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**Vasopressin Causes Endothelium-Dependent Relaxations of the Canine Basilar Artery**

Z.S. Katusic, J.T. Shepherd, and P.M. Vanhoutte

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**SUMMARY.** The effect of synthetic 8-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 contractions of femoral arteries 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)*

**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^-8 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 vasoreactive substances.

**Integrity of the Endothelium**

The basilar arteries were examined under light microscopy, using polychromatic staining (Van Reempts 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
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 x 10^-6 M) during contraction obtained with prostaglandin F_2α [2 x 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); [L-(2-mercapto-β-cyclopentamethylenepropionic acid), 2-(O-methyl) tyrosine]-arginine vasopressin, [d(CH)₅Tyr(Me)AVP (Ciba-Geigy)]; norepinephrine-bitartarate (Sigma); prostaglandin F₃α (PGF₃α, 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.

**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,
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></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)₅Tyr(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 x 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 intra-carotid 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 (Heistad and Kontos, 1983).
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, 1973). 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.

References


INDEX TERMS: Adenosine diphosphate • Thrombin • \(V_1\)-vasopressinergic receptors • Endothelium • Vascular smooth muscle • Cerebellar blood vessels • Coronaries

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