Effect of Small Molecule Vasopressin V_{1a} and V₂ Receptor Antagonists on Brain Edema Formation and Secondary Brain Damage following Traumatic Brain Injury in Mice

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Abstract

The attenuation of brain edema is a major therapeutic target after traumatic brain injury (TBI). Vasopressin (AVP) is well known to play a major role in the regulation of brain water content and vasoendothelial functions and to be involved in brain edema formation. Therefore, the aim of the current study was to analyze the antiedematous efficacy of a clinically relevant, nonpeptidic AVP V_{1a} and V₂ receptor antagonists. C57Bl6 mice were subjected to controlled cortical impact (CCI) and V_{1a} or V₂ receptors were inhibited by using the highly selective antagonists SR-49059 or SR-121463A either by systemic (intraperitoneal, IP) or intracerebroventricular (ICV) application. After 24 h, brain edema, intracranial pressure (ICP), and contusion volume were assessed. Systemically applied AVP receptor antagonists could not reduce secondary lesion growth. In contrast, ICV administration of AVP V_{1a} receptor antagonist decreased brain edema formation by 68%, diminished post-traumatic increase of ICP by 46%, and reduced secondary contusion expansion by 43% 24 h after CCI. The ICV inhibition of V₂ receptors resulted in significant reduction of post-traumatic brain edema by 41% 24 h after CCI, but failed to show further influence on ICP and lesion growth. Hence, centrally applied vasopressin V_{1a} receptor antagonists may be used to reduce brain edema formation after TBI.

Key words: arginine vasopressin; brain edema; CBF; traumatic brain injury

Introduction

TRAUMATIC BRAIN INJURY (TBI) is one of the most frequent causes of mortality and morbidity in children and in young adults in industrialized countries.¹ Outcome depends on the characteristics of the primary lesion and the degree of secondary brain damage.^{2,3} The primary tissue damage emerges immediately at the moment of injury because of mechanical forces on the brain parenchyma and can therefore not be treated. The secondary expansion of the primary lesion, however, is driven by a complex sequence of molecular events and is, hence, amenable to treatment. Unfortunately, even today, many mechanisms leading to secondary brain damage are not fully understood, thereby constraining the development of novel treatment options for brain injured patients.^{4,5}

Arginine vasopressin (AVP), also known as antidiuretic hormone or argipressin, is a cyclic nonapeptide synthesized in the hypothalamus and transported via the axonal fiber system to the neurohypophysis, where it is released into the bloodstream.⁶ Existence of extrahypophyseal vasopressinergic pathways within the central nervous system (CNS) have been demonstrated and suggest the independent release and function of systemic versus central

vasopressin.^{6,7} Besides its major role in controlling water and solute excretion in the kidney, AVP is also involved in the regulation of brain ion homeostasis, cerebrospinal fluid production, brain capillary water permeability, and microvascular resistance.^{8–13} AVP has also been reported to regulate cell volume and water permeability of subpial astrocytes.^{14,15} These effects are mediated by three seven-transmembrane G-protein-coupled AVP receptors V_{1a}, V_{1b}, and V₂. In the CNS, most of the AVP-mediated signaling is processed via the V_{1a} receptor subtype, whereas V₂ receptors and V_{1b} receptors are expressed to a lesser extent.^{16–19}

Centrally released AVP seems to play a causal role in the increase of brain water content and the formation of vasogenic brain edema, predominantly via its V₁ receptor.^{20–23} Moreover, AVP is elevated in plasma and cerebrospinal fluid of patients with various forms of brain injury—e.g., TBI, cerebral ischemia, and subarachnoid hemorrhage.^{24–26} Because of these properties, we previously investigated the neuroprotective role of AVP V_{1a} and V₂ inhibition after experimental TBI.²¹ We observed a decrease in brain edema formation, intracranial pressure (ICP), and contusion volume by a peptidic AVP V₁ receptor antagonist given intracerebroventricularly (ICV); a V₂ receptor antagonist had no effect.

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Because peptidic receptor inhibitors have multiple disadvantages, however, especially when considering their potential clinical use, we aimed to investigate whether nonpeptidic small molecule AVP V_{1a} and V_2 receptor antagonists have also a therapeutic potential after experimental TBI. We therefore used the highly specific nonpeptide AVP receptor antagonists SR-49059 (V_{1a} receptor antagonist) and SR-121463A (V_2 receptor antagonist) and investigated their effect on post-traumatic brain edema, ICP, contusion volume, cerebral blood flow (CBF), and blood pressure after controlled cortical impact (CCI) in mice.^{27,28}

Methods

Animals

In this study, male C57/BL6 mice (23–29 g) purchased from Charles River, Sulzfeld, Germany were used. All mice had free access to water and food. Animal experiments were performed in accordance with the institutional guidelines of the animal care institutions of the University of Munich and approved by the Bavarian government (protocol number 55.2-1-54-2531-117-05).

Anesthesia

Animals were anesthetized as described previously.²⁹ Briefly, anesthesia was induced with 4% isoflurane in a gas-tight chamber and maintained by intraperitoneal (IP) injection of midazolam (5 mg/kg KG, Dormicum,® Roche, Basel, Switzerland), fentanyl (0.05 mg/kg, Janssen-Cilag, Neuss, Germany), and medetomidine (0.5 mg/kg, Pfizer, Karlsruhe, Germany). Body temperature was maintained at 37°C by a feedback-controlled heating pad (FHC, Bowdoin, ME). Anesthesia was terminated by IP injection of atipamezole (2.5 mg/kg; Pfizer, Karlsruhe, Germany), naloxone (1.2 mg/kg; Inresa, Freiburg, Germany), and flumazenil (0.5 mg/kg; Hoffmann-La-Roche, Grenzach-Wyhlen, Germany). For short-term anesthesia (<30 min) animals received 30% O₂ by a face mask. For long-term anesthesia, animals were intubated with an orotracheal tube and mechanically ventilated with 30% O₂ (Minivent, Hugo Sachs, Germany) as described previously.²⁹

Physiological monitoring

For the investigation of systemic changes in vital parameters after application of SR-49059 and SR-121463A, we measured end-tidal pCO₂, noninvasive blood pressure (NIBP), and regional cerebral blood flow (rCBF) in two separate experimental series. After induction of anesthesia, mice were intubated, mechanically ventilated, and fixed in a stereotactic frame with a nose clamp. Microcapnometry (CI240, Columbus Instruments, Columbus, OH) was used to maintain arterial pCO₂ between 35 and 40 mm Hg.²⁹ Arterial blood gases were assessed with a blood gas analyzer (Chiron 860, Bayer, Germany) at the end of each experiment.

Systolic blood pressure (SBP) was measured by a NIBP monitoring system (RTBP 2000, Kent Scientific, Torrington, CT) with a custom-made adjustable tail-cuff device.²⁹

rCBF was assessed in the parietal cortex contralateral to the injury by laser-Doppler fluxmetry (Periflux 4001 Master, Perimed, Sweden).³⁰ For this purpose, a laser-Doppler probe was attached to the temporal bone (Fig. 1). Data were collected and recorded with a PC data acquisition system (A/D converter PCI 9112, Adlink Technology, Taiwan; Software: DasyLab 5.0, IED GmbH, Hamburg, Germany).

CCI

After induction of anesthesia, animals were fixed in a stereotactic frame with a nose clamp, the scalp was incised midline, and a craniotomy of 4 × 4 mm was performed posterolateral to the bregma

FIG. 1. Schematic drawing of a mouse skull with the location of trephination, contusion, and the positions of the laser-Doppler probe, measurement of intracranial pressure (ICP), and the intracerebroventricular (ICV) injection of the vasopressin receptor antagonists.

over the right hemisphere leaving the dura mater intact (Fig. 1). CCI (Mouse-Katjuscha 2000, L. Kopacz, University of Mainz, Germany) was performed immediately after opening the skull as described previously.^{21,31,32} Briefly, the impact was applied perpendicular to the dura with the following parameters: piston diameter: 3 mm; penetration depth: 1 mm; velocity: 8 m/sec; and impact duration: 150 msec. The initially removed bone flap was glued back (Histoacryl,® Braun-Melsungen, Melsungen, Germany), and the scalp was sutured. After antagonizing anesthesia mice were put into an incubator heated to 35°C until recovery of spontaneous motor activity.

Measurement of ICP

ICP was measured 24 h after trauma using a microprobe with a pressure transducer microchip at the tip (Ø 0.9 mm, MIPM, Mammendorf, Germany). The microprobe was inserted 1 mm rostral and 1 mm lateral to the bregma of the traumatized hemisphere at a depth of 3 mm (Fig. 1).³¹

Determination of brain water content

Mice were sacrificed 24 h after CCI by cervical dislocation under deep isoflurane anesthesia. Brains were sampled in toto, cerebellum, brain stem, and olfactory bulb removed, and hemispheres divided using a brain matrix (Kent Scientific). The brains were weighted immediately to obtain their wet weight (w.w.) and afterward dried at 110°C for 24 h, followed by the measurement of the dry weight (d.w.). Brain water content was then calculated by the following formula: $[(w.w.-d.w.)/w.w.] \times 100$.²¹

Quantification of contusion volume

Animals were euthanized 24 h after CCI in deep isoflurane anesthesia. Brains were removed, immediately frozen in dry ice, and stored at –20°C. Coronal sections with a thickness of 10 µm were sliced every 500 µm with a cryostat (CryoStar HM 560, Microm, Germany), dry fixed, stained with Nissl staining, and photographed with a digital camera system connected to a microscope. The contused brain tissue was then measured on the digital photographs using standard image analysis software (Olympus DP-soft, Germany) by an investigator blinded to the treatment of the animals.

Contusion volume (V) was calculated as described previously based on the contused area (A) on 15 consecutive coronal brain sections 500 μm (d) apart using the following formula: $V = d \times (A_1 \times 0.5 + A_2 + A_3 \dots + A_{15} \times 0.5)$.

Drug administration

The inhibition of V_{1a} and V_2 receptors was performed by using the selective antagonists SR-49059 (V_{1a} receptor antagonist) and SR-121463A (V_2 receptor antagonist) (Sanofi Aventis, Toulouse, France).^{27,28}

For IP administration, 1 or 10 μg SR-49059/g body weight (BW) were dissolved in 18.5 μL water/g BW with 10% dimethylformamide (DMF) (Serradeil-Le Gal et al, 1993). SR-121463A was applied IP at 0.1 and 1 $\mu\text{g/g}$ BW and dissolved in 18.5 μL saline/g BW.²⁸ Compounds and vehicles were applied 10 min after TBI.

For ICV administration, 1.08 μg , 540 ng, and 270 ng SR-49059 were dissolved in 2 μL water with 10% DMF resulting in dosages of 40, 20, and 10 ng/g BW, respectively. SR-121463A was applied at 108 ng dissolved in 2 μL saline. The dissolved compounds (or vehicle) were given ICV 3 min after TBI contralateral to the trauma site (0.9 mm left, 0.1 mm posterior, and 3.1 mm deep relative to the bregma) over a period of 2 min—i.e., at a rate of 1 $\mu\text{L}/\text{min}$ (Fig. 1). Because ICV injections necessitate scalp closure after injection, we used this protocol to keep the anesthesia time equal in all groups.

Experimental design

The current study consists of five different experimental series. To determine potential physiological changes induced by the receptor antagonists or their respective vehicles, we performed two experimental series (A+B) to measure $p\text{CO}_2$, NIBP, and rCBF after administration of the compound and performing CCI. $p\text{CO}_2$, NIBP, and rCBF were measured every 5 min during the whole experiment. As soon as these parameters reached a stable baseline value, receptor antagonists or vehicles were administered in a blinded manner. At 20 minutes later, trephination, and after another 10 minutes, CCI was performed as described above. Subsequent to CCI, the parameters were measured for a further 30 min until the animals were sacrificed.

(A) For physiological parameters after IP administration, four groups were formed: 10% DMF (control, $n=10$), 10 μg SR-49059/g (BW) ($n=10$), saline (control, $n=5$), and 1 μg SR-121463A/g (BW) ($n=8$).

(B) For physiological parameters after ICV administration, another four groups were investigated: 10% DMF (control, $n=8$), 1.08 μg SR-49059 ($n=7$), saline (control, $n=5$), and 108 ng SR-121463A ($n=7$).

Three treatment series were performed (C–E). After IP injection solely contusion volume were measured. In animals receiving ICV application, we analyzed in addition to contusion volume brain edema formation and ICP levels.

(C) Contusion volume 24 h after CCI and IP administration of the receptor antagonists were investigated in seven different groups: 15 min survival ($n=8$), 24 h saline (control, $n=6$), 10% DMF (control, $n=8$), 1 μg SR-49059/g (BW) ($n=7$), 10 μg SR-49059/g (BW) ($n=8$), 0.1 μg SR-121463A/g (BW) ($n=8$) and 1 μg SR-121463A/g (BW) ($n=7$).

(D) Contusion volume 24 h after CCI and ICV administration of the receptor antagonists were investigated in five different groups: 15 min survival ($n=7$), 24 h saline (control, $n=7$), 10% DMF (control, $n=7$), 1.08 μg SR-49059 ($n=7$), and 108 ng SR-121463A ($n=7$).

(E) For measurement of changes in post-traumatic brain edema and ICP 24 h after CCI and ICV administration of the receptor antagonists, we performed a series of six groups: sham ($n=8$), 24 h saline (control, $n=7$), 1.08 μg SR-49059 ($n=7$), 540 ng SR-49059 ($n=7$), 270 ng SR-49059 ($n=7$), and 108 ng SR-121463A ($n=7$).

In all described experiments, animals were randomly assigned to the different treatment groups and the investigator, who performed animal surgery, was blinded in terms of the treatment.

Statistical analysis

Measurements over time (BP, rCBF) were tested versus baseline with Friedman repeated measures analysis of variance (ANOVA) on Ranks followed by Student-Newman-Keuls All Pairwise Multiple Comparison Procedure as a *post hoc* test. Differences between groups were tested by the Kruskal-Wallis test for nonparametric one-way ANOVA followed by Student-Newman-Keuls All Pairwise Multiple Comparison Procedure as a *post hoc* test. Differences between the two groups were tested using the Mann-Whitney-Wilcoxon test for multiple comparisons on ranks for independent samples. All results are presented as mean $\pm$ standard error of the mean (SigmaStat 3.0, Jandel Scientific, Erkrath, Germany). $p < 0.05$ was considered significant.

Results

Effect of systemically (IP) applied AVP antagonists

Physiological changes. For determination of physiological changes induced by AVP receptor antagonists or vehicles, we measured $p\text{CO}_2$, NIBP, and rCBF after administration of the compounds and after additional cerebral contusion as described above. rCBF showed no significant differences in those animals receiving IP 10% DMF, saline, or SR-121463A, while the group of SR-49059 showed a significant decrease in rCBF (Fig. 2). After IP administration of the agents, SBP remained significantly higher in the group receiving the V_2 receptor antagonist SR-121463A from 20 min after IP administration until the end of the experiment. The other groups (10% DMF, V_{1a} receptor antagonist SR-49059, and saline) showed comparable BP curves with no significant differences (data not shown).

Contusion volume. As shown previously, all mice showed a contusion at the impact site with small petechial hemorrhages within. Contrecoup injuries, intracranial hematomas, or other major pathologies were not observed.^{21,31,32} At 24 h after CCI, all groups

FIG. 2. Time course of regional cerebral blood flow (% baseline) 30 min before (baseline) and up to 30 min after controlled cortical impact (CCI) in controls (saline) and V_{1a} respectively V_2 receptor antagonist treated animals (intraperitoneal [IP]). The receptor antagonist was injected at 0 min. SEM, standard error of the mean.

showed significant growth of contusion volume compared with the primary brain lesion in animals that were sacrificed 15 min after CCI ($p < 0.001$). Comparing the receptor antagonist-treated groups (IP) with nontreated and control groups 24 h after injury, no significant change in contusion volume could be observed (data not shown). Only a small, but not significant decrease in contusion volume was observed in both groups with the AVP V_{1a} receptor antagonist (24 h saline: $31.4 \pm 0.5 \text{ mm}^3$, $n=6$; $1 \mu\text{g}$ SR-49059/g (BW): $30.3 \pm 0.9 \text{ mm}^3$, $n=7$; $10 \mu\text{g}$ SR-49059/g (BW): $28.3 \pm 1.9 \text{ mm}^3$, $n=8$) (data not shown). Because this approach did not affect the main outcome parameter of our study (contusion volume), it was regarded ineffective and was not investigated further.

Effect of ICV applied antagonists

Physiological changes. After ICV application of $2 \mu\text{L}$ 10% DMF, SR-49059, saline, or SR-121463A, no significant alteration of pCO_2 , NIBP, or rCBF could be observed before and after CCI (Fig. 3). Compared with 10% DMF, SR-49059, and saline, however, SBP is higher in the SR-121463A treated group under baseline conditions from 10 min after ICV application until CCI, but reached no level of significance (Fig. 3). CBF was not affected by ICV administration of neither V_{1a} and V_2 receptor antagonists nor their vehicle. End-tidal pCO_2 remained at $40.5 \pm 1.2 \text{ mm Hg}$ during the measurement of these physiological parameters and was not influenced by the agents.

Contusion volume. All groups showed significant increase of contusion volume after 24 h compared with the primary brain damage (15 min after CCI, $p < 0.001$): 15 min: $20.1 \pm 0.9 \text{ mm}^3$ ($n=7$), 24 h saline: 30.6 ± 0.9 ($n=7$), 10% DMF: 30.7 ± 1.1 ($n=7$), $1.08 \mu\text{g}$ SR-49059: $25.7 \pm 1.5 \text{ mm}^3$ ($n=7$), and 108 ng SR-121463A: 31.1 ± 0.8 ($n=7$) (Fig. 4). A significant reduction of contusion volume by SR-49059 was observed in comparison with ICV injection of 10% DMF or saline. Compared with the contusion growth between 15 min and 24 h after trauma from 20.1 ± 0.9 to $30.6 \pm 0.9 \text{ mm}^3$ ($+10.5 \text{ mm}^3$) in saline treated mice, the contusion in animals treated with $1.08 \mu\text{g}$ SR-49059 expanded only to $25.7 \pm 1.5 \text{ mm}^3$ ($+5.6 \text{ mm}^3$, -47%).

FIG. 3. Time course of systolic blood pressure (mm Hg) after intracerebroventricular application of V_{1a} or V_2 receptor antagonist. The receptor antagonist was injected at 0 min. SEM, standard error of the mean; CCI, controlled cortical impact.

FIG. 4. Contusion volume 15 min and 24 h after controlled cortical impact receiving saline and respectively in the experimental groups (24 h) with additional intracerebroventricular application of V_{1a} or V_2 receptor antagonist. Treatment was initiated 3 min after contusion. Concentration of compounds in ng/g body weight (mean body weight 27 g).

No significant difference could be observed between the injection of saline and 10% DMF. The inhibition of the AVP V_2 receptor with SR-121463A did not reduce contusion volume after 24 h (Fig. 4).

Brain edema formation and ICP. Normal brain water content in nontraumatized mice was 78.2%. TBI significantly increased brain water content of the traumatized right hemisphere by 2.6% to $80.8 \pm 0.6\%$ ($p < 0.001$), while the brain water content of the contralateral hemisphere did not change significantly ($77.9 \pm 0.2\%$) (Fig. 5). ICV injection of AVP V_{1a} receptor antagonists ($1.08 \mu\text{g}$, 40 ng/g BW SR-49059, $n=7$) reduced the post-traumatic increase of brain water content in a dose-dependent manner from 2.6% to 1.8% ($80.8 \pm 0.2\%$ vs. $79.0 \pm 0.6\%$;

FIG. 5. Brain water content in both hemispheres (traumatized, not traumatized) 24 h after controlled cortical impact in normal mice (sham) or animals receiving intracerebroventricular injection of saline, V_{1a} respectively V_2 receptor antagonist. Treatment was initiated 3 min after contusion. Concentration of compounds in ng/g body weight (mean body weight 27 g). SEM, standard error of the mean.

$p < 0.05$)—i.e., by 68%. Animals treated with 540 ng SR-49059 (20 ng/g BW, $n = 7$) also showed a significant reduction of brain water content in the traumatized hemisphere by 27% ($80.8 \pm 0.2\%$ vs. $80.1 \pm 0.2\%$; $p < 0.05$), whereas injection of 270 ng SR-49059 (10 ng/g BW, $n = 7$) and 10% DMF ($n = 7$) ($80.5 \pm 0.7\%$ vs. $80.3 \pm 0.3\%$) showed no significant change compared with untreated animals. Surprisingly, animals receiving 108 ng SR-121463A ($n = 7$) also showed significant reduction of brain water content by 41% in comparison with untreated animals ($80.8 \pm 0.2\%$ vs. $79.7 \pm 0.1\%$; $p < 0.05$) (Fig. 5).

At 24 h after TBI, ICP was significantly higher than in non-injured animals (23.8 ± 1.2 mm Hg instead of 5.7 ± 0.2 mm Hg; $p < 0.001$). ICV administration of SR-49059 (V_{1a} receptor antagonist) reduced the post-traumatic increase of ICP up to 46% in a dose-dependent manner (Fig. 6). At 24 h after TBI, we measured a significantly lower ICP in animals treated with 1.08 μ g SR-49059 (40 ng/g BW, $n = 7$) (15.5 ± 1.1 mm Hg; $p < 0.05$) and 540 ng SR-49059 (20 ng/g BW, $n = 7$) (17.6 ± 1.6 mm Hg; $p < 0.05$), while injection of 270 ng SR-49059 (10 ng/g BW, $n = 7$) (23.4 ± 2.3 mm Hg) and 10% DMF ($n = 7$) (22.3 ± 2.1 mm Hg) showed equally to the results in brain edema formation no significant change compared with untreated animals. Although mice treated with 108 ng SR-121463A ($n = 7$) showed reduced brain water content, the reduction of ICP in these animals (20.9 ± 1.6 mm Hg) was not significant (Fig. 6).

Discussion

The present study showed that the inhibition of central vasopressin V_{1a} receptors by small molecule inhibitors attenuates the secondary growth of a cortical necrosis after experimental TBI. Inhibition of AVP V_{1a} receptors reduced post-traumatic brain edema formation up to 68% in a dose-dependent manner and the subsequent maximum ICP increase by 46%. Consequently, central inhibition of AVP V_{1a} receptors leads to a decrease in secondary brain damage by 47% and, hence, to a smaller brain tissue necrosis 24 h after TBI compared with untreated control animals. Inhibition

of AVP V_2 receptors showed far less pronounced effects. Previously, we showed similar neuroprotective effects of centrally applied peptidic AVP V_1 receptors antagonists.²¹ These inhibitors, however, had a questionable selectivity and multiple other disadvantages—e.g., difficult chemical optimization. Because these properties may compromise the translational potential of these peptidic inhibitors, we currently investigated the small molecule inhibitors SR-49059 (V_1 receptor antagonist) and SR-121463A (V_2 receptor antagonist). These antagonists are highly specific, have a high selectivity toward AVP V_1 receptors when compared with a plethora of other receptors, ion channels, and enzymes, and display an excellent safety profile in several animal species and in humans.^{33,34} Accordingly, these small molecule inhibitors may have an improved potential to reach the clinical scenario.

Besides the pronounced neuroprotective effect of small-molecule V_{1a} receptor antagonists, an intriguing finding of the current study is that central inhibition of V_{1a} receptors is by far more protective than systemic inhibition. One of the main reasons for this effect seems to be the CBF lowering effect of systemic V_{1a} receptor inhibition. This indicates two interesting points, namely: (1) vasopressin is involved in the maintenance of CBF after TBI and (2) reduction of CBF is detrimental after TBI, two findings that are well in line with previous findings.^{9,10,35}

In addition, our findings also indicate that only the central inhibition of V_{1a} receptors is neuroprotective. Accordingly, the most pronounced effect was observed when AVP V_{1a} receptor antagonists were applied ICV: central V_{1a} receptors were inhibited while peripheral/intravascular V_{1a} receptors maintained CBF and prevented post-traumatic cerebral ischemia. Central application of drugs may be problematic in patients because ICV injections may have serious side effects—e.g., ventriculitis/meningitis; however, when a reasonable risk/benefit ratio is present, there is no doubt that intrathecal injections are viable clinical options. Accordingly, despite their purely central activity and the resulting unusual route of application, the currently used AVP V_{1a} receptor antagonists may still have a good clinical potential when carefully tested and evaluated.

Like the current results, other experimental studies also point out a role of AVP in the pathophysiology of various brain injuries—e.g., after focal cerebral ischemia, global cerebral ischemia, subarachnoid or intracerebral hemorrhage,^{23,26,36} and TBI^{20–23,36–40}; however, the precise mechanisms on how AVP protects the brain are still not fully understood. We know that the amount of AVP mRNA or AVP protein is increased after brain injury^{25,41–43} and that AVP significantly increases vascular permeability via its V_{1a} receptor subtype when applied centrally.^{23,36,39,44,45} Accordingly, we hypothesize that the main neuroprotective mechanism of AVP receptor inhibition is most likely mediated by the reduction of brain edema formation. If we consider that vasopressin has also been reported to induce glial swelling, it is also likely that the central inhibition of AVP V_{1a} receptors does not only attenuate vasogenic but also cytotoxic brain edema formation.^{14,22,46,47} Therefore AVP V_{1a} receptor inhibition may have a multifactorial effect on the formation of post-traumatic brain edema.

Naturally, our study also has some inherent limitations, such as: (1) the used species, because the pathophysiology of contusion expansion may be different in mice compared with humans; (2) the used compound, because despite thorough testing, the currently used antagonists may have so far unknown activities beyond the inhibition of AVP V_1 receptors; and (3) the therapeutic approach, because other mechanisms also not influenced by central AVP V_{1a} receptor inhibition—e.g., CBF—may be involved in secondary

FIG. 6. Intracranial pressure 24 h after controlled cortical impact in normal mice (sham) or animals receiving saline, V_{1a} respectively V_2 receptor antagonist intracerebroventricularly. Treatment was initiated 3 min after contusion. Concentration of compounds in ng/g body weight (mean body weight 27 g). SEM, standard error of the mean.

contusion expansion. Accordingly, further experiments in different species and in different TBI models will be necessary before any clinical use should be taken into consideration.

Conclusion

The current study suggests that central inhibition of vasopressin V_{1a} receptors with clinically applicable small molecule antagonists reduces brain edema formation, ICP, and secondary brain damage after TBI in mice. Thus, inhibition of cerebral AVP V_{1a} receptors might represent a novel therapeutic target for TBI.

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Author Disclosure Statement

No competing financial interests exist.

References

- Corrigan, J.D., Selassie, A.W., and Orman, J.A. (2010). The epidemiology of traumatic brain injury. *J. Head Trauma Rehabil.* 25, 72–80.
- Giacoppo, S., Bramanti, P., Barresi, M., Celi, D., Foti Cuzzola, V., Palella, E., and Marino, S. (2012). Predictive biomarkers of recovery in traumatic brain injury. *Neurocrit. Care* 16, 470–477.
- Pearl, G.S. (1998). Traumatic neuropathology. *Clin. Lab. Med.* 18, 39–64.
- Borgens, R.B., and Liu-Snyder, P. (2012). Understanding secondary injury. *Q. Rev. Biol.* 87, 89–127.
- Kermer, P., Klocker, N., and Bahr, M. (1999). Neuronal death after brain injury. Models, mechanisms, and therapeutic strategies in vivo. *Cell Tissue Res.* 298, 383–395.
- Buijs, R.M. (1980). Immunocytochemical demonstration of vasopressin and oxytocin in the rat brain by light and electron microscopy. *J. Histochem. Cytochem.* 28, 357–360.
- Ripphagen, C.L., and Pittman, Q.J. (1986). Arginine vasopressin as a central neurotransmitter. *Fed. Proc.* 45, 2318–2322.
- Faraci, F.M., Mayhan, W.G., and Heistad, D.D. (1990). Effect of vasopressin on production of cerebrospinal fluid: possible role of vasopressin (V₁)-receptors. *Am. J. Physiol.* 258, R94–R98.
- Faraci, F.M., Mayhan, W.G., Schmid, P.G., and Heistad, D.D. (1988). Effects of arginine vasopressin on cerebral microvascular pressure. *Am. J. Physiol.* 255, H70–H76.
- Fernandez, N., Martinez, M.A., Garcia-Villalon, A.L., Monge, L., and Dieguez, G. (2001). Cerebral vasoconstriction produced by vasopressin in conscious goats: role of vasopressin V(1) and V(2) receptors and nitric oxide. *Br. J. Pharmacol.* 132, 1837–1844.
- Raichle, M.E., and Grubb, R.L., Jr. (1978). Regulation of brain water permeability by centrally-released vasopressin. *Brain Res.* 143, 191–194.
- Rosenberg, G.A., Kyner, W.T., Fenstermacher, J.D., and Patlak, C.S. (1986). Effect of vasopressin on ependymal and capillary permeability to tritiated water in cat. *Am. J. Physiol.* 251, F485–F489.
- Niermann, H., miry-Moghaddam, M., Holthoff, K., Witte, O.W., and Ottersen, O.P. (2001). A novel role of vasopressin in the brain: modulation of activity-dependent water flux in the neocortex. *J. Neurosci.* 21, 3045–3051.
- Del Bigio, M.R., and Fedoroff, S. (1990). Swelling of astroglia in vitro and the effect of arginine vasopressin and atrial natriuretic peptide. *Acta Neurochir. Suppl. (Wien)* 51, 14–16.
- Hertz, L., Chen, Y., and Spatz, M. (2000). Effects of arginine vasopressin on water space in astrocytes and in whole brain. *Am. J. Physiol. Endocrinol. Metab.* 278, E1175–E1176.
- Lolait, S.J., O'Carroll, A.M., Mahan, L.C., Felder, C.C., Button, D.C., Young, W.S., III, Mezey, E., and Brownstein, M.J. (1995). Extra-pituitary expression of the rat V_{1b} vasopressin receptor gene. *Proc. Natl. Acad. Sci. U. S. A.* 92, 6783–6787.
- Kato, Y., Igarashi, N., Hirasawa, A., Tsujimoto, G., and Kobayashi, M. (1995). Distribution and developmental changes in vasopressin V₂ receptor mRNA in rat brain. *Differentiation* 59, 163–169.
- Ostrowski, N.L., Lolait, S.J., and Young, W.S., III (1994). Cellular localization of vasopressin V_{1a} receptor messenger ribonucleic acid in adult male rat brain, pineal, and brain vasculature. *Endocrinology* 135, 1511–1528.
- Brinton, R.E., Gee, K.W., Wamsley, J.K., Davis, T.P., and Yamamura, H.I. (1984). Regional distribution of putative vasopressin receptors in rat brain and pituitary by quantitative autoradiography. *Proc. Natl. Acad. Sci. U. S. A.* 81, 7248–7252.
- Hockel, K., Scholler, K., Trabold, R., Nussberger, J., and Plesnila, N. (2012). Vasopressin V(1a) receptors mediate posthemorrhagic systemic hypertension thereby determining rebleeding rate and outcome after experimental subarachnoid hemorrhage. *Stroke* 43, 227–232.
- Trabold, R., Krieg, S., Scholler, K., and Plesnila, N. (2008). Role of vasopressin V(1a) and V2 receptors for the development of secondary brain damage after traumatic brain injury in mice. *J. Neurotrauma* 25, 1459–1465.
- Vakili, A., Kataoka, H., and Plesnila, N. (2005). Role of arginine vasopressin V₁ and V₂ receptors for brain damage after transient focal cerebral ischemia. *J. Cereb. Blood Flow Metab.* 25, 1012–1019.
- Rosenberg, G.A., Scremin, O., Estrada, E., and Kyner, W.T. (1992). Arginine vasopressin V₁-antagonist and atrial natriuretic peptide reduce hemorrhagic brain edema in rats. *Stroke* 23, 1767–1774.
- Xu, M., Su, W., Huang, W.D., Lu, Y.Q., Xu, Q.P., and Chen, Z.J. (2007). Effect of AVP on brain edema following traumatic brain injury. *Chin. J. Traumatol.* 10, 90–93.
- Mather, H.M., Ang, V., and Jenkins, J.S. (1981). Vasopressin in plasma and CSF of patients with subarachnoid haemorrhage. *J. Neurol. Neurosurg. Psychiatry* 44, 216–219.
- Joynt, R.J., Feibel, J.H., and Sladek, C.M. (1981). Antidiuretic hormone levels in stroke patients. *Ann. Neurol.* 9, 182–184.
- Serradeil-Le Gal, C., Wagnon, J., Garcia, C., Lacour, C., Guiraudou, P., Christophe, B., Villanova, G., Nisato, D., Maffrand, J.P., Le Fur G., et al. (1993). Biochemical and pharmacological properties of SR 49059, a new, potent, nonpeptide antagonist of rat and human vasopressin V_{1a} receptors. *J. Clin. Invest.* 92, 224–231.
- Serradeil-Le Gal, C., Lacour, C., Valette, G., Garcia, G., Foulon, L., Galindo, G., Bankir, L., Pouzet, B., Guillon, G., Barberis, C., Chicot, D., Jard, S., Vilain, P., Garcia, C., Marty, E., Raufaste, D., Brossard, G., Nisato, D., Maffrand, J.P., and Le Fur, G. (1996). Characterization of SR 121463A, a highly potent and selective, orally active vasopressin V₂ receptor antagonist. *J. Clin. Invest.* 98, 2729–2738.
- Thal, S.C., and Plesnila, N. (2007). Non-invasive intraoperative monitoring of blood pressure and arterial pCO₂ during surgical anesthesia in mice. *J. Neurosci. Methods* 159, 261–267.
- von Baumgarten L., Trabold, R., Thal, S., Back, T., and Plesnila, N. (2008). Role of cortical spreading depressions for secondary brain damage after traumatic brain injury in mice. *J. Cereb. Blood Flow Metab.* 28, 1353–1360.
- Zweckberger, K., Stoffel, M., Baethmann, A., and Plesnila, N. (2003). Effect of decompression craniotomy on increase of contusion volume and functional outcome after controlled cortical impact in mice. *J. Neurotrauma* 20, 1307–1314.
- Zweckberger, K., Eros, C., Zimmermann, R., Kim, S.W., Engel, D., and Plesnila, N. (2006). Effect of early and delayed decompressive craniectomy on secondary brain damage after controlled cortical impact in mice. *J. Neurotrauma* 23, 1083–1093.
- Brouard, R., Laporte, V., Serradeil Le Gal C., Pignol, R., Jang, H., Donat, F., Lockwood, G., Fournie, D., and Dreux, F. (1998). Safety, tolerability, and pharmacokinetics of SR 49059, a V_{1a} vasopressin receptor antagonist, after repeated oral administration in healthy volunteers. *Adv. Exp. Med. Biol.* 449, 455–465.
- Serradeil-Le, G.C., Wagnon, J., Simiand, J., Griebel, G., Lacour, C., Guillon, G., Barberis, C., Brossard, G., Soubrie, P., Nisato, D., Pascal, M., Pruss, R., Scatton, B., Maffrand, J.P., and Le, F.G. (2002). Characterization of (2S,4R)-1-[5-chloro-1-[(2,4-dimethoxyphenyl)sulfonyl]-3-(2-methoxy-phenyl)-2-oxo-2,3-dihydro-1H-indol-3-yl]-4-hydroxy-N,N-dimethyl-2-pyrrolidine carboxamide (SSR149415), a selective and orally active vasopressin V_{1b} receptor antagonist. *J. Pharmacol. Exp. Ther.* 300, 1122–1130.

35. Trabold, R., Schueler, O.G., Eriskat, J., Plesnila, N., Baethmann, A.J., and Back, T. (2006). Arterial hypotension triggers perifocal depolarizations and aggravates secondary damage in focal brain injury. *Brain Res.* 1071, 237–244.
36. Doczi, T., Laszlo, F.A., Szerdahelyi, P., and Joo, F. (1984). Involvement of vasopressin in brain edema formation: further evidence obtained from the Brattleboro diabetes insipidus rat with experimental subarachnoid hemorrhage. *Neurosurgery* 14, 436–441.
37. Manaenko, A., Fathali, N., Khatibi, N.H., Lekic, T., Hasegawa, Y., Martin, R., Tang, J., and Zhang, J.H. (2011). Arginine-vasopressin V1a receptor inhibition improves neurologic outcomes following an intracerebral hemorrhagic brain injury. *Neurochem. Int.* 58, 542–548.
38. Liu, X.F., Shi, Y.M., and Lin, B.C. (1991). Mechanism of action of arginine vasopressin on acute ischemic brain edema. *Chin. Med. J. (Engl.)* 104, 480–483.
39. Dickinson, L.D., and Betz, A.L. (1992). Attenuated development of ischemic brain edema in vasopressin-deficient rats. *J. Cereb. Blood Flow Metab.* 12, 681–690.
40. Armstead, W.M. (2001). Vasopressin induced cyclooxygenase dependent superoxide generation contributes to K(+) channel function impairment after brain injury. *Brain Res.* 910, 19–28.
41. Pascale, C.L., Szmydynger-Chodobska, J., Sarri, J.E., and Chodobski, A. (2006). Traumatic brain injury results in a concomitant increase in neocortical expression of vasopressin and its V(1a) receptor. *J. Physiol. Pharmacol.* 57, Suppl 11, 161–167.
42. Barreca, T., Gandolfo, C., Corsini, G., Del, S.M., Cataldi, A., Rolandi, E., and Franceschini, R. (2001). Evaluation of the secretory pattern of plasma arginine vasopressin in stroke patients. *Cerebrovasc. Dis.* 11, 113–118.
43. Huang, W.D., Pan, J., Xu, M., Su, W., Lu, Y.Q., Chen, Z.J., Jiang, T.Y., and Yang, Y.M. (2008). Changes and effects of plasma arginine vasopressin in traumatic brain injury. *J. Endocrinol. Invest* 31, 996–1000.
44. Nagao, S., Kagawa, M., Bemana, I., Kuniyoshi, T., Ogawa, T., Honma, Y., and Kuyama, H. (1994). Treatment of vasogenic brain edema with arginine vasopressin receptor antagonist—an experimental study. *Acta Neurochir. Suppl. (Wien)* 60, 502–504.
45. Doczi, T., Szerdahelyi, P., Gulya, K., and Kiss, J. (1982). Brain water accumulation after the central administration of vasopressin. *Neurosurgery* 11, 402–407.
46. Chen, Y., McNeill, J.R., Hajek, I., and Hertz, L. (1992). Effect of vasopressin on brain swelling at the cellular level: do astrocytes exhibit a furosemide—vasopressin-sensitive mechanism for volume regulation? *Can. J. Physiol. Pharmacol.* 70, Suppl, S367–S373.
47. Bemana, I., and Nagao, S. (1999). Treatment of brain edema with a nonpeptide arginine vasopressin V1 receptor antagonist OPC-21268 in rats. *Neurosurgery* 44, 148–155.

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