JOURNAL OF NEUROTRAUMA 37:665–674 (February 15, 2020)

DOI: 10.1089/neu.2019.6653

Vasopressin V_{1a} Receptors Regulate Cerebral Aquaporin 1 after Traumatic Brain Injury

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Abstract

Brain edema formation contributes to secondary brain damage and unfavorable outcome after traumatic brain injury (TBI). Aquaporins (AQP), highly selective water channels, are involved in the formation of post-trauma brain edema; however, their regulation is largely unknown. Because vasopressin receptors are involved in AQP-mediated water transport in the kidney and inhibition of V_{1a} receptors reduces post-trauma brain edema formation, we hypothesize that cerebral AQPs may be regulated by V_{1a} receptors. Cerebral *Aqp1* and *Aqp4* messenger ribonucleic acid (mRNA) and AQP1 and AQP4 protein levels were quantified in wild-type and V_{1a} receptor knockout ($V_{1a}^{-/-}$) mice before and 15 min, 1, 3, 6, 12, or 24 h after experimental TBI by controlled cortical impact. In non-traumatized mice, we found AQP1 and AQP4 expression in cortical neurons and astrocytes, respectively. Experimental TBI had no effect on *Aqp4* mRNA or AQP4 protein expression, but increased *Aqp1* mRNA ($p < 0.05$) and AQP1 protein expression ($p < 0.05$) in both hemispheres. The *Aqp1* mRNA and AQP1 protein regulation was blunted in V_{1a} receptor knockout mice. The V_{1a} receptors regulate cerebral AQP1 expression after experimental TBI, thereby unraveling the molecular mechanism by which these receptors may mediate brain edema formation after TBI.

Keywords: arginine vasopressin; brain edema; cerebral aquaporin; traumatic brain injury; vasopressin V_{1a} receptor knockout mouse

Introduction

Traumatic brain injury (TBI) leads to the formation of vasogenic and cytotoxic brain edema. Brain edema in turn increases intracranial pressure, which negatively impacts clinical outcome.[1–5] Approximately 50% of post-trauma brain edema develops secondarily to the initial mechanical brain damage and is thus amenable to treatment.[6–8] The molecular mechanisms that underlie TBI-induced brain edema, however, are still poorly understood, and successful translational research of causal therapeutic interventions is still missing.

Arginine vasopressin (AVP), also known as anti-diuretic hormone, is essential for both systemic water and blood pressure regulation via its peripheral vasopressin V_2 and V_{1a} receptors, respectively. In the kidney, AVP maintains systemic water homeostasis by regulating the expression of aquaporin 2 (AQP2) thru its V_2 receptors.[9] Moreover, AVP seems to be involved in various cerebral disorders—e.g. elevated AVP plasma levels are positively correlated with the severity of ischemic stroke and TBI[10–12] and pharmacological inhibition or genetic deletion of V_{1a} receptors reduces post-trauma brain edema and improves functional outcome after experimental TBI.[13–16] The molecular link between vasopressin receptors and brain edema formation is, however, missing so far.

The AQPs are highly selective water channels that mediate the bidirectional, energy-independent movement of water molecules across lipophilic cell membranes.[17] The AQP family consists of three subgroups: (1) the AQPs (AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, and AQP8), which are referred to as ''pure'' water channels; (2) the aquaglyceroporins (AQP3, AQP7, AQP9, and AQP10), which contribute to the diffusion of glycerol, urea, and certain monocarboxylates; and (3) the so-called super-AQPs (AQP11 and AQP12), which are present in the cytoplasm.

In the rodent brain, AQP1, AQP4, and AQP9 are relevant in brain disorders.[18–20] The AQP1 and AQP4 are highly water-permeable and most likely relevant for post-trauma cytotoxic brain edema,[17] but the underlying pathophysiology and their regulation is still understood insufficiently. Based on our previously published results demonstrating a link between AVP receptors and brain

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edema formation,[14] and the fact that AQP1 and AQP4 directly mediate brain swelling, we hypothesize that cerebral AQPs might be regulated by AVP. To test this hypothesis, we investigated the regulation of AQP1 and AQP4 after TBI in wild-type (WT) and V_{1a} receptor knockout mice ($V_{1a}^{-/-}$).

Methods

Animals

Homozygous male $V_{1a}^{-/-}$ mice, their WT littermates,[21] and WT C57BL/6 mice (Charles River, Sulzfeld, Germany) with a body weight of 18–25 g were used in this study. The animals were housed at 22°C with 60% relative humidity and had free access to food and water. All animal experiments were performed in accordance with the Animal Research Reporting of In Vivo Experiments criteria and were approved by the ethics board of the Government of Upper Bavaria (protocol number 55.2-1-54-2531-117-05).

Anesthesia and TBI induction

Anesthesia was induced with 4% isoflurane in a chamber and maintained by administration of 2% isoflurane in 30% O_2/68% N_2O delivered via face mask. Core body temperature was maintained at 37°C using a feedback-controlled heating pad connected to a rectal probe. To induce trauma, a craniotomy was prepared over the right parietal cortex as described previously.[22] Controlled cortical impact (CCI) was delivered perpendicular to the surface of the brain using a CCI applicator that was custom-made for use in mice (Mouse Katjuscha 2000, L. Kopacz, University of Mainz, Germany) using the following parameters: 8 m/sec velocity, 3 mm diameter, 1 mm brain displacement, and 150 msec duration. After CCI, the skull was closed by affixing the removed bone flap using tissue glue (Vetbond, 3M, St. Paul, MN). The animals recovered from anesthesia in an incubator heated at 33°C.

Mouse brain preparation

The mice were deeply anesthetized and sacrificed using cervical dislocation. The sagittal suture was opened, and the brain was dissected from the spinal cord and skull base. For RNA extraction, the brains were placed on a cooled RNase-free brain matrix. Two coronal sections were prepared 2 mm rostral and 2 mm caudal relative to the site of trauma. The sections were divided into quadrants, and the traumatized upper-right and non-traumatized lower-left quadrants were used for analysis. For protein preparation, each quadrant was snap-frozen in liquid nitrogen.

For immunohistochemistry, animals were deeply anesthetized with an intraperitoneal injection of 0.4 mL chloral hydrate (3.6%) and transcardially perfused with a solution containing 4% paraformaldehyde (PFA). The brains were removed and post-fixed for 24 h in 4% PFA at 4°C, rinsed in water for 1 h, and stored in 70% ethanol. Thereafter, brains were embedded in paraffin and sectioned according to the established protocol (Supplementary Table 1).

Primer

Primers were designed using the National Center for Biotechnology Information and Ensemble databases and were synthesized by Eurofins MWG Operon (Ebersberg, Germany) (Supplementary Tables 2–4). The specificity of the AQP1 and AQP4 primers was proofed in WT mouse liver, pancreas, kidney, and brain tissues by qualitative polymerase chain reaction (PCR).

Quantitative real-time PCR

The RNA was isolated using the RNeasy Lipid Tissue Mini-Kit (Qiagen, Hilden, Germany). Purity of RNA was defined as a 260 nm/230 nm ratio ≥2 and a 260 nm/280 nm ratio of 1.8–2.[23] Total RNA (1 μg) was used to synthesize cDNA by reverse transcription (QuantiTect, Qiagen). The optimal annealing temperature for quantitative real-time PCR (qRT-PCR) was determined using melting curve analysis (LightCycler 1.5, Roche Heidelberg, Germany); the annealing temperature for amplifying *Aqp1* and *Aqp4* was 57°C and 61°C, respectively. As an internal control, *β-actin* was used because its expression is not altered by CCI.[24] To minimize sample variability, each sample was adjusted to *β-actin* using the standard curve method (Supplementary Table S5 and Formula 1).[25]

Western blotting analysis

Protein samples were prepared by sonicating frozen cortices for 30 sec in a lysis buffer containing a protease inhibitor cocktail.[26] To detect AQP1 and AQP4, 10 μg and 1 μg protein, respectively, were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis; thereafter, proteins were transferred to a polyvinylidene fluoride membrane (PerkinElmer, Baesweiler, Germany). The membranes were incubated in polyclonal antibodies against AQP1 (Chemicon, Ventura, CA; 1:1000) or AQP4 (Chemicon; 1:3000) together with a monoclonal antibody against β-actin (Sigma-Aldrich, Buchs, Switzerland; 1:2500) in Odyssey blocking buffer (LI-COR Biosciences, Bad Homburg, Germany) overnight at 4°C. After washing, the membranes were incubated with anti-rabbit Alexa-Fluor-680 (Molecular Probes, Eugene, OR; 1:10 000) and anti-mouse IR-Dye-800 (Roche) for 2 h at room temperature; fluorescence intensity was quantified using an Odyssey infrared scanner (LI-COR).[26]

Immunohistochemistry

Paraffin-embedded mouse brain sections were processed as described previously.[26] The sections were deparaffinized, and antigen retrieval was performed by boiling the sections for 15 min in 0.1 M citrate buffer (sodium citrate tribasic dihydrate, Sigma-Aldrich Co., St. Louis, MO). After blocking with 0.3% bovine serum albumin in phosphate-buffered saline (PBS) and 0.1% Triton X-100 for 1.5 h, the slides were rinsed three times in PBS for 10 min and incubated with the following primary antibodies overnight at 4°C: mouse anti-NeuN (1:500), mouse anti-GFAP (1:400), rabbit anti-AQP1 (1:100), and rabbit anti-AQP4 (1:200) (Chemicon International, Temecula, CA or EMD Millipore, Billerica, MA).

After washing, AQP1-labeled sections were incubated with a goat anti-rabbit secondary antibody coupled with Alexa-Fluor-680 (1:1 000; Molecular Probes), and AQP4-labeled sections were incubated with a goat anti-rabbit secondary antibody coupled with Alexa-Fluor-800 (1:1 000; Roche) for 2 h at room temperature. The slides were air-dried, and the fluorescence signal was measured in the ipsilateral and contralateral cortices using an Odyssey infrared scanner at 21 μm/pixel resolution (LI-COR Biosciences).

For immunohistochemistry, sections from WT and $V_{1a}^{-/-}$ mice were incubated with secondary anti-rabbit antibodies coupled with Alexa-Fluor-594 or Alexa-Fluor-468 antibodies (Molecular Probes, 1:500). The slides were mounted using VECTASHIELD with 4',6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA), and fluorescence was visualized using an epifluorescence microscope (BX41, Olympus, Center Valley, PA). Specific labeling was confirmed by negative staining when the respective primary antibody was omitted.

Experimental design

Each animal was assigned randomly to either no surgery (naïve) or surgery with CCI. After CCI, tissue was collected at specific time

points ranging from 15 min to 24 h. Surgery was performed by a researcher who was blind with respect to the genotype of the animal. Time points of tissue collection were assigned randomly immediately after CCI. To quantify the levels of *Aqp1* and *Aqp4* messenger ribonucleic acid (mRNA), qRT-PCR was performed on brain tissue obtained from naïve WT and $V_{1a}^{-/-}$ mice and from WT and $V_{1a}^{-/-}$ mice 6 (only WT) and 24 h after CCI ($n = 10$–11 mice each). The AQP1 and AQP4 protein levels were measured using Western blot analysis of brain tissue obtained from naïve WT mice and from CCI-induced mice 6, 12, and 24 h after CCI ($n = 5$ each).

Immunohistochemistry was performed on brain sections obtained from naïve WT and $V_{1a}^{-/-}$ mice as well as from WT and $V_{1a}^{-/-}$ mice 15 min (only WT), 1, 3, 12, and 24 h after CCI ($n = 5$ each). All analyses were performed by an investigator blinded to treatment and/or the genotype of investigated animals.

Statistical analysis

Groups were compared using analysis of variance (ANOVA) on ranks followed by *post hoc* testing for the qRT-PCR and immunohistochemistry data using SigmaStat 2.0 (Jandel Scientific, Erkrath, Germany). Western blot data were analyzed using ANOVA on ranks followed by the Dunnett multiple comparison test (GraphPad InStat 3.05, GraphPad Software, San Diego, CA). Differences were significant if $p < 0.05$.

Results

AQP1 and AQP4 mRNA and protein levels

The specificity of AQP1 and AQP4 primers was confirmed in various mouse tissues by the expected PCR products of 222 and 144 base pairs, respectively (Fig. 1A and Supplementary Table S4). The *Aqp1* mRNA was detected in liver, pancreas, kidney, and brain tissue, whereas *Aqp4* mRNA was detected in liver, kidney, and brain tissue, but not in the pancreas—an expression pattern consistent with previous reports.[27] The AQP1 protein and two AQP4 isoforms (M23 and M1) were detected in the mouse brain at the expected molecular weight of approximately 28 kDa (Fig. 1B).

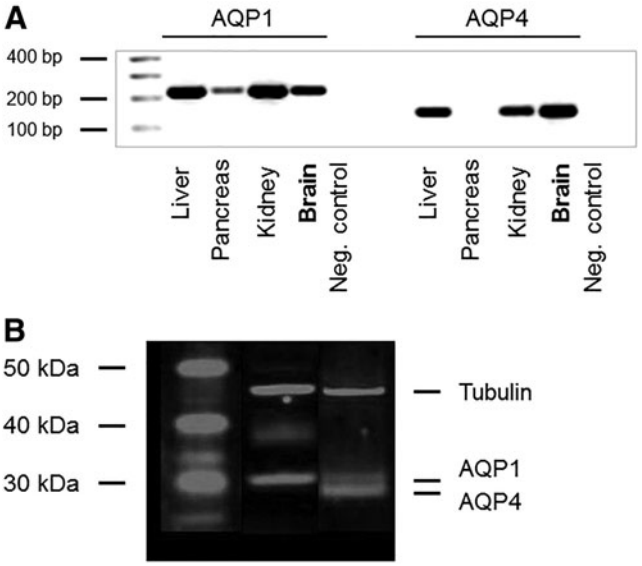

FIG. 1. Aquaporin (AQP) 1- and AQP4-specific primers and antibodies. (**A**) The AQP1 and AQP4 specific primers produced polymerase chain reaction products with the expected sizes (222 and 144 base pairs, respectively) in the indicated mouse tissues. Water was used as negative control. (**B**) Western blot analysis using antibodies against AQP1 (middle) and AQP4 (right) yielded protein bands at the expected molecular weight of approximately 28 kDa. Note the presence of both AQP4 isoforms—namely, M1 and M23. Tubulin was used as a loading control.

AQP4 expression in the healthy mouse brain

The AQP4 was expressed ubiquitously in cortical and subcortical glial cells and in the choroid plexus as well as in the meninges, hippocampal stratum radiatum, corpus callosum, and glia limitans (Fig. 2A). High-resolution images of the healthy mouse brain showed AQP4 on astrocyte end-feet (Fig. 2B), and thus well in line with previous findings. No labeling was detected when the primary antibody was omitted, confirming antibody specificity (data not shown).

AQP1 expression in the healthy mouse brain

The AQP1 immunostaining was positive in the choroid plexus, cortical layers, and hippocampal CA1/CA3 regions in the healthy WT mouse brain (Fig. 3A). Co-staining revealed that AQP1 was

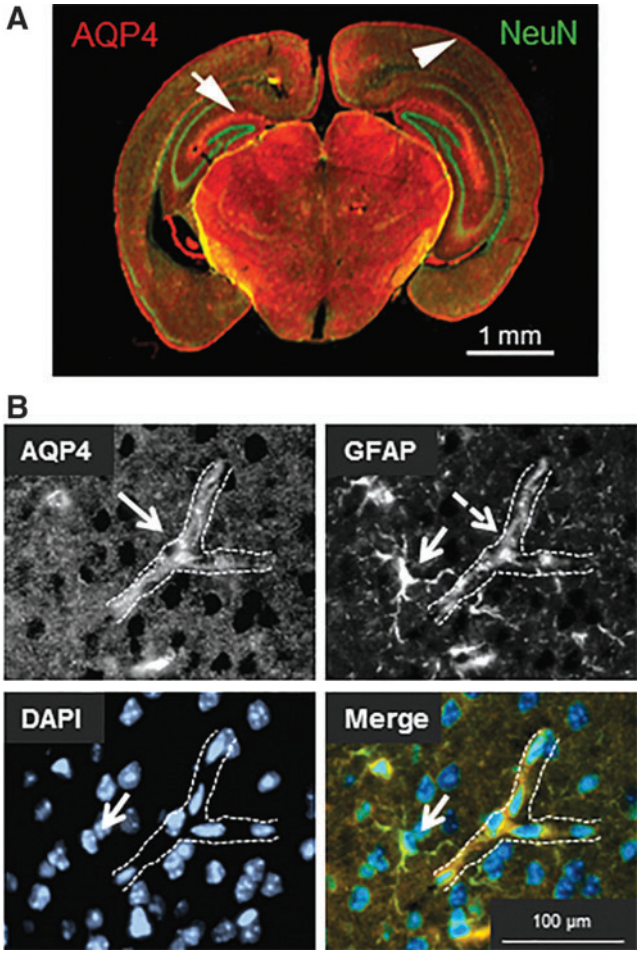

FIG. 2. Aquaporin (AQP) 4 expression in healthy mouse brain. (**A**) The AQP4 (red) was detected ubiquitously in cortical and subcortical glial cells. (**B**) High-resolution images confirm AQP4 expression in astrocytes in the healthy mouse cerebral cortex. The scale bars in A and B represent 1 mm and 100 μm, respectively.

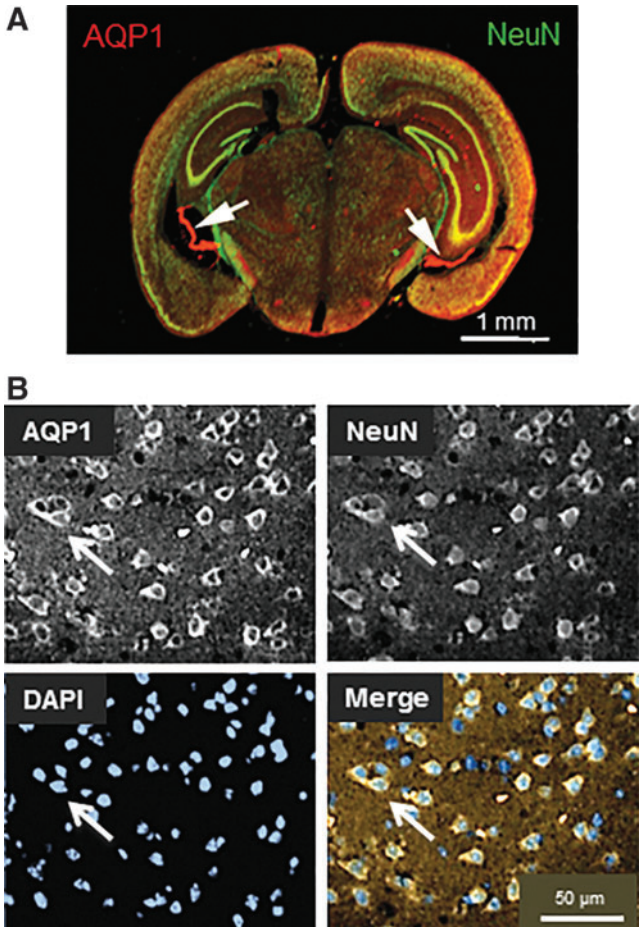

FIG. 3. Aquaporin (AQP) 1 expression in healthy mouse brain. (**A**) The AQP1 (red) was detected in the epithelium of the choroid plexus and co-localized with the neuronal marker NeuN (green) throughout the cortex. (**B**) High-resolution images and co-staining (AQP1, DAPI, NeuN) confirm AQP1 expression in cortical neurons of naïve wild-type mice. Thus, AQP1 expression was found for the first time in murine cortical neurons (arrows). The scale bars in A and B represent 1 mm and 50 μm, respectively.

co-expressed with the neuronal marker NeuN, indicating AQP1 expression in cortical neurons. High-resolution images of healthy mouse brains confirmed AQP1 in cortical neurons as indicated by the merged staining (AQP1, DAPI, NeuN) (Fig. 3B).

Aqp4 mRNA and protein levels after CCI in WT mouse brains

The Aqp4 mRNA was not regulated 6 and 24 h after CCI in WT mouse brains ($n=10$) (Fig. 4A). On the protein level, AQP4 was transiently increased in the traumatized hemisphere 12 h after CCI. No changes were observed in the hemisphere contralateral to the trauma ($n=5$) (Fig. 4B; Supplementary Figs. S1 and S2). β-actin was used as a loading control.

Aqp1 mRNA and protein levels in WT mouse brains after CCI

The *Aqp1* mRNA levels were increased fourfold 24 h after CCI in WT mouse brains ($n=10$) (Fig. 5A). The AQP1 protein was measured in the ipsilateral and contralateral hemispheres of naïve as well as 15 min and 24 h after CCI in WT mice (Fig. 5B, 5C). The number of AQP1-positive neurons was increased in both hemispheres 15 min after CCI ($p<0.05$). At 24 h after CCI, the number of AQP1-positive neurons had returned to baseline in the ipsilateral hemisphere but remained increased in the contralateral hemisphere ($p<0.05$) ($n=5$ per group).

V_{1a} receptors regulate Aqp1 mRNA and protein levels after CCI

The *Aqp1* mRNA did not differ between naïve WT ($n=10$) and $V_{1a}^{-/-}$ mouse brains ($n=11$). The *Aqp1* mRNA levels increased fourfold in WT mouse brains 24 h after CCI ($p=0.031$) but remained unchanged in $V_{1a}^{-/-}$ mouse brains (Fig. 6A). In the $V_{1a}^{-/-}$ mouse brain, AQP1 was suppressed in cortical neurons 1 h after CCI compared with the WT mouse brain (Fig. 6B). This difference was not because of neuronal cell death in the $V_{1a}^{-/-}$ mouse brain, because expression of the neuronal marker NeuN was not reduced. The AQP1 protein was quantified in the contralateral hemisphere (Fig. 6C). Ipsilateral AQP1 immunoreactivity in the vicinity of the lesion was not reliable because of bleeding, and AQP1 expression in erythrocytes. AQP1 was increased 1 and 24 h after CCI in the contralateral hemisphere of WT mice, but not in $V_{1a}^{-/-}$ mice ($n=5$ per group) (Supplementary Figure S3).

Discussion

We investigated whether arginine vasopressin V_{1a} receptors regulate cerebral AQPs after experimental TBI induced by CCI. Therefore, we measured *Aqp1* and *Aqp4* mRNA and their protein levels in the brains of WT and $V_{1a}^{-/-}$ mice at baseline as well as after experimental TBI. Our findings provide the first evidence that arginine vasopressin V_{1a} receptors regulate the short- and long-term expression of cerebral AQP1—i.e., specifically, the upregulation of *Aqp1* mRNA at 24 h and AQP1 protein at 15 min, 1 h, and 24 h post-injury.

For the first time, AQP1 was identified in cortical neurons in WT and $V_{1a}^{-/-}$ mouse brains, suggesting that V_{1a} receptors may contribute to neuronal swelling via AQP1 on neurons. Our results do not provide any evidence that V_{1a} receptors regulate AQP4. Thus, V_{1a} receptors and AQP1 are presumably auspicious target proteins for the post-trauma anti-edematous treatment without interfering with AQP4 function.

Animal model

Controlled cortical impact is a well-characterized contusion model and well-suited for investigating the formation of post-trauma brain edema, because it recapitulates the clinical manifestations, including combined vasogenic and cytotoxic brain edema.[5,28,29]

In the V_{1a} receptor knockout mouse, the V_{1a} receptor gene was inactivated by deleted portions of the first exon and intron. In the literature, $V_{1a}^{-/-}$ mice have been described with 7% lower blood pressure, 9% less blood volume, and a reduced adrenocorticotropic response[21]—hence, systemic parameters that might influence brain edema formation.

Thus, previously we monitored physiological parameters including mean arterial blood pressure, intracranial pressure, and cerebral blood flow (pre- and post-injury) without measurable differences between WT and $V_{1a}^{-/-}$ mice. Moreover, brain water content, weight, and function did not differ at baseline, while V_{1a} receptor deficiency resulted in less brain edema, less secondary necrosis, less weight loss, and full recovery regarding weight and

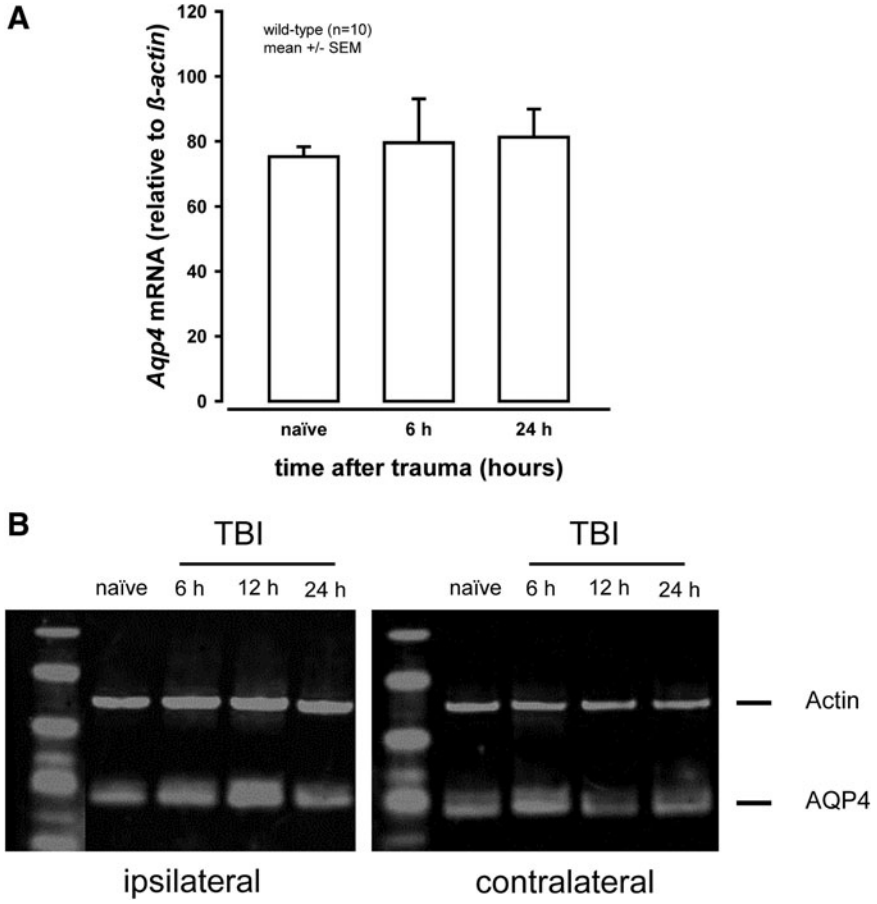

FIG. 4. Aquaporin (*Aqp*) *4* messenger ribonucleic acid (mRNA) and AQP4 protein levels after controlled cortical impact (CCI) in wild-type (WT) mice. (**A**) The *Aqp4* mRNA levels were not altered in WT mice after CCI. (**B**) The Western blot analysis revealed increased AQP4 protein levels in the trauma-induced lesion 12 h after CCI in WT mouse brains, while remained unchanged in the contralateral hemisphere. β-actin was used as a loading control. SEM, standard error of the mean; TBI, traumatic brain injury.

functional outcome compared with WT after experimental TBI, emphasizing the V_{1a} receptor-mediated neuroprotection (Supplementary Tables S6 and S7).[14,21,30] As an essential requirement to prove our hypothesis, we found for the first time that basal *Aqp1* and *Aqp4* mRNA as well as AQP1 and AQP4 protein levels do not differ between WT and $V_{1a}^{-/-}$ mice.

Taking all findings together, the lack of V_{1a} receptors most probably does not interfere with potential systemic and cerebral factors that might change post-trauma brain edema formation, and thus, $V_{1a}^{-/-}$ mice are a suitable model to investigate the V_{1a} receptor-dependent regulation of cerebral AQPs after TBI.

AVP in brain pathologies

The AVP plays a role in the pathophysiology of TBI, ischemic and hemorrhagic stroke via V_{1a} but not V_2 receptors.[10,13,30–36] Focusing translational TBI research, increased AVP plasma levels correlated positive with TBI severity in humans,[11,12] suggesting at least a partial contribution to the post-trauma pathophysiology. Moreover, patients having neurological diseases with increased intracranial pressure (ICP) had elevated AVP levels in their cerebrospinal fluid (CSF), indicating a relevance in the pathophysiology of TBI.[37] Experimental studies in rats after TBI further underline the crucial role of AVP secretion from the neurohypophysis, from microglia, from central macrophages, and from the cerebrovascular endothelium in the post-traumatic pathophysiology with the maximum of AVP synthesis 1 day after the brain injury, well in line with the maximum of brain edema formation.[14,38]

Vasopressin V_{1a} receptors: Distribution and their role in brain edema formation

The AVP V_{1a} receptors are located peripherally on smooth muscle cells of small arteries, arterioles, and post-capillary venules, thereby mediating vasoconstriction to regulate systemic blood pressure and to maintain cardiovascular homeostasis. The raising question is whether V_{1a} receptors also regulate cerebral vessels, and thus have impact for post-trauma brain edema formation—or the other way around, whether the lack of V_{1a} receptors might result in an increased venous drainage reducing brain edema formation, which is AQP-independent.

To our knowledge, the V_{1a} receptor expression on cerebral vasculature in the human and mouse brain is still not clarified fully. In the rat brain, however, V_{1a} receptors are distributed widely on neurons of the frontoparietal cortex of all brain layers, on superficial astrocytes, vessels, and two types of vasopressin-responsive cells have been described—namely, neurons and perivascular cells.[39–42]

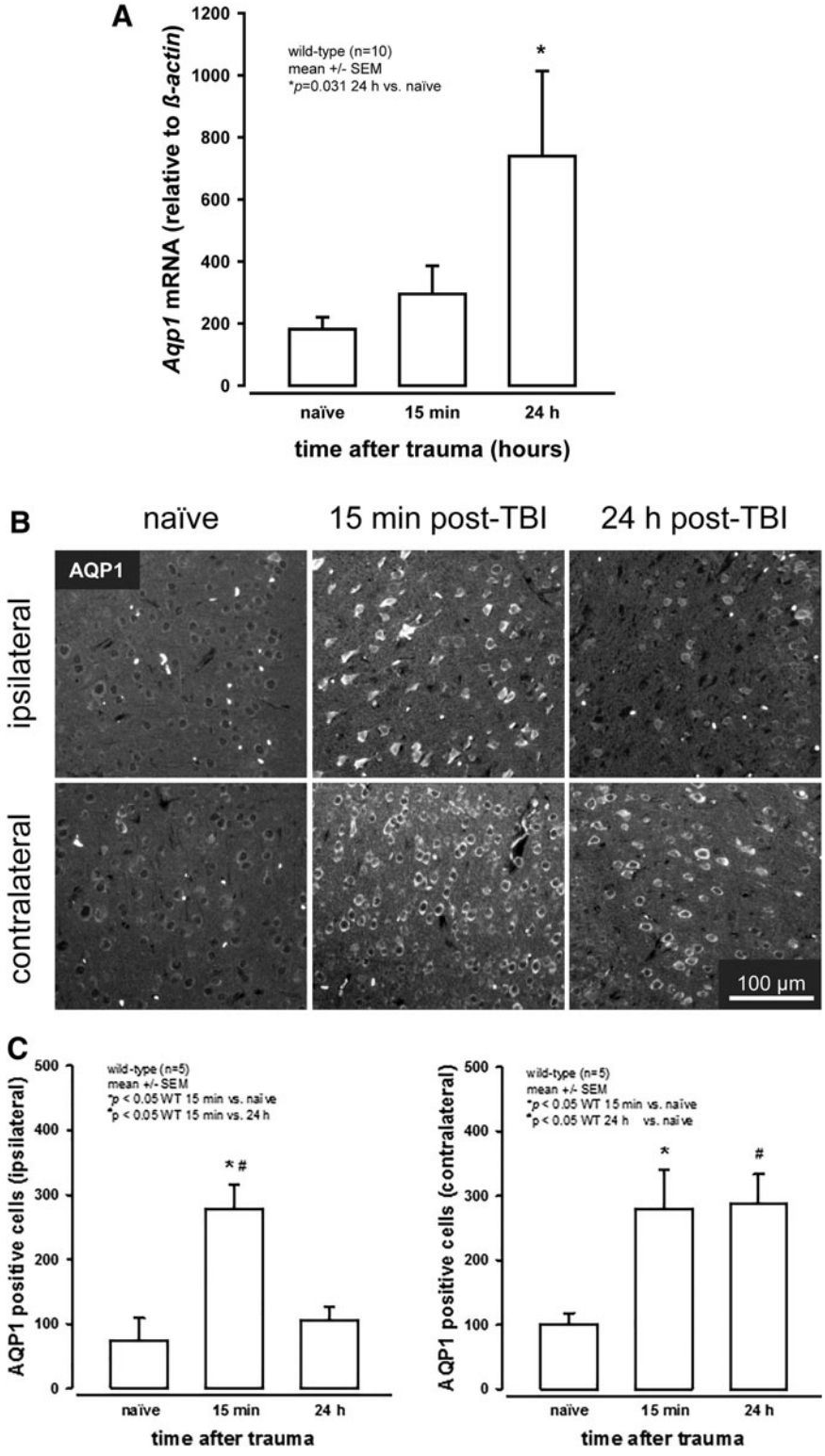

FIG. 5. Aquaporin (*Aqp*) *1* messenger ribonucleic acid (mRNA) and AQP1 expression after controlled cortical impact (CCI) in wild-type (WT) mice. **(A)** *Aqp1* mRNA levels were increased fourfold 24 h after CCI in WT mice. (**B**) The AQP1 protein was measured in the ipsilateral (upper row) and contralateral (lower row) hemispheres of naïve (left) as well as 15 min (middle) and 24 h (right) after CCI in WT mice. (**C**) The number of AQP1-positive neurons was increased in both hemispheres 15 min after CCI. At 24 h after CCI, the number of AQP1-positive neurons had returned to baseline in the ipsilateral hemisphere (left) but remained increased in the contralateral hemisphere (right). SEM, standard error of the mean; TBI, traumatic brain injury.

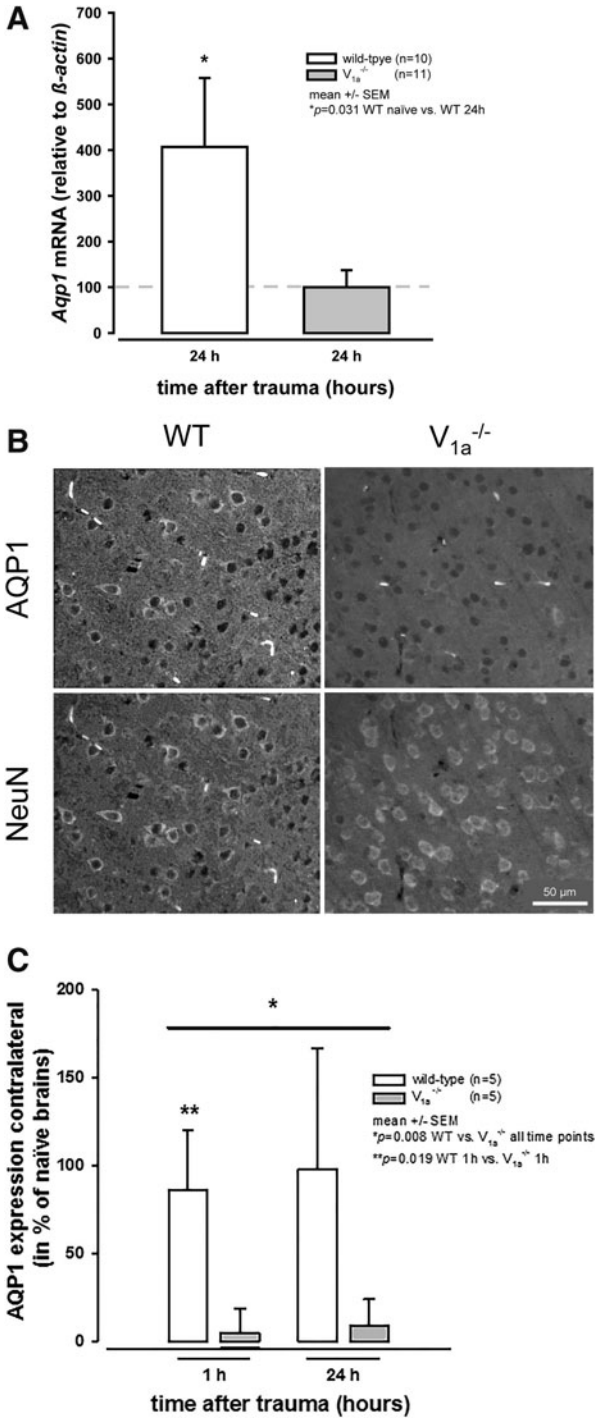

FIG. 6. Vasopressin V_{1a} receptor regulate aquaporin (*Aqp*) *1* messenger ribonucleic acid (mRNA) and AQP1 protein after controlled cortical impact (CCI). (**A**) The *Aqp1* mRNA was increased fourfold 24 h after CCI in wild-type (WT) ($n=10$) but did not change in $V_{1a}^{-/-}$ mouse brains ($n=11$). The *Aqp1* mRNA did not differ between naïve WT and $V_{1a}^{-/-}$ mice. (**B**) The $V_{1a}^{-/-}$ mice (right) had suppressed AQP1 on cortical neurons 1 h after CCI compared with WT (left) mice. The AQP1 did not differ between naïve WT and $V_{1a}^{-/-}$ mice. (**C**) AQP1 was increased 1 and 24 h after CCI in the contralateral hemisphere of WT mice, but not in $V_{1a}^{-/-}$ mice ($n=5$ per group). The AQP1 was only quantified reliably in the contralateral hemisphere, because AQP1 immunoreactivity was perilesional increased from bleeding and its expression on erythrocytes, thus a major confounder. SEM, standard error of the mean.

After experimental TBI in rats, augmented expression of V_{1a} receptors was found on the cerebrovascular endothelium of microvessels and large-diameter blood vessels in the frontoparietal, ipsilateral cortex between 2 and 4 days after brain injury.[39] Whether of relevance in the mouse brain, it is not within the time frame of the presented experiments. Thus, simply vascular effects can most probably be excluded in our $V_{1a}^{-/-}$ mice, but *in vivo* experiments might help to clarify vascular effects and venous drainage after experimental TBI in mice.

The early upregulation of V_{1a} receptors on axonal processes with enlarged varicosities 1–2 h post-injury as well as on astrocytes in the injured parenchyma starting at 8 h and peaking 4–6 days after TBI in rats using the Freeney weight-drop–model,[39,43] which results in a predominantly focal injury, thus not fully comparable to our contusion model,[28] is of major interest because these varicosities might store AQP1 to be installed in the neuronal cell membrane. Interestingly, V_{1a} receptors were not only upregulated, but also redistributed during the evolving brain edema from the astrocytic cell bodies to their end-feet—thus, the location of AQP4.[39] Whether these findings are transferable to the mouse brain needs to be clarified with special focus on the enlarged axonal varicosities.

Rationale for studying cerebral AQP1 and AQP4

AQP1, AQP4, and AQP9 are relevant in rodent cerebrovascular brain pathologies.[18,20,44,45] Here, we investigated the highly water-permeable AQP1 and AQP4 channels[17] as playing a role in cytotoxic brain edema whereas AQP9 is low water-permeable and relevant for the energy metabolism.[44,46,47]

AQP1 in cortical neurons contributes to formation of cytotoxic brain edema

The AQP1 is expressed in the epithelium of the choroid plexus playing a role in the production and circulation of CSF as well as intracranial pressure regulation.[48,49] Here, we provide the first evidence that AQP1 is expressed in cortical neurons. Although the function of AQP1 in neurons currently is unknown, AQP1 most probably contributes to post-trauma neuronal swelling, and thus the cytotoxic brain edema formation of the ipsilateral hemisphere after experimental TBI by short- and long-term regulation via V_{1a} receptors as proven here.

The demonstrated link between AQP1 and AVP may lie in the presence of four phosphorylation sites on AQP1, which serve as targets for short-term regulation via protein kinase A (PKA) and protein kinase C (PKC) as reviewed previously.[20,50,51] The PKC is activated by V_{1a} receptors and in *Xenopus* oocytes water and ion permeability of AQP1 are increased via the PKC-dependent phosphorylation of threonine-157 and threonine-239.[52]

Moreover, the phosphorylation of tyrosine-253 in the C-terminal domain seems to serve as a master switch, controlling the ion permeability of AQP1 by cyclic guanosine monophosphate.[53,54] This switch is activated indirectly by AVP via V_{1a} receptors through increased intracellular calcium concentration.[55] Thus, the phosphorylation-induced rapid, reversible installation of AQP1 into cell membranes is most probably AVP and V_{1a} receptor-dependent, well in line with the presented results of V_{1a} receptor-dependent AQP1 upregulation within 15 min after experimental TBI.

The long-term upregulation of AQP1 by V_{1a} receptors is mediated by several pathways, including c-Jun N-terminal kinase (JNK). Activation of JNK by AVP signaling leads to blood–brain barrier damage and vasogenic brain edema.[56] Our findings that V_{1a} receptors regulate both the short-term and long-term expression of

AQP1 suggest V_{1a} receptors and AQP1 as a novel therapeutic target for treating post-trauma cytotoxic brain edema.

Role of AQP4 for edema formation

The AQP4 has broad beneficial and detrimental effects in brain edema formation.[57–61] Here we demonstrated that AQP4 is most probably not regulated by V_{1a} receptors after experimental TBI and is ubiquitously expressed in cortical and subcortical astrocytes, primarily in a polarized pattern on perivascular end-feet. This polarized expression changes when astrocytes are activated after cerebral ischemia or brain trauma, including AQP4 redistribution from the astrocyte end-feet to the cell body, but its functional relevance for the formation and resolution of brain edema remains unclear.[62–65]

Interestingly, V_{1a} receptors undergo an antidromic redistribution from the astrocytic cell body to the perivascular end-feet after experimental TBI in rats,[39] conceivably supporting that AQP4 is not regulated by V_{1a} receptors. Recent works, however, showed that AQP4 is associated and build a complex with the sulfonylurea receptor 1-transient receptor potential melastatin 4 (Sur1-Trpm4) monovalent cation channel in astrocytes.[66] This association suggests that AQP4 plays a rather passive role in the edema process and inhibiting Sur1-Trpm4 by glyburide might prevent edema formation.[67–69] Certainly, AQP4 is most important in brain edema formation and part of a molecular complex of ionic channels giving the driving force for water movement.[66,70,71]

Taken together, identifying the heterogenous functions of AQP4 after experimental TBI remains difficult. Here we demonstrated that deletion of V_{1a} receptors does not influence *Aqp4* mRNA and AQP4 protein levels, thus does not hamper beneficial AQP4-related clearance of solutes, water, and macromolecules that might cause exacerbation of brain edema and post-trauma neurodegeneration.

V_{1a} receptor knockout mice and brain edema formation

Our previously published experiments showed decreased brain edema formation in the traumatized hemisphere of V_{1a} receptor knockout mice compared with WT mice. Further, V_{1a} receptor knockout mice showed decreased secondary brain injury 24 h post-CCI, reduced weight loss, and a better functional outcome 7 days post-CCI in comparison with WT controls.[14] Together with the current results of V_{1a} receptor dependent AQP1 regulation, we suggest V_{1a} receptors and AQP1 as relevant mechanisms for the formation of post-trauma cytotoxic brain edema.

Limitations

Our study has a few limitations that need closer consideration. First, we did not measure plasma sodium and potassium levels in the present study; however, these measurements were performed by us in previous investigations and we could demonstrate that plasma levels of these electrolytes were not affected by inhibition of V_{1a} receptors. Thus, we could exclude systemic and renal effects of V_{1a} receptor inhibition.[16] Second, we did not investigate post-traumatic redistribution of AQP4 in astrocytes by immunohistochemistry because AQP4 was not regulated by V_{1a} receptors and the main scope of our investigation was regulation of AQP4 and not its redistribution.

Finally, we did not perform *in vivo* imaging of cerebral vessels to identify in which vascular compartment V_{1a} receptor inhibition reduces brain edema formation. Such experiments, which will need to be performed in the future, are, however, extremely complex and time consuming and were therefore beyond the scope of the current investigation.

Conclusions

We report for the first time that arginine vasopressin V_{1a} receptors, which are involved in the formation of post-trauma brain edema, regulate the expression of cerebral AQP1. Thus, V_{1a} receptors together with AQP1 may represent novel therapeutic targets for the prevention and management of post-trauma brain edema.

Acknowledgments

We thank Dr. Gozoh Tsujimoto (Kyoto University, Japan) for providing the V_{1a} receptor knock-out mice; Eckart Thein and staff for breeding our knock-out animals; Heike Beck and Manuela Schneider for discussions regarding qRT-PCR; and Uta Mamrak and Alke Schropp for providing excellent technical assistance. The presented results are part of doctoral thesis of KR.

Funding Information

JB was supported by the National Institutes of Health (NIH) grant R01HD061946 and by the Swiss National Science Foundation grant 3100A0-122166. NP was supported by ERA-NET NEURON/BMBF CnsAflame (Funding # 01EW1502B).

Author Disclosure Statement

No competing financial interests exist.

Supplementary Material

Supplementary Figure S1
Supplementary Figure S2
Supplementary Figure S3
Supplementary Table S1
Supplementary Table S2
Supplementary Table S3
Supplementary Table S4
Supplementary Table S5
Supplementary Table S6
Supplementary Table S7
Formula 1

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