Humoral Regulation of Blood Flow to Choroid Plexus: Role of Arginine Vasopressin

Frank M. Faraci, William G. Mayhan, William J. Farrell, and Donald D. Heistad

The goal of this study was to examine humoral mechanisms that regulate blood flow to the choroid plexus. We determined the effects of arginine vasopressin on blood flow (microspheres) to the choroid plexus in anesthetized and awake rabbits. In anesthetized rabbits, blood flow to the choroid plexus was 342 ± 31 (mean ± SEM) ml/min/100 g under control conditions. Intravenous infusion of vasopressin at 4 and 40 mU/kg increased plasma vasopressin levels from 11 ± 1 to 55 ± 15 and 441 ± 120 pg/ml, respectively, and blood flow to the choroid plexus decreased by 48 ± 6% and 70 ± 4%. Cerebral blood flow was not affected by infusion of vasopressin. Similar responses to infusion of vasopressin were observed in awake rabbits. The V₁ antagonist [d(CH₂)₅Tyr(Me)AVP] (10 μg/kg i.v.) had no effect on resting blood flow, but abolished the effect of vasopressin on blood flow to the choroid plexus. Vasoconstrictor responses of the choroid plexus to intravenous infusion of phenylephrine were not attenuated by the V₁ antagonist. Thus, circulating vasopressin, at plasma levels that are observed under physiological and pathophysiological conditions, has marked effects on blood flow to the choroid plexus. These effects appear to be mediated through a V₁ receptor. We speculate that vasopressin may play an important role in regulation of blood flow to the choroid plexus and perhaps in the regulation of cerebrospinal fluid production. (Circulation Research 1988;63:373–379)

The choroid plexus is the major site of formation of cerebrospinal fluid. Sympathetic nerves and other adrenergic stimuli have important effects on blood flow to the choroid plexus and on cerebrospinal fluid production. In contrast, relatively little is known about effects of humoral stimuli on blood flow to the choroid plexus.

Humoral mechanisms may be more important in regulating blood flow to the choroid plexus compared with other areas of the brain because blood vessels of the choroid plexus have fenestrated endothelium and are relatively permeable. Thus, unlike most intracranial blood vessels, in which a blood-brain barrier is present, blood-borne humoral agents reach smooth muscle in blood vessels of the choroid plexus.

Arginine vasopressin has important effects on vasomotor tone and on water permeability in the renal tubules. The choroid plexus may be exposed to vasopressin from several sources. Plasma and cerebrospinal fluid levels of vasopressin increase substantially during stimuli such as hemorrhage and intracranial hypertension. Blood vessels of the choroid plexus appear to be innervated by vasopressin-containing neurons that originate from the hypothalamus, suggesting that a high concentration of vasopressin may surround these blood vessels.

A relatively high density of V₁ receptors are present in the choroid plexus, suggesting that vasopressin may have important effects on blood flow. Although most vascular responses to vasopressin are mediated by V₁ receptors, the effects of vasopressin on the choroid plexus are difficult to predict. In pial arteries, vasopressin is a constrictor that acts through a V₁ receptor. In contrast, in the basilar artery, vasopressin produces relaxation through V₁ receptors.

The first goal of the present study was to determine effects of circulating vasopressin on blood flow to the choroid plexus. We examined blood flow responses to levels of vasopressin that are observed under physiological and pathophysiological conditions in both anesthetized and awake rabbits and tested the hypothesis that vasopressin has differential effects on cerebral blood flow and on blood flow to the choroid plexus. Our second goal was to determine whether changes in blood flow to the choroid plexus occur through a V₁-receptor-mediated mechanism.
Materials and Methods

Anesthetized Rabbits

Experiments were performed on 25 New Zealand white rabbits (2–3.5 kg) that were individually housed with free access to food and water prior to the experiments. The rabbits were anesthetized with thiopental (30–40 mg/kg i.v.) followed by chloralose (40–50 mg/kg i.v.). Supplemental chloralose was administered at approximately 10 mg/kg/hr. The trachea was cannulated, and the animals were ventilated mechanically with air and supplemental oxygen. A catheter was placed into a femoral artery for measurement of systemic pressure and to sample arterial blood. A femoral vein was cannulated for infusion of drugs. Catheters were inserted into the left atrial appendage for injection of microspheres and in both brachial arteries for withdrawal of reference blood samples during microsphere injection. The rabbits received a constant infusion of saline (approximately 0.5 ml/min) to maintain hydration and to suppress endogenous elevation of plasma vasopressin, which may occur during anesthesia or following surgery. Skeletal muscle paralysis was produced with gallamine triethiodide (5 mg/kg).

Unanesthetized Rabbits

Six rabbits were anesthetized with intravenous sodium thiopental. Catheters were inserted into the brachial and femoral vessels as described above. A pigtail polyethylene catheter was inserted into the right femoral artery and advanced up the aorta into the left ventricle for injection of microspheres. After surgery, the animals were allowed to recover from anesthesia for at least 2 hours in a Plexiglas box. Following this recovery period, the animals were observed to be awake and alert, and the experimental protocol was begun. The rabbits did not exhibit behavioral signs of discomfort during the protocol. We have used this approach to study awake animals previously.

Measurement of Blood Flow

Blood flow was measured using radioactive microspheres (15 μm diameter) labeled with 46Sc, 99mTc, 153Gd, 85Sr, 113Sn, and 141Ce. We have previously validated the use of microspheres for the measurement of blood flow to the choroid plexus in rabbits. Before each injection, the vial containing microspheres was vigorously shaken for several minutes. We injected 0.5–1.3 x 10^6 spheres into the left atrium or left ventricle in 10–20 seconds, followed by a saline flush. Starting 10 seconds before the injection of microspheres and continuing for 1.5 minutes thereafter, reference arterial blood samples were withdrawn from both brachial arteries using a withdrawal pump.

At the end of the experiment, anesthetized rabbits were killed with intravenous potassium chloride. The awake rabbits were killed with an overdose of pentobarbital. The brain was removed and placed in buffered formalin for 1–3 days before dissection into regional samples. The samples were weighed and placed into plastic tubes. Radioactivity of tissue samples and reference arterial blood samples was determined using a three-inch sodium iodide well-type gamma counter. Isotope separation was performed using standard techniques. Blood flow (BF) was calculated as BF = (C_T x 100 x Q_R) / C_R, where Q_R is the reference sample flow rate, and C_T and C_R are counts in tissue and reference samples, respectively. Vascular resistance was calculated as mean arterial pressure divided by blood flow.

Experimental Protocol

In nine anesthetized rabbits (Group 1), we measured blood flow and plasma vasopressin levels six times: twice under control conditions, 2 minutes after intravenous infusion of three doses of [Arg^8]vasopressin (Peninsula, Belmont, California) (0.4, 4, and 40 mU/kg, infused over 2 minutes), and after a 1-hour recovery period. In some rabbits, systemic pressure increased during the high dose of vasopressin, and small amounts of venous blood were withdrawn to maintain aortic pressure constant. Plasma vasopressin levels were measured by radioimmunoassay as described in detail previously. Arterial blood gases were measured with each measurement of blood flow.

In nine anesthetized rabbits (Group 2), we measured blood flow six times as described in Group 1 with the following exception. Between the two control measurements, the vasopressin (V_1) antagonist 1(β-mercaptop-β, β-cyclopentamethylene propionic acid) 2-(O-methyl) tyrosine arginine-vasopressin [d(CH_2)_5Tyr(Me)AVP] was administered intravenously (10 μg/kg). The goal of these experiments was to determine if changes in blood flow to the choroid plexus in response to vasopressin were mediated by a V_1 receptor.

In seven rabbits (Group 3), we tested the effect of phenylephrine on blood flow to the choroid plexus in the presence and absence of the V_1 antagonist d(CH_2)_5Tyr(Me)AVP. Blood flow was measured under control conditions and during intravenous infusion of phenylephrine (25 μg/kg/min). During infusion of phenylephrine, aortic pressure was maintained at control levels by withdrawal of venous blood. After a 30-minute recovery period, during which time the shed blood was slowly rein infused, d(CH_2)_5Tyr(Me)AVP was administered intravenously (10 μg/kg). Blood flow was then measured again under control conditions and during infusion of phenylephrine. The purpose of these experiments was to test the specificity of the V_1 antagonist in blocking vasoconstrictor responses of the choroid plexus.

In six awake rabbits (Group 4), we measured blood flow six times as described in the protocol for Group 1. The purpose of these experiments was to examine the response of the choroid plexus to vasopressin in conscious animals.
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Changes in blood flow to the choroid plexus (open bars) and cerebrum (filled bars) in anesthetized rabbits in response to intravenous infusion of arginine vasopressin. The response to vasopressin is shown under control conditions (left panel) and following administration of the V₁ antagonist (right panel). Values during vasopressin are compared with the preceding control value. Values are mean±SEM.

Statistics
Statistical analysis was performed using a one-way analysis of variance with Tukey's test for critical difference. A p value less than 0.05 was considered significant. All values are expressed as mean±SEM.

Results

Effects of Vasopressin on Blood Flow to Choroid Plexus in Anesthetized Rabbits

In anesthetized rabbits, plasma vasopressin levels averaged 11 ± 1 pg/ml under control conditions. Plasma levels increased to 14 ± 2, 55 ± 15, and 441 ± 120 pg/ml after infusion of the low, middle, and high dose of vasopressin, respectively. Following a 1-hour recovery period, plasma vasopressin decreased to 17 ± 4 pg/ml.

Under control conditions, blood flow to the choroid plexus was approximately 10-fold greater than blood flow to the cerebrum (Table 1). The low dose of vasopressin had no significant effect on blood flow to the choroid plexus. Infusion of the middle and high dose of vasopressin caused a dose-related decrease in blood flow to the choroid plexus (Figure 1). The middle dose of vasopressin reduced blood flow to the choroid by 48 ± 6%. The high dose of vasopressin decreased blood flow to the choroid plexus by 70 ± 4% and increased vascular resistance of the choroid approximately fourfold (from a control value of 0.26 ± 0.02 to 1.06 ± 0.17 mm Hg/ml·min·100 g, p<0.05). Blood flow to the choroid plexus returned to control levels following one hour of recovery. In contrast to the choroid plexus, blood flow to the cerebrum was not affected by infusion of vasopressin (Table 1).

Effect of V₁ Antagonist

The vasopressin antagonist, d(CH₂)₅Tyr(Me)AVP, had no effect on resting blood flow to the choroid plexus or the brain (Table 2). In the presence of the antagonist, infusion of vasopressin had no effect on blood flow to the choroid plexus (Figure 1, Table 2). These results suggest that the vasoconstrictor response of the choroid plexus to vasopressin was mediated through a V₁ receptor mechanism.

Effect of Phenylephrine on Blood Flow to Choroid Plexus

Infusion of phenylephrine significantly decreased blood flow to the choroid plexus under control conditions (Figure 2). Blood flow to the cerebrum was not affected by phenylephrine (34 ± 4 vs. 36 ± 5 ml/min/100 g). Following administration of the vasopressin antagonist, vasoconstrictor responses of the choroid plexus to phenylephrine were preserved (Figure 2). These results suggest that the V₁ antagonist blocks the response of the choroid plexus to vasopressin without producing nonspecific blockade of vasoconstrictor responses.

| TABLE 1. Effect of Arginine Vasopressin on Blood Flow to Choroid Plexus in Anesthetized Rabbits |
|---------------------------------------------|----------------|----------------|----------------|----------------|----------------|
| Aortic pressure (mm Hg)                     | Control        | 82 ± 3         | 80 ± 3         | 80 ± 3         | 81 ± 4         | 77 ± 3         |
| Blood flow (ml/min/100 g)                   | Control        | 80 ± 3         | 80 ± 3         | 80 ± 3         | 81 ± 4         | 77 ± 3         |
| Choroid Plexus                             | 324 ± 42       | 330 ± 35       | 280 ± 42       | 178 ± 30*      | 96 ± 20*       | 389 ± 98       |
| Cerebrum                                   | 30 ± 2         | 33 ± 2         | 35 ± 2         | 36 ± 2         | 39 ± 4         | 40 ± 3         |
| Arterial PCO₂ (mm Hg)                       | 34 ± 1         | 35 ± 1         | 36 ± 1         | 34 ± 1         | 36 ± 1         | 36 ± 1         |
| Arterial PO₂ (mm Hg)                        | 144 ± 10       | 138 ± 7        | 137 ± 7        | 158 ± 22       | 134 ± 9        | 136 ± 11       |
| Arterial pH                                 | 7.40 ± 0.01    | 7.39 ± 0.01    | 7.37 ± 0.02    | 7.38 ± 0.02    | 7.34 ± 0.02    | 7.34 ± 0.02    |

Values are mean±SEM (n = 9). *Significantly different from preceding control at p<0.05.
TABLE 2. Effect of Arginine Vasopressin on Blood Flow to Choroid Plexus After V₁ Antagonist

<table>
<thead>
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<th>Control</th>
<th>V₁ antagonist</th>
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<th>Recovery</th>
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<td>Aortic pressure (mm Hg)</td>
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<td>71 ± 5</td>
<td>72 ± 5</td>
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<td>Arterial PCO₂ (mm Hg)</td>
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Values are mean ± SEM (n = 9). *Significantly different from control at p < 0.05. The V₁ antagonist (10 μg/kg) was administered after the first control measurement.

**Effect of Vasopressin on Blood Flow to Choroid Plexus in Awake Rabbits**

In awake rabbits, plasma vasopressin averaged 12 ± 2 pg/ml under control conditions. This value increased to 14 ± 1, 51 ± 13, and 463 ± 125 pg/ml after infusion of the low, middle, and high dose of vasopressin, respectively. Following 1 hour of recovery, plasma vasopressin had decreased to 18 ± 5 pg/ml.

Infusion of the low dose of vasopressin had no effect on blood flow to the choroid plexus. Infusion of the middle and high doses of vasopressin caused a dose-related decrease in blood flow to the choroid plexus (Figure 3). Cerebral blood flow (Table 3) was not affected by infusion of vasopressin in awake animals.

**Discussion**

There are three major new findings in the present study. First, arginine vasopressin, at circulating levels seen under physiological and pathophysiological conditions, produces a marked decrease in blood flow to the choroid plexus in both anesthetized and awake rabbits. Second, vasopressin selectively alters blood flow to the choroid plexus without affecting blood flow to the cerebrum. Third, decreases in blood flow to the choroid plexus appear to be mediated through a V₁-receptor mechanism.

**Measurement of Blood Flow**

We have previously examined the validity of using microspheres to measure blood flow to the choroid plexus in rabbits. By measuring blood flow to the choroid plexus with different sized microspheres, we determined that there is no significant shunting of 15-μm microspheres in the choroid plexus.

Since the choroid plexus in rabbits is relatively small, the number of microspheres trapped in the tissue may also be relatively small. The presence of small numbers of microspheres may increase the variance of the method but does not prevent detection of changes in blood flow. In our recent study, we determined that the average variance for consecutive measurements of blood flow to the choroid plexus was only slightly higher than that for the total brain (11% versus 6%). It has been suggested recently that small numbers of microspheres in tissue samples increase the variance of blood flow measurements less than originally suggested.

**Vascular Effects of Vasopressin**

Several studies indicate that vasopressin produces constriction of cerebral vessels in vitro. In contrast, the canine basilar artery relaxes in response to vasopressin in vitro through an endothelium-dependent mechanism.
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Blood Flow, ml·min⁻¹·100g⁻¹

Figure 3. Changes in blood flow to the choroid plexus (open bars) and cerebrum (filled bars) in awake rabbits in response to intravenous infusion of vasopressin. Values are mean±SEM.

In the present study, circulating vasopressin had no effect on cerebral blood flow. These results are in agreement with previous reports that showed no effect of vasopressin on total or regional cerebral blood flow.²⁴⁻²⁵ Peptides such as vasopressin do not readily cross the blood-brain barrier.²⁶ Thus, it is unlikely that significant amounts of circulating vasopressin pass through cerebral endothelium and reach the smooth muscle of cerebral blood vessels.

In contrast to blood vessels in most regions of the brain, vessels of the choroid plexus have fenestrated endothelium and are relatively permeable.²⁻³⁻⁵ These morphological characteristics and the results of the present study suggest that vasopressin readily reaches smooth muscle of choroidal blood vessels, where it has marked effects on blood flow. We recently observed increases in blood flow to the choroid plexus during infusion of adenosine and decreases in blood flow during infusion of α-adrenergic agonists without affecting blood flow to the cerebrum.⁶ These findings support the concept that circulating humoral agents can have important effects on blood flow to the choroid plexus without changing total cerebral blood flow.

Role of V₁ Receptors

We used d(CH₂)₅Tyr(Me)AVP to block effects of arginine vasopressin on blood flow to the choroid plexus. The specificity of this antagonist has been established in several preparations and in several vascular beds.²⁷⁻³⁰ In cerebral blood vessels, Katunisic et al²⁹ demonstrated selectivity and competitive antagonism of this antagonist for the V₁ receptor.

A recent study indicates that the choroid plexus contains a relatively high density of V₁ receptors.¹³ We obtained evidence for the selective action of d(CH₂)₅Tyr(Me)AVP on blood vessels of the choroid plexus. In the choroid plexus, the V₁ antagonist blocked the constrictor effect of vasopressin while the constrictor effect of phenylephrine was preserved.

Recent experiments have suggested that vasopressin may activate V₂ receptors as well as V₁ receptors on vascular smooth muscle or endothelium.²⁸⁻³¹ The V₂ receptor appears to mediate the antidiuretic response to vasopressin in the kidney. Administration of vasopressin in the presence of V₁-receptor blocker or administration of a V₂ agonist decreased total peripheral resistance in dogs.³¹ Increases in plasma vasopressin levels produced by dehydration had no effect on blood flow to kidney, liver, and bone under normal conditions but produced increases in blood flow to these tissues following V₁-receptor blockade.³⁸ These vasodilator responses were abolished by combined V₁- and V₂-receptor blockade. Thus, administration of a V₁ antagonist unmasked V₂-mediated vasodilator responses to vasopressin in some vascular beds.³⁸

In the present study, treatment with d(CH₂)₅Tyr(Me)AVP abolished the reduction in blood flow to the choroid plexus in response to vasopressin, and there was no increase in choroidal blood flow. Thus, we found no evidence for unmasking of a V₂-mediated blood flow response to vasopressin in the choroid plexus.

Functional Implications

The choroid plexus is the major site of cerebrospinal fluid formation.¹⁻² Blood flow to the choroid plexus probably is a major determinant of production of cerebrospinal fluid.²⁻³ Other agonists such as norepinephrine have been shown to decrease both blood flow to the choroid plexus and cerebrospinal fluid production.⁴⁻⁶

Although we have shown that vasopressin has potent effects on blood flow to the choroid plexus,
there is very little information concerning effects of vasopressin on cerebrospinal fluid production. Incubation of the isolated choroid plexus with vasopressin (10^{-12} to 10^{-9} M) in vitro produces morphological changes in the epithelial cells which are suggestive of changes in fluid transport. Davson and Segal infused a very high dose of vasopressin (Pitressin, 0.3 U/min) intracarotid in rabbits, and found a reduction in the formation of cerebrospinal fluid. These studies suggest that vasopressin may affect the formation of cerebrospinal fluid by the choroid plexus.

Recent evidence suggests that vasopressin-containing neurons project from the hypothalamus to blood vessels that supply the choroid plexus. This anatomical relationship raises the possibility of central neural regulation of choroidal blood flow and production of cerebrospinal fluid. The possibility of hypothalamic regulation of blood flow to the choroid plexus is especially attractive because some regions of the hypothalamus may detect changes in the osmolarity of cerebrospinal fluid.

Vasopressin levels in both the blood and in the cerebrospinal fluid can vary substantially. Plasma vasopressin levels may increase to approximately 40 pg/ml during dehydration. In this study, increases in plasma vasopressin to approximately 50 pg/ml reduced blood flow to the choroid plexus by almost 50%. Thus, osmotic stimuli such as dehydration may increase levels of vasopressin sufficiently to affect blood flow. Other stimuli may increase plasma vasopressin to levels as high as several hundred picograms per milliliter to greater than 1,000 pg/ml. These stimuli include hypoxia, hemorrhage, shock, emesis, and intracranial hypertension. Our results imply that these plasma levels of vasopressin have marked effects on blood flow to the choroid plexus.

The implications of reduction in blood flow to the choroid plexus during hemorrhage, shock, or emesis are not clear. Vasopressin-induced reductions in blood flow to the choroid plexus, however, may play an important protective role during intracranial hypertension. We speculate that increases in the level of circulating vasopressin may reduce blood flow to the choroid plexus and the production of cerebrospinal fluid when intracranial pressure is elevated, and thereby tend to reduce intracranial pressure.

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