Cyclic Guanosine Monophosphate Inhibition of Contraction May be Mediated through Inhibition of Phosphatidylinositol Hydrolysis in Rat Aorta

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SUMMARY. The purpose of this study was to determine whether cyclic guanosine monophosphate inhibits contraction through inhibition of phosphatidylinositol hydrolysis. Sodium nitroprusside and atriopeptin II, agents which activate soluble and particulate guanylate cyclase, respectively, inhibited norepinephrine-induced contraction and accumulation of inositol monophosphate, a measure of phosphatidylinositol hydrolysis. Acetylcholine, an agent which elevates smooth muscle cyclic guanosine monophosphate levels through release of an endothelial-derived relaxing factor, induced similar inhibitory effects on contraction and inositol monophosphate accumulation in the presence but not absence of the endothelium. The cyclic nucleotide analogue 8-bromo cyclic guanosine monophosphate also inhibited contraction and inositol monophosphate accumulation. These results suggest that cyclic guanosine monophosphate may inhibit contraction through inhibition of phosphatidylinositol hydrolysis. Furthermore, the inhibition of phosphatidylinositol hydrolysis was independent of the mechanism by which cyclic guanosine monophosphate elevation occurred. (Circ Res 58: 407-410, 1986)

THERE is considerable evidence that intracellular cyclic guanosine monophosphate (cGMP) inhibits contraction of vascular smooth muscle (for a review, see Rapoport and Murad, 1983a). However, the mechanism by which cGMP inhibits contraction is not clear. It has been proposed that the responses of a variety of tissues, including smooth muscle, to external stimuli may be mediated through hydrolysis of phosphatidylinositols (Berridge and Irvine, 1984). It has also been suggested that cyclic nucleotides may function as feedback inhibitors of excitatory processes, such as platelet aggregation and contraction, through inhibition of phosphatidylinositol hydrolysis (Takai et al., 1984). The present study investigates in intact rat aorta whether agents that elevate cGMP and inhibit norepinephrine-induced contraction also inhibit the hydrolysis of phosphatidylinositol.

Methods

Relaxation Studies

Rats (Sprague-Dawley, male, 280–360 g) were decapitated, and their thoracic aortas were removed and cleaned of extraneous fatty tissue. The aortas were then cut into helical strips (approximately 2 mm × 1 cm) with care taken not to rub the intimal surfaces with any object. The intimal surfaces of additional strips were rubbed with a scalpel to remove the endothelium (Rapoport and Murad, 1983b). These tissues did not relax in response to acetylcholine (Rapoport and Murad, 1983b). Strips were then mounted in organ baths containing 37°C Krebs-Ringer bicarbonate solution which had the following composition (mm): NaCl, 118.5; KCl, 4.74; MgSO₄, 1.18; KH₂PO₄, 1.18; CaCl₂, 2.5; NaHCO₃, 24.9; glucose, 10. Resting tension of 0.2 g-force was maintained throughout the experiment, or as indicated. Tissues were allowed to equilibrate for 2 hours before the addition of any drugs.

Tissues then were contracted with 0.3 μM norepinephrine in the presence of 10 mM LiCl. LiCl was added, since inositol monophosphate assays were performed in the presence of LiCl (see below). Additional strips were exposed to atriopeptin II, sodium nitroprusside, or acetylcholine for 4 minutes or to 8-bromo cGMP for 14 minutes prior to 10 mM LiCl and then, after an additional 1 minute, to 0.3 μM norepinephrine for 30 minutes.

Inositol Monophosphate Studies

Inositol monophosphate was assayed according to the procedure of Brown and Brown (1983). Briefly, rat thoracic aortas with or without endothelium were exposed for 3 hours to Krebs-Ringer bicarbonate solution which was gassed with 95% O₂-5% CO₂ and had the following composition (mm): NaCl, 118.5; KCl, 4.74; MgSO₄, 1.18; KH₂PO₄, 1.18; CaCl₂, 2.5; NaHCO₃, 24.9; glucose, 10. Lithium chloride was added to inhibit inositol monophosphate hydrolysis. Aortas without endothelium were gently blotted and weighed prior to exposure to [³H]inositol. Tissues were then transferred to flasks containing fresh Krebs-Ringer bicarbonate solution and norepinephrine, sodium nitroprusside, atriopeptin II, 8-bromo cGMP, or acetylcholine were added as above in the presence of 10 mM LiCl. Lithium chloride was added to inhibit inositol monophosphate phosphatase (Berridge et al., 1982).

Aortas were then washed with 8 mg/ml of 10% trichloroacetic acid, centrifuged (low speed, 15 minutes, and after extraction of the supernatants with ether (2.0 ml, 5 times), the supernatants were loaded onto anion exchange columns (175 mg; BioRad AG1-X8). Similar results were
obtained when tissues were homogenized in chloroform:methanol:water (5:10:4) followed by the addition of chloroform:methanol:water (10:10:5), according to the method of Brown and Brown (1983) (data not shown). The columns were washed with 70 ml of water, and the inositol monophosphate was eluted with 8 ml of 0.2 M ammonium formate in 100 mM formic acid. Scintillation cocktail (10 ml) was added to the eluate and the samples were counted. Recovery of inositol monophosphate through the ether extraction and column chromatography procedures was 80%. Recovery of phosphatidylinositol in this fraction was approximately 0.3%. The norepinephrine-induced increase in inositol monophosphate formation was dependent upon the presence of lithium (unpublished observation). The pellets of the tissues with endothelium were resuspended in 1.0 N NaOH and assayed for protein according to the method of Lowry et al. (1951). Differences between means within each experiment were evaluated by analysis of variance. If analysis of variance demonstrated a significant difference among means, Student’s unpaired t-test was then used to determine which pairs of means were significantly different. Significance was accepted at the 0.05 level of probability.

Materials

Acetylcholine-HCl, 8-bromo cGMP, l-norepinephrine-HCl, and sodium nitroprusside were obtained from Sigma, atriopeptin II was from Peninsula Laboratories, and myo-[2-3H(N)]-inositol (16.5 Ci/mmol) was from New England Nuclear.

Results

Exposure of rat aorta to 0.3 μM norepinephrine for 30 minutes in the presence of 10 mM LiCl induced contraction and elevated inositol monophosphate levels (Fig. 1; Table 1). Pretreatment with 1 μM nitroprusside, 10 nm atriopeptin II, or 0.3 mM 8-bromo cGMP inhibited both the norepinephrine-induced contraction and accumulation of inositol monophosphate (Fig. 1; Table 1). Exposure to nitroprusside, atriopeptin II, and 8-bromo cGMP did not

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Inositol monophosphate (counts/min per mg wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>28 ± 7 (4)*</td>
</tr>
<tr>
<td>Norepinephrine (0.3 μM)</td>
<td>148 ± 4 (3)†</td>
</tr>
<tr>
<td>Norepinephrine (0.3 μM + nitroprusside (1 μM))</td>
<td>59 ± 10 (4)†</td>
</tr>
<tr>
<td>None</td>
<td>32 ± 1 (4)*</td>
</tr>
<tr>
<td>Norepinephrine (0.3 μM)</td>
<td>222 ± 26 (4)†</td>
</tr>
<tr>
<td>Norepinephrine (0.3 μM + atriopeptin II (10 mM))</td>
<td>57 ± 9 (3)†</td>
</tr>
<tr>
<td>None</td>
<td>43 ± 12 (4)*</td>
</tr>
<tr>
<td>Norepinephrine (0.3 μM)</td>
<td>154 ± 29 (4)†</td>
</tr>
<tr>
<td>Norepinephrine (0.3 μM + 8-bromo cGMP (0.3 mM))</td>
<td>92 ± 3 (4)†</td>
</tr>
<tr>
<td>None</td>
<td>43 ± 5 (4)*</td>
</tr>
<tr>
<td>Norepinephrine (0.3 μM)</td>
<td>162 ± 21 (4)†</td>
</tr>
<tr>
<td>Acetylcholine (10 μM)</td>
<td>41 ± 16 (4)*</td>
</tr>
<tr>
<td>Norepinephrine (0.3 μM + acetylcholine (10 μM))</td>
<td>175 ± 27 (4)†</td>
</tr>
<tr>
<td>None</td>
<td>55 ± 8 (6)*</td>
</tr>
<tr>
<td>Norepinephrine (0.3 μM)</td>
<td>307 ± 35 (8)†</td>
</tr>
<tr>
<td>Acetylcholine (10 μM)</td>
<td>41 ± 16 (8)*</td>
</tr>
<tr>
<td>Norepinephrine (0.3 μM + acetylcholine (10 μM))</td>
<td>187 ± 25 (8)†</td>
</tr>
</tbody>
</table>

Aortas with and without endothelium were incubated in [3H]inositol, washed, and allowed to rest or were exposed, in some cases, to 0.3 μM norepinephrine for 30 minutes. Additional tissues were pretreated with 1 μM nitroprusside, 10 nm atriopeptin II, or 10 μM acetylcholine for 5 minutes or 0.3 mM 8-bromo cGMP for 15 minutes before administration of norepinephrine. LiCl (10 mM) was added to all tissues 1 minute before norepinephrine. Shown are mean ± se. Numbers in parentheses = number of aortas.

*Significantly less than tissues exposed to norepinephrine.
†Significantly greater than tissues exposed to nitroprusside, atriopeptin II, 8-bromo cGMP, or acetylcholine.
of tissues, including smooth muscle (Berridge and Irvine, 1984). Inositol monophosphate accumulation has also been proposed as a measure of hydrolysis of phosphatidylinositols (Berridge et al., 1982). In the present study, 0.3 μM norepinephrine increased the accumulation of inositol monophosphate. These results are consistent with those of others who have also recently demonstrated that 10 μM norepinephrine increased inositol monophosphate levels in rat aorta (Legan et al., 1985) and that epinephrine elevated phosphatidic acid labeling, another measure of hydrolysis of phosphatidylinositols (Berridge and Irvine, 1984), in rabbit aorta (Villalobos-Molina et al., 1982).

The increased accumulation of inositol monophosphate due to norepinephrine was inhibited by agents which elevate cGMP through different mechanisms. Sodium nitroprusside is thought to act directly on the smooth muscle to activate, presumably, soluble guanylate cyclase (Rapoport and Murad, 1983a). Atriopeptin II has been shown to activate particulate guanylate cyclase (Waldman et al., 1984; Winquist et al., 1984; Tremblay et al., 1985) and acetylcholine to act on the endothelium to cause release of a factor which elevates cGMP within the smooth muscle (Rapoport and Murad, 1983b). Thus, it appears that the cGMP levels elevated through these different mechanisms have a similar inhibitor effect on hydrolysis of phosphatidylinositols. In support of the hypothesis that the inhibition of inositol monophosphate accumulation was due to the elevated levels of cGMP in the observation that 8-bromo cGMP inhibited inositol monophosphate accumulation.

The present results are consistent with the proposal of Takai et al. (1984) that cGMP acts through inhibition of phosphatidylinositol breakdown, presumably through activation of cGMP-dependent protein kinase. Sodium nitroprusside, acetylcholine and atriopeptin II have been demonstrated to activate cGMP-dependent protein kinase in rat aorta (Fiscus et al., 1984; Fiscus et al., 1985). It has also been demonstrated that sodium nitroprusside, 8-bromo cGMP, and acetylcholine, as well as other endothelium-dependent vasodilators, induce similar protein phosphorylation patterns in intact rat aorta (Rapoport et al., 1982, 1983, 1984). The identities of these proteins are not known; however, one or more of these proteins may regulate the hydrolysis of phosphatidylinositols. The inhibition of hydrolysis would then, presumably, decrease the concentration of intracellular Ca++ through several mechanisms which include decreased release of Ca++ from the sarcoplasmic reticulum due to lack of inositol triphosphate formation (Berridge and Irvine, 1984), reduced permeability to extracellular Ca++ due to decreased amounts of phosphatidic acid (Michell, 1975), and decreased activation of protein kinase C due to decreased formation of diacylglycerol (Takai et al., 1984).

**Discussion**

The present results represent the first demonstration, to our knowledge, that agents which elevate cGMP inhibit agonist-elevated inositol monophosphate accumulation in smooth muscle. Hydrolysis of phosphatidylinositols is thought to be an initial event which follows receptor activation in a variety
We thank Mary Benedict for her skilled technical assistance, Drs. Carl Johnson and Arnold Schwartz for helpful suggestions and review of the manuscript, and Liz Wendelmoot and Robin Wright for assistance in preparation of the manuscript.

This work was supported by grants from the Veterans Administration, the American Heart Association, Southwestern Ohio Chapter, and BRSG S07 RR05408-24 from the National Institutes of Health.

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Received September 9, 1985; accepted for publication December 11, 1985.

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INDEX TERMS: Vascular smooth muscle • Inositol phosphates • cGMP • Contraction • Vasodilators
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doi: 10.1161/01.RES.58.3.407

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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