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Studies on the effects of viprostol in isolated small blood vessels and thoracic aorta of the rat

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1 The effects of viprostol, prostaglandin E_2 (PGE_2) and nitroglycerin were studied in basilar artery, small mesenteric artery and the vein parallel to it as well as thoracic aorta of the rat.

2 In KCl-contracted basilar artery, viprostol produced a concentration-related biphasic response, contraction at concentrations $< 3 \times 10^{-6}$ M and relaxation at concentrations $> 3 \times 10^{-6}$ M. PGE_2 produced a concentration-related contraction while nitroglycerin produced a concentration-related relaxation.

3 In KCl-contracted small mesenteric artery, viprostol produced a biphasic response which was similar to that in the basilar artery. PGE_2 produced a contraction and nitroglycerin produced relaxation in a concentration-dependent manner.

4 In KCl-contracted small mesenteric vein, in contrast to basilar and mesenteric artery, viprostol produced only a concentration-related relaxation in the range of 1×10^{-6} to 1×10^{-4} M. PGE_2 produced a contraction and nitroglycerin produced a concentration-related relaxation.

5 In KCl-contracted thoracic aorta, PGE_2 produced a biphasic response, relaxation at concentrations $< 3 \times 10^{-7}$ M and a concentration-related contraction at concentrations $> 3 \times 10^{-7}$ M. Viprostol only produced a concentration-related contraction at concentrations $> 1 \times 10^{-6}$ M, which was significantly less in magnitude than the contraction produced by PGE_2. Nitroglycerin produced a concentration-related relaxation as seen in the small vessels.

6 In conclusion, the present data demonstrate that viprostol is a vasorelaxant agent which effectively dilates KCl-contracted basilar, small mesenteric artery and vein, but not the thoracic aorta of rat. The potent antihypertensive action of viprostol is probably due to its relaxant effect on the small arteries and veins but not on the large conduit artery.

Introduction

Viprostol has been demonstrated to have a potent and prolonged blood pressure lowering effect in many models of hypertension (Cervoni *et al.*, 1984). This long-lasting antihypertensive effect can be achieved by various routes of administration, including transdermal application in animal studies (Cervoni *et al.*, 1984) and in clinical trials (Given *et al.*, 1984; Olivari *et al.*, 1984; Uribarri *et al.*, 1984). In clinical studies, viprostol has been demonstrated to lower arterial blood pressure significantly in man following transdermal and/or intravenous administration. The antihypertensive action of viprostol has been suggested to be the result of peripheral vasodilatation (Cervoni *et al.*, 1984). However, this conclusion was based mainly on the relaxant effect of viprostol on noradrenaline perfused rabbit isolated central ear artery preparations and the increased blood flow to femoral, carotid, coronary, superior mesenteric and renal vascular beds upon intra-arterial injection of viprostol in dogs (Cervoni *et al.*, 1984). The relaxant effect of viprostol on the larger size arterial beds alone probably does not explain its potent and prolonged antihypertensive activity. Ideally, viprostol would have to dilate small resistance-sized arteries to achieve its potent blood pressure lowering effects. The purpose of this study was, therefore, to evaluate the effects of viprostol on cerebral artery and the small mesenteric artery and vein. These three blood vessels are haemodynamically important for their resistance (arterial) or capacitance (venous) function in both central and peripheral vascular beds. Since viprostol is an analogue of prostaglandin E_2 (PGE_2), the effect of PGE_2 on these

vessels was also examined and compared to viprostol. For comparison, a conduit artery, such as thoracic aorta was also included in the study.

Methods

Basilar artery, small mesenteric artery and/or vein preparations

Sprague-Dawley rats (300–350 g) were decapitated with a guillotine. The whole brain, as well as the segment of the ileum containing the mesenteric arcades originating from the superior mesenteric artery, were removed and placed in oxygenated modified Krebs solution of the following composition (mM): NaCl 119, KCl 4.6, $MgCl_2$. $6H_2O$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 15, glucose, 6.0 and $CaCl_2$ 1.5. The brain basilar artery and the arterial and corresponding venous segment of the tertiary branch of the mesenteric artery were isolated and cut into 1.5–2.0 mm long ring segments (the outer diameters of the basilar artery, small mesenteric artery or vein were $<150\,\mu M$). Each ring was threaded onto a tissue holder and transferred to a microvessel apparatus. The microvessel apparatus used in this study was a modification of the model of Högestätt *et al.* (1983). In brief, our apparatus featured a built-in microscope and two three-axes micrometers which greatly enhance the ability to manipulate the tissue holders when threading the vessels onto the tungsten wire in the apparatus. One of the tissue holders was attached to a force-displacement transducer (FT03) which was connected to a Grass polygraph (Model 7D) for recording the contraction of the tissue. The opposite tissue holder, which was attached to the other micrometer, was moveable and was used for threading the vessel onto the wire. Each ring segment was carefully threaded onto a tungsten wire on the tip of the tissue holder which was then inserted into position on the micrometer of the apparatus. With the aid of the microscope and the micrometers, the tip of the tungsten wire from the other tissue holder was gently passed into the lumen of the vessel. Vessels were preloaded with an initial 50 mg of optimal tension by fine adjustment of the micrometer and allowed to equilibrate for 90 min, during which time the bathing medium was changed at 20 min intervals. Histological examination of the arteries removed from the tissue holder at the end of the study indicated that the vessels maintained their intact endothelium (Lai *et al.*, unpublished observations).

Passive force and contractile responses to potassium

To determine the optimal initial loading tension, basilar arteries and small mesenteric arteries and the corresponding veins from rats were allowed to equilibrate for 60 min with zero resting tension. After the equilibration period, 100 mM K^+ was introduced into the bath to determine the maximum effect. Following the washout, the vessels were then stretched to successively greater tension levels at 25 mg increments. As each increment of passive force was applied to the vessels, the contractile response to 100 mM K^+ was determined. The maximum K^+-induced contractile force was reached when the applied passive force on the vessel was approximately 50 mg. When the passive force was increased to 125 mg, there was no further increase in the K^+-induced contractile tension. Thus, the optimal initial tension placed on these vessels was determined to be approximately 50 mg and was used throughout these studies.

Thoracic aortic ring preparations

Rats were killed as above and the thoracic aorta was quickly excised and placed in oxygenated Krebs solution of the following composition (mM): NaCl 112.9, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$. $7H_2O$ 1.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.4 and glucose, 11.5. The aorta was cleaned of connective tissue and cut into rings approximately 2–3 mm in length. Each ring was attached via stainless steel wire hooks to a tissue holder (Model M-89, MRA Corp., Clearwater, FL) and to a force-displacement transducer (Model UC3, Gould-Statham, Burco, Dayton, OH). The bathing medium and tissues were maintained at 37°C in a 50 ml capacity glass chamber (MRA Corp.). An initial tension of 1 g was placed on each tissue. The tissues were allowed to equilibrate for 60–90 min, during which time the bathing medium was replaced every 20 min. Aortic ring tension was monitored by a Grass polygraph (Model 7D).

Contraction and/or relaxation responses of viprostol, prostaglandin E_2 and nitroglycerin

Cumulative concentration-response curves were determined for viprostol, PGE_2, nitroglycerin or vehicle in tissues precontracted with a submaximal concentration of KCl (20–25 mM) which could maintain a steady state of contraction at or near 50–75% of the maximal contraction. % contraction was calculated as the % increase in control contraction, while relaxation of the contracted tissue back to baseline was considered as 100% relaxation. The vehicle for viprostol and PGE_2 was ethanol/Krebs solution. At the concentrations of 1×10^{-5}, 3×10^{-5} and 1×10^{-4} M of viprostol or PGE_2, in the organ bath, the respective concentration of ethanol was 0.1, 0.3 and 0.37%. The vehicle for nitroglycerin was distilled water.

Statistics

For each concentration, an analysis of variance was performed and comparisons between means were made by use of Student's t test, at the $P<0.05$ significant level, with the variability obtained from the analysis of variance.

Drugs

Viprostol (Cl 115,347; (D,L)-15-deoxy-16-hydroxy-16 (α/β)-vinyl-prostaglandin E_2 methyl ester) was synthesized in Lederle Labs, Medical Research Division, American Cyanamid Company; 1-prostaglandin E_2 (Ono Pharmaceutical Co., Osaka, Japan); nitroglycerin tablet (Eli Lilly & Co., Indianapolis, IN).

Viprostol and PGE_2 were dissolved in pure ethanol and stored in the regrigerator as stock solution. Fresh dilutions were made daily in Krebs solution.

Results

KCl evoked a concentration-dependent contraction in basilar artery (BA), mesenteric artery (MA) and mesenteric vein (MV) with respective maximal-developed tension (mg) of 239.2 ± 43.3 ($n = 6$), 200.8 ± 38.3 ($n = 6$) and 120.0 ± 16.2 ($n = 6$). The EC_{50} values (mM) estimated for these vessels were 27.3 ± 1.4 ($n = 6$), 45.7 ± 2.5 ($n = 6$) and 28.8 ± 2.9 ($n = 6$) for BA, MA and MV, respectively. These respective concentrations of K^+ were used to contract the individual vessels for the following relaxation studies.

In KCl-contracted BA (Figure 1), viprostol produced a concentration-related biphasic response, contraction at concentrations $< 3 \times 10^{-6}$ M and relaxation at concentrations $> 3 \times 10^{-6}$ M. PGE_2 produced a concentration-related contraction, while nitroglycerin produced a concentration-related relaxation with an EC_{50} of $2.8 \pm 0.2 \times 10^{-5}$ M ($n = 6$). The ethanol vehicle had no effects at concentrations up to 0.1%. At concentrations of 0.3 and 0.37% ethanol in the bathing solution an increase in tension of 4 ± 2 and 28 ± 16%, respectively, was noted. However, these increases were not statistically significant.

In KCl-contracted MA, viprostol, PGE_2 and nitroglycerin produced effects similar to those in the BA (data not shown). Viprostol produced a biphasic response while PGE_2 produced a contraction. On the other hand, nitroglycerin produced a relaxation with an EC_{50} of $8.3 \pm 1.0 \times 10^{-5}$ M ($n = 4$). Vehicle, however, had no significant effect on baseline tension. No increase in tension at the corresponding 3×10^{-5} and 1×10^{-4} M concentrations was noted (as in the BA preparation).

In KCl-contracted MV (Figure 2), in contrast to BA and MA, viprostol produced only a concentration-

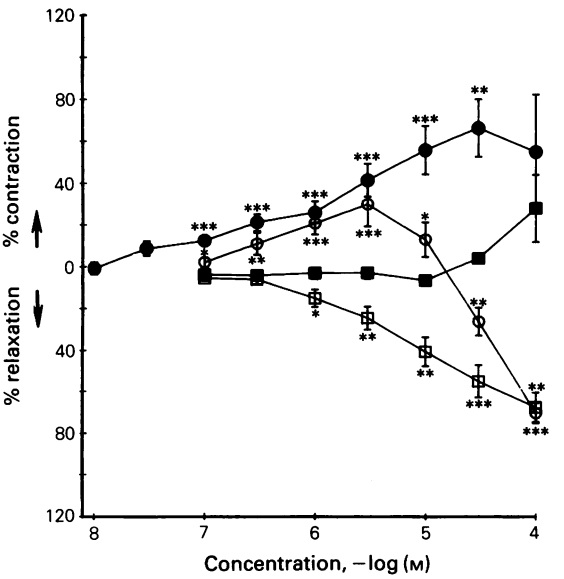

Figure 1 Vasoeffect of viprostol (○), prostaglandin E_2 (●) and nitroglycerin (□) on KCl-contracted isolated basilar arteries of the rat. Each value is the mean response of 7–8 arteries; vertical lines indicate s.e.mean. Significantly different from the responses of the control (■), $*P<0.05$, $**P<0.01$, $***P<0.001$.

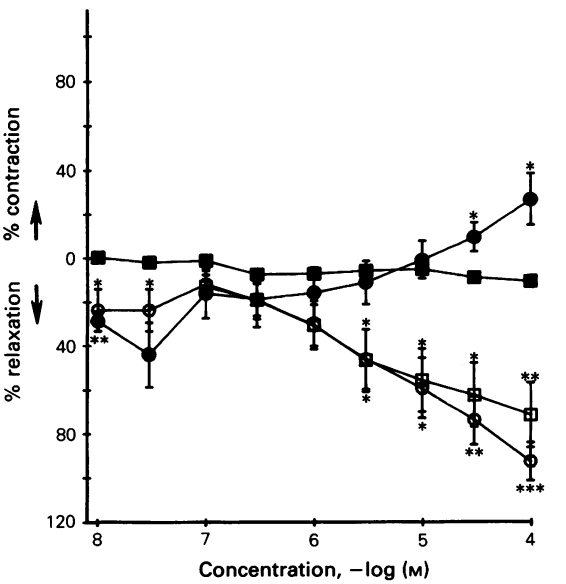

Figure 2 Vasoeffect of viprostol (○), prostaglandin E_2 (●) and nitroglycerin (□) on KCl-contracted rat isolated small mesenteric veins which parallel the arterial segments. Each value is the mean response of 6 preparations; vertical lines indicate s.e.mean. Significantly different from the responses of the control (■), $*P<0.05$, $**P<0.01$, $***P<0.001$.

related relaxation in the range of 3×10^{-7} to 1×10^{-4} M. As in BA and MA, PGE_2 produced a contraction. However, the contraction was smaller than in BA and MA. Nitroglycerin in MV, as in BA and MA, also produced a concentration-related relaxation with an EC_{50} of $5.0 \pm 0.7 \times 10^{-6}$ M ($n = 4$). Vehicle had no effect on baseline tension.

In KCl-contracted thoracic aortic rings (Figure 3), PGE_2 produced a biphasic response, relaxation at concentrations $< 3 \times 10^{-7}$ M and a concentration-related contraction at concentrations greater than 3×10^{-7} M. However, viprostol only produced a concentration-related contraction at concentrations $> 1 \times 10^{-6}$ M, which was significantly less in magnitude than the contraction produced by PGE_2. Nitroglycerin, again, produced a concentration-related relaxation with an EC_{50} of $2.6 \pm 0.4 \times 10^{-7}$ M ($n = 4$). When this EC_{50} was compared to those of BA, MA and MV, it was noted that nitroglycerin was significantly more active in thoracic aorta ($P < 0.05$) than in the smaller vessels.

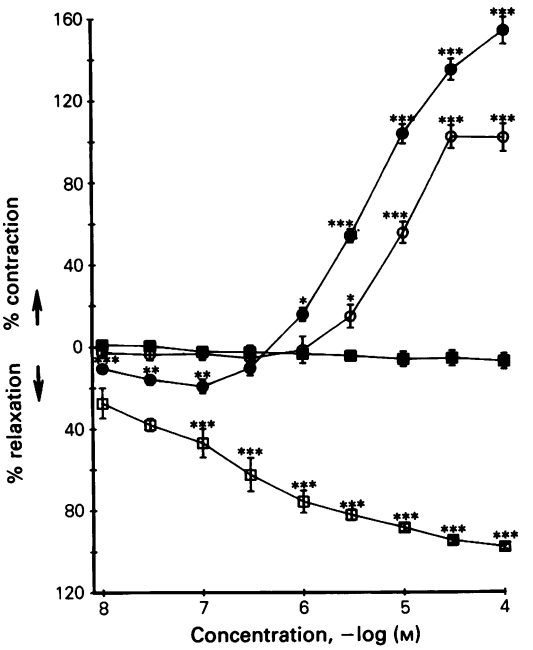

Figure 3 Vasoeffect of viprostol (○), prostaglandin E_2 (●) and nitroglycerin (□) on KCl-contracted isolated thoracic aorta of the rat. Each value is the mean response of 4 aortic rings; vertical lines indicate s.e.mean. *Significantly different from the responses of the control (■), $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Discussion

The present results demonstrate that viprostol is an effective vasorelaxant. It dilates KCl-contracted isolated BA and MA at concentrations $> 3 \times 10^{-6}$ M, and MV at concentrations $> 1 \times 10^{-7}$ M, but did not relax thoracic aorta of the rat. Further, this study has demonstrated that although viprostol is an analogue of PGE_2, the vasoeffect of viprostol in the rat is qualitatively different from that of PGE_2.

The effect of viprostol appears to display tissue specificity. For example, in the basilar and small mesenteric artery, viprostol produced a concentration-related contraction followed by a concentration-related relaxation. In the small mesenteric vein, viprostol produced only relaxation, while in the thoracic aorta it produced only contraction. On the contrary, PGE_2 contracted brain basilar and small mesenteric artery or vein and the thoracic aorta. The constrictor effects of PGE_2 have been demonstrated in various types of isolated vascular smooth muscle preparations (Strong & Bohr, 1967; Levy, 1973). The present data, in general, are in agreement with the results presented in the literature, except that we observed a small but significant relaxation at concentrations less than 0.1 μM in thoracic aorta. The significance of this vasorelaxation, if any, is not presently known. Also, the present data demonstrate that the relaxant effect of nitroglycerin is independent of the vessel studied. It dilated the large conduit artery, small resistance artery, as well as the small capacitance vein. However, it is noted that nitroglycerin is more potent in the larger artery than in the small blood vessels. This observation is in agreement with the study of Tsukada *et al.* (1986), that small vessels are less sensitive to nitroglycerin than are large vessels.

The more potent vasoconstrictor and less potent vasodilator effect of PGE_2 as compared to that of viprostol is an interesting observation. We do not have an explanation for the difference at the present time. However, this effect could be associated with the nature of the chemical structure *per se* since viprostol, an analogue of PGE_2, has a hydroxyl and a vinyl group at the C_{16} position. It is speculated that the vasodilator effect of viprostol is due to the functional groups in the C_{16} position and its longer duration of hypotensive effect is the result of the ability of the compound to resist degradation by the enzyme, 15-prostaglandin dehydrogenase. This merits further consideration and study.

In conclusion, the antihypertensive effect of viprostol seen in rats is probably the result of its relaxant effects on small blood vessels, but not the larger conduit artery.

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