Vasopressin V1a Receptors Mediate Posthemorrhagic Systemic Hypertension Thereby Determining Rebleeding Rate and Outcome After Experimental Subarachnoid Hemorrhage

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Background and Purpose—Arginine vasopressin V1 receptors have been suggested to be involved in the pathophysiology of acute brain injury. Therefore, we aimed to determine the role of arginine vasopressin V1 receptors after experimental subarachnoid hemorrhage (SAH).

Methods—Sprague-Dawley rats subjected to SAH by endovascular puncture received either vehicle or a V1 receptor antagonist intravenously from 1 minute before until 3 hours after SAH. Intracranial pressure, cerebral blood flow, and mean arterial blood pressure were monitored until 60 minutes after SAH. Brain water content was quantified 24 hours after SAH and neurological function and mortality were assessed daily for 7 days after hemorrhage.

Results—In control rats, SAH induced high intracranial pressure, a brief increase in plasma arginine vasopressin, a subsequent increase in systemic blood pressure (Cushing response), a high rebleeding rate (30%), severe neurological deficits, and a 7-day mortality rate of 50%. V1 receptor antagonist-treated animals exhibited a far less pronounced Cushing response, a less severe increase of intracranial pressure, did not exhibit rebleedings, had less severe brain edema formation and neurological deficits, and a mortality rate of only 20% (all P<0.05 versus vehicle).

Conclusions—Inhibition of arginine vasopressin V1a receptors reduces the severity of SAH and prevents rebleedings by blunting the posthemorrhagic hypertonic response (Cushing reflex), thereby reducing mortality and secondary brain damage after experimental SAH. Because the severity of the initial bleeding and rebleedings are major factors contributing to an unfavorable outcome after SAH, inhibition of V1a receptors may represent a novel strategy to treat SAH. (Stroke. 2012;43:227-232.)

Key Words: antidiuretic hormone (ADH) ▪ arginine vasopressin (AVP) ▪ brain edema ▪ Cushing response ▪ experimental ▪ rat ▪ subarachnoid hemorrhage ▪ V1a receptors

Subarachnoid hemorrhage (SAH), caused in most cases by the rupture of an intracranial aneurysm, is associated with a particularly high mortality of >50%.1 Most patients die within the first 48 to 72 hours after hemorrhage due to the severity of the initial hemorrhage, rebleedings, and/or the sequelae of early brain injury.2,3 The mechanisms underlying the early mortality of SAH are not well understood and, hence, therapeutic options are very limited.4

Arginine vasopressin (AVP), also known as antidiuretic hormone, is a nonapeptide synthesized in the hypothalamus and released into the bloodstream by the pituitary gland. The classical physiological actions of AVP are mediated by vascular V1a receptors, which cause vasoconstriction with a subsequent increase in systemic blood pressure, and by renal V2 receptors, which mediate reabsorption of water from the descending duct of the loop of Henle, thereby reducing diuresis. Besides these systemic actions, vasopressin has also been suggested to be involved in the control of ion and water homeostasis in the brain due to the presence of AVP receptors in the central nervous system.5 The exact mechanisms of this central function, however, remain to be investigated in detail.6

During pathological conditions, for example, cerebral ischemia, traumatic brain injury, or SAH, AVP is elevated in the plasma and cerebrospinal fluid of affected patients7–9 and V1 receptors were found to be overexpressed in an experimental model of traumatic brain injury.10 Pharmacological
blockage of V1 receptors resulted in a significantly improved functional outcome and reduced brain damage in animal models of cerebral ischemia and traumatic brain injury.11,12 It remains to be elucidated whether AVP V1 receptors also have a pathophysiological role after SAH. Therefore, the aim of the current study was to investigate the therapeutic potential of AVP V1 receptors in an experimental model of SAH.

Materials and Methods
The current experiments were approved by the Animal Care Committee of the Government of Upper Bavaria (Protocol Number 221-2531-09/05).

Animal Preparation and Monitoring
Male Sprague-Dawley rats (250–300 g body weight; Charles River Laboratory, Sulzfeld, Germany) were anesthetized and monitored as previously described.13 Briefly, animals were intubated and mechanically ventilated with 30% O2 and anesthesia was induced and maintained for 60 minutes after SAH by intraperitoneal injections of midazolam, fentanyl, and medetomidine. Brain and body temperature were monitored and kept at 37°C. Regional cerebral blood flow was monitored by laser-Doppler flowmetry, and intracranial pressure (ICP) was continuously measured using an intraparenchymal microsensor until 60 minutes after SAH. The tail artery was cannulated for continuous measurement of mean arterial blood pressure (MABP) and for blood gas analysis and the right jugular vein for drug administration. Three hours after SAH, animals were briefly reanesthetized with 1.5% isoflurane in 70% nitrous oxide for removal of the jugular catheter.

Induction of SAH
SAH was induced by endovascular puncture as previously described.14 Briefly, the right carotid artery was exposed and a 3-0 monofilament was advanced into the internal carotid artery until SAH was indicated by a sudden increase of ICP and decrease of local blood flow (Figure 1A–D, open circles). Thirty percent (3 of 10) of vehicle-treated rats subjected to SAH had at least 1 rebleeding, whereas no rebleedings were observed in animals treated with the V1 receptor antagonist (Figure 2A).

Determination of Rebleedings
Rebleedings were determined by a characteristic secondary increase in ICP by >15 mm Hg after recovery from primary hemorrhage (Supplemental Figure I; http://stroke.ahajournals.org).

Neurological Evaluation and Body Weight
Neurological deficits and body weight were assessed by an investigator blinded to the treatment of the animals before SAH induction (Day 0) and on postoperative Days 1, 2, 3, 5, and 7. For the quantification of neurological deficits, we used a 6-point neurological score (5= no apparent deficit; 0= no spontaneous motor activity) and the Beam Balance Test as previously described.15,16

Quantification of Brain-Water Content
Animals were euthanized 24 hours after SAH and their brains were quickly removed. The hemispheres were separated and weighed to assess their wet weight before being dried for 24 hours at 110°C to determine their dry weight. Hemispheric water content (%) was calculated using the following formula [(wet weight−dry weight)/ wet weight]×100.

Application of the V1 Receptor Antagonist
The selective antagonist [deamino-Pen1, O-Me-Tyr2, Arg8]-vasopressin17 (V-1880; Lot 053K13071; Sigma, St Louis, MO) was used to inhibit V1 receptors.11,12 Forty micrograms of the drug dissolved in 400 μL saline (vehicle) was applied as a bolus (100 μL) given immediately before SAH followed by a 3-hour continuous infusion (100 μL/hour). Control animals received only vehicle. Sham-operated animals were assigned to either drug protocol without induction of SAH.

Experimental Groups
Rats were randomly assigned to 1 of the following groups: (1) application of the V1 receptor antagonist + SAH (n=8); (2) application of the V1 receptor antagonist + sham surgery (n=3); (3) application of vehicle + SAH (n=8); or (4) application of vehicle + sham surgery (n=3). Twenty-four hours after SAH, animals were euthanized for quantification of brain-water content. One animal in each of the initial SAH groups died and had to be replaced.

In a second series, rats were randomly assigned to 1 of the 2 following groups: (1) application of the V1 receptor antagonist + SAH (n=10) and (2) application of vehicle + sham surgery (n=10). Mortality, neurological deficits, and body weight were assessed for 7 days before the animals were euthanized using deep isoflurane anesthesia.

All investigators were blinded to the treatment of the animals until completion of data analysis.

Measurement of AVP in Plasma
A total volume of 0.8 mL arterial blood taken from the tail artery was collected in prechilled tubes containing 30 μL heparin and centrifuged at 3500 rpm for 60 seconds at 4°C. The plasma was collected and stored at −80°C until further use. AVP was measured as previously described by using a highly sensitive radioimmunoassay.18 AVP was measured before and 5, 30, and 120 minutes after SAH (n=6 per group).

Statistical Analysis
Statistical analysis was performed using the SigmaStat 3.1 statistical software package (SPSS Science Inc, Chicago, IL) and STATISTICA 9.1 (StatSoft, Hamburg, Germany). Data were analyzed using Student t test (physiological data) or the Mann-Whitney U rank sum test. Mortality between groups was compared using the Cox F test. Statistical significance of results was assumed at P<0.05. Data are presented as mean±SEM if not otherwise indicated.

Results
Physiological Parameters
Application of the V1 receptor antagonist in sham-operated animals had no effect on MABP, ICP, or cerebral blood flow compared with vehicle (Supplemental Figure II). In animals subjected to SAH, arterial blood gases and associated parameters did not vary between groups (Supplemental Table I). SAH in vehicle-treated animals induced an immediate increase in ICP (up to approximately 75 mm Hg), a rapid increase in MABP (by approximately 30–40 mm Hg), and concomitant changes in cerebral perfusion pressure and regional cerebral blood flow (Figure 1A–D, open circles). Inhibition of V1 receptors after SAH resulted in a less severe increase in ICP (approximately 50 mm Hg), only a minor increase of MABP (approximately 10 mm Hg, P<0.05 versus vehicle), and a less severe decrease in cerebral perfusion pressure compared with vehicle-treated SAH animals (Figure 1A–D, closed circles).

Rebleeding
Thirty percent (3 of 10) of vehicle-treated rats subjected to SAH had at least 1 rebleeding, whereas no rebleedings were observed in animals treated with the V1 receptor antagonist (Figure 2A).

Brain Edema Formation
Sham-operated animals showed normal brain water content 24 hours after surgery (79.0%±0.1% in vehicle-treated and 79.1%±0.1% in V1 antagonist-treated rats; Figure 2B, sham).
SAH resulted in an increase in brain water content to 79.8% ± 0.2% in vehicle-treated animals; this increase was prevented in rats treated with the V1 antagonist (79.0% ± 0.1%, P < 0.05 versus vehicle; Figure 2B, SAH).

Neurological Deficits and Body Weight
V1 receptor antagonist-treated animals exhibited less severe neurological deficits after SAH compared with animals receiving vehicle infusion. Statistical significance (P < 0.05 versus vehicle) was reached on Days 1 through 3 when neurological function was quantified using the 6-point scale (Figure 3A) and on Day 5 (P < 0.05 versus vehicle) using the Beam Balance Test (Figure 3B).

Posthemorrhagic body weight dropped to 78% ± 4% and 83% ± 3% of baseline on Day 3 after SAH in the vehicle and V1 antagonist groups, respectively. This was followed by a gradual recovery to 82% ± 5% and 89% ± 3% of baseline 7 days after SAH (nonsignificant) in the vehicle and V1 antagonist groups, respectively.

Mortality
There was a significantly lower mortality rate in the SAH + V1 receptor antagonist group (2 of 10 [20%]) compared with the SAH + vehicle group (5 of 10 [50%]) during the 7-day posthemorrhagic observation period (P < 0.05). All animals that survived the first 72 hours after SAH survived for the remaining 7-day observation period (Figure 3C). Of the 3 animals in the SAH + vehicle group that displayed a secondary increase in ICP (indicative of rebleeding), 2 died within 48 hours after SAH. There was no mortality in sham-operated animals.

Plasma Vasopressin
To investigate whether AVP is involved in posthemorrhagic systemic hypertension (Cushing response; Figure 1A), we measured AVP plasma levels during the Cushing response (5–15 minutes after SAH). AVP was indeed elevated during the hypertensive episode and returned to baseline levels thereafter (Figure 4A; P < 0.05).

Figure 1. Time course of dynamic changes in (A) mean arterial pressure (delta MABP); (B) intracranial pressure (ICP); (C) cerebral perfusion pressure (CPP); and (D) ipsilateral regional cerebral blood flow until 60 minutes after SAH in vehicle and V1 receptor antagonist-treated animals. SAH resulted in acute MABP and ICP elevation that was significantly reduced by prehemorrhagic application of a V1 antagonist (P < 0.05 vs vehicle). CPP and regional cerebral blood flow were acutely reduced after SAH followed by a gradual recovery during the observation period without intergroup differences. SAH indicates subarachnoid hemorrhage.
Temporal Profile of the Cushing Response After SAH

To investigate the exact temporal profile of the Cushing response, we performed high-resolution mean arterial pressure measurements (1 Hz). SAH triggered a bimodal mean arterial pressure response: a short early elevation (Figure 4B, vertical arrow) followed by a long-lasting increase, which leveled off slowly (Figure 4B, horizontal arrow). Inhibition of AVP V1a receptors blunted the second large mean arterial pressure increase indicating that AVP and AVP V1a receptors are responsible for a large proportion of the Cushing response (Figure 4C).

Discussion

In this study, we demonstrate that selective pharmacological inhibition of AVP V1a receptors after SAH reduces the posthemorrhagic increase in systemic blood pressure (Cushing response), thereby attenuating the primary bleeding and preventing the detrimental effect of rebleedings. As a result,
brain edema formation is blunted, mortality is reduced from 50% to 20%, and animals have a better functional outcome.

Our data indicate that the underlying mechanism of this finding is the involvement of AVP and AVP V₁a receptors in the ICP-induced increase in systemic blood pressure (Cushing reflex) immediately after SAH. A sharp increase in ICP triggers the release of AVP into the bloodstream; consecutive activation of vascular V₁a receptors results in systemic arterial constriction and a subsequent hypertensive response, which exacerbates SAH and potential rebleedings. Inhibition of AVP V₁a receptors blunts the Cushing response, thereby resulting in less severe initial bleeding, prevention of rebleedings, and a better outcome.

In the current experiments, SAH produced not only an acute increase in ICP and a decrease in regional cerebral blood flow, but also reactive hypertension that lasted for approximately 20 minutes post-SAH. This reactive hypertension is part of a specific hemodynamic response to raised ICP present in all mammals, including humans, the so-called Cushing reflex. Current understanding indicates that catecholamines are mainly responsible for this association between ICP and blood pressure; AVP was not previously believed to be involved in this phenomenon. Interestingly, however, SAH resulted in an immediate increase of plasma AVP that paralleled the Cushing response. Our findings are supported by previous reports showing that experimental injection of blood into the subarachnoid space resulted in a prolonged increase of AVP plasma levels and in an enhanced central release of AVP and by data in patients in whom AVP plasma levels were increased in cerebrospinal fluid and serum early after SAH. Based on the finding that inhibition of V₁a receptors significantly reduced the posthemorrhagic Cushing response, our current data support the concept that the immediate posthemorrhagic increase in ICP causes a rapid increase in plasma AVP, which activates V₁a receptors and results in subsequent arterial hypertension. These findings indicate that the Cushing reflex may, to a large extent, be mediated by V₁a receptors. Indeed, MABP recordings with high temporal resolution during SAH show that the Cushing response has a small initial and a large secondary component. Because the secondary component is completely blunted by V₁a receptor inhibition, our data strongly suggest that AVP mediates the major component of the Cushing response.

Changes in MABP after SAH, ranging from severe hypertension to hypotension, are well documented from clinical practice. Arterial hypertension was shown to be independently associated with death and severe disability 3 months after SAH. Therefore, it is not surprising that the prevention of posthemorrhagic hypertension significantly reduced mortality and resulted in a better functional outcome in animals treated with the V₁a receptor antagonist. The significant reduction of the posthemorrhagic hypertensive response observed in V₁a receptor antagonist-treated animals was the reason for a less extensive initial hemorrhage (as indicated by the lower peak ICP) and for the prevention of secondary rebleedings; there was no secondary acute increase in ICP detectable in the receptor antagonist-treated group. Rebleeding was, however, observed in 30% of the vehicle-treated animals.

Figure 4. A, AVP plasma levels before and after SAH. SAH results in a 36-fold increase in plasma AVP 5 minutes after SAH, that is, at the time when posthemorrhagic hypertension is observed (5–15 minutes after SAH). B, Mean arterial blood pressure (MAP; gray line) and intracranial pressure (ICP; black line) before, during, and after SAH. In animals receiving vehicle, the ICP-induced arterial hypertension (Cushing response) has 2 phases: a short initial 1 (vertical arrow) and a long-lasting second 1 (horizontal arrow). C, Same experiment as in B but with systemic application of a nonblood–brain barrier-permeable AVP V₁a receptor antagonist before SAH. Inhibition of vascular AVP V₁a receptors blunts the second phase of the Cushing reflex suggesting that AVP V₁a receptors are responsible for the largest part of this response. AVP indicates arginine vasopressin; SAH, subarachnoid hemorrhage.
animals and two thirds of these animals died within the 7-day observation period.

Previously, we and others suggested that inhibition of the V₁ receptor may reduce brain edema formation after cerebral ischemia and traumatic brain injury directly through inhibition central V₁ receptors. Although we cannot fully exclude that this mechanism may also be responsible for part of the neuroprotective effect of V₁ receptor inhibition observed in the current study, our findings suggest that AVP has a very specific, so far unrecognized, role in the pathophysiology of SAH, in which systemic blood pressure has an immediate and direct effect on the primary insult and the frequency of rebleedings, both strong predictors of outcome after SAH. Therefore, the direct effect of V₁ receptor inhibition on brain edema formation seems to be of only subordinate significance in SAH.

Taken together our results show that AVP V₁a receptors mediate systemic hypertension after SAH, thereby exacerbating the initial bleeding, causing rebleedings, and, hence, secondary brain damage, functional deficits, and additional mortality. Inhibition of AVP V₁a receptors reduces the severity of hemorrhage and prevents rebleeding. Because these 2 factors are among the most important determinants for outcome after SAH, AVP V₁ receptor antagonist may have significant therapeutic potential for the prevention and/or amelioration of SAH-induced brain damage.

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Disclosures

None.

References

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**Supplemental table**

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Data are expressed as mean ± SEM (n=10 in each group), pH: arterial pH, PaCO₂: arterial pCO₂, PaO₂: arterial O₂.
Supplemental figure 2

A

MAP (mmHg)

mean ± SEM
n=3 in each group

B

ICP (mmHg)

mean ± SEM
n=3 in each group

C

rCBF ipsilateral (% baseline)

mean ± SEM
n=3 in each group
Supplemental figure legends

**Supplemental figure 1** Recording of intracranial pressure (ICP) in a single animal. Re-bleedings were detected by a secondary ICP increase larger than 15 mmHg.

**Supplemental figure 2** Mean arterial blood pressure (MAP) (A), intracranial pressure (ICP) (B), and regional cerebral blood flow (rCBF) (C) in animals treated with vehicle or a V₁-receptor antagonist. Application of the V₁-receptor antagonist did not effect any of the measured parameters (n=3 per group).