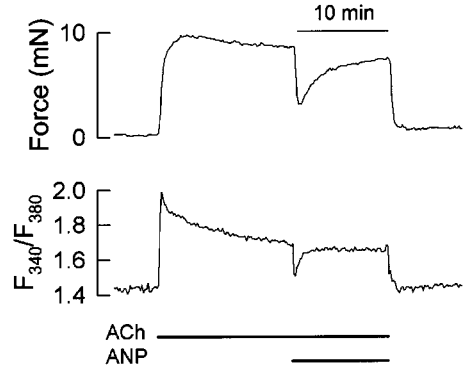

Fig. 3. Representative tracing of the effect of atrial naturetic peptide (ANP; 200 mM) on isometric force *(top)* and F_{340}/F_{380} *(bottom)* in a tracheal smooth muscle strip contracted with 0.04 μM ACh.

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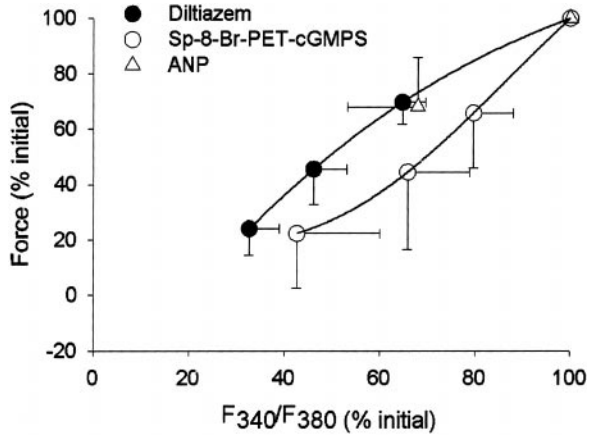

Fig. 4. Relationships between F_{340}/F_{380} (an index of intracellular Ca^{2+} concentration) and isometric force in porcine tracheal smooth muscle strips initially contracted with ACh (to ~30% of maximal force) and then relaxed with diltiazem (0.3, 1, 3 μM), ANP (200 nM), or the *Sp* diastereoisomer of β-phenyl-1,N^2-etheno-8-bromoguanosine-3′,5′-cyclic monophosphorothionate (*Sp*-8-Br-PET-cGMPS; 1, 3, and 10 μM). Values are means ± SD; n = 10 animals for diltiazem, n = 5 animals for other compounds.

compounds caused a significant rightward shift of the F_{340}/F_{380}-force relationship, such that, for a given force, the F_{340}/F_{380} was greater in the presence of the compound (Figs. 4 and 5). The F_{340}/F_{380} producing 50% of initial force (calculated by interpolation of the fitted relationship between F_{340}/F_{380} and force) was 87 ± 5% of the initial F_{340}/F_{380} for SNP, 76 ± 3% for DEA-NO, 68 ± 10% for *Sp*-8-Br-PET-cGMPS, and 50 ± 8% of the initial F_{340}/F_{380} for diltiazem. These values for SNP, DEA-NO, and *Sp*-8-Br-PET-cGMPS were all significantly greater than that for diltiazem ($P < 0.001$). Thus these agents relaxed the strips both by decreasing $[Ca^{2+}]_i$ and by decreasing the force developed for a given $[Ca^{2+}]_i$ (the Ca^{2+} sensitivity). The value for SNP

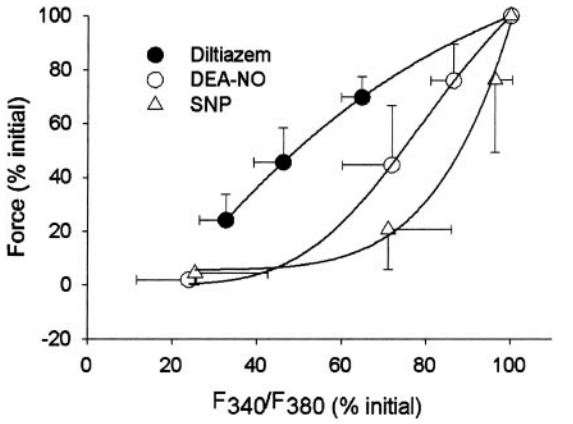

Fig. 5. Relationships between F_{340}/F_{380} (an index of intracellular Ca^{2+} concentration) and isometric force in porcine tracheal smooth muscle strips initially contracted with acetylcholine (to ~30% of maximal force) and then relaxed with diltiazem (0.3, 1, and 3 μM), SNP (0.1, 0.4, and 4 μM), or diethylammonium (*Z*)-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (DEA-NO; 0.003, 0.01, and 0.1 μM). Lines show curves fit with nonlinear regression. Values are means ± SD, n = 10 animals for diltiazem, n = 6 animals for other compounds.

was significantly greater than those for both DEA-NO and *Sp*-8-Br-PET-cGMPS ($P < 0.05$); i.e., SNP caused a significantly greater rightward shift of the F_{340}/F_{380}-force relationship compared with these other two compounds.

Effect of ANP or SNP on $[cGMP]_i$

Addition of 200 nM ANP to the tissues induced a significant time-dependent increase in $[cGMP]_i$. The increase in $[cGMP]_i$ was significant beginning at 1 min, reached a maximal value at ~1.5 min, and then declined to levels sustained above baseline values at 10 min (Fig. 6*A*). The $[cGMP]_i$ at 10 min after ANP increased 3.3-fold above baseline values (from 0.27 ± 0.11 to 0.90 ± 0.41 pmol/mg protein). Addition of 400 nM SNP significantly increased $[cGMP]_i$ compared with baseline values only at 10 min after administration, a 1.6-fold increase (from 0.27 ± 0.13 to 0.43 ± 0.28 pmol/mg protein) (Fig. 6*B*).

DISCUSSION

The major finding of this study is that, during muscarinic stimulation, compounds that stimulate pGC relaxes airway smooth muscle exclusively by decreasing $[Ca^{2+}]_i$, whereas compounds that stimulate sGC decrease both $[Ca^{2+}]_i$ and Ca^{2+} sensitivity.

cGMP can relax airway smooth muscle via several mechanisms that can be broadly classified as affecting

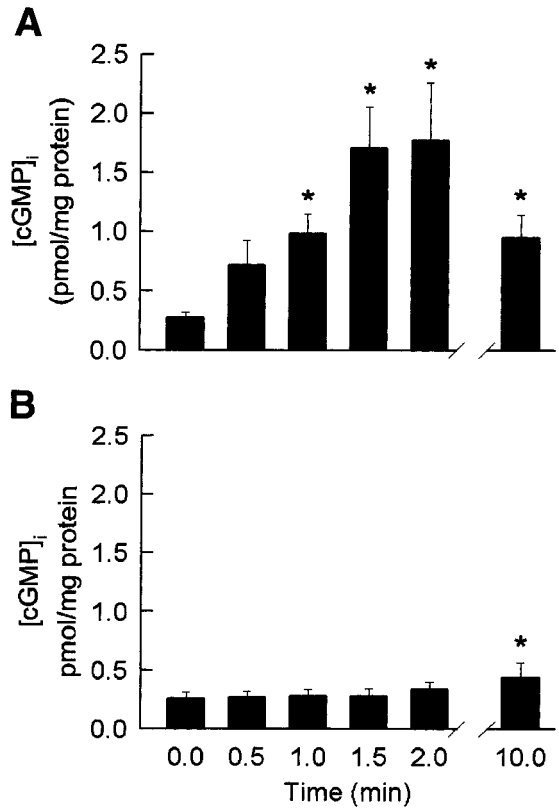

Fig. 6. Time course for the effect of 200 nM (*A*) or 400 nM SNP (*B*) on $[cGMP]_i$ in tracheal smooth muscle strips stimulated with 0.04 μM ACh. Values are means ± SD; n = 9 animals. *Statistically significant difference from baseline, ANOVA with Dunnett's test, $P < 0.05$.

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either $[Ca^{2+}]_i$ or the force developed for a given $[Ca^{2+}]_i$. All actions are presumably mediated via the phosphorylation of target proteins by cGK (8). Several mechanisms tend to decrease $[Ca^{2+}]_i$, including enhanced Ca^{2+} sequestration into the sarcoplasmic reticulum (26), inhibition of currents through L-type Ca^{2+} channels (33), stimulation of Ca^{2+}-activated K^+ channels (42), and inhibition of receptor-mediated signal transduction (22). cGMP also decreases Ca^{2+} sensitivity. In phasic smooth muscle, the likely mechanism is the phosphorylation of telokin by PKC, which then activates smooth muscle protein phosphatase and thus accelerates the dephosphorylation of the regulatory myosin light chain (34, 41). However, telokin is scarce in tonic smooth muscle such as airway smooth muscle (34). Our laboratory's prior data in canine tracheal smooth muscle suggest that the predominant mechanism by which cGMP decreases Ca^{2+} sensitivity is an inhibition of muscarinic receptor-mediated signal transduction by processes that remain to be defined (20).

In our study, stimulation of pGC by ANP produced a biphasic response in both force and $cGMP_i$, with an initial rapid transient phase, followed by a plateau. For this reason, we studied only a single concentration of ANP, rather than obtaining a full dose-response relationship as for the other compounds. This concentration was found to produce a maximal response in preliminary studies (data not shown). This maximal concentration produced only a relatively modest reduction in force (~30%). Of interest, the peak in force response preceded the peak in the response of $[cGMP]_i$, suggesting that there is not a simple relationship between the measured $[cGMP]_i$ and the force response. Responses to ANP were measured during a relatively low degree of muscarinic stimulation (~30% of maximal response), because we noted in preliminary data that there was little response to ANP during exposure to high concentrations of ACh. All of these features are consistent with prior investigations of ANP in airway smooth muscle (16, 17, 40).

When $[cGMP]_i$ was increased by activation of sGC, we noted differences in the relationship between $[cGMP]_i$ and relaxation when compared with that measured in response to ANP. SNP (400 nM) produced greater relaxation compared with 200 nM ANP (79 vs. 32%) yet was accompanied by a much smaller increase in $[cGMP]_i$ (1.6- vs. 3.3-fold increase at 10 min after stimulation). In addition, this increase did not reach statistical significance until 10 min after SNP exposure, whereas the force response was more rapid (Fig. 2). In a previous investigation (37), our laboratory found that 0.1 μM DEA-NO produced a similar steady-state increase in $[cGMP]_i$ compared with ANP (2.7-fold) in porcine tracheal smooth muscle, yet, like SNP, this concentration of DEA-NO was sufficient to cause complete relaxation in the present study. Thus, for a given increase in $[cGMP]_i$, relaxation produced by stimulation of pGC appears to be less than relaxation produced by stimulation of sGC, a finding similar to those of prior studies (16, 40). This difference is associated with a differential pattern of effect on the relationship between force and $[Ca^{2+}]_i$, assessed in this study by comparison of the F_{340}/F_{380}-force relationship measured by progressive inhibition of Ca^{2+} influx through L-type voltage-sensitive Ca^{2+} channels with diltiazem with that measured during stimulation of pGC or sGC. This relationship was not affected by exposure to ANP, demonstrating that stimulation of pGC during muscarinic stimulation produced relaxation solely by decreasing $[Ca^{2+}]_i$. In contrast, the relationship was altered by both SNP and DEA-NO, such that less force was maintained at a given $[Ca^{2+}]_i$, demonstrating that these agents produced relaxation both by decreasing $[Ca^{2+}]_i$ and by decreasing Ca^{2+} sensitivity.

This differential effect on Ca^{2+} sensitivity between compounds that stimulate pGC and those that stimulate sGC could occur if the latter have effects in addition to increasing $[cGMP]_i$. There is evidence for these actions in prior studies in airway smooth muscle for several NO donors (6, 9, 31, 32, 36). For example, our laboratory showed that, although inhibitors of sGC can block DEA-NO-induced relation of porcine tracheal smooth muscle, they do not block the action of SNP (37). For this reason, we also examined the effects of a cell-permeant cGMP analog on Ca^{2+} sensitivity, confirming that intracellular concentrations of cGMP sufficient to produce significant relaxation by itself decreases both $[Ca^{2+}]_i$ and Ca^{2+} sensitivity, producing a pattern of results very similar to that observed in response to DEA-NO. Of interest, the effect of SNP on Ca^{2+} sensitivity, as quantified by the F_{340}/F_{380} producing 50% of initial force, was greater than the effect of the cGMP analog or DEA-NO, suggesting that SNP may indeed have effects on Ca^{2+} sensitivity in addition to those caused by increases in $[cGMP]_i$. The mechanism of this additional effect is unknown, but it may be related to the generation of superoxide (30) or peroxynitrite (1, 35) by SNP. The ability of the cGMP analog to affect Ca^{2+} sensitivity suggests that the increases in $[cGMP]_i$ produced by ANP should have also decreased Ca^{2+} sensitivity. Indeed, the relatively lesser effect of ANP on force compared with agents that stimulate sGC is explained in part by this lack of effect on Ca^{2+} sensitivity.

Why then did the substantial increases in $[cGMP]_i$ produced by ANP not affect Ca^{2+} sensitivity? It is possible that cGMP produced via stimulation of pGC at the cell membrane does not have access to regions of the cell that regulate Ca^{2+} sensitivity. This could arise from either a physical barrier to diffusion or a functional barrier such as that presented by a high level of phosphodiesterase activity. Precedent for the concept of regional differences in intracellular concentrations of mediators that regulate contraction comes from the superficial buffer barrier hypothesis. This posits that superficial sarcoplasmic reticulum creates a gradient between $[Ca^{2+}]$ in the immediate subplasmalemmal area and the more interior portions of the cytosol (39). Whether such gradients may exist for mediators such as cGMP is not known. If present, it would require very high local concentrations of cGMP during ANP stimu-

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lation, because relatively high concentrations were measured in homogenates derived from the whole cell. Another explanation may lie in the quite different kinetics of the responses to ANP compared with the other compounds studied (compare Figs. 2 and 3). With ANP, only one aspect of cGMP-induced relaxation may be activated. For example, the NO donors may produce a steady-state relaxation that includes both a rapid inhibition of Ca^{2+} channels (42) and more delayed effects on pathways that control Ca^{2+} sensitivity (6, 24). Because of its more transient nature, ANP may activate only the initial rapid effect on Ca^{2+} channels (an effect facilitated by the membrane location of pGC). A final possible explanation is the presence of ANP-receptor subtypes that activate second-messenger systems other than cGMP, such as G proteins. For example, NPR-C receptors couple to pertussis toxin-sensitive G proteins in smooth muscle (27, 28). The effects of such activation on the regulation of Ca^{2+} sensitivity, and indeed the presence of such receptors in airway smooth muscle, are unknown.

In summary, activation of guanylyl cyclase relaxes airway smooth muscle stimulated by a muscarinic agonist via different mechanisms that depend on the identity of the enzyme stimulated. Stimulation of pGC relaxes the muscle exclusively by decreasing $[Ca^{2+}]_i$, whereas compounds that stimulate sGC decrease both $[Ca^{2+}]_i$ and Ca^{2+} sensitivity. This observation may explain at least in part why ANP, which stimulates pGC, can only partially relax airway smooth muscle, whereas compounds that stimulate sGC can produce complete relaxation.

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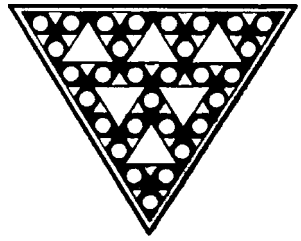

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