Endothelium-Independent Contractions of Human Cerebral Arteries in Response to Vasopressin

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We studied the effects of vasopressin in isolated segments from branches (500–700 μm in external diameter) of human middle cerebral arteries obtained during autopsy of 15 patients who had died 3–8 hours before. Paired segments, one normal and the other de-endothelialized by gentle rubbing, were mounted for isometric recording of tension in organ baths. In 11 normal segments, vasopressin produced concentration-dependent contractions with an EC50 of 7.0 × 10⁻⁹ M. Removal of the endothelium from 12 segments did not significantly affect vasopressin-induced contractions. Vasopressin produced further contractions in arterial segments with (n=4) or without (n=5) endothelium precontracted with KCl. In segments precontracted with prostaglandin F2α, acetylcholine caused relaxation only of those with endothelium. At 10⁻⁸ M (n=11), the vasopressin V-1 receptor antagonist d(CH₂)₅Tyr(Me)AVP produced a 60-fold shift to the right of the control response curve for vasopressin. Increasing the concentration of the receptor antagonist to 10⁻⁶ M (n=7) further displaced the control curve in a parallel manner. These results indicate that vasopressin exerts a powerful constrictor action on isolated human cerebral arteries by direct stimulation of V-1 receptors located predominantly on smooth muscle cells. It appears that this contractile response is not modulated by the presence of an intact endothelial cell layer. (Stroke 1990;21:1689–1693)

Vasopressin causes powerful constriction in a variety of vascular regions1,2 and is now thought to serve as a major fast-acting pressure control system in hypotensive states.3,4 The vasopressin receptor designated V-1 seems to mediate the cardiovascular actions of the peptide, whereas its antiuretic action is mediated through V-2 cyclic adenosine monophosphate–dependent receptors.5,6 Some cardiovascular actions of vasopressin are mediated by V-2–like receptors in both humans7 and dogs.8 A common finding in the vascular effects of this peptide is the heterogeneity of responsiveness, depending on region and species.9,10 With regard to the cerebral vessels, the debate goes on whether pharmacologic studies of vasopressin in various animal species can be extrapolated to humans.11 Integrity of the endothelial cell layer is a factor that may account for variations in the responses of different vascular beds to neurohumoral agents.12,13 Therefore, we designed this study to determine the direct effects of arginine vasopressin on isolated human cerebral arteries, with special emphasis on endothelium-dependent responses. We also studied the concentration–response relation of the cerebral arteries to vasopressin after treatment with d(CH₂)₅Tyr(Me)AVP, a compound that is devoid of agonistic pressor activity and antagonizes the vasopressor effects of vasopressin.14,15

Materials and Methods

Human cerebral arteries were obtained during autopsy of 15 patients (nine men and six women, aged 23–79 years) who had died 3–8 hours before. The cause of death varied; eight patients were victims of automobile accidents, four had died of myocardial infarction, and three had died of cancer of the stomach. The arteries were immediately placed in chilled Krebs-Henseleit solution, and cylindrical segments 3 mm long were cut from branches of the middle cerebral artery for isometric recording of tension. Outside diameter of the segments was measured using an ocular micrometer within a Wild M8 zoom microscope (Heerbrugg, Switzerland) and ranged from 500 to 700 (mean±SEM 623±37) μm.
In some arterial segments the endothelium was removed mechanically by inserting a roughened stainless steel wire into the lumen and gently rolling the segment on wet filter paper.

Two stainless steel pins 100 µm in diameter were introduced through the arterial lumen. One pin was fixed to the wall of the organ bath, while the other was connected to a strain gauge. The recording system included a Universal transducing cell (UC3, New Orleans, La.), a Statham microscale accessory (U15, Glen Burnie, Md.) and a Beckman recorder (Type RS, Fullerton, Calif.). Each arterial segment was set up in a 4-ml bath containing modified Krebs-Henseleit solution of the following millimolar composition: NaCl 115, KC1 4.6, KH2PO4 1.2, MgSO4 1.2, CaCl2 2.5, NaHCO3 25, glucose 11.1, and disodium ethylenediaminetetraacetic acid 0.01. The solution was equilibrated with 95% O2 and 5% CO2 to give a pH of 7.3-7.4. Temperature was held at 37° C. To establish the resting tension for maximal force development, the arterial segments were exposed repeatedly to 60 mM KC1. Basal tension was increased gradually until contractions were maximal. The optimal resting tension was 1 g. The segments were allowed to attain a steady level of tension during a 2-hour accommodation period before testing.

After each experiment the arterial segments were carefully opened flat and stained with AgNO3 to visualize the endothelium. Only those with >70% of the endothelium were considered as normal segments. Segments from which the endothelium had been removed never showed more than 5% of their intima covered with endothelium either before or after the experiment.

Concentration–response curves for vasopressin were determined in a cumulative manner, and control [in the absence of d(CH2)5Tyr(Me)AVP] and experimental [in the presence of d(CH2)5Tyr(Me)AVP] responses were obtained from different segments. The antagonist (10^-8 or 10^-6 M) was added to the organ bath 15 minutes before the concentration–response curve was determined. Concentrations of vasopressin that produced 50% of the maximum contraction (EC50 values) were determined for each arterial segment, and from these values the geometric means for EC50 with 95% confidence intervals were calculated. Responses to the cumulative addition of acetylcholine (10^-7 to 10^-6 M) were determined in some arterial segments after submaximal contractions induced by 10^-6 to 3×10^-6 M prostaglandin F2a (PGF2a).

Drugs used were acetylcholine chloride, arginine vasopressin, [β-mercapto-β,β-cyclopentamethylene-propionyl-O-Me-tyr²-Arg⁶] vasopressin [d(CH2)5Tyr (Me)AVP], papaverine hydrochloride, and PGF2a (Sigma Chemical Co., St. Louis). Drugs were dissolved in distilled water and added to the organ bath in volumes of <70 µl. Stock solutions of the drugs were freshly prepared every day.

Data are expressed as mean±SEM. The results were evaluated statistically by means of Student’s t test. A probability value of less than 0.05 was considered significant.

### Results
Cumulative application of vasopressin produced a constrictor response that was concentration-dependent (Figure 1). The maximum tension developed as well as the EC50 were similar (p>0.05) in arterial segments with and without endothelium (Table 1). There was no significant difference in the contraction

### Table 1. EC50 Values and Maximal Responses of Vasopressin in Human Cerebral Artery Segments

<table>
<thead>
<tr>
<th>EC50 (M)</th>
<th>Geometric mean</th>
<th>95% confidence interval</th>
<th>Maximal response (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With endothelium</td>
<td>11</td>
<td>7.0x10^-10</td>
<td>2.6x10^-10 to 1.8x10^-9</td>
</tr>
<tr>
<td>Without endothelium</td>
<td>12</td>
<td>4.2x10^-10</td>
<td>1.9x10^-10 to 9.2x10^-9</td>
</tr>
<tr>
<td>With d(CH2)5Tyr(Me)AVP.</td>
<td>10^-6 M</td>
<td>3.2x10^-8</td>
<td>1.5x10^-8 to 6.8x10^-8</td>
</tr>
<tr>
<td></td>
<td>10^-5 M</td>
<td>1.9x10^-4</td>
<td>1.0x10^-4 to 3.5x10^-4</td>
</tr>
</tbody>
</table>

*p<0.05, 0.001, respectively, different from segments with or without endothelium by Student's t test.
of segments with and without endothelium to the addition of 60 mM KCl (904 ± 325 versus 805 ± 207 mg, p > 0.05).

Because they did not differ significantly, the concentration–response curves of arterial segments with and without endothelium were combined to determine the control curve for vasopressin. The presence of $10^{-8}$ M d(CH$_2$)$_3$Tyr(Me)AVP in the organ bath displaced the control curve for vasopressin to the right, but differences in the maximal tensions developed were not significant (p > 0.05) (Figure 2, Table 1). Increasing the concentration of d(CH$_2$)$_3$Tyr(Me)AVP to $10^{-6}$ M further displaced the control curve for vasopressin (Figure 2, Table 1).

To determine whether vasopressin induces relaxation in previously contracted arteries, increasing concentrations of this peptide were added to nine segments precontracted with KCl. Figure 3 shows that vasopressin caused further contractions in both normal and endothelium-denuded arterial segments previously contracted with 60 mM KCl. In these segments the ability of the smooth muscle to relax was tested by adding $10^{-4}$ M papaverine when maximal contractions had been attained with vasopressin. Under these conditions papaverine caused almost complete relaxation of both normal and endothelium-denuded segments (Figure 3).

Acetylcholine was added during steady-state contractions produced by PGF$_{2a}$ (Figure 4). Relaxation was observed in 14 of 20 normal arterial segments tested even though examination of whole-mount sections confirmed the presence of >80% of the endothelium in all 20 segments. In 12 rubbed arterial segments with >90% of the endothelial cell layer removed, acetylcholine had no effect on the contraction induced by PGF$_{2a}$.

Discussion

Our results indicate that vasopressin is a potent agonist for the contraction of cerebral smooth muscle through activation of specific V-1 vasopressinergic receptors. The maximal tensions attained with vasopressin are similar to those induced with KCl and the EC$_{50}$ values for vasopressin are lower than those for
5-hydroxytryptamine, angiotensin II, and norepinephrine in the same vascular preparations. Our data also show that the vasopressin-induced contraction is not linked to the presence of an intact endothelium, thus indicating that vasodilator substances secreted by endothelial cells under basal, spontaneous conditions do not counteract the potent contractile effects of vasopressin on smooth muscle cells. In agreement with our results, previous experiments have shown that vasopressin causes endothelium-independent contraction in human mesenteric arteries and in the aorta and renal and carotid arteries of rats.

In contrast, in experiments in isolated basilar and circumflex coronary arteries from dogs, vasopressin caused concentration-dependent relaxations during contractions evoked by PGF$_2$α, after removal of the endothelium these inhibitory responses were significantly reduced. These latter findings indicate that the relaxation induced by vasopressin in canine basilar and coronary arteries may be mediated by the activation of specific V-1 vasopressinergic receptors on endothelial cells, resulting in the release of an endothelium-derived relaxing factor (EDRF). Conversely, we observed only constriction in human cerebral arteries, even when the segments were precontracted with KCl. At 60 mM KCl, one can assume that the vascular muscle was well depolarized. Presumably, vasopressin acts by increasing Ca$^{2+}$ influx into the muscle to above and beyond the influx produced by depolarization. Integrity of the endothelium had no effect on this response. Our results indicate the lack of specific V-1 receptors on endothelial cells or the absence of an effective release of EDRF induced by vasopressin. Therefore, it is likely that the contractile effects of vasopressin on human cerebral arteries are due to direct stimulation of specific receptors located on smooth muscle cells. In addition to regional and species differences, size of the vessels used may be a factor in variations in the responses of these studies. The dog basilar and left circumflex coronary arteries used by Katusic et al are large main arteries compared with the small human arteries that we analyzed. In relation to this, previous studies in anesthetized cats have shown that vasopressin selectively dilates large cerebral arteries (the circle of Willis and its major branches) and raises the resistance of small vessels. In anesthetized goats vasopressin decreases blood flow in a branch of the middle cerebral artery. It is concluded that vasopressin produces contraction only in small cerebral arteries, particularly those near the site at which extracranial vessels become intracranial vessels.

In contrast to the absence of a modulatory role for endothelium in the vasopressin-induced contraction, acetylcholine caused endothelium-dependent relaxation with a pattern similar to that originally described in most mammalian arteries. The lack of relaxation in response to acetylcholine observed in cerebral vessels without endothelium does not reflect a nonspecific effect on smooth muscle cells since the responsiveness to K$^+$ and papaverine was similar in normal and endothelium-denuded arterial segments. The acetylcholine-induced relaxation in middle cerebral artery segments that we observed confirm similar findings in human basilar artery rings.

Antagonists of arginine vasopressin have been used to study the cardiovascular effects of vasopressin and to characterize the receptors involved. d(CH$_2$)$_5$Tyr(Me)AVP has been reported to be a potent inhibitor of the pressor responses to vasopressin in anesthetized rats. Furthermore, this compound does not interfere with the vasconstrictor response to angiotensin II or norepinephrine. We demonstrate that d(CH$_2$)$_5$Tyr(Me)AVP is an effective antagonist of the response to arginine vasopressin in human cerebral arteries. At 10$^{-8}$ M, the antagonist produced a 60-fold shift to the right of the concentration-response curve for vasopressin, presumably due to a competitive agonist-antagonist interaction. Our results parallel those previously observed in human mesenteric arteries using the same V-1 vasopressin antagonist and in human cerebral veins using dPVDAVP, another arginine vasopressin antagonist. Our results are, however, somewhat different from those of Fox et al, who reported the absence of a rightward shift of the concentration-response curves for arginine vasopressin in the rat tail artery after exposure to d(CH$_2$)$_5$Tyr(Me)AVP. The absence of competitive antagonism in the latter report probably depended on the conditions of the experiments and the type of artery used. These factors are particularly relevant in the assessment of the vascular effects of vasopressin and emphasize the need for caution in extrapolating the effects from experimental animals to humans.

Immunocytochemical studies have provided strong evidence for the role of arginine vasopressin as a putative neurotransmitter or neuromodulator within the brain. The question of whether this neuropeptide can be released in amounts sufficient to influence arterial lumen or blood flow under physiologic or pathologic conditions is as yet hypothetical. Indeed, there are experimental observations showing that a sudden increase in intracranial pressure in cats, produced by either the injection of blood into the subarachnoid space or cerebral compression, leads to a rapid rise in the concentration of vasopressin in the blood. It has also been reported that 24% of a series of 42 patients with subarachnoid hemorrhage had an increased concentration of vasopressin in either the plasma or the cerebrospinal fluid, and sometimes in both fluids. Moreover, in patients with severe congestive heart failure, the plasma levels of arginine vasopressin can be up to 20 times normal. Consequently, the cerebral vasoconstriction that we observed should be taken into consideration in certain pathophysiologic states in which vasopressin is released in amounts that could interfere with the blood supply to the brain.
In conclusion, we demonstrate that vasopressin exerts a powerful constrictor action on isolated human cerebral arteries by direct stimulation of V-1 vasopressin receptors predominantly located on smooth muscle cells. It appears that in human cerebral arteries the contractile response to vasopressin is not modulated by the presence of an intact endothelium.

References


Key Words • cerebral arteries • endothelium, vascular • vasopressins
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