Activation of Guanosine 3′,5′-Cyclic Monophosphate (cGMP)-Dependent Protein Kinase in Rat Vas Deferens and Distal Colon is Not Accompanied by Inhibition of Contraction

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ABSTRACT

There is good evidence that in vascular smooth muscle, the relaxant effects of sodium nitroprusside (SNP) are mediated by increases in cGMP levels and activation of cGMP-dependent protein kinase (PKG). However, in rat vas deferens and rat distal colon, cGMP-elevating agents such as SNP and atrial natriuretic factor (ANF) have been shown to elevate cGMP without inducing relaxation. The lack of relaxation might be explained by either lack of activation of PKG by these agents or low levels of PKG in these tissues. The object of the present study was to investigate these possibilities by simultaneously monitoring cGMP levels, PKG activity and contractility in isolated strips of rat vas deferens, rat proximal colon and distal colon exposed to high concentrations of SNP or ANF. Verification of the specificity of the assay for PKG was obtained using MonoQ chromatography to resolve soluble smooth muscle extracts, followed by immunoblotting with a PKG-specific antibody to identify the kinase. In rat vas deferens, 5 mM SNP increased cGMP levels (14-fold) and PKG activity ratios (3.4-fold) but did not inhibit phenylephrine-induced contractions. In both rat proximal and rat distal colon, 100 nM ANF significantly elevated cGMP levels and PKG activity ratios, but only in the proximal colon was inhibition of spontaneous contractions observed. Total PKG activity was much lower (~16 pmol PO₄/min/mg protein) in rat vas deferens, which was not relaxed by SNP, than in rabbit aorta (~148 pmol PO₄/min/mg), which was relaxed. However, in the rat proximal colon, despite low PKG levels (~11 pmole/min/mg), ANF did inhibit contractions. Thus the inability of the cGMP-elevating agents SNP and ANF to inhibit contractions in rat vas deferens and rat distal colon cannot be explained by either of the possibilities suggested above.

It is generally accepted that the vascular smooth muscle-relaxing effects of drugs such as SNP and nitroglycerin are mediated via increases in tissue levels of cGMP. The early literature in support of this conclusion has been extensively reviewed elsewhere (Ignarro and Kadowitz, 1985; Waldman and Murad, 1987) and will not be described here. All of the criteria generally used to determine whether a response is mediated by a cyclic nucleotide appear to have been satisfied for the vasorelaxant effects of these agents and for other cGMP-elevating agents such as ANF.

However, it is not so clear that cGMP plays a role as a mediator of relaxation in other types of smooth muscles. For example, SNP markedly elevated cGMP levels in rat vas deferens and in estrogen-primed rat myometrium but had no relaxant effect in these preparations (Diamond and Janis, 1978; Diamond, 1983). In the vas deferens, high concentrations of SNP increased cGMP levels by as much as 16-fold but did not prevent or reverse contractions produced by submaximal concentrations of PE. This is in contrast to the situation in the rat aorta, where much smaller increases in cGMP (less than 2-fold) produced by SNP are accompanied by significant relaxation (Lincoln and Fisher-Simpson, 1983). More recently, a similar dissociation between cGMP elevation and relaxation was reported in the rat distal colon (Suthamnatpong et al., 1993). In that study, it was found that elevation of cGMP by nitric oxide and ANF in rat proximal colon was accompanied by relaxation, whereas a similar elevation of cGMP produced by these agents in the distal colon was not accompanied by relaxation. Thus it appears that smooth muscles can be classified as either “responsive” or “nonresponsive” with respect to whether they are relaxed by increases in tissue levels of cGMP.

The underlying mechanism by which an elevation of cGMP can cause vascular smooth muscle relaxation has not been completely elucidated. It has been suggested that activation of a specific PKG plays an important role in the relaxation of blood vessels by cGMP-elevating agents such as SNP and ANF.

ABBREVIATIONS: BPDEtide, peptide substrate RKISASEFDRPLR; KB, Krebs’ bicarbonate; PE, phenylephrine; PKA, cAMP-dependent protein kinase; ANF, atrial natriuretic factor; PKI, protein kinase inhibitor; PKG, cGMP-dependent protein kinase; SNP, sodium nitroprusside; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.
ANF (Lincoln, 1989). Several lines of evidence support this hypothesis. For example, Francis et al. (1988), using a number of cyclic nucleotide analogs, observed a good correlation between the ability of these analogs to activate PKG and their ability to relax isolated strips of pig coronary artery. Cornwell and Lincoln (1989), using another approach, demonstrated that the ability of ANF and 8-bromo-cyclic GMP (8Br-cGMP) to lower intracellular calcium levels in primary cultures of rat aortic cells depended on the presence of PKG in these cells. After several cell passages, the levels of PKG were markedly diminished in these cells, and they were no longer responsive to ANF or 8Br-cGMP. Adding purified PKG back to the cells, using osmotic lysis, restored the ability of the cells to respond to ANF and 8Br-cGMP, which indicated that PKG was required for the action of these compounds. Direct evidence for a role of PKG was provided by early studies in which PKG activity was measured in rat aortic strips relaxed by ANF (Fiscus et al., 1985). A good correlation between relaxation and activation of the kinase was reported. More recent studies from our own laboratory, using a new assay for PKG, demonstrated similar correlations between activation of PKG and relaxation of isolated strips of rabbit aorta by SNP and nitroglycerin (Patel and Diamond, 1997).

In an interesting study using isolated, perfused rat hearts, Lincoln and Keely (1981) reported that both ACh and SNP increased cGMP levels in the hearts but that only ACh activated the PKG and only ACh exerted a negative inotropic effect in these preparations. It was concluded that activation of PKG was required for cGMP-induced negative inotropy and that SNP had no effect on contractility because it elevated cGMP in a compartment that did not have access to the kinase. If activation of PKG is a necessary step in the chain of events leading to relaxation of vascular smooth muscle by cGMP-elevating agents, as discussed above, then the failure of some smooth muscles to relax in the face of elevated cGMP levels might be explained by a lack of activation of PKG (or insufficient levels of PKG) in these nonresponsive tissues. The object of the present study was to investigate this possibility by simultaneously monitoring cGMP levels, PKG activity and contractility in isolated strips of rat vas deferens, proximal colon and distal colon exposed to high concentrations of SNP or ANF.

Materials and Methods

Preparation of rat vas deferens and experimental protocol. Male Wistar rats (275–300 g) were sacrificed by carbon dioxide exposure, and the proximal colon (defined as the ascending colon up to the transverse colon) and the distal colon (defined as the descending colon) were removed and placed in warm (37°C) Tyrode’s buffer of the following composition (mM): KCl (2.7), NaH2PO4 (0.4), MgCl2 (1.8), NaCl (136.9), NaHCO3 (11.9) and D-glucose (5.6). The segments were flushed thoroughly with Tyrode’s buffer (to remove fecal material). After cleaning, 2.0-cm segments (two from the proximal and two from the distal colon, per rat) were set up for tension recording in 20-ml organ baths containing Tyrode’s buffer and aerated with 95% O2/5% CO2. The segments were set up such that the anal end was attached to an anchor in the organ bath and the oral end tied (via a thread) to a force-displacement transducer (Grass Model FT03C) connected to a Grass Model 7D polygraph recorder. The segments were equilibrated with 1-g preload tension for at least 30 min, during which time the buffer was changed every 10 min.

Thirty minutes after the segments started to contract spontaneously, one segment from the proximal colon and one from the distal colon were exposed to 0.1 μM ANF for 2 min. Then they were frozen between tongs precooled in liquid nitrogen and were stored for biochemical analysis. The control segments received an equivalent volume (200 μl) of the vehicle (0.05 M acetic acid) and were frozen, as above, 2 min later. Thus, with this protocol, each treated segment had its paired control segment.

Estimation of cGMP. cGMP was determined as outlined previously (Patel and Diamond, 1997). Basically, frozen samples were homogenized in ice-cold 6% TCA, and the TCA mixture was added until the tissue was visibly solubilized. cGMP was then determined in samples, using a commercially available cGMP radioimmunoassay kit (BIOTRAK cGMP-SPA). Tissue cGMP levels were calculated as picomoles of cGMP per gram wet weight of tissue.

PKG and PKA assay. At least 50 mg (rat vas deferens) or 80 mg (rat colon) of tissue was homogenized (15 sec at medium speed) in 1 ml of the vehicle (0.05 M acetic acid) and was frozen, as above, 2 min later. The homogenate was then homogenized (15 sec at medium and 5 sec at high-speed agitation) in 5 vol of ice-cold buffer of the following composition: 100 mM potassium phosphate (pH 6.8), 1.0 mM 3-isobutyl-1-methylxanthine, 10.0 mM EDTA and 10.0 mM 2-mercaptoethanol. The homogenate was transferred to cold centrifuge tubes and then centrifuged at 12,000 × g for 15 min at 4°C. The supernatant, containing the soluble fraction, was placed on ice and assayed immediately, in duplicate, for PKG and PKA activity as described previously (Patel and Diamond, 1997).

Column chromatography of PKG and PKA in smooth muscle extracts. Both PKG and PKA were separated using a Pharmacia MonoQ anion exchange column (HR5/5) coupled to a FPLC system (Pharmacia LKB Biotech, Uppsala, Sweden), and each fraction was then assayed for PKG and PKA activity as described previously (Patel and Diamond, 1997). Fractions that showed maximal PKG and PKA activity were then immunoblotted for the presence of PKG.

Western blots. Immunoblotting of resolved proteins, after SDS-PAGE, with a polyclonal affinity-purified antibody raised against a peptide sequence derived from the C-terminus of the Iα isoform of PKG, was performed as described previously (Patel and Diamond, 1997). The antibody was a gift from Dr. S. Pelech, Kinetek Pharmaceutical Corp., Vancouver, B.C., Canada.
Protein determination. Protein concentrations in tissue fractions and in samples applied to the MonoQ column were determined using a commercially available assay (Bio-Rad) on the basis of the method of Bradford (1976).

Statistical analysis. Values in the drug-treated groups were compared with their respective controls using a Student’s t test statistical program (SigmaStat V 1.0, Jandel Scientific, San Mateo, CA). A probability (P) of less than .05 was accepted as the level of significance. In all experiments, mean values were compared with treatment values on a paired basis. All values are expressed as the mean ± S.E.

Materials. ANF (rat, 1–28 amino acids) was obtained from Bachem California (Torrance, CA). A list of the reagents used in the kinase assays, SDS PAGE and Western blots can be found in Patel and Diamond (1997). All other reagents were obtained from commercial sources.

Results

MonoQ chromatography of PKG in rat vas deferens, proximal colon and distal colon. PKG and PKA activity in crude soluble fractions from the muscle preparations was resolved by MonoQ anion exchange chromatography. In rat vas deferens (fig. 1C, upper panel), three peaks of kinase activity were resolved, one cGMP-dependent (peak activity at fraction 27) and two cAMP-dependent (peak activities at fractions 10 and 34, respectively). Judging on the basis of previously reported elution profiles (Hei et al., 1991) the cAMP-dependent peaks presumably reflect type I (fraction 10) and type II (fraction 34) PKA activities. The cGMP-dependent peak is seen in the presence of a PKA inhibitor, PKI, which indicates that it represents PKG activity, not that of PKA.

Similarly, in rat distal colon (fig. 1A) and proximal colon (fig. 1B), three distinct peaks were resolved: two for PKA and one for PKG. Judging on the basis of the MonoQ profiles, all three tissues contain more type II PKA than type I PKA. In contrast to the results in vas deferens and distal colon, the type II PKA activity in the proximal colon was greater than the PKG activity, even when the PKG-specific substrate BD-DEtide was used. The data also show that 5 μM cAMP can activate PKG. This is evidenced by the increase in phosphotransferase activity in vas deferens fractions 26 to 28 in the presence of 5 μM cAMP (▼, fig. 1C), which coincides with the cGMP-dependent peak seen in the presence of a PKA inhibitor. Activation of PKG by cAMP might also account for the small shoulders seen just below the type II PKA peaks in proximal colon (fractions 24–27) and distal colon (fractions 25 and 26) in the presence of 5 μM cAMP. Finally, it is apparent from the MonoQ profiles that there is very little cyclic nucleotide-independent phosphorylation under the conditions used for measurement of PKG.

Western blotting of PKG in rat vas deferens, proximal colon and distal colon. To confirm the identity of PKG in those fractions that exhibited maximal PKG activity, we performed Western blots. The blots (fig. 1, lower panel) show that maximal immunoreactivity was found in those fractions that had the greatest PKG activity and in the lane that contained the PKG Iα holoenzyme standards. There was no immunoreactivity in lanes that contained PKA standards or PKG activity (mmol/min/μl) vs. MonoQ fraction number

Fig. 1. Crude soluble extracts of rat distal colon (panel A), proximal colon (panel B) and vas deferens (panel C) were fractionated on a MonoQ chromatography column with a linear gradient of 0 to 400 mM NaCl (upper panels). Each fraction was assayed for cGMP- and cAMP-dependent phosphotransferase activity. PKG activity was measured in the presence of 1 μM PKI and in the presence (●) and absence (■) of 5 μM cGMP. PKA activity was measured in the presence (○) and absence (□) of 5 μM cAMP, in the absence of PKI. The amount of protein loaded was at least 15 mg. Results shown here are from a single experiment and are representative of results obtained in two similar experiments. The lower panel illustrates the results of immunoblotting experiments on selected fractions from the MonoQ profiles shown in panels A, B and C. Fractions corresponding to PKA and PKG peaks from each type of smooth muscle were resolved by SDS-PAGE for Western blotting with an antibody raised to the C-terminus of type Iα PKG as described in “Materials and Methods.” Lanes 1, 2 and 3 correspond to fractions 8, 28 and 30 from distal colon. Lanes 4, 5 and 6 correspond to purified PKA holoenzyme, PKA α catalytic subunit and type Iα PKG, respectively. Lanes 7, 8 and 9 correspond to fractions 9, 27 and 30 from proximal colon, and lanes 10, 11 and 12 correspond to fractions 10, 27 and 34 from vas deferens. Positions of proteins of known molecular mass are indicated by their molecular mass values (kDa) at the left of the figure.
in the tissue fractions that contained type I PKA activity, which indicated that the antibodies were PKG-specific. The presence of slight immunoreactivity in lanes 3 (fraction 31 from fig. 1A) and 9 (fraction 30 from fig. 1B), which correspond to type II PKA activity, is probably due to the fact that in these tissues, there is overlap between the PKG peaks and the peaks that correspond to type II PKA.

Effect of SNP on PE-induced contractions, cGMP levels and PKG activity ratio in rat vas deferens. One of the objectives of this study was to determine whether SNP could activate PKG in the rat vas deferens. The protocol for this part of the study is illustrated in figure 2. Three control responses to PE were obtained in each muscle strip. These PE-induced contractions were consistent and reproducible (see left panels of fig. 2). Thirty minutes after the control responses were obtained, one muscle strip from each animal was pretreated with SNP (0.1 mM or 5 mM) and then challenged again with PE. The other strip from each animal was used as a control (i.e., it was not exposed to SNP but was challenged again with PE). Muscles were then clamp-frozen and retained for biochemical analyses. As shown in figure 2 (and table 1), SNP had no effect on the contractile responses to PE in these preparations. In preliminary experiments, it was found that treatment of vas deferens with 5 mM SNP for up to 15 min had no effect on contractile responses to 3 μM PE (data not shown). This agrees with the results of previous studies from this laboratory (Diamond and Janis, 1978; Diamond, 1983).

As shown in table 1, both concentrations of SNP produced significant increases in cGMP levels and in PKG activity in the vas deferens. For example, the 5 mM concentration produced a 14-fold increase in cGMP and a 3.4-fold increase in the kinase activity ratios. It should be noted that there was no significant change in the total PKG activity (i.e., activity measured in the presence of sufficient added cGMP to activate the enzyme maximally). The observed increases in activity ratios were due to significant increases in endogenous PKG activity (i.e., activity measured in the absence of added cGMP). This is a further indication that the increase in kinase activity caused by SNP is in fact due to activation of PKG, and not a cyclic nucleotide-independent protein kinase, because if the activation had been due to a cyclic nucleotide-independent kinase, there would also have been an increase in total kinase activity.

Effect of ANF on spontaneous contractions, cGMP levels and PKG activity ratios in rat proximal and distal colon. The protocol for this set of experiments is illustrated in figure 3. The effects of 100 nM ANF on spontaneous contractions in isolated strips of rat proximal and distal colon were monitored. As shown in figure 3, 100 nM ANF almost completely inhibited spontaneous contractions in the proximal colon but had no apparent effect in the distal colon. As determined visually, there was no detectable effect of 100 nM ANF in any of the seven experiments performed on the distal colon, whereas in 6 out of 7 segments of proximal colon, spontaneous contractions were completely inhibited for at least 1 min by that concentration of ANF. In the seventh segment, the magnitude of the spontaneous contrac-

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>cGMP (pmol/g tissue)</th>
<th>cGMP / PKG Activity Ratio</th>
<th>PKG Activity Ratio</th>
<th>Tension (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.6 ± 0.1</td>
<td>1.4 ± 0.4 / 12 ± 1</td>
<td>0.12 ± 0.02</td>
<td>610 ± 90</td>
</tr>
<tr>
<td>SNP (0.1 mM)</td>
<td>4.8 ± 0.8*</td>
<td>3.3 ± 0.6 / 16 ± 4</td>
<td>0.19 ± 0.02*</td>
<td>570 ± 40</td>
</tr>
<tr>
<td>Control</td>
<td>1.4 ± 0.3</td>
<td>2.5 ± 0.3 / 29 ± 4</td>
<td>0.08 ± 0.01</td>
<td>550 ± 50</td>
</tr>
<tr>
<td>SNP (5.0 mM)</td>
<td>20 ± 3*</td>
<td>8.9 ± 3.1 / 27 ± 6</td>
<td>0.27 ± 0.03*</td>
<td>560 ± 40</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. Means were compared with their controls by using a paired Student’s t test.

* Significantly different from control (P < .05).
nM ANF as indicated for 2 min and then were frozen. Treated segments were frozen 2 min later.

In preliminary and distal colon. Rabbit aortic strips were prepared as pre-

arations from rabbit aorta, rat vas deferens and rat proximal 

and distal colon. Rabbit aortic strips were prepared as pre-

arations from rabbit aorta, rat vas deferens and rat proximal and distal colon.

Total PKG activity was significantly higher in rabbit aorta (148 ± 17 pmol/min/mg) than in the rat vas deferens (16.4 ± 1.6 pmol/min/mg) and in the rat proximal (10.6 ± 0.8 pmol/min/mg) and distal colon (11.4 ± 1.1) pmol/min/mg. In addition, PKG activity levels were significantly higher in rat vas deferens than in rat proximal or distal colon.

**Discussion**

As noted in the introduction, there is extensive evidence to suggest that the vascular smooth muscle-relaxing effects of compounds such as SNP, nitroglycerin and ANF are mediated by increases in tissue levels of cGMP and subsequent activation of a PKG. However, in some types of smooth muscle, including rat vas deferens, myometrium and distal colon, SNP and ANF have been shown to markedly increase tissue levels of cGMP without relaxing the preparations. The objective of the present study was to determine whether the failure of these “nonresponsive” tissues to relax in the face of elevated cGMP levels was due to 1) an inability of the elevated cGMP to activate PKG in these tissues, presumably because the cGMP was in a compartment that does not have access to the kinase, or 2) the possibility that total levels of PKG in these tissues may be too low to exert a relaxant effect even when activated.

Early attempts to measure activation of PKG in rat vas deferens were unsuccessful (Diamond et al., 1983). This may have been due to the use of a high assay temperature (30°C), which has been reported to promote dissociation of cGMP from its binding sites on PKG, thereby inactivating it (McCune and Gill, 1979). Fiscus et al. (1984) reported that PKG activation could be demonstrated only when the assay was conducted at 0°C and with an abbreviated incubation time (2.5 min) to limit dissociation of cGMP from PKG. The availability of an improved assay for PKG (Jiang et al., 1992), using BPDEtide as the substrate, has now provided us with an assay sensitive enough to allow measurement of PKG activity at lower temperatures, even in tissues, such as the rat vas deferens, that have low levels of the enzyme.

Verification of the specificity of the assay for PKG was obtained using MonoQ column chromatography and immuno blotting, as described in “Results.” The MonoQ column chromatography of crude soluble extracts from rat vas deferens and rat colon provided evidence that the phosphotransferase activity measured in our experiments was, in fact, due to PKG. Using BPDEtide as the substrate, we found that neither PKA nor other cyclic nucleotide-independent protein kinases contributed significantly to the kinase activity measured in the presence of the PKA inhibitor PKI. Western blots performed using fractions from the MonoQ columns demonstrated that the strongest immunoreactivity to a PKG-specific antibody was found in the fractions that contained the cGMP-dependent peaks. These results are similar to those obtained in our laboratory in studies on PKG activity in rabbit aortic preparations (Patel and Diamond, 1997). Taken as a whole, the results support the conclusion that the phosphotransferase activity being measured in these tissues is due to PKG-mediated phosphorylation of BPDEtide.

Using this improved technique, we investigated the possibility that a lack of activation of PKG may explain the inability of cGMP-elevating agents to relax some types of
smooth muscle. As discussed in the results, SNP produced a dose-dependent increase in cGMP levels in the rat vas deferens, and this effect was accompanied by a significant activation of PKG. With the highest concentration of SNP used (5 mM), we observed marked elevation of cGMP (14-fold) and activation of PKG (3.4-fold). This was not accompanied by inhibition of PE-induced contractions in these preparations. Much smaller elevations of cGMP (2.7-fold) and activation of the kinase (1.4-fold) in rabbit aortic strips were accompanied by significant relaxation of the blood vessels (Patel and Diamond, 1997). Thus the lack of relaxation seen in rat vas deferens in response to SNP-induced increases in cGMP cannot be explained on the basis of failure of the elevated cGMP to activate the kinase. During the course of these studies, we noted that total tissue levels of PKG were much lower in rat vas deferens than in the rabbit aorta, and we considered the possibility that the vas deferens might not be relaxed by SNP, even though PKG was activated, because total tissue levels of the kinase were insufficient to initiate a relaxant effect. However, subsequent results obtained in studies with rat proximal and distal colon argue against this possibility. In confirmation of a previous report by Suthamnatpong et al. (1993), we found that 100 nM ANF produced a similar degree of elevation of cGMP in both the rat proximal and the rat distal colon but that only the proximal colon was relaxed by ANF. In addition, we found that the degree of activation of PKG by ANF was almost identical in the two tissues. Therefore, as was the case in the vas deferens, elevation of cGMP and activation of PKG were not accompanied by relaxation in the distal colon. Total tissue levels of PKG in the proximal and distal colon were found to be approximately equal, and both were significantly lower than the corresponding levels in the rat vas deferens. If it is accepted that relaxation of the proximal colon by ANF is mediated via activation of PKG, then low levels of PKG in the rat vas deferens cannot be used to explain the lack of relaxation seen in that tissue when PKG is activated by SNP. Thus neither failure to activate PKG nor insufficient total levels of the kinase can explain the inability of cGMP-elevating agents to relax the rat vas deferens and distal colon.

Table 2: Effect of ANF on contractility, cGMP levels and PKG activity ratios in spontaneously-contracting rat proximal and distal colon

<table>
<thead>
<tr>
<th>Tissue</th>
<th>n</th>
<th>cGMP (pmol/g tissue)</th>
<th>cGMP / cGMP (pmol PO4/min/mg protein)</th>
<th>PKG Activity Ratio</th>
<th>Inhibition of Spontaneous Contractions?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal Colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>7</td>
<td>0.42 ± 0.04</td>
<td>0.91 ± 0.21 / 11 1 ± 2</td>
<td>0.07 ± 0.01</td>
<td>No</td>
</tr>
<tr>
<td>ANF (100 nM)</td>
<td>7</td>
<td>1.1 ± 0.1*</td>
<td>1.40 ± 0.17 / 10 ± 1</td>
<td>0.14 ± 0.01*</td>
<td></td>
</tr>
<tr>
<td>Proximal Colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>0.47 ± 0.03</td>
<td>0.82 ± 0.13 / 10 ± 1</td>
<td>0.08 ± 0.01</td>
<td>Yes</td>
</tr>
<tr>
<td>ANF (100 nM)</td>
<td>7</td>
<td>1.5 ± 0.2*</td>
<td>1.2 ± 0.2 / 7.5 ± 0.9</td>
<td>0.18 ± 0.03*</td>
<td></td>
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</table>

Values are mean ± S.E. Means were compared with their controls by using a paired Student’s t test. * Significantly different from control (P < .05).
distal colon after activation of PKG. Although PKG is generally believed to be a soluble enzyme, it is possible that it is translocated under certain circumstances (i.e., from the soluble to the particulate fraction) and that, in fact, this translocation is necessary for it to exert a physiological effect. For example, Lincoln and co-workers (Cornwell et al., 1991; Pryzwansky et al., 1995) have suggested that PKG must be co-localized to its substrates, as well as activated by cGMP, before the substrates can be phosphorylated and a functional effect seen. If such a co-localization or translocation occurs in vascular smooth muscle, and not in rat vas deferens and distal colon, then this could provide an explanation for the lack of relaxation observed in the latter preparations in response to cGMP-elevating agents. It is also possible that the necessary substrate(s) for PKG are not present in these “non-responsive” tissues. Further studies along these lines might help to clarify the role of PKG in the control of smooth muscle tension.

In summary, the results of the present studies provide the first demonstration of direct activation of PKG by cGMP-elevating agents in rat vas deferens, proximal colon and distal colon. However, as noted above, only in the proximal colon was activation of PKG accompanied by relaxation. At the present time, we have no explanation for the lack of relaxation observed in rat vas deferens and distal colon after the activation of PKG.

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References


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