Prostaglandins and Potassium Relaxation in Vascular Smooth Muscle of the Rat

The Role of Na-K ATPase

WARREN E. LOCKETTE, R. CLINTON WEBB, AND DAVID F. BOHR

SUMMARY

We explored the hypothesis that prostaglandin-induced vasodilation is caused by activation of the electrogenic sodium-potassium pump which results in membrane hyperpolarization and relaxation of vascular smooth muscle. Helical strips of rat tail artery relax in response to potassium after norepinephrine-induced contractions in physiological salt solution containing a low-potassium concentration. The amplitude of this potassium-induced relaxation is used as an index of sodium-potassium ATPase activity. It was observed that PGA1, PGE2, and PGF2α (10⁻⁶ g/ml) significantly enhanced the magnitude of potassium-induced relaxation. PGA2 and PGI2 (10⁻⁶ g/ml) had no significant effect. PGE2 caused relaxation of contractions induced by either 25 mM KCl or norepinephrine (10⁻⁹ g/ml), and these relaxations were inhibited by 10⁻⁴ M ouabain. Indomethacin (5.3 x 10⁻⁶ g/ml) and meclofenamate (10⁻⁶ g/ml) reduced the magnitude of potassium-induced relaxation by more than 30% of control. PGF2α (10⁻⁴ g/ml) reversed the inhibition of potassium relaxation by meclofenamate. These observations suggest that prostaglandins induce vascular smooth muscle relaxation by stimulation of the sodium pump and that endogenous prostaglandins normally potentiate potassium relaxation.

shown that potassium-induced relaxation may be used as a functional indicator of Na-K ATPase activity. In the current study, a comparison was made of the effects of prostaglandins and prostaglandin synthetase inhibitors on potassium-induced relaxation of isolated vascular smooth muscle.

**Methods**

Male Sprague-Dawley rats (250–350 g) were anesthetized with sodium pentobarbital (approximately 50 mg/kg, ip). Tail arteries were excised, dissected free of loose connective tissue, and cut into helical strips (0.7 x 8.0 mm). These strips were attached to Grass force transducers (FT03) and suspended vertically in a muscle bath with physiological salt solution (PSS) of the following composition (in mmol/liter): NaCl, 130; KCl, 4.7; Na2HPO4, 1.18; MgSO4 • 7H2O, 1.7; CaCl2 • 2H2O, 1.6; NaHCO3, 14.9; dextrose, 5.5; and CaNa2 versenate, 0.026. PSS was gassed with 95% O2-5% CO2 and maintained at 37°C. Low potassium PSS had the same composition as the normal PSS except for a reduction in potassium concentration to 1.0 mM; no compensation for altered tonicity was made. The tail arteries were equilibrated for 1 hour, and a passive stretch of 500 mg was maintained throughout the experiment to achieve near maximal contractile forces.

The procedure for the evaluation of potassium-induced relaxation was designed so that each strip served as its own control. Data were analyzed with a paired t-test and results expressed as mean ± SE. The strips were incubated in low potassium (1.0 mM) PSS for 15 minutes (cf. Fig. 1) before adding norepinephrine (Levophed bitartrate, Winthrop Laboratories) to the bath to give a concentration of 10^-7 g/ml. Two minutes later, when the contraction had reached a steady level, KCl was injected into the muscle bath to elevate the bath concentration from 1.0 mM to 3.5 or 6.0 mM. These specific conditions were selected to give a small potassium-induced relaxation in the absence of the agent being tested for its ability to enhance the relaxation. This permitted the recording of a large increment in relaxation when the intervention (e.g., treatment with a prostaglandin) potentiated the relaxation mechanism. The magnitude of the relaxation was quantified as a percent of the total contraction that existed just before the addition of potassium to the bath. The effects of prostaglandins, indomethacin, and meclofenamate on potassium-induced relaxation were determined in most experiments by adding these agents to the muscle bath 12 minutes before inducing the potassium relaxation. Control relaxations (Fig. 1, upper tracing) were observed before and after the relaxations in the presence of these agents (Fig. 1, lower tracing).

The effect of ouabain on relaxation produced by PGE2, also was determined. Helical strips were made to contract in response to either 25 mM KCl (final [K+] = 29.7 mM) or norepinephrine (10^-9 g/ml) in the presence or absence of 10^-4 M ouabain. PGE2 was then added, and the relaxation was quantified as a percent change from the total contractile response. Experiments with 25 mM KCl (final [K+] = 29.7 mM) were carried out in the presence of 10^-5 M phentolamine (Regitine, CIBA) to eliminate the effects of norepinephrine which would have been released from intrinsic nerve endings by KCl.

The prostaglandins were supplied by Dr. John Pike of the Upjohn Company, Kalamazoo, Michigan. PGA1, PGA2, and PGE2 were kept in ethanolic stock solution of 10 mg/ml. The stock solution was diluted and added to the PSS in the muscle bath to give a final concentration of prostaglandins of 10^-6 g/ml. The bath concentration of ethanol did not exceed 0.1%, and, although at that concentration ethanol had no effect on the mechanical responses of the strips, it was used in the bath in control observations. PGE2 (tromethamine salt) was stored in an aqueous stock solution of 10 mg/ml. Indomethacin (Merck) was dissolved in ethanol such that the final bath concentration of ethanol was 0.5%; this concentration of ethanol inhibited potassium relaxation and, therefore, was used in control experiments for indomethacin. Ouabain (Nutritional Biochemicals) and meclofenamate (Parke-Davis) were stored as aqueous solutions.

Statistical analysis was performed by the use of a paired t-test. In all cases, a p value of less than 0.05 was selected to denote statistical significance between groups.
Results

Effects of Prostaglandins on Potassium Relaxation

The tracings in Figure 1 illustrate the procedure used to evaluate Na-K ATPase activity by the magnitude of a transient, potassium-induced relaxation. Two minutes after the addition of norepinephrine to the bath, a transient relaxation was induced by raising the concentration of potassium in the bath to 3.5 mM. After a brief period of relaxation, a spontaneous return of tension was observed (Fig. 1, upper tracing).

Addition of PGF$_2\alpha$ (10$^{-6}$ g/ml) 5 minutes after exposure to low potassium PSS resulted in a small, sustained contraction in all strips (Fig. 1, lower tracing). The presence of PGF$_2\alpha$ increased the magnitude and duration of relaxation in the subsequent response to added potassium. The relaxation induced by potassium in the presence of PGF$_2\alpha$ was transient, being followed by a spontaneous return of tension. After the washout of PGF$_2\alpha$ from the muscle bath, relaxation in response to potassium returned to the control magnitude. The magnitude of relaxation in response to potassium was increased as the concentration of PGF$_2\alpha$ was increased (Fig. 2A). The total contractions induced by norepinephrine 10$^{-7}$ g/ml, and PGF$_2\alpha$ also were increased as the concentration of PGF$_2\alpha$ was increased (Fig. 2B).

Comparative Effects of Prostaglandins

Figure 3 compares the effects of the various prostaglandins on potassium relaxation. All data on the prostaglandins are expressed as percent change from the control potassium relaxation response. For example, in Figure 1, the control relaxation (upper tracing) was 38%, the relaxation in the presence of PGF$_2\alpha$ (lower tracing) was 83%; our data describe this as a 118% potentiation of potassium relaxation by PGF$_2\alpha$.

At 10$^{-6}$ g/ml, PGA$_1$, PGE$_2$, and PGF$_2\alpha$ significantly potentiated potassium-induced relaxation of tail artery strips; PGA$_2$ and PGE$_1$ did not significantly alter the magnitude of potassium-induced relaxation. PGF$_2\alpha$ (10$^{-6}$ g/ml) caused a greater enhancement of potassium-induced relaxation in these experiments than in the experiments reported in Figure 2. There is no apparent reason for the greater effect that was observed.

The magnitude of the contractile response to norepinephrine (10$^{-7}$ g/ml) in low potassium PSS was significantly increased above control values by PGA$_1$, PGA$_2$, PGE$_2$, and PGF$_2\alpha$ (Table 1). PGE$_1$ did not significantly change the contractile response to norepinephrine. These observations suggest that the augmentation by PGA$_1$, PGE$_2$, and PGF$_2\alpha$ of the potassium-induced relaxation (Fig. 3) and its reduction by indomethacin and meclofenamate (Fig. 3 and 4) are not a simple reflection of an alteration in the magnitude of the norepinephrine contractile response.
Prostaglandin Synthetase Inhibitors and Potassium Relaxation

Indomethacin and meclofenamate, inhibitors of prostaglandin synthesis, cause a decrease in the amplitude of potassium-induced relaxation (Figs. 3 and 4). Indomethacin resulted in a decrease of potassium-induced relaxation for all concentrations of KCl from 1.0 mM to 15 mM. PGF₂α (10⁻⁶ g/ml) reversed the inhibition of potassium relaxation by meclofenamate (Fig. 4). Meclofenamate had no significant effect on the contractile responses of helical strips to norepinephrine; however, indomethacin reduced the contractile responses to norepinephrine by approximately 30% (Table 1).

Relationship of Potassium Relaxation to the Magnitude of Contraction

In the studies with prostaglandins and indomethacin, there is some parallelism between the percent relaxation induced by potassium and the magnitude of the norepinephrine response (cf. Table 1 and Figs. 2 and 3). To determine whether the magnitude of the norepinephrine response per se was responsible for the observed results, norepinephrine concentrations of 10⁻⁷ g/ml and 10⁻⁶ g/ml were used to produce the variations in the magnitude of contraction (Fig. 5). The percent relaxation induced by potassium was much greater when a smaller concentration of norepinephrine was used. Under these conditions, the percent relaxation is smaller when the magnitude of contraction is greater.

Table 1

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Control NE (10⁻⁷ g/ml)</th>
<th>Agonist alone</th>
<th>Agonist + NE (10⁻⁷ g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGA₁ (10⁻⁶ g/ml)</td>
<td>839 ± 40</td>
<td>179 ± 32</td>
<td>941 ± 42*</td>
</tr>
<tr>
<td>PGA₂ (10⁻⁶ g/ml)</td>
<td>708 ± 75</td>
<td>272 ± 54</td>
<td>996 ± 45*</td>
</tr>
<tr>
<td>PGE₁ (10⁻⁶ g/ml)</td>
<td>908 ± 81</td>
<td>129 ± 25</td>
<td>925 ± 77</td>
</tr>
<tr>
<td>PGE₂ (10⁻⁶ g/ml)</td>
<td>874 ± 62</td>
<td>379 ± 50</td>
<td>968 ± 63*</td>
</tr>
<tr>
<td>PGF₂ (10⁻⁶ g/ml)</td>
<td>777 ± 45</td>
<td>467 ± 39</td>
<td>898 ± 54*</td>
</tr>
<tr>
<td>Indomethacin (5.3 × 10⁻⁶ g/ml)</td>
<td>655 ± 87</td>
<td>0</td>
<td>459 ± 51*</td>
</tr>
<tr>
<td>Meclofenamate (10⁻⁶ g/ml)</td>
<td>765 ± 34</td>
<td>0</td>
<td>755 ± 38</td>
</tr>
</tbody>
</table>

This table compares the magnitudes of contractile responses induced by NE in the control condition to those induced by NE when the various agonists were present in the muscle bath. The first column identifies the experimental situation (the agonist being tested for its effect on potassium-induced relaxation). The second column gives the magnitude of the contractile response to NE (10⁻⁷ g/ml) in the untreated condition (as in Fig. 1, upper tracing). The third column gives the magnitudes of contraction induced by the various agonists being tested (as in Fig. 1, lower tracing); and the fourth column gives the total contraction produced when NE was added to the bath in the presence of the various agonists (as in Fig. 1, lower tracing). The augmentation by PGA₁, PGE₁ and PGF₂ of the potassium-induced relaxation (Fig. 3) and its reduction by indomethacin and meclofenamate (Figs. 3 and 4) are not a simple reflection of an alteration of the magnitude of the norepinephrine contractile responses.

* Statistical differences (P < 0.05) between contractile responses to NE in the control condition (column 2) and in the experimental situation (column 4).

The Effect of Ouabain on Relaxation in Response to PGE₂

Prostaglandins have a biphasic effect on vascular smooth muscle. Low concentrations of prostaglandins induce relaxation of isolated strips, and high concentrations of prostaglandins induce contraction of vascular smooth muscle when added to the mus-
cle bath. When the artery strips were caused to contract in response to the additions of 25 mM KCl (final [K+] = 29.7 mM) to normal PSS, the subsequent addition of low concentrations of PGE2 (10^-12 to 10^-7 g/ml) produced relaxation. Maximum relaxation occurred at a PGE2 concentration of 10^-8 g/ml. Ouabain (10^-4 M), a specific inhibitor of Na-K ATPase, prevented PGE2 induced relaxation (Fig. 6).

PGE2 (10^-8 g/ml) also produced relaxation of artery strips (n = 4) contracted with norepinephrine (10^-9 g/ml). The magnitude of the relaxation (expressed as percent change from the contractile response) was 66 ± 7%. Treatment with 10^-4 M ouabain caused a significant reduction (P < 0.05) in the magnitude of the relaxation in response to PGE2 (51 ± 2%). This level of inhibition of the relaxant effect of PGE2 by ouabain represented a change of 21 ± 7% from the untreated condition.

Discussion

Certain prostaglandins, depending on the species and the tissue examined, have been shown to augment tissue blood flow by acting directly on vascular smooth muscle to cause vasodilation (Conway, 1975; Hedqvist, 1972; Ferreira and Vane, 1974); therefore, prostaglandins may participate in the regulation of blood pressure and tissue perfusion (Messina et al., 1976). Consistent with this hypothesis are the findings that the levels of prostaglandin-like substances found in venous blood of the resting dog hindlimb are increased by muscular exercise or arterial occlusion (Herbacznka-Cedra et al., 1974; Weiner et al., 1977). Furthermore, prostaglandins have been shown to attenuate vasoconstrictor responses of blood vessels to angiotensin II (Aiken and Vane, 1972).

The mechanisms by which prostaglandins produce vasodilation are not known; the current data indicate that prostaglandins may induce relaxation of vascular smooth muscle by increasing the electrogenic transport of sodium and potassium by Na-K ATPase. This interpretation is based on the observation that potassium-induced relaxation of isolated vascular strips is a functional indicator of Na-K ATPase activity (Anderson, 1976; Bonaccorsi et al., 1977; Haddy, 1975; Webb and Bohr, 1978). Bonaccorsi et al. (1977) showed that helical strips of rat tail artery relax in response to potassium after serotonin-induced contractions in potassium-free PSS. Ouabain, a Na-K ATPase inhibitor (Schwartz et al., 1975), eliminates the relaxation response to the addition of potassium to potassium-free PSS. Webb and Bohr (1978) have shown that potassium-induced relaxation parallels the known effects of variations in the following parameters on Na-K ATPase: (1) extracellular potassium concentration; (2) intracellular sodium concentration; (3) ouabain concentration; (4) magnesium concentration; and (5) temperature.

Our studies indicate that PGF2α, PGA1, and PGE2 significantly enhance potassium-induced relaxation, i.e., Na-K ATPase activity, whereas PGA2 and PGE1 have no significant effect. Other reports on various systems suggest that prostaglandins may enhance Na-K ATPase activity. Toda (1974) has observed that relaxation of isolated canine cerebral arterial strips by potassium was increased by PGF2α in a concentration-dependent manner. Limas and Cohn (1974) have reported that a ouabain-sensitive Na-K ATPase in microsomal fractions isolated from dog mesenteric arteries was activated by PGE1, PGE2, and PGA2, but not PGF2α. Prostaglandins also have been shown to stimulate the active transport of sodium in the toad bladder, frog skin, and kidney (Hinman, 1972). Forster et al. (1976) have reported that arachidonic acid, a prostaglandin precursor, reversed ouabain-induced cardiac arrhythmias; this reversal was blocked by indomethacin. On the basis of our results, it seems likely that vascular smooth muscle relaxation in response to PGA1, PGE2, and PGF2α is the result of membrane hyperpolarization produced by increasing the electrogenic transport of sodium and potassium by Na-K ATPase.

Further support for the role of an electrogenic pump in prostaglandin-mediated vascular relaxation is gained from the observation that prostaglandins do not augment the relaxant effects of all vasodilators. Feigen et al. (1978) observed that in infusion of arachidonic acid into the renal vascular bed of dogs produced an increased renal blood flow. Both indomethacin and meclofenamate markedly reduced the vasodilator effect, suggesting that the response to arachidonic acid was dependent on

**Figure 6** The effect of ouabain on PGE2-induced relaxation. Helical strips of rat tail artery were made to contract in response to 25 mM KCl (final [K+] = 29.7 mM) in the presence or absence of ouabain (10^-4 M). PGE2-induced relaxation of the KCl-contracted strips. Relaxation was quantified as the percent change from the magnitude of the contractile response induced by 25 mM KCl (final [K+] = 29.7 mM). PGE2-induced relaxation of the contracted strips was inhibited by ouabain (n = 4).
endogenously produced prostaglandins. Indomethacin and meclofenamate did not affect the vasodilator properties of nitroglycerin (Chapnick et al., 1977; Feigen et al., 1978). Nitroglycerin produces relaxation of vascular smooth muscle through alteration of passive ionic fluxes; this relaxant effect is not dependent on Na-K ATPase (Kreye, 1978).

Since prostaglandins caused contraction at the concentrations used in these experiments, it is important to determine whether changes in potassium relaxation are the result of changes in the magnitude of the contractile properties of the vascular smooth muscle. It appears that the augmentation by PGA1, PGE2, and PGF2α of the potassium-induced relaxation and its reduction by indomethacin and meclofenamate are not simple reflections of an alteration of the magnitude of the norepinephrine contractile responses. Whereas the magnitude of potassium relaxation varied directly with the magnitude of the total norepinephrine contraction when these agents were used, the magnitude of potassium relaxation varied inversely with the magnitude of the norepinephrine contraction when the latter was altered by changing norepinephrine concentration. Furthermore, meclofenamate decreased potassium relaxation without altering the magnitude of the norepinephrine response, and the contractile response to PGA1 plus norepinephrine was greater than the contractile response to norepinephrine alone, and there was not significant augmentation by this prostaglandin of the potassium-induced relaxation (Table 1 and Fig. 3).

At low concentrations, PGE2 causes relaxation of vascular smooth muscle. We have shown that PGE2-induced relaxation of vascular smooth muscle is blocked by ouabain; this suggests that PGE2 requires an active Na-K ATPase to produce relaxation. Kadar and Sunhara (1969) have shown that PGE1 inhibits norepinephrine contractions of isolated dog mesenteric arteries and veins, and PGE2α enhanced responses to norepinephrine. The inhibition of the response to norepinephrine by PGE1 was abolished by pretreatment of the tissues with ouabain; PGE2α enhancement of norepinephrine responses were unaffected by ouabain. In light of the biphasic response to prostaglandins, it seems likely that the contractile effect of PGE2α under the conditions described by Kadar and Sunhara, masked the relaxant action which we have reported and which also has been reported by Toda (1974).

Though indomethacin and meclofenamate inhibited potassium-induced relaxation, it is not certain whether this inhibition of the electrogenic pump is caused by an inhibition of the prostaglandin synthetase system, or whether it is a result of a direct effect of these agents on Na-K ATPase. Because both indomethacin and meclofenamate inhibit potassium-induced relaxation, and because prostaglandins reverse meclofenamate inhibition of potassium-induced relaxation, it is likely that these agents act on endogenous prostaglandin synthesis to exert their effect on Na-K ATPase activity.

The molecular mechanisms by which prostaglandins alter the transport of sodium and potassium in vascular smooth muscle is not evident. It may be related to the activation of adenylate cyclase by prostaglandins (Hinman, 1972), and thus to the cellular concentrations of adenosine 3',5'-monophosphate (cyclic AMP). Cyclic AMP-mediated hyperpolarization of vascular smooth muscle has been suggested to be due to stimulation of an electrogenic pump (Somlyo et al., 1972), and cyclic AMP and theophylline have been observed to increase the activity of Na-K ATPase in microsomal fractions isolated from vascular smooth muscle (Limas and Cohn, 1974). Alternatively, the prostaglandins may alter the electrogenicity of the sodium pump, or they may produce a shift in passive ion gradients which causes a change in pump activity. Whatever the molecular mechanism, it appears that, when certain prostaglandins cause relaxation of vascular smooth, they may do so by increasing Na-K ATPase activity, and that endogenous prostaglandins in this muscle may have a tonic vasodilator influence.

**References**


Synthesis and Pharmacology of a Noncompetitive Antagonist of Angiotensin-Induced Constrictions of Vascular Smooth Muscle

[Sarcosyl]¹-[Cysteinyl (S-Methyl)]⁸-Angiotensin II

RICHARD J. FREER, JAMES C. SUTHERLAND, JR., AND ALAN R. DAY

SUMMARY The synthesis of an angiotensin II (A II) antagonist, sarcosyl¹-cysteinyl[S-methyl]⁸-angiotensin II [Sar¹-Cys(Me)⁸-A II], showing partial organ selectivity and properties of a noncompetitive antagonist, is described. The compound was found to be an extremely potent antagonist on vascular smooth muscle both in vitro (pA₂ for rabbit aorta = 9.2) and in vivo on rat blood pressure (dose ratio of 10³ for ED₂₅ mm Hg during 1 μg/kg per min infusion of antagonist). It was without effect on norepinephrine responses in both assay systems. In contrast, it was a considerably weaker antagonist on visceral smooth muscle (pA₂ for guinea pig ileum = 8.5; pA₂ for rat uterus = 7.9). Interestingly, in the vascular smooth muscle preparations, the compound also exhibited elements of a noncompetitive antagonist in that both the slope and maximum of the A II dose-response curves were reduced markedly. Qualitatively similar results were obtained with sarcosyl¹-alanyl⁸-angiotensin II (Saralasin) on rabbit aorta. Moderate depression of maximum response was seen in guinea pig ileum but not in rat uterus. These effects on vascular smooth muscle were reversible in vitro but only partially reversible in vivo. Circ Res 46: 720-725, 1980

SINCE the report of Marshall et al. (1970) that the reversed analogue, phenylalanyl⁴-tyrosyl⁸-angiotensin II, was a competitive inhibitor of angiotensin II (A II), a host of other analogues has been prepared which also inhibit the action of this hormone (see Peach, 1977; Regoli et al., 1974; Khoshla et al., 1974a). The structure-activity data generated to date for antagonists (as well as for agonists) have clearly indicated a pharmacologically heterogeneous population of receptors for A II in a variety of target cells. For example, an alkylating analogue of A II was shown to irreversibly antagonize the action of the parent hormone on guinea pig ileum but was without effect on isolated rat uterus or, in vivo, in the rat blood pressure assay (Paiva et al., 1972). In addition, des-Asp¹-Ile⁸-angiotensin II (Ile⁷-A III) is a more effective inhibitor of steroidogenesis induced by angiotensin III (A III, des-Asp¹-angiotensin II) than of an A II-induced effect (Sarstedt et al., 1975). Other antagonists have also been described which show some organ selectivity (Regoli et al., 1974). Many examples also exist in the agonist series (again, see Peach, 1977; Khoshla et al., 1974a, for discussion) including the postulated roles for angiotensin III (Goodfriend and Peach, 1975). It is a fact that this analogue is an extremely potent...
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Circ Res. 1980;46:714-720
doi: 10.1161/01.RES.46.5.714

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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