Actions of C-Type Natriuretic Peptide and Sodium Nitroprusside on Carbachol-Stimulated Inositol Phosphate Formation and Contraction in Ciliary and Iris Sphincter Smooth Muscles

Ke-Hong Ding and Ata A. Abdel-Latif

**Purpose.** To investigate the effects of C-type natriuretic peptide (CNP) and sodium nitroprusside (SNP) on cyclic guanosine monophosphate (cGMP) accumulation and on carbachol (CCh)-stimulated inositol 1,4,5-triphosphate (IP₃) production and contraction in ciliary muscle (CM) and iris sphincter (Sph) isolated from bovine and other mammalian species.

**Methods.** Ciliary muscle and sphincter isolated from cows, cats, dogs, rabbits, monkeys, and humans were used. Bovine specimens were used in the present work. Accumulation of cGMP and cyclic adenosine monophosphate (cAMP) in tissue extracts was measured by radioimmunoassay, IP₃ production was measured by ion-exchange chromatography, and changes in tension were recorded isometrically.

**Results.** In general, CNP and SNP exerted differential inhibitory effects on muscarinic-receptor-induced responses in CM and Sph isolated from the various species. Thus in bovine CM, SNP stimulated cGMP formation in a time- and concentration-dependent manner and dose dependently inhibited CCh-induced IP₃ production and contraction. These effects were inhibited by LY 83583, a soluble guanylyl cyclase inhibitor, and mimicked by 8-Br-cGMP, a cell-membrane permeable analogue of cGMP. The inhibitory effects of the soluble cGMP analogue are tissue and species specific. Sodium nitroprusside had no effect on the muscarinic responses in bovine Sph, but it attenuated CCh-induced contractions in Sph isolated from cats, dogs, and rabbits. In bovine Sph, CNP increased cGMP accumulation in a time- and dose-dependent manner and dose dependently inhibited CCh-induced IP₃ production and contraction. LY 83583 had no effect on the muscarinic responses. C-type natriuretic peptide attenuated CCh-induced contraction in CM isolated from monkey and human, but it had no influence on this response in CM isolated from cows, cats, and dogs.

**Conclusions.** In bovine CM, SNP effects are probably mediated through soluble guanylyl cyclase, whereas in Sph the CNP effects are mediated through membrane-bound guanylyl cyclase, which is associated with the type-B natriuretic peptide receptor. Agents that strongly increase intracellular cGMP levels, including SNP and CNP, produce significant inhibition of CCh-induced IP₃ production and contraction. These effects are tissue and species specific. The results indicate that the cGMP signaling system, similar to the cAMP system, has a major inhibitory influence on the muscarinic responses in smooth muscles of the iris-ciliary body. The agents CNP and SNP, which stimulate cGMP accumulation in the ocular smooth muscles, could reduce intraocular pressure, presumably by increasing uveoscleral outflow induced by relaxation of the CM. However, the relationships between the CNP- and SNP-induced inhibition of the muscarinic stimulation and the reported intraocular pressure-lowering effects of the cGMP-elevating agents remain to be determined. Invest Ophthalmol Vis Sci. 1997;38:2629-2638.

An important mechanism by which the peripheral nervous system regulates muscle tone is a reciprocal interaction between the parasympathetic (cholinergic) nervous system, which liberates acetylcholine, and the sympathetic (adrenergic) nervous system, which liberates norepinephrine. In the smooth muscles of the iris-ciliary body, adrenergic nerves can regulate,
through changes in intracellular cyclic adenosine monophosphate (cAMP) levels, the muscarinic stimulation of phosphoinositide turnover and contraction and, in many instances, the inhibition of muscle contraction (relaxation).1 In addition to muscarinic and β-adrenergic receptors, the smooth muscles of the iris–ciliary body contain receptors for substance P, endothelin, and prostaglandins that also function through the cAMP and phosphoinositide systems, and receptors for atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP) that function through the cyclic guanosine monophosphate (cGMP) system.2-5 Although it is well established that, in nonvascular smooth muscle such as that in the iris and the trabecula, elevation of intracellular cAMP concentrations can lead to inhibition of agonist-stimulated phosphoinositide metabolism and contraction, there is little evidence that cGMP is similarly involved in the mechanism of inhibition of these responses.1 Cyclic AMP6-9 and cGMP2-4,10,11 serve as second messengers in regulating aqueous humor dynamics and in mediating their responses to various pharmacologic agents.2-4,10,11 Ocular administration of cGMP-elevating agents and soluble cGMP analogues decreases intraocular pressure (IOP),5,8,11 and at least part of the effect of these agents was shown to involve an increase in the outflow facility of aqueous humor.6,10,11 These effects could be mediated by the relaxation of the ciliary muscle (CM).5,8,11 Agents that elevate cGMP could reduce IOP, presumably by increasing uveoscleral outflow induced by relaxation of the CM. Because the effects of nitrovasodilators and natriuretic peptides are mediated, at least in part, by an increase in the intracellular level of cGMP2-5,11 in the current study, we have compared the effects of sodium nitroprusside (SNP), an activator of cytoplasmic soluble guanylyl cyclase,12,13 and CNP, an activator of particulate guanylyl cyclase11 associated with the type-B natriuretic peptide receptor (NPR-B) in human ocular cells,5 on carbachol (CCh)-induced inositol 1,4,5-triphosphate (IP3) production and contraction and on cGMP formation in iris sphincter (Sph) and CM isolated from bovine and other mammalian species. We found that SNP and CNP stimulate cGMP formation and inhibit CCh-induced IP3 production and contraction in the ocular smooth muscles and that these effects are tissue and species specific. The agents SNP and CNP, through cGMP, could reduce IOP by presumably increasing uveoscleral outflow induced by relaxation of the CM.

MATERIALS AND METHODS

The general methods used here were essentially the same as described previously.15 The CCh and SNP were obtained from Sigma Chemical Company, St. Louis, Missouri; CNP was from Peninsula Labora-
tories, Belmont, California; 8-Bromo-cGMP and LY 83583 were from Calbiochem, San Diego, California; [125I]cGMP and [125I]cAMP radioimmunoassay kits from Advanced Magnetics, Cambridge, Massachusetts; and myo-[3H]inositol (specific radioactivity 15.5 Ci/mm) was purchased from Amersham, Arlington Heights, Illinois. All other chemicals used were of reagent grade.

Preparation of Iris Sphincter and Ciliary Muscle

Bovine and rabbit eyes were obtained from a local slaughterhouse. Cat and dog eyes were obtained through the courtesy of the Richmond County Animal Control (Augusta, GA), Macaca mulata eyes through the courtesy of Dr. Mohamad Sharawy, and human eyes from the Medical College of Georgia Eye Bank. The human eyes were obtained 7 to 14 hours after death. Eyes were enucleated immediately after death and transported to the laboratory packed in ice. The iris Sph and CM were dissected under a binocular microscope and the muscles placed in modified Krebs–Ringer bicarbonate buffer of the following composition (in millimoles): NaCl, 118; NaHCO3, 25; KCl, 4.7; KH2PO4, 1.2; MgSO4, 1.2; CaCl2, 1.25; glucose, 10. The pH of the buffer was adjusted and maintained at 7.4 with 97% O2–3% CO2. The tissues were kept at 4°C and used for study within 1 hour. In general, each Sph or CM came from the same eye and was cut into four to six equal strips, with one strip serving as a control and the other as experimental. In our opinion, methods for securing animal tissues were humane, included properly obtained approval, and complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Incubation of Iris Sphincter and Ciliary Muscle With Myo-[3H]Inositol and Analysis of Inositol Phosphates

To label the tissue with myo-[3H]inositol, the Sph or CM strips were incubated for 90 minutes at 37°C in 1 ml Krebs–Ringer bicarbonate buffer, containing 10 μCi myo-[3H]inositol. The tissues were washed four times with 3 ml nonradioactive Krebs–Ringer bicarbonate buffer and then suspended individually in 1 ml fresh nonradioactive buffer. LiCl (10 mM, final concentration) was added to each incubation; and 10 minutes later, CCh or other test agents were added and incubations continued for the time indicated. The incubations were terminated by the addition of 1 ml 10% (wt/vol) trichloroacetic acid (TCA). The tissues were homogenized and centrifuged and the supernatant extracted with anhydrous diethyl ether. The water-soluble tissue extract was neutralized to pH 7 with NaOH, and myo-[3H]inositol phosphates were analyzed by anion-exchange...
TABLE 1. Inhibitory Effects of Various Cyclic Guanosine Monophosphate- and Cyclic Adenosine Monophosphate-Elevating Agents on Muscle Contraction in Ciliary Muscle and Sphincter Isolated From Different Species

<table>
<thead>
<tr>
<th>Additions</th>
<th>Bovine</th>
<th>Cat</th>
<th>Dog</th>
<th>Rabbit (Sph)</th>
<th>Monkey (CM)</th>
<th>Human (CM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sph</td>
<td>CM</td>
<td>Sph</td>
<td>CM</td>
<td>Sph</td>
<td>CM</td>
</tr>
<tr>
<td>Sodium nitroprusside (1 mM)</td>
<td>0</td>
<td>75</td>
<td>18</td>
<td>36</td>
<td>66</td>
<td>12</td>
</tr>
<tr>
<td>C-type natriuretic peptide (1 µM)</td>
<td>83</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Isoproterenol (5 µM)</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>52</td>
<td>77</td>
<td>33</td>
</tr>
<tr>
<td>Forskolin (10 µM)</td>
<td>100</td>
<td>73</td>
<td>100</td>
<td>50</td>
<td>100</td>
<td>43</td>
</tr>
<tr>
<td>PGE2 (1 µM)</td>
<td>0</td>
<td>95</td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>33</td>
</tr>
</tbody>
</table>

The methods used were the same as described in Materials and Methods. Concentrations of carbachol were 0.5 and 2.5 µM for sphincter (Sph) and ciliary muscle (CM), respectively. Each value represents an average of three determinations, and SEM for each value was <1.5. The values are given as percentage of inhibition of carbachol-induced contraction. Maximal tensions for carbachol-induced contractions for Sph muscles isolated from bovine, cat, dog, and rabbit were (mg tension/mg wet weight tissue): 21.0 ± 0.7, 39.0 ± 1.6, 28.0 ± 2.0, and 42.4 ± 1.3, respectively, and for CM isolated from bovine, cat, dog, monkey, and human were 5.4 ± 0.07, 9.2 ± 0.7, 15.6 ± 1.0, 11.4 ± 0.3, and 21.4 ± 1.0, respectively.

Calculations and Statistical Analysis
To correct for variation in tissue size, the data for [3H]inositol phosphates and cGMP or cAMP were normalized to tissue protein content. Data are expressed as mean values ± SEM. Statistical differences between the two mean values were determined by a paired Student’s t-test. When P was <0.05, the difference in values was considered to be significant.

RESULTS
Inhibitory Effects of cGMP- and cAMP-Elevating Agents on Carbachol-Induced Contraction
Sodium nitroprusside, an activator of soluble guanylyl cyclase, had no effect on the contractile response in bovine Sph, but inhibited that of cat, dog, and rabbit by 18%, 66%, and 72%, respectively (Table 1). In contrast, SNP inhibited the contractile response of CM isolated from bovine, cat, dog, monkey, and human by 75%, 36%, 12%, 26%, and 62%, respectively. C-type natriuretic peptide, an activator of membrane-bound guanylyl cyclase, inhibited the contractile response in iris Sph isolated from bovine, cat, dog, and rabbit, by 83%, 12%, 12%, and 41%, respectively, and in CM isolated from monkey and human, by 11% and 20%, respectively. It had no effect on CM isolated from bovine, cat, and dog. With the exception of isoproterenol in the bovine CM and prostaglandin E2 in Sph isolated from bovine, cat, and dog, and CM from monkey, the cAMP-elevating agents exerted significant inhibitory effects in both muscles isolated from all species.

These data demonstrate that cGMP-elevating agents exert differential inhibitory effects on CCh-induced contraction in Sph and CM isolated from bovine, cat, dog, rabbit, monkey, and human. In the following...
Concentration–Contraction Response Curves of the Effect of C-Type Natriuretic Peptide, in the Absence and Presence of LY 83583, on Isolated Bovine Iris Sphincter Muscle Precontracted with Carbachol

C-type natriuretic peptide, an activator of type-B natriuretic peptide receptor (NPR-B), produced significant, concentration-dependent relaxation in the isolated Sph (Fig. 2). Relaxation was observed at concentrations as low as 1 nM; and at 1 μM, the peptide caused an approximate 83% reduction in the contractile response. LY 83583, which has no inhibitory effect on membrane-associated guanylyl cyclase, had no effect on muscle relaxation induced by CNP in this tissue. These data suggest that CNP is a potent relaxant in the bovine iris Sph and that this effect may be mediated through the membrane-bound guanylyl cyclase.

Concentration–IP₃ Response Curves of the Effect of Sodium Nitroprusside and C-Type Natriuretic Peptide on Isolated Bovine Ciliary Muscle and Iris Sphincter Precontracted with Carbachol

To determine whether the cGMP-elevating agents can inhibit CCh-induced production of IP₃ involved in Ca²⁺ mobilization and contraction in smooth muscle in the ocular tissues, we investigated the effects of different concentrations of SNP and CNP on CCh-induced IP₃ accumulation in the bovine CM and SPh. The results of these studies are given in Figures 3 and 4, respectively. Addition of different concentrations of SNP to CM prelabeled with [³H]inositol dose depen-
CNP- and SNP-Induced cGMP Formation and Muscle Relaxation

CNP- and SNP-induced cGMP formation and muscle relaxation was studied in isolated bovine ciliary muscle and iris sphincter precontracted with carbachol. Muscles were prelabeled with [3H]inositol (10 μCi/ml) for 90 minutes at 37°C. The labeled muscles were washed with nonradioactive buffer and incubated in buffer that contained 10 mM LiCl for 10 minutes. Carbachol (25 μM) was added, followed by the addition of different concentrations of sodium nitroprusside as indicated. After 10 minutes of incubation, the inositol phosphates were extracted from the tissues and analyzed by anion-exchange chromatography, as described in Materials and Methods. The basal level of IP3 production in ciliary muscle was 1189 ± 80 disintegrations per minute (dpm)/mg protein and in iris sphincter was 2140 ± 150 disintegrations per minute (dpm)/mg protein. Values are means ± SEM. There are three experiments for each data point.

Sodium nitroprusside (SNP) and C-type natriuretic peptide (CNP) inhibition of CCh-induced IP3 production and contraction is mediated through cGMP formation, in the following experiments we investigated the kinetics of the effects of the cGMP-elevating agents on cGMP formation in bovine ocular tissues.

### Kinetics of the Effect of Sodium Nitroprusside and C-Type Natriuretic Peptide on cGMP Formation in the Ocular Tissues

SNP and CNP inhibition of CCh-induced IP3 production and contraction is mediated through cGMP formation, in the following experiments we investigated the kinetics of the effects of the cGMP-elevating agents on cGMP formation in bovine ocular tissues.

#### Time Course of the Effect of Sodium Nitroprusside on cGMP Formation in Bovine Ciliary Muscle

Figure 5 depicts the time course of the effect of SNP on cGMP formation in bovine CM. Sodium nitroprusside (100 μM) induced cGMP formation in a time-dependent manner with a half-life (t½, effective time for half-maximum response) value of 4.3 minutes. This NO donor induced a 56% increase in cGMP accumulation by 1 minute and reached a 390% increase within 10 minutes. There was little change in cGMP formation after 10 minutes of incubation.

#### Effects of Different Concentrations of Sodium Nitroprusside on cGMP Formation in Bovine Ciliary Muscle and Sphincter

In CM, SNP at 1 μM and 1 mM increased cGMP formation by 50% and 428%, respectively (Fig. 6). The EC50 value for cGMP accumulation was 13.3 μM. In contrast, SNP (10 μM and 1 mM) had no significant effect on cGMP formation in bovine Sph. These data demonstrate that SNP increases cGMP formation in bovine CM in a time- and concentration-dependent manner.

#### Time Course of the Effect of C-Type Natriuretic Peptide on cGMP Formation in Bovine Sphincter

Figure 7 depicts the time course of the effect of CNP on cGMP formation in bovine Sph. At 1 μM, it time...
FIGURE 5. Time course of sodium nitroprusside's effect on cyclic guanosine monophosphate (cGMP) formation in bovine ciliary muscle. Ciliary muscles were incubated in buffer for 15 minutes at 37°C, then incubated in buffer that contained 0.1 mM isobutylmethylxanthine for 10 minutes. Sodium nitroprusside (0.1 mM) was then added and incubations continued for various time intervals, as indicated. Cyclic guanosine monophosphate was assayed in the TCA-soluble extract by means of radioimmunoassay, as described in Materials and Methods. The basal value for cGMP formation was 6.73 ± 0.16 pmol/mg protein. Values are means ± SEM. There are three experiments for each data point.

FIGURE 6. Concentration–cyclic guanosine monophosphate (cGMP) response curves of the effect of sodium nitroprusside on cGMP formation in bovine ciliary muscle and iris sphincter. Conditions of incubation were the same as described in Figure 5, except that different concentrations of sodium nitroprusside were added, as indicated, and the time of incubation with sodium nitroprusside was 10 minutes. The basal value for cGMP formation in the iris sphincter was 0.92 ± 0.17 pmol/mg protein. Values are means ± SEM. There are three experiments for each data point. cGMP = cyclic guanosine monophosphate.

FIGURE 7. Time course of C-type natriuretic peptide's effect on cyclic guanosine monophosphate (cGMP) formation in bovine iris sphincter. Conditions of incubation were the same as described for Figure 5, except that iris sphincter muscle and C-type natriuretic peptide (1 μM) were used in this experiment. Values are means ± SEM. There are three experiments for each data point.

FIGURE 8. Concentration–cyclic guanosine monophosphate (cGMP) response curves of the effect of C-type natriuretic peptide on cGMP formation in bovine ciliary muscle and iris sphincter. Conditions of incubation were the same as described for Figure 6, except that different concentrations of C-type natriuretic peptide were added, as indicated. Values are means ± SEM. There are three experiments for each data point.

Effects of Different Concentrations of C-Type Natriuretic Peptide on cGMP Formation in Bovine Ciliary Muscle and Sphincter

In bovine Sph, at 1 nM to 1 μM, CNP-stimulated cGMP formation was concentration dependent (Fig. 8). At 1 μM, the increase in cGMP accumulation was 550%, which is considerably greater than that observed for 1 mM SNP in CM. These data on cGMP formation correlate well with the potency of the relaxing effects formation by 1 minute and reached a 525% increase within 10 minutes.

FIGURE 5. Time course of sodium nitroprusside's effect on cyclic guanosine monophosphate (cGMP) formation in bovine ciliary muscle. Ciliary muscles were incubated in buffer for 15 minutes at 37°C, then incubated in buffer that contained 0.1 mM isobutylmethylxanthine for 10 minutes. Sodium nitroprusside (0.1 mM) was then added and incubations continued for various time intervals, as indicated. Cyclic guanosine monophosphate was assayed in the TCA-soluble extract by means of radioimmunoassay, as described in Materials and Methods. The basal value for cGMP formation was 6.73 ± 0.16 pmol/mg protein. Values are means ± SEM. There are three experiments for each data point.

dependently stimulated cGMP accumulation with a $t_{1/2}$ of 2.4 minutes. This $t_{1/2}$ value is approximately two times lower than that obtained for the stimulatory effect of SNP on cGMP accumulation in CM. C-type natriuretic peptide induced a 120% increase in cGMP formation by 1 minute and reached a 525% increase within 10 minutes.
of SNP and CNP in the ocular tissues. C-type natriuretic peptide had no effect on cGMP formation in the bovine CM (Fig. 8).

Effects of LY 83583 on Sodium Nitroprusside- and C-Type Natriuretic Peptide-Induced Stimulation of cGMP Formation in Bovine Ciliary Muscle and Sphincter

As shown in Figure 9, 10 μM and 50 μM LY 83583 significantly inhibited SNP-induced cGMP formation in CM by 53% and 76%, respectively, but had no effect on CNP-induced cGMP formation in the Sph. These results suggest that the increase in cGMP level generated by SNP in bovine CM was produced by the soluble form of guanylate cyclase, whereas CNP-induced cGMP by CNP in the Sph was produced by the membrane-bound form of the enzyme.

Concentration–Contraction Response Curves of the Effect of 8-Br-cGMP on Isolated Bovine Ciliary Muscle and Sphincter

8-Br-cGMP is a cell-membrane permeable analogue of cGMP often used to mimic the effects of cGMP-elevating agents in various cellular responses. As can be seen in Figure 10, this cGMP analogue relaxed the contractile response in a concentration-dependent manner, with an EC50 value of 9.8 μM. At 1 mM concentration, this agent induced ~63% and 10% relaxation in CM and Sph, respectively. Similarly, 8-Br-cGMP inhibited the IP3 response in CM in a concentration-dependent manner with an IC50 value of 3 μM (data not shown). These findings confirm the involvement of cGMP in the inhibitory actions of cGMP-elevating agents on CCh stimulation of IP3 production and muscle contraction in bovine Sph and CM.

Inhibitory Effects of 8-Br-cGMP on Muscle Contraction in Sphincter and Ciliary Muscle Isolated from Different Species

The finding that 1 mM 8-Br-cGMP induced ~63% and ~10% relaxation in bovine CM and Sph, respectively.

| TABLE 2. Inhibitory Effects of 8-Br-Cyclic Guanosine Monophosphate on Muscle Contraction in Sphincter (Sph) and Ciliary Muscle (CM) Isolated From Different Species |
|-----------------------------|--------|--------|
| **Species**                 | **% Inhibition of Carbachol-Induced Contraction** |
| Bovine                      | 10     | 63     |
| Cat                         | 100    | 70     |
| Dog                         | 88     | 25     |
| Rabbit                      | 63     | —      |

The methodology used was the same as described in the Figure 10 legend. Concentration of 8-Br-cyclic guanosine monophosphate was 1 mM. Concentrations of carbachol were 0.5 and 2.5 μM for Sph and CM, respectively. Each value represents an average of three determinations, and SEM for each value was < 1.5. Values for maximal tensions for carbachol-induced contraction for Sph and CM were the same as given in Table 1.
Effects of Sodium Nitroprusside and C-Type Natriuretic Peptide on cAMP Formation

To rule out the possibility that cAMP may be involved in the observed relaxant effects of SNP and CNP in the ocular muscles, we carried out experiments to determine whether the cGMP-elevating agents could increase cAMP levels in these tissues. The results obtained revealed that neither SNP (0.1 mM and 1 mM) nor CNP (0.1 µM and 1 µM) stimulated cAMP accumulation in the CM and Sph muscles, respectively (data not shown). These observations show that cAMP accumulation is not involved in SNP- and CNP-induced relaxation and IP3 inhibition in bovine CM and Sph, respectively.

DISCUSSION

These results demonstrate for the first time the relaxing effects of CNP in iris Sph isolated from bovine, cat, dog, and rabbit, and in CM from monkey and human (Table 1). In a wide variety of tissues, including ocular, much interaction (cross-talk) occurs between the second messenger pathways. Cross-talk between the cAMP and IP3-Ca2+ and muscle contraction–relaxation in the smooth muscles of the iris–ciliary body has already been reported. In the current study, we have shown that in CM and Sph isolated from bovine, such cGMP-elevating agents as SNP and CNP increase intracellular cGMP concentration, and inhibit CCh-induced IP3 production and contraction. In addition, we have demonstrated that these effects are tissue and species specific (Table 1). Thus, we have found that in bovine CM, but not in Sph, SNP stimulated cGMP accumulation and inhibited CCh-induced IP3 production and contraction. The finding that in CM the effects of SNP were inhibited by LY 83583 could suggest that these responses may be mediated through the cytoplasmic soluble guanylyl cyclase. In contrast, in the bovine Sph, but not in CM, CNP stimulated cGMP accumulation and inhibited CCh-induced IP3 production and contraction. However, that these effects were not inhibited by LY 83583 could suggest that in this tissue, the observed CNP effects may be mediated through membrane-bound guanylyl cyclase. In contrast to the bovine Sph, SNP inhibited CCh-induced contraction in Sph isolated from cat, dog, and rabbit (Table 1). Furthermore, CNP inhibited CCh-induced contraction in CM isolated from monkey and human by 11% and 20%, respectively. Thus, the distinct differential effects of SNP and CNP on the biochemical and physiological responses in Sph and CM are probably unique to the cow. Therefore, for the current studies on the mechanism of the inhibitory effects of cGMP-elevating agents on CCh-induced IP3 production and contraction in CM and Sph, we selected the cow. As has been mentioned, natriuretic peptides, including CNP, can stimulate the formation of cGMP through the activation of membrane-bound guanylyl cyclases. These particulate guanylyl cyclases are themselves specific receptors for the natriuretic peptides. Two types of receptor enzymes are known: the type-A natriuretic peptide receptor (NPR-A), which is selective for ANP, and the type-B natriuretic peptide receptor (NPR-B), which is selective for CNP. Because the Sph responded to CNP, but not to ANP (KHD and AAL, data not shown), we can conclude that this muscle contains the NPR-B. As can be seen in Table 1, CM from cow, cat, and dog lacked the NPR-B, whereas this receptor was found in CM isolated from monkey and human. In support of this, Pang et al, working with human ocular cells, concluded that NPR-B is the primary functional NPR in the trabecular meshwork and CM cells. Takashima et al reported that ANP, CNP, and brain natriuretic peptide (BNP) lower IOP in the rabbit eye and that CNP was more effective than ANP or BNP. In cerebral arterial smooth muscle cells CNP but not ANP increased cGMP formation. These investigators suggested that smooth muscle cells in cerebral arteries express only NPR-B, whereas cells from peripheral arteries can express NPR-A and NPR-B. In canine arteries and veins, CNP but not ANP is a relaxing factor of isolated peripheral veins. The above findings suggest a physiological role for CNP in the regulation of the smooth muscles of the iris–ciliary body.

In support of these conclusions, in the present study we have shown that in bovine CM, the SNP stimulated cGMP formation in a time- and concentration-dependent manner with a t1/2 value of 4.3 minutes and an EC50 value of 13.5 µM (Figures 5 and 6, respectively). Sodium nitroprusside dose-dependently inhibited CCh-induced IP3 production (Fig. 3) and contraction (Fig. 1). These data are in agreement with previous reports in which SNP was shown to attenuate CCh-induced contraction in feline CM, bovine CM, and rabbit iris Sph. The mechanism of the lack of an effect of SNP on the contractile response in the bovine Sph, compared with its relaxant effect in Sph isolated from cat, dog, and rabbit (Table 1) is not clear. Sodium nitroprusside is thought to produce NO, which activates soluble guanylyl cyclase to produce cGMP, which relaxes the muscle through activation of cGMP-dependent protein kinase (G-kinase). The findings that LY 83583 (Fig. 1) and 8-Br-cGMP (Fig. 10) inhib-
significantly inhibited SNP-induced cGMP formation and contraction in this muscle. In addition, LY 83583 inhibited CCh-induced calcium mobilization in the bovine CM (Fig. 10). The discrepancy observed between the relaxing effects of the soluble cGMP analogue (10%) and the CNP agonist (83%) in the bovine Sph (Fig. 10, Table 1) could be because of the low permeability of this tissue to 8-Br-cGMP. This possibility is supported by the finding that the soluble cGMP analogue inhibited CCh-induced contraction by 10%, 100%, and 88%, and 63% in sphincters isolated from cow, cat, dog, and rabbit, respectively (Table 2). The inhibitory effects of the soluble cGMP analogue are tissue and species specific. In addition, NPR-B, which is selective for CNP, appears to be more efficiently coupled to the activation of the particulate guanylyl cyclase in bovine Sph than in that from other species (Table 1).

The data obtained on the effects of CNP on the biochemical and physiological responses in the Sph also support the involvement of cGMP in the inhibitory actions of CNP on these responses. In the eye of any species, there is little information available on CNP. In bovine Sph, CNP dose dependently inhibited (relaxed) CCh-induced contraction (Fig. 2) and CCh-stimulated IP₃ production (Fig. 4), and time- and dose-dependently stimulated cGMP formation (Figs. 7 and 8). The findings that the inhibitory effects of CNP on the biochemical and physiological responses were not reversed by LY 83583 and were not elicited by SNP suggest that the observed inhibitory effects of this peptide in the bovine Sph are mediated through the particulate guanylyl cyclase. In human trabecular meshwork cells and in human CM cells, CNP increased the accumulation of cGMP, and this correlated with suppression by the peptide of CCh-induced calcium mobilization in these cells. In this study CNP, did not affect resting cell calcium concentration but lowered CCh-activated calcium mobilization. In rabbit, intravital injection of the (BNP) increased outflow facility (but did not affect aqueous humor flow or uveoscleral outflow), reduced IOP, and significantly increased cGMP concentrations in the aqueous humor of the eye. Although CNP had no effect on the contractile response in CM isolated from bovine, cat, and dog, it did attenuate CCh-induced contraction in CM isolated from monkey and human (Table 1). C-type natriuretic peptide–induced increase of cGMP in human trabecular meshwork cells and in human ciliary muscle cells correlated with suppression of CCh-induced calcium mobilization in the cell. Lowering of intracellular Ca²⁺ concentration by cGMP and subsequent relaxation of the CM could be involved in the IOP-lowering effects of the cGMP-elevating agents. C-type natriuretic peptide and SNP, which stimulate cGMP accumulation in the ocular smooth muscles, could reduce IOP, presumably by increasing uveoscleral outflow induced by relaxation of the CM.

In conclusion, the data reported here show that CNP and Sph, agents that strongly increase intracellular cGMP levels, as do SNP and CNP, produce significant inhibition of CCh-induced IP₃ production and contraction. These effects are tissue and species specific. The relaxing effects of these agents could be related to an inhibition of the G-protein-regulated phospholipase C through a mechanism mediated by cGMP-dependent protein kinase. In addition, the results indicate that the cGMP signaling system, as with the cAMP system, has a major inhibitory influence on the stimulated phosphoinositide turnover and contraction in the smooth muscles of the iris–ciliary body. C-type natriuretic peptide and SNP, which stimulate cGMP accumulation in the ocular smooth muscles, could reduce IOP by increasing uveoscleral outflow induced by relaxation of the CM. However, the relationships between CNP- and SNP-induced inhibition of muscarinic–cholinergic contraction in these tissues and the reported IOP-lowering effects of cGMP-elevating agents in the eye remain to be determined.

Key Words
C-type natriuretic peptide, ciliary and sphincter smooth muscles, cyclic guanosine monophosphate, muscle relaxation, sodium nitroprusside

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References


