Vasopressin Causes Endothelium-Dependent Relaxations of the Canine Basilar Artery

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SUMMARY. The effect of synthetic arginine vasopressin (vasopressin) was studied in isolated canine basilar, left circumflex coronary, and femoral arteries of the dog. Vascular rings with and without endothelium were suspended for isometric tension recording in physiological salt solution. The removal of the endothelium was confirmed by the absence of relaxations induced by either thrombin (basilar arteries) or acetylcholine (coronary and femoral arteries). In the basilar artery, vasopressin induced concentration-dependent inhibition of myogenic tone. In basilar and coronary arteries, the hormone caused concentration-dependent relaxations during contractions evoked by prostaglandin F2α. In femoral arteries, vasopressin caused contraction. After removal of the endothelium, the inhibitory responses to vasopressin were abolished in basilar arteries and significantly reduced in left circumflex coronary arteries. The relaxations of basilar arteries caused by adenosine diphosphate were not affected by endothelium removal. The V₁-vasopressinergic antagonist d(CH₂)₅Tyr (Me)AVP prevented the inhibitory response to vasopressin, but did not alter endothelium-dependent relaxations of basilar arteries caused by adenosine diphosphate. These results demonstrate that the endothelial cells mediate relaxation induced by vasopressin via specific V₁-vasopressinergic receptors. (Circ Res 55: 575-579, 1984)

VASOPRESSIN causes contraction of vascular smooth muscle in vivo (Altura, 1973; Nakano, 1974; Monos et al., 1978; Vanhoutte, 1978). Small arteries of the rabbit brain constrict when exposed to vasopressin (Uchida et al., 1967). However, contraction is not observed in larger isolated cerebral arteries (Allen et al., 1974; Altura and Altura, 1984). Studies in the cat and rat demonstrate that intracarotid infusion of large doses of vasopressin can increase cerebral blood flow (Kozniewska et al., 1979; Kozniewska and Skolasinska, 1982). The presence or absence of endothelium can help to explain apparent discrepancies between the responses to a variety of neurohumoral substances observed in vivo or in vitro, or in small or large blood vessels (Furchgott et al., 1981; Furchgott, 1983; Vanhoutte and Rimele, 1983). The present experiments were designed to determine whether or not vasopressin affects endothelial and smooth muscle cells in canine blood vessels.

Methods

The experiments were performed on rings (4 mm long) of basilar, left circumflex coronary, and femoral arteries taken from dogs of either sex (20–30 kg) anesthetized with sodium pentobarbital (30 mg/kg, iv). The brain, heart, and femoral arteries were removed and placed in physiological salt solution (millimolar composition: NaCl, 118.3; KCl, 4.7; CaCl₂, 2.5; MgSO₄, 1.2; KH₂PO₄, 1.2; NaHCO₃, 25.0; CaEDTA, 0.026; glucose, 11.1). Basilar arteries were dissected free under magnification. In some rings, the endothelium was mechanically removed by a brief, gentle rubbing of the intimal surface (De Mey and Vanhoutte, 1981, 1982; Cohen et al., 1983). Each ring was connected to a force transducer (Gould UTC-2) and suspended in an organ chamber filled with 25 ml of physiological salt solution (37°C, pH 7.4), gassed with 95% O₂-5% CO₂. Isometric tension was continuously recorded (Hewlett-Packard 7418 A).

The rings cut from basilar arteries were allowed to stabilize at a resting tension of 200–400 mg for 1 hour. The tension was increased to 3 g (Allen et al., 1974). This was followed by another 1-hour period of equilibrium. The rings of left circumflex coronary and femoral arteries were stretched to the optimal point of their length-tension relationship using a standard concentration (3 x 10⁻⁷ M) of norepinephrine in femoral, and of prostaglandin F₂α (2 x 10⁻⁷ M) in left circumflex coronary arteries (De Mey and Vanhoutte, 1980; Cohen et al., 1983). After this procedure, the preparations were allowed to equilibrate for 45 minutes.

Concentration-response (relaxation or contraction) curves for vasopressin and adenosine diphosphate (ADP) were obtained in a cumulative fashion. The relaxations induced by vasopressin and ADP were expressed as percent of the maximal relaxation induced by papaverine (10⁻⁴ M). The preparations were washed at least three times with 25 ml of physiological salt solution and allowed to equilibrate for 30 minutes after each exposure to vasoactive substances.

Integrity of the Endothelium

The basilar arteries were examined using light microscopy, using polychromatic staining (Van Reemts and Borgers, 1975; De Mey and Vanhoutte, 1981). Examination of transverse sections confirmed the presence and absence of endothelial cells in control and rubbed rings, respectively. Preliminary experiments indicated that
FIGURE 1. Effects of increasing doses of bovine thrombin on contractions of canine basilar arteries, with and without endothelium, induced by prostaglandin F$_2$α (2 × 10$^{-6}$ M). Thrombin caused transient relaxation (shown), followed by contraction in arteries with endothelium; in denuded rings thrombin caused only contraction. The changes in tension are expressed as percent of the response to prostaglandin F$_2$α, and are shown as means ± SEM.

Thrombin caused potent transient relaxations (followed by contractions) in basilar arteries where the endothelium was present, but not in endothelium-denuded rings (De Mey and Vanhoutte, 1982; White et al., 1984) (Fig. 1). In further experiments, the functional integrity of the endothelium in these arteries was confirmed by the presence of an instantaneous relaxation induced by 1 U/ml of thrombin; in the left circumflex coronary and femoral arteries it was confirmed by the presence of the relaxation induced by acetylcholine (3 × 10$^{-6}$ M) during contraction obtained with prostaglandin F$_2$α (2 × 10$^{-6}$ M) (De Mey and Vanhoutte, 1981; Cohen et al., 1983).

Drugs

The following pharmacological agents were used: acetylcholine chloride (Sigma); adenosine 5'-diphosphate (Sigma); synthetic 8-arginine vasopressin (Bachem); [18-mercapto-ß-cyclopentamethylenepropionic acid], 2-[O-methyl] tyrosine] arginine vasopressin, [d(CH$_2$)$_3$Tyr (Me)AVP (Ciba-Geigy)]; norepinephrine-bitartarate (Sigma); prostaglandin F$_2$α (PGF$_2$α; Sigma); bovine thrombin (Sigma); and sodium pentobarbital (Fort Dodge Laboratories). Stock solutions of the drugs were freshly prepared every day. Drugs were dissolved in distilled water such that volumes of less than 0.5 ml were added to the organ chambers.

FIGURE 2. Original tracing demonstrating the inhibitory effect of low concentrations of vasopressin on myogenic tone in a ring of the canine basilar artery with intact endothelium (top panel). Relaxation was observed at 10$^{-10}$ M and increased up to 3 × 10$^{-8}$ M concentration. In the absence of endothelium (lower panel), these doses caused slight increases in tension. With concentrations in excess of 3 × 10$^{-8}$ M, there was an increase in tension in the ring with intact endothelium, whereas, in that without endothelium, slight relaxation occurred. At the end of the experiment, papaverine 10$^{-4}$ M caused complete relaxation of both rings.

Statistical Analysis

The data are expressed as means ± SEM; n refers to the number of dogs. Statistical comparisons between responses of rings from the same artery, with and without endothelium, or in the presence and absence of antagonist,

FIGURE 3. Cumulative concentration-response curves to arginine vasopressin in control and endothelium-denuded rings of canine basilar artery. The relaxations were obtained in rings which developed myogenic tone. The data are shown as means ± SEM (n = 7) and are expressed as percent of the maximal relaxations induced by papaverine (10$^{-6}$ M 100% = 1.92 ± 0.33 g and 1.57 ± 0.18 g for control and endothelium-denuded rings, respectively. *The difference between the two types of preparations is statistically significant (P < 0.05).
TABLE 1
Effect of Endothelium Removal on Isometric Contractions Evoked by Prostaglandin F2α (2 × 10^-8 M) in Canine Basilar, Left Circumflex Coronary, and Femoral Arteries*

<table>
<thead>
<tr>
<th>Artery</th>
<th>Control</th>
<th>Denuded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basilar</td>
<td>3.81 ± 0.25 (10)</td>
<td>3.32 ± 0.35 (10)</td>
</tr>
<tr>
<td>Coronary</td>
<td>4.01 ± 0.71 (6)</td>
<td>4.83 ± 1.20 (6)</td>
</tr>
<tr>
<td>Femoral</td>
<td>12.62 ± 1.53 (4)</td>
<td>10 ± 1.58 (4)</td>
</tr>
</tbody>
</table>

* Data expressed as increases in tension (g) above baseline and shown as means ± SEM. The number of experiments is given in parentheses.

Results

In unstimulated rings of basilar artery which developed spontaneous myogenic tone, the tone was inhibited in a concentration-dependent manner by vasopressin (10^-12 to 10^-7 M). The inhibitory effect of vasopressin was abolished by the removal of the endothelium (Figs. 2 and 3). At higher concentrations, relaxation changed to contraction. Since the effects of the lower doses are of physiological interest, we have concentrated on these, and have not investigated the causes of increase in tension at the higher concentrations of the hormone. In rings of the basilar arteries devoid of myogenic tone, vasopressin produced no significant change in tension.

Vasopressin (10^-10 to 10^-6 M) caused concentration-dependent relaxations in control rings of basilar and left circumflex coronary arteries made to contract by prostaglandin F2α, but only further contraction in femoral arteries. Removal of the endothelium did not significantly affect the response of either artery to prostaglandin F2α (Table 1). However, it abolished the inhibitory response to vasopressin in basilar arteries, and significantly reduced it in left circumflex coronary arteries (Fig. 4). Endothelium removal did not significantly affect the further increases in tension caused by vasopressin in femoral arteries contracted with either prostaglandin F2α (Fig. 4) or norepinephrine (3 × 10^-7 M; data not shown).

On second exposure (after 30 minutes) of basilar arteries to increasing concentrations of vasopressin the relaxation was significantly reduced (by more than 30%), and the threshold-concentration increased (approximately 10-fold). The endothelium-dependent response to thrombin was unaltered in preparations made tachyphylactic to vasopressin (data not shown).

Paired rings from the same dogs were exposed to vasopressin in control solution and in solution containing the V1-vasopressinergic antagonist d(CH2)5 Tyr(Me)AVP. The antagonist was added 10 minutes after contraction with prostaglandin F2α (2 × 10^-6 M) had been initiated. It did not affect the response to the prostaglandin; at 10^-8 M it reduced, and at 10^-6 M it abolished, the vasopressin-induced relaxations (Fig. 5).

ADP caused dose-dependent relaxations in control rings made to contract with prostaglandin F2α (2 × 10^-6 M). Removal of endothelium significantly reduced the inhibitory effect of ADP. The ADP-
induced relaxation was not affected by d(CH₂)₅Tyr (Me)AVP (10⁻⁶ M) (Fig. 6).

**Discussion**

The study demonstrates that vasopressin relaxes the canine basilar artery only if the endothelium is present; the endothelium-mediated response resulted in relaxations comparable to the maximal inhibitory effect of the smooth muscle relaxant papaverine. In the absence of endothelial cells, vasopressin does not affect the vascular smooth muscle of larger canine arteries, illustrating the heterogeneity of the direct constrictor effect of the peptide (Uchida et al., 1967; Nakano, 1974; Monos et al., 1978; Vanhoutte, 1978; Altura and Altura, 1984). In the presence of a V₁-vasopressinergic antagonist (Kruszynski et al., 1980; Sawyer and Manning, 1984) the endothelial-dependent relaxations were antagonized in a concentration-dependent manner. A concentration that abolished the relaxation with vasopressin did not affect the relaxation to adenosine diphosphate, illustrating the selectivity of the action of the antagonist. Thus, the endothelial cells of this cerebral artery appear to have V₁-vasopressinergic receptors which, when activated by a concentration of the peptide as low as 3 × 10⁻¹¹ M, trigger an inhibitory signal to the underlying smooth muscle cells.

The concentration of vasopressin causing endothelium-dependent relaxations is similar to that measured in the blood under physiological conditions and during hemorrhage, septic shock, or acute intracranial hypertension (Rap and Chwalbinska-Moneta, 1978; Cowley et al., 1980; Wilson et al., 1981; Cowley et al., 1983). In the dog, the vasopressin released into the circulation during hemorrhage has limited access to the cerebrospinal fluid (Wang et al., 1981), which suggests that the peptide does not cross the blood-brain barrier. Perivascular application of vasopressin to the surface of the brain arterioles does not affect pial arteriolar diameter or arteriolar blood flow (Lassoff and Altura, 1980). Thus, the increase in cerebral flow noted with intracarotid injections of vasopressin (Kozniewska et al., 1979; Kozniewska and Skolasinska, 1982) could be explained by its action on the endothelial cells.

The present study confirms that vasopressin causes relaxation of the coronary arteries (Turlapaty and Altura, 1982), and demonstrates that this relaxation is due in part to an endothelium-mediated process. Vasopressin is reported to increase the coronary vascular resistance in vivo (Schmid et al., 1983). This implies differential responsiveness to vasopressin in large coronary arteries and coronary resistance vessels. However, in the cerebral circulation, unlike other vascular beds, pial arteries significantly contribute to total vascular resistance (Hestad and Kontos, 1983).
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In the femoral artery, endothelial cells do not modulate the contractile response to vasopressin. The findings with the femoral artery confirm that vasopressin has a potent constrictor action on peripheral blood vessels (Altura and Altura, 1974). The differential effects of vasopressin on cerebral and peripheral arteries favor the interpretation that the increased levels of circulating vasopressin during hemorrhage and septic shock could favor the redistribution of blood from the periphery to the cerebral circulation and help to maintain cerebral blood flow.

The present study is the first to demonstrate that a hormone, in concentrations comparable to those detected in the blood of intact animals and man, can cause endothelium-dependent inhibition of vascular smooth muscle.

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References


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