Regional Differences in the Vasodilator Response to Vasopressin in Canine Cerebral Arteries In Vivo

Yoshio Suzuki, MD; Shin-ichi Satoh, PhD; Hirofumi Oyama, MD; Masakazu Takayasu, MD; Masato Shibuya, MD

Background and Purpose: The aim of this study was to investigate the regional differences in the in vivo vasodilator responses to vasopressin, which is thought to stimulate the release of nitric oxide from the endothelium, in canine cerebral arteries by angiography.

Methods: Angiograms were performed through a catheter inserted directly into the right vertebral artery and were taken periodically after the infusion of vasopressin. The diameters of various segments of the major arteries were measured using a computerized image analysis system.

Results: The bolus administration of vasopressin (10 pmol to 1 nmol) into the vertebral artery produced a long-lasting, dose-dependent vasodilation in the major cerebral arteries centering around the circle of Willis. One nanomole of vasopressin appeared to be the optimal dose for producing maximal vasodilation. The internal diameters of the basilar, posterior communicating, and internal carotid arteries experienced the most dilation (approximately 150% of control) 2 minutes after the infusion of 1 nmol of vasopressin, followed by those of the middle cerebral, the intracranial portion of the vertebral, and the anterior spinal arteries (approximately 130% of control). The extracranial portion of the vertebral artery (109.8±4.8% of control, n=4) was less sensitive to 1 nmol of vasopressin. Pretreatment with an intracerebral injection of 10 μmol of NG-monomethyl L-arginine suppressed the vasodilator effect of vasopressin and substance P, whereas it did not affect the response to vasoactive intestinal peptide.

Conclusions: These results suggest that the arteries composing the circle of Willis at the base of the brain are more sensitive to nitric oxide release induced by vasopressin compared with other intracranial and extracranial arteries. (Stroke 1993;24:1049-1054)

Key Words • nitric oxide • vasodilation • vasopressins • dogs

It is generally accepted that vasopressin produces an antidiuretic effect in the kidney and induces vasocostriction in vascular smooth muscle. Recent studies, however, have demonstrated that vasopressin has a vasodilator effect on various vascular beds such as the cerebral, coronary, and pulmonary arteries, and that vasopressin decreases resistance in the large arteries in the cerebral and coronary circulation.1-5 The vasodilator activity of vasopressin appears to be mediated through the release of nitric oxide from the endothelium, while the vasoconstrictive effect of vasopressin is due to the direct stimulation of specific receptors in the smooth muscle.6-8 It has been shown in in vitro studies that the cerebral arteries from the different regions of the brain may differ in their responsiveness to vasopressin, since the proximal and distal branches of the middle cerebral artery did not show a vasodilator response to vasopressin similar to that of the basilar, posterior communicating, and brain-stem arteries.8,9 The vasodilation induced by the injection of vasopressin into the vertebral artery has been characterized angiographically by its potent and long-lasting action on the cerebral arteries compared with that induced by substance P or acetylcholine.2 This in vivo finding makes it possible to investigate by angiography any regional differences in the sensitivity and responsiveness to nitric oxide stimulated by a vasopressin injection into the major cerebral arteries. To our knowledge there have been no reports on the in vivo heterogeneity of the responsiveness to vasoactive substances.

In this study, we have focused on the regional differences in the responsiveness to vasopressin in the major cerebral arteries centered around the circle of Willis at the base of the brain. The changes in the internal diameters of the intracranial vertebral, extracranial vertebral, anterior spinal, basilar, posterior communicating, internal carotid, and middle cerebral arteries have been compared angiographically after the intraarterial injection of vasopressin.

Materials and Methods

Animal Preparation

Our protocol followed the guidelines for the care and use of animals in the physiological sciences as approved.

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From the Department of Neurosurgery, Nagoya University School of Medicine, Nagoya, Japan.

Correspondence to Department of Neurosurgery, Nagoya University School of Medicine, 65 Tsurumai, Showa, Nagoya, 466, Japan (Dr Suzuki).
by the Physiological Society of Japan. Mature mongrel dogs of either sex, weighing 8 to 20 kg, were used for this study. They were anesthetized with pentobarbital (20 to 30 mg/kg) intravenously, intubated through the trachea, and ventilated with room air delivered by a respirator (model B2, Igarashi lka Kogyo Co). The ventilation rate (15 cycles per minute) and tidal volume (20 mL/kg) were adjusted to maintain the arterial blood gases and pH within physiological limits. A catheter was placed in the left femoral artery to monitor the mean arterial blood pressure and heart rate. To prevent dehydration, which induces an increase in endogenous plasma vasopressin levels, a slow intravenous infusion of saline was maintained during the procedures.

**Measurement of Vascular Diameter by Angiography**

The internal diameter of various segments of the intracranial and extracranial arteries was determined by vertebral angiography. A catheter was inserted directly into the right vertebral artery just before the foramen of the transverse process of the cervical vertebrae (C-6) at the base of the neck. Angiograms were performed using 3.0 mL of 65% iothalamate meglumine and were taken periodically at a fixed magnification after 10 pmol to 10 nmol of vasopressin, which was dissolved in 2.0 mL of physiological saline, or 2.0 mL of normal saline was infused into the vertebral artery over 5 seconds. The diameters of various segments of the major arteries were measured using a computerized image analysis system (IMAGE 1.27, Macintosh IIcx) and were compared to determine the effects of the peptides on vascular activity.20 The extracranial vertebral artery (the proximal portion before the C-1 transverse foramen), the intracranial vertebral artery (near the verteobasilar junction), the anterior spinal artery at the level of C-2, the middle third of the basilar artery, the posterior communicating artery, the internal carotid artery, and the middle cerebral artery (the proximal portion of the M-1 segment) were selected for this study.

The modulating effects of L-NMMA, which inhibits the formation of nitric oxide from l-arginine, on the vasodilation induced by vasopressin, substance P, and vasoactive intestinal peptide (VIP) were compared. Ten micromoles of L-NMMA were injected intracisternally 30 minutes before the administration of vasopressin, substance P, and VIP. This dose of L-NMMA, which almost maximally decreases the basal diameters of the major cerebral arteries, was determined empirically from a preliminary study. The solutions of substance P and VIP were injected intracisternally because they induced an obvious vasodilation only when administered from the extraluminal side. The injection of solutions into the cisterna magna was done after the withdrawal of the same amount of cerebrospinal fluid as was used for the L-NMMA, substance P, or VIP solution to maintain a constant intracranial pressure. Care was taken to keep the animals in a head-down position, which enhanced the contact of the preparations with the major arteries of the brain.

**Materials**

Synthetic Arg-vasopressin, substance P, and VIP were purchased from Peptide Institute Inc, Osaka, Japan. L-NMMA was obtained from Calbiochem, La Jolla, Calif. All other chemicals were reagent grade.

**Statistical Analysis**

All data are expressed as mean±SEM. Differences were analyzed with analysis of variance and Dunnett’s test, as well as Student’s t test. Values of P<.05 were considered statistically significant.

**Results**

The intravertebral administration of 2.0 mL of saline solution caused no appreciable change by angiography in the diameter of the major cerebral arteries. A vasopressin bolus over doses ranging from 10 pmol to 1 nmol produced a dose-dependent vasodilation. Doses higher than 1 nmol were less effective than 1 nmol in producing vasodilation; therefore, 1 nmol of vasopressin appeared to be the optimal dose for producing the maximal vasodilator effect, without affecting the systemic blood pressure (Fig 1). A 1-nmol dose of vasopressin significantly dilated the major cerebral arteries, with the maximum dilation occurring 2 minutes after injection. At seven representative points along the major arteries, the difference in the vasodilator response to vasopressin was compared quantitatively (Fig 2). The percent vasodilation was greatest in the internal carotid, posterior communicating, and basilar arteries and was approximately 150%. The percent vasodilation in the middle, anterior spinal, and intracranial vertebral arteries was somewhat smaller at approximately 130%, while the extracranial vertebral artery showed an increase of approximately 110%. The extracranial portion of the vertebral artery was less sensitive to vasopressin than the intracranial portion of the same artery and was significantly less sensitive than the anterior spinal artery at the same level of the cervical region (Fig 3). This strong vasodilation at each point continued until at least 5 minutes after the injection of vasopressin (Table).

Pretreatment with an intracisternal injection of 10 μmol of L-NMMA for 30 minutes decreased the basal diameters of the basilar arteries (78.7±3.7% of control, n=6) and significantly inhibited the vasodilation induced by the intra-arterial injection of 1 nmol of vasopressin (Fig 4). Substance P and VIP induced a long-lasting vasodilation on angiography only when injected intracisternally. The pretreatment with 10 μmol of
L-NMMA also inhibited the vasodilator effect of 10 nmol of substance P, but it did not suppress the effect of 1 nmol of VIP (Fig 5).

There were no significant changes in the mean arterial blood pressure after the intracisternal injection of L-NMMA, substance P, or VIP.

**Discussion**

This in vivo study demonstrated that there is a regional difference in the responsiveness to vasopressin in the major cerebral arteries. The arteries composing the circle of Willis at the base of the brain appeared to be more sensitive to vasopressin than the other vessels tested. The internal carotid, posterior communicating, and basilar arteries dilated the most, followed by the middle cerebral artery and the intracranial portion of the vertebral artery. The extracranial portion of the vertebral artery, the artery in this study farthest from the circle of Willis, was clearly less sensitive to vasopressin than the intracranial arteries and the anterior spinal artery at the same level of the cervical region. This evidence suggests that vasopressin has a relatively selective action on the cerebral arteries, including the spinal arteries.

**Effects of Vasopressin on Diameter of Cerebral Arteries in Dogs**

<table>
<thead>
<tr>
<th>Artery and drug</th>
<th>2 Minutes</th>
<th>5 Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (n=4-5)</td>
<td>99.1±2.7</td>
<td>94.6±1.9</td>
</tr>
<tr>
<td>Vasopressin (n=4-5)</td>
<td>128.3±7.5*</td>
<td>127.2±3.9*</td>
</tr>
<tr>
<td>ICA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (n=3-4)</td>
<td>94.4±4.4</td>
<td>95.0±9.3</td>
</tr>
<tr>
<td>Vasopressin (n=3-4)</td>
<td>150.0±14.2*</td>
<td>150.7±12.7†</td>
</tr>
<tr>
<td>PComA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (n=4-5)</td>
<td>98.5±2.9</td>
<td>98.1±3.7</td>
</tr>
<tr>
<td>Vasopressin (n=3-4)</td>
<td>145.7±7.2*</td>
<td>133.3±9.6†</td>
</tr>
<tr>
<td>BA</td>
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<tr>
<td>Vehicle (n=7)</td>
<td>91.5±3.4</td>
<td>96.8±1.6</td>
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<tr>
<td>Vasopressin (n=5-6)</td>
<td>152.9±9.6*</td>
<td>135.5±10.8*</td>
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<tr>
<td>VA (extracranial)</td>
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<td></td>
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<tr>
<td>Vehicle (n=3)</td>
<td>104.2±2.3</td>
<td>99.6±3.2</td>
</tr>
<tr>
<td>Vasopressin (n=4)</td>
<td>132.3±7.9†</td>
<td>131.1±8.9†</td>
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<tr>
<td>ASA</td>
<td></td>
<td></td>
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<tr>
<td>Vehicle (n=3)</td>
<td>98.4±3.3</td>
<td>98.3±1.7</td>
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<tr>
<td>Vasopressin (n=4-5)</td>
<td>130.5±2.5*</td>
<td>135.3±6.9*</td>
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<td>VA (extracranial)</td>
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<tr>
<td>Vehicle (n=4)</td>
<td>98.8±1.2</td>
<td>100.0±2.0</td>
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<tr>
<td>Vasopressin (n=4)</td>
<td>109.8±4.8‡</td>
<td>111.9±3.8‡</td>
</tr>
</tbody>
</table>

Values are mean±SEM for number of animals indicated, expressed as percent change in diameter. MCA, middle cerebral artery; ICA, internal carotid artery; PComA, posterior communicating artery; BA, basal artery; VA, vertebral artery; ASA, anterior spinal artery.

*P<.01, †P<.05 vs control by Student’s t test.
‡P=not significant.
Angiography through the vertebral artery, which is the main artery supplying the cerebral circulation in dogs, could often reveal not only the posterior but also the anterior circulation supplied by the internal carotid artery. Since the internal carotid artery could respond to vasopressin to the same extent as the basilar and posterior communicating arteries, we assumed that enough blood flow was supplied by the vertebral artery to communicate with the anterior circulation. For a more precise evaluation, the influence of vasopressin on the anterior circulation may need to be investigated using a carotid artery. Because vasopressin was injected locally as a bolus in the vertebral artery, the concentration of vasopressin that reaches the various cerebral arteries may not be the same. However, the internal carotid artery, which is relatively far from the vertebral artery, could respond to vasopressin well. This fact indicates that a 1-nmol concentration of vasopressin is sufficient to stimulate endothelium-dependent vasodilation.

The vasodilation induced by vasopressin may be the result of the release of nitric oxide from the endothelium, as has been shown in the canine basilar artery in vitro. The mechanical removal of the endothelium and V-1 antagonists reduced the vasodilator response to vasopressin in vitro. The relaxation in response to vasopressin was reduced in the presence of L-NMMA, an arginine analogue that inhibits the activity of nitric oxide synthase, and was restored by the addition of L-arginine but not D-arginine. In this study, the intracisternal injection of L-NMMA suppressed the vasodilator activities of vasopressin and substance P but not VIP. The response to substance P, unlike that of VIP, has been found to be dependent on the presence of an intact endothelium. These findings support the previous in vitro evidence and suggest that the intracisternal injection of L-NMMA inhibits the synthesis of nitric oxide in the endothelium from the extraluminal side. It is likely that vasopressin decreases the resistance of the large arteries around the circle of Willis through the release of nitric oxide from the endothelium. This result may be consistent with evidence that suggests that nitric oxide predominantly contributes to the adjustment of blood flow in large vessels.

A recent study has shown that nitric oxide is synthesized in the nerve terminals innervating the cerebral arteries as well as in the endothelium. However, it seems unlikely that vasopressin from the intraluminal side diffuses through the smooth muscle wall to reach the nerve terminals and thereby modulates the release of nitric oxide from the nerve terminals. The intracisternal injection of L-NMMA, which probably suppresses the synthesis of nitric oxide in both the endothelium and nerve terminals, decreased the diameter of the basilar artery by approximately 21%. This suggests that the synthesis and release of nitric oxide in the vascular wall may contribute to the maintenance of basal vascular tone.

Although no vasopressin-induced vasoconstriction was observed in this study, it has been reported from in

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**Fig 4.** Line graph shows effect of 10 μmol of N^G^-monomethyl L-arginine (L-NMMA) on vasodilator activity of 1 nmol of vasopressin on canine basilar artery. L-NMMA was injected intracisternally 30 minutes before intravertebral injection of vasopressin.

**Fig 5.** Bar graph shows effect of 10 μmol of N^G^-monomethyl L-arginine (L-NMMA) on vasodilator activity of 10 nmol of substance P (SP) and 1 nmol of vasoactive intestinal peptide (VIP) on canine basilar artery. L-NMMA was injected intracisternally 30 minutes before intracisternal injection of substance P and VIP. Effect of L-NMMA on these peptides was compared at 20 minutes after injection of these peptides.
vitro experiments using isolated canine vascular strips that vasopressin causes a vasoconstriction in more than half of the proximal part of the middle cerebral artery. In the small branches of the middle cerebral arteries, vasopressin did not produce any significant vasoactive changes. In vitro experiments that involve both the intraluminal and extraluminal access of vasopressin to the arteries might induces mixed responses by stimulating the smooth muscles, endothelium, or nerve fibers in the vascular wall. The difference in responsiveness between the in vitro and in vivo studies may be explained by the different access of vasopressin to the arteries. However, the intracisternal injection of vasopressin (extraluminal application) in a preliminary in vivo study produced only a vasodilation in the proximal part of the middle cerebral artery on angiography (data not shown). Another consideration is that the data obtained from in vivo studies are usually affected by more factors of both a systemic and local nature. For example, the nitric oxide that is released from the endothelium of major arteries and promoted by the intraarterial or intracisternal injection of vasopressin probably dilates the smooth muscle not only near the released site but also a little farther downstream because it is carried by the bloodstream. The half-life of nitric oxide is known to be less than 10 seconds, but it may play a role in controlling the vascular tone somewhat distal to the released site.

The possible existence of vasopressin in the endothelium or nerve terminals of the blood vessels has been demonstrated by several morphological studies. Recent biochemical data have supported this possibility since vasopressin can be extracted from rat and bovine vascular tissue. Interestingly, blood vessels from hypophysectomized and Brattleboro rats also contains vasopressin at levels similar to those of intact control rats. These additional lines of evidence suggest that vasopressin contributes to the regulation of the regional blood flow not only as a circulating hormone, but also as a mediator of local origin.

Arginine vasopressin is released by the neurohypophysis and has been shown to be a potent constrictor of smooth muscle in many vascular beds, including the cerebral circulation. However, several recent studies have shown that arginine vasopressin can produce relaxation of canine cerebral vascular muscle via an endothelium/nitric oxide–dependent mechanism. Since previous studies have focused on examining the effects of arginine vasopressin on canine cerebral arteries using in vitro methodologies, the goal of the present studies by Suzuki et al was to examine regional differences in responses of cerebral arteries to arginine vasopressin in vivo. These investigators examined the effects of infusion of arginine vasopressin into the vertebral artery on diameter of the canine basilar, posterior communicating, internal carotid, middle cerebral, intracranial portion of the vertebral, and anterior spinal arteries using angiographic methodology. In addition, these investigators examined the role of nitric oxide in responses of cerebral arteries to arginine vasopressin using N^G^-monomethyl-L-arginine. The authors found that arginine vasopressin produced greater dilatation of the basilar, posterior communicating, and internal carotid arteries than that observed in the middle cerebral, intracranial portion of the vertebral, and anterior spinal arteries. In addition, N^G^-monomethyl-L-arginine inhibited dilatation in response to vasopressin and substance P but did not affect dilatation in response to vasoactive intestinal polypeptide. Thus, the results of the studies by Suzuki et al suggest important regional differences in responses of large canine cerebral arteries to arginine vasopressin and an important

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**References**


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