Real-Time Monitoring of Changes in Brain Extracellular Sodium and Potassium Concentrations and Intracranial Pressure after Selective Vasopressin-1a Receptor Inhibition following Focal Traumatic Brain Injury in Rats

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Abstract

Brain swelling and increased intracranial pressure (ICP) following traumatic brain injury (TBI) contribute to poor outcome. Vasopressin-1a receptors (V1aR) and aquaporin-4 (AQP4) regulate water transport and brain edema formation, perhaps in part by modulating cation fluxes. After focal TBI, V1aR inhibitors diminish V1aR and AQP4, reduce astrocytic swelling and brain edema. We determined whether V1aR inhibition with SR49059 after lateral controlled-cortical-impact (CCI) injury affects extracellular Na^+ and K^+ concentrations ($[\text{Na}^+]_e$; $[\text{K}^+]_e$). Ion-selective Na^+ and K^+ electrodes (ISE) and an ICP probe were implanted in rat parietal cortex, and $[\text{Na}^+]_e$, $[\text{K}^+]_e$, and physiological parameters were monitored for 5 h post-CCI. Sham-vehicle-ISE, CCI-vehicle-ISE and CCI-SR49059-ISE groups were studied, and SR49059 was administered 5 min to 5 h post-injury. We found a significant injury-induced decrease in $[\text{Na}^+]_e$ to 80.1 ± 15 and 87.9 ± 7.9 mM and increase in $[\text{K}^+]_e$ to 20.9 ± 3.8 and 13.4 ± 3.4 mM at 5 min post-CCI in CCI-vehicle-ISE and CCI-SR49059-ISE groups, respectively ($p < 0.001$ vs. baseline; *ns* between groups). Importantly, $[\text{Na}^+]_e$ in CCI-SR49059-ISE was reduced 5–20 min post-injury and increased to baseline at 25 min, whereas recovery in CCI-vehicle-ISE required more than 1 hr, suggesting SR49059 accelerated $[\text{Na}^+]_e$ recovery. In contrast, $[\text{K}^+]_e$ recovery took 45 min in both groups. Further, ICP was lower in the CCI-SR49059-ISE group. Thus, selective V1aR inhibition allowed faster $[\text{Na}^+]_e$ recovery and reduced ICP. By augmenting the $[\text{Na}^+]_e$ recovery rate, SR49059 may reduce trauma-induced ionic imbalance, blunting cellular water influx and edema after TBI. These findings suggest SR49059 and V1aR inhibitors are potential tools for treating cellular edema post-TBI.

Key words: AQP4; cations; TBI; V1a receptor; vasopressin

Introduction

TRAUMATIC BRAIN INJURY (TBI) IS A LEADING cause of death and disability.¹ Following TBI, increased brain swelling leads to elevation of intracranial pressure (ICP), a frequent cause of death and poor prognosis.^{2–6} Clinically, the degree of brain swelling assessed by computed tomography shortly following injury is highly correlated with outcome.⁷ This suggests that therapies aimed at treating brain swelling must be delivered immediately following injury to retard the development of edematous swelling and prevent death and disability. The contribution of post-traumatic brain edema to brain swelling remains a critical problem without an ef-

fective clinical treatment that addresses the cause of edema. The efficacy of symptomatic treatments such as decompressive craniotomy remain controversial,^{3–6} and therefore, better understanding of the underlying processes may lead to new pharmacological approaches to abate and thus treat brain swelling.⁸

Our previous work supports the hypothesis that post-traumatic brain edema is predominantly cellular. We posit that astrocytic swelling, a characteristic response to injury,⁹ may prove to be a target for drug treatments designed to blunt water influx at the cellular level and thus ameliorate cytotoxic brain edema by reversing or reducing swelling. Astrocytes are key players in maintaining cerebral water homeostasis by balancing ionic gradients and

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respond to injury with varying molecular and morphological changes such as swelling, hypertrophy, or proliferation, often described as astrogliosis.¹⁰

The hormone arginine vasopressin (AVP) was identified as a potential mediator of astrocytic cell swelling and subsequent fulfilling edematous changes following cerebral injury.^{11–17} It is thought that centrally-released AVP orchestrates the development of brain edema by inducing water and ionic movement.^{18,19} Increased AVP levels activate the V1a receptor (V1aR), facilitate water transport across astrocytic cell membranes, and are associated with brain edema formation.

In animal models of TBI, V1aR expression increases, supporting the role of AVP in TBI^{20,21} and subsequent cellular swelling. Further, experimental models of intracerebral hemorrhage, middle cerebral artery occlusion, ischemia, and cold and traumatic brain injury have demonstrated that V1aR inhibition reduces brain edema.^{22–31}

The underlying mechanism of this response has been attributed to either modulation of vasopressin in the brain parenchyma¹⁷ or its interaction with aquaporin-4 (AQP4). AQP4 is the predominant water channel in the brain and has a significant role in the pathophysiology of brain edema.^{32–36} Evidence suggests V1aR regulate the expression and function of AQP4,^{17,22,37} although the mechanism is still elusive.^{17,22,29–31}

Recently, we explored the hypothesis that selective V1aR inhibition by the non-peptide antagonist SR49059^{30,38} can reduce brain edema in animal models of middle cerebral artery occlusion^{22,39} and TBI.³⁰ Overall, we showed that V1aR inhibition decreases brain AQP4 expression, brain edema, and astrocytic cell swelling, and reduces expression of V1aR.^{30,40} Importantly, these studies showed a clear correlation between the time course of the trauma-induced shift in intracerebral Na⁺ concentrations and the increase in percent brain water content.^{22,30,31,39,41} Our findings were consistent with previous reports demonstrating similar shifts between water and Na⁺ content.³¹ These data provide a conceptual framework to explain the pathophysiology of cellular swelling that we demonstrate is intimately linked to trauma induced ionic dysfunction, whereas a decrease in extracellular Na⁺ ([Na⁺]_e) and a concomitant increase in K⁺ ([K⁺]_e) concentrations creates the osmotic gradient that drives water flow into the intracellular compartment, thereby setting the stage for increased brain edema and raised ICP.^{2,42,43}

We tested the hypothesis that administration of a V1aR-selective inhibitor, SR49059, immediately after focal TBI would alter regulation of [Na⁺]_e and [K⁺]_e in the extracellular space of brain, blunt the increase in ICP, and speed post-injury recovery of [Na⁺]_e and [K⁺]_e to their normal values. [Na⁺]_e and [K⁺]_e were measured using ion selective electrodes (ISE), continuously, over a 5 h period, in a rat controlled-cortical-impact (CCI) model of focal TBI.

Materials and Methods

Ion-selective electrodes

Cortical [Na⁺]_e and [K⁺]_e were monitored in real-time using customized ISE (Microelectrodes, Inc., Bedford, NH). Three different electrodes were used: a NAS11-18 glass Na⁺-selective electrode (0.5 mm diam.), a flexible valinomycin-based K⁺-selective electrode (1.0 mm diam.), and a flexible 3 M KCl-filled double junction reference electrode (1.0 mm diam.). The Na⁺- and K⁺-selective ISE have a selectivity of ~1000:1 for Na⁺ over K⁺ and K⁺ over Na⁺, respectively.

Prior to experiments, both ISE were calibrated at 37°C and pH 7.35 in mock-cerebrospinal solutions with mixed Na⁺ and K⁺ concentrations ranging from 101.4 – 54.5 mEq/L and 3 – 56.1 mEq/L, respectively. Solutions were designed with a constant ionic strength and an osmolality of 300 mOsm/L. ISE potentials were recorded after low pass filtering at 5 Hz with a PowerLab 4/30 data acquisition system (ADInstruments Inc., Colorado Springs, CO), and ISE potentials (in mV) were converted to concentrations of Na⁺ and K⁺ (in mEq/L) in real-time based on the calibration curve for each electrode (MATLAB R2011a, MathWorks, Natick, MA). Preliminary experiments demonstrated that calibrations were not significantly influenced by electrode use.

Animal preparation, anesthesia and monitoring

All animals received humane care in compliance with the *Guide for the Care and Use of Laboratory Animals*, 8th edition (National Academy Press, Washington, DC, 2011), and all experimental, surgical and post-operative procedures were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee. Animals were housed in the vivarium with 12 h light/12 h dark cycles and given access to pellet food and water ad libitum.

Adult Sprague-Dawley rats (350–400 g) were prepared following the experimental timeline described in Figure 1. General anesthesia was induced with isoflurane (4.5%) in an induction chamber. After induction, animals were intubated, and anesthesia was maintained with mechanical ventilation with a gas mixture of N₂O (67%), O₂ (33%), and isoflurane (1.5–2.0%). Rectal temperature was continuously maintained at 37.0 ± 0.5°C using a heating pad (Harvard Apparatus, Holliston, MA). A catheter (PE 50, Becton Dickinson and Company, Sparks, MD) was placed in the femoral artery to measure mean arterial blood pressure (mABP) continuously and provide access for arterial blood gas sampling. mABP also was used to manage isoflurane concentration and ventilation rate as needed to maintain mABP under anesthesia. The femoral vein also was cannulated to administer either vehicle, 1.5% dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO) in 0.9% saline, or drug, SR49059, with an infusion pump (sp210w syringe pump, KD Scientific, Holliston, MA). DMSO allows diffusion of drugs through the blood–brain barrier and into the brain.⁴⁴

At 30 and 15 min before injury and 15 min and 5 h post-injury, arterial blood gas parameters (pH, partial pressure of oxygen [pO₂], partial pressure of carbon dioxide [pCO₂] and plasma Na⁺ and K⁺ concentrations) were measured using a blood gas analyzer (ABL800 Flex, Radiometer America Inc., OH). mABP and ICP (1 mm diam. probe; Codman and Shurtleff, Inc., MA) were monitored continuously using the PowerLab 4/30 data acquisition system.

Surgical Preparation

Rats were placed in a stereotactic frame (David Kopf Instruments, Tujunga, CA), a midline scalp incision was made, and the skin and periosteum were retracted. Using a surgical microscope, a 10-mm diameter craniotomy was made midway between bregma and lambda on the right side, with the medial edge of the craniotomy 1 mm lateral to midline. Na⁺ and K⁺ ISE were implanted stereotactically, at the border of the craniotomy, 1.5-mm deep in the right parietal cortex, below the dura mater and, ipsilateral to the designated injury site (Fig. 2). The Na⁺ ISE was positioned at bregma 0.00 mm, lateral 3.00 mm, and the K⁺ ISE at bregma 6.00 mm, lateral 2.00 mm. The reference electrode was placed contralateral at bregma 3.00 mm, lateral +2.00 mm, via a burr hole. The ICP probe was placed at bregma 6.00 mm, lateral 4.00 mm, ipsilateral to the injury, 3 mm into the brain parenchyma to increase

FIG. 1. Experimental timeline. Time of controlled cortical impact (CCI) was designated as 0 min. Sequential steps in the timeline were: 1) Calibration of ion-selective electrode (ISE) to measure extracellular Na⁺ and K⁺ concentrations ([Na⁺]_e; [K⁺]_e); 2) animal prepared for surgery (prep) and recording of mean arterial blood pressure (mABP; continuously) and arterial blood gases (ABG; -30, -15, +15 min and +5 h); 3) ISE, reference electrode, and intracranial pressure (ICP) probe implanted; and ISE allowed to stabilize for >45 min; 4) baseline ISE readings of [Na⁺]_e and [K⁺]_e averaged over 10 min; 5) CCI or sham CCI at 0 min; 6) continuous intravenous infusion of SR49059 or vehicle from +5 min to 5 h-post CCI; and 7) continuous monitoring of brain [Na⁺]_e, [K⁺]_e, ICP and mABP continued until the end of the experiment.

the signal-to-noise ratio. The coordinates for the insertion sites of the ISE and ICP probe were selected as they target the perilesional cortex, adjacent to the core necrotic site, where cellular edema predominates in brain injury. Representative brain slices confirmed the perilesional positioning of the electrode implantation sites

(Fig. 3). To obviate the potential of acute effects of tissue damage due to ISE and ICP probe placement, the electrodes were allowed to stabilize for at least 45 min before data acquisition. Further, following stabilization, baseline data were recorded and averaged for 10 min prior to injury.

FIG. 2. Schematic diagram of stereotactic localization of ion-selective electrode (ISE), reference electrode (Ref), and intracranial pressure (ICP) probe. Brain slices depict placement of: Na⁺ ISE at bregma 0.00 mm, lateral -3.00 mm; reference electrode at bregma -3.00 mm, lateral +2.00 mm; K⁺ ISE at bregma -6.00 mm, lateral -2.00 mm; and ICP probe at bregma -6.00 mm, lateral -4.00 mm. Brain slices modified from Paxinos atlas. ISE and ICP signals were filtered, digitized, and displayed.

FIG. 3. Representative rat brain slices illustrating ion-selective electrode (ISE), reference electrode, and intracranial pressure (ICP) probe insertion sites and controlled cortical impact (CCI) at different bregma levels with histology (Toluidine blue stain tile-scan). (A) Sham animal with no electrodes; bregma -3.00 mm level. R, right side; L, left side. (B) CCI animal without electrodes; bregma -3.00 mm. (C) CCI animal with ISE (*) and ICP probe inserted; bregma 0 mm slice showing Na⁺ ISE site. (D) Same CCI animal, bregma -3.00 mm, showing CCI and reference electrode site on contralateral side. (E) Same CCI animal, bregma -6.00 mm, showing K⁺ ISE and ICP probe sites.

CCI Injury

Focal TBI was produced by an established CCI model described previously.³⁰ A severe CCI injury was induced at time 0 min using a pneumatic impactor (5 mm diam.) mounted at a 20° angle from the vertical plane. A single impact at a velocity of 6 m/sec, deformation depth of 1.5 mm, and dwell time of 0.3 sec was delivered to the right parietal cortex. After injury, the excised skull section, notched to accommodate ISE, was immediately replaced and sealed with bone wax. Sham animals were exposed to identical surgical procedures without CCI. Any animal demonstrating significant intraparenchymal or extradural hemorrhage was excluded from the study.

Experimental design

Rats ($n=5$ /group) were randomly assigned to three groups: a) untreated CCI injury (CCI-vehicle-ISE); b) SR49059-treated CCI injury (CCI-SR49059-ISE); and c) sham injury (sham-vehicle-ISE). SR49059 (2.76 mg/kg, Sanofi Recherche, Montpellier, France) dissolved in 1.5% DMSO, added to 0.9% saline, was administered intravenously for 5 h at 960 μ L/h (total 4.8 mL) beginning 5 min after CCI. The required dose was based on evidence obtained in previous studies.^{24,30,31,38} In the vehicle groups, 1.5% DMSO in 0.9% saline was infused instead. In addition, to assess the effect of ISE insertion on ICP, we compared a sham-vehicle group in which only an ICP probe was used ($n=3$) with the sham-vehicle-ISE experimental group with ISE, reference electrode, and ICP probe. Real-time recordings of $[Na^+]_e$, $[K^+]_e$, ICP, and mABP were obtained throughout the study (PowerLab 4/30), and values at selected times were analyzed.

Histological assessment

To confirm the peri-lesional positioning of electrode implantation sites (Fig. 3), animals were sacrificed at 5 h post-CCI (or post-

infusion for shams) with an overdose of isoflurane (5%), and were transcardially perfused with 4% paraformaldehyde. Serial 40 - μ m coronal sections were cut (0.20 to 6.00 mm posterior to bregma), collected, mounted and processed for toluidine blue staining. Briefly, sections were dehydrated/rehydrated in alcohols, washed, stained in 0.1% Toluidine Blue (Sigma-Aldrich, St. Louis, MO), and mounted. Images targeting localization of ISE, reference electrode and ICP probe placement (Figs. 2–3) were acquired using an Olympus BX51 microscope ($4\times$ objective lens) coupled to an Olympus DP72 CCD camera and Olympus cellSens acquisition software.

Statistical analysis

The effect of treatment on $[Na^+]_e$, $[K^+]_e$ and changes in ICP (Δ ICP) were assessed using two-way repeated measures analysis of variance (treatment, time, treatment $\times$ time; repeated measures with respect to time) and post-tests conducted by the Student–Newman–Keuls (SNK) method (Fig. 4, 5, and 6). For ICP studies, preliminary analyses (Results) evaluated the effect of ISE placement on ICP at time 0 min (Student *t*-test) and verified that ICP in the CCI-vehicle-ISE, CCI-SR49059-ISE, and sham-vehicle-ISE groups were not significantly different at time 0 min (one-way analysis of variance). Data are expressed as mean $\pm$ SEM, and $p < 0.05$ was considered to be statistically significant.

Results

Physiology monitoring

There were no significant changes in mABP (at baseline and up to 5 h) or arterial blood gas measurements including pH, pCO_2 , pO_2 , plasma Na⁺ and plasma K⁺ measured at -15 min, $+15$ min and $+5$ h between experimental groups.

FIG. 4. Controlled cortical impact (CCI) significantly reduced $[Na^+]_e$ from its time 0 min (baseline) values of 141.6 ± 5.1 mEq/L in the CCI-vehicle- ion-selective electrode (ISE) and 135.3 ± 3.9 mEq/L in CCI-SR49059-ISE groups to 80.1 ± 15 and 87.9 ± 7.9 mEq/L, respectively, after injury but before drug administration (5 min). Groups were indistinguishable prior to drug treatment. SR49059, a selective vasopressin-1a receptor blocker, accelerated $[Na^+]_e$ recovery following injury. $[Na^+]_e$ in CCI-SR49059-ISE animals increased to a value not significantly different from baseline at 25 min, whereas equivalent recovery in CCI-vehicle-ISE animals required 2 h post-injury; dashed box highlights difference in recovery time course. $[Na^+]_e$ in the sham-vehicle-ISE group did not significantly change over time (time 0 min baseline, 139.6 ± 4.4 mEq/L), and $[Na^+]_e$ was not distinguishable between groups at baseline. Data are plotted as mean $\pm$ SEM ($n=5$ /group); *, $p < 0.05$ vs 0 min. Two-way repeated measures analysis of variance and Student–Newman–Keuls *post hoc* test.

FIG. 5. Controlled cortical impact (CCI) significantly increased $[K^+]_e$ from its time 0 min (baseline) value of 5.2 ± 0.6 mEq/L in the CCI-vehicle- ion-selective electrode (ISE) and 5.7 ± 0.7 mEq/L in the CCI-SR49059-ISE groups to 20.9 ± 3.8 mEq/L and 13.4 ± 3.4 mEq/L, respectively, after injury but before drug administration (5 min). Groups were indistinguishable prior to drug treatment. Infusion of SR49059 beginning at 5 min post-CCI did not alter the time required for recovery of $[K^+]_e$. $[K^+]_e$ was indistinguishable from baseline after 45 min of recovery in both groups. In the sham-vehicle-ISE group, $[K^+]_e$ did not significantly change over time during the experiment. Data are plotted as mean $\pm$ SEM ($n=5$ /group). *, $p < 0.05$ vs 0 min, Two-way repeated measures analysis of variance and Student–Newman–Keuls *post hoc* test.

FIG. 6. Time course of changes in intracranial pressure (Δ ICP). (A) Effect of SR49059 in controlled cortical impact (CCI). CCI caused a sustained elevation of Δ ICP in CCI-vehicle- ion-selective electrode (ISE) group (35 – 300 min; *, $p < 0.05$ vs 0 min) but block of vasopressin-1a receptor by SR49059 abolished the CCI-induced increase in Δ ICP (CCI-SR49059-ISE group; ns vs 0 min). Sham-vehicle-ISE group serves as time control for the effects of ISE implantation (180 – 300 min, *, $p < 0.05$ vs 0 min). Comparing between groups at each time point, Δ ICP was significantly greater in CCI-vehicle-ISE than in sham-vehicle-ISE (a, $p < 0.05$) or CCI-SR49059-ISE (b, $p < 0.05$), but CCI-SR49059-ISE and sham-vehicle-ISE were not distinguishable at any time point. Data are plotted as mean $\pm$ SEM ($n = 5$ /group). (B) Effect of ISE implantation on time course of Δ ICP in sham injury groups. Δ ICP did not significantly change over time in sham-vehicle group without ISE ($n = 3$). Sham-vehicle-ISE group is reproduced from Panel A (*, $p < 0.05$ vs 0 min; a, $p < 0.05$ between groups). Two-way repeated measures analysis of variance and Student–Newman–Keuls *post hoc* test were used for both A and B.

Cortical $[Na^+]_e$ and $[K^+]_e$ after CCI and effect of V1aR inhibition on recovery

Baseline measurement of $[Na^+]_e$ and $[K^+]_e$ in CCI-vehicle-ISE, CCI-SR49059-ISE and sham-vehicle-ISE were not significantly different prior to injury. $[Na^+]_e$ was 139.6 ± 4.4 mEq/L for the CCI-vehicle-ISE group, 135.3 ± 3.9 mEq/L for the CCI-SR49059-ISE group and 141.6 ± 5.1 mEq/L for the sham-vehicle-ISE group

($n = 5$ /group). $[K^+]_e$ was 5.2 ± 0.6 mEq/L for the CCI-vehicle-ISE group, 5.7 ± 0.7 mEq/L for the CCI-SR49059-ISE group and 4.7 ± 0.7 mEq/L for the sham-vehicle-ISE group ($n = 5$ /group).

Immediately following CCI, $[Na^+]_e$ rapidly decreased by ~35–40% and $[K^+]_e$ rapidly increased ~135–300%, and both slowly returned to normal values (Fig. 4). At 5-min post-CCI, $[Na^+]_e$ was 80.1 ± 15 mEq/L in the CCI-vehicle-ISE group and 87.9 ± 7.9 mEq/L in the CCI-SR49059-ISE group ($p < 0.001$ vs. 0 min for each group; ns between groups). The selective V1aR inhibitor SR49059 accelerated the time course of $[Na^+]_e$ recovery following injury. $[Na^+]_e$ in CCI-SR49059-ISE animals was significantly reduced between 5 and 20 min post-injury but recovered to a level indistinguishable from baseline at 25 min post injury. In contrast, $[Na^+]_e$ in CCI-vehicle-ISE animals remained depressed between 5 and 60 min post injury, and equivalent $[Na^+]_e$ recovery to baseline was not detected until 2 h post-injury. In the sham-vehicle-ISE group, $[Na^+]_e$ did not significantly change during the 5 h experiment.

While CCI decreased $[Na^+]_e$, $[K^+]_e$ markedly increased (Fig. 5). At 5-min post-CCI but before drug administration, $[K^+]_e$ was 20.9 ± 3.8 mEq/L in the CCI-vehicle-ISE group and was 13.4 ± 3.4 mEq/L in the CCI-SR49059-ISE group ($p < 0.001$ vs. 0 min for each group; ns between groups), and as found for $[Na^+]_e$, $[K^+]_e$ was not significantly different between the groups at this time point. In contrast to the response of $[Na^+]_e$, SR49059 administration did not significantly affect the time for recovery of $[K^+]_e$ to its baseline level. In both groups, $[K^+]_e$ remained significantly elevated for the 45 min post-injury and then returned to a value indistinguishable from baseline. $[K^+]_e$ in the sham-vehicle-ISE group did not significantly change during the 5 h experiment.

ICP monitoring

To clarify the effect of ISE insertion on ICP, we compared ICP at time 0 min in a sham-vehicle group without ISE to that in the sham-vehicle-ISE group. In sham-vehicle (ICP probe only) animals, ICP was 7.0 ± 1.2 mmHg at 0 min, whereas ICP was 10.9 ± 0.9 mmHg in the ISE group. Thus, implanting two ISE and a reference electrode caused an increase of ICP of 3.9 mmHg at 0 min ($p < 0.05$, *t*-test). Nevertheless, ICP before injury was indistinguishable in the sham-vehicle-ISE, CCI-vehicle-ISE, and CCI-SR49059-ISE groups (ns , one-way analysis of variance); this was expected because the three groups were treated identically until the time of injury (Fig. 1).

Because SR49059 accelerated the time course of recovery of $[Na^+]_e$ after CCI (Fig. 3), we determined whether inhibition of V1aR with SR49059 would alter the time course of Δ ICP after injury at time 0 min. Δ ICP in the uninjured sham-vehicle-ISE group served as a time control for the effects of ISE implantation. Figure 6A demonstrates that CCI caused an increase in Δ ICP in the CCI-vehicle-ISE group, and the elevation of Δ ICP was maintained over the entire 5 h study, compared with either time 0 (*, $p < 0.05$) or the sham-vehicle-ISE time control (a, $p < 0.05$). In marked contrast, Δ ICP did not significantly change from its time 0 min value when V1aR were blocked with SR49059 (CCI-SR49059-ISE group) and was indistinguishable from the sham-vehicle-ISE time control. Comparing CCI-vehicle-ISE and CCI-SR49059 groups at each time point showed that SR49059 also significantly reduced Δ ICP from that observed without SR49059 (b, $p < 0.05$), except at 60 min ($p \approx 0.07$). Thus, beginning infusion of SR49059 five min after CCI prevented the CCI-induced elevation of ICP that otherwise lasted for the 5 h duration of the study.

The time course of the sham-vehicle-ISE group revealed a slow fall in Δ ICP that reached significance between 180 and 300 min. These data suggest that the 3.9 mm Hg elevation of ICP at time 0 min with ISE implantation noted previously is likely to have slowly resolved over time. In contrast, in a separate sham-vehicle group without implanted ISE (Fig. 6B), Δ ICP did not significantly change over the 5 h time course of the study.

Discussion

$[Na^+]_e$ and $[K^+]_e$ in CCI

Large extracellular ionic changes rapidly occur after focal brain injury by a controlled cortical impact to the parietal cortex of rats. $[Na^+]_e$ decreased by ~35–40% and $[K^+]_e$ increased ~135–300% within 5 min of injury, and both slowly returned to normal values. Analogous alterations in $[Na^+]_e$ and $[K^+]_e$ occur in models of MCA occlusion,^{31,37} mimicking stroke and diffuse injury (weight-drop, Marmarou model).^{42,43} However, to our knowledge, extracellular cation changes and their recovery have not been described in CCI models.

One factor contributing to this rapid and opposing change in cation concentrations could be opening of Na^+ and K^+ channels in response to post-traumatic neuronal depolarization and the resulting storm of neurotransmitter release. In particular, studies in patients have shown a significant rise in levels of glutamate and extracellular K^+ , whereas TBI-induced alterations in ionic and neurotransmitter regulation are thought to be important factors leading to brain swelling and elevated ICP,^{45,46} reflecting either intracellular swelling or reactivity in blood vessels following TBI.

Because cortical extracellular fluid (ECF) volume fraction is ~15%,^{47,48} much smaller changes in intracellular cation concentrations are sufficient to explain the extracellular perturbations. Nevertheless, an excess cellular Na^+ uptake relative to K^+ loss with associated counter ions could create an osmotic gradient, drive water in cells, especially astrocytes, and lead to brain edema.^{30,40,49,50} For example, total tissue brain Na^+ as measured by flame photometry remained elevated at 5 h after CCI.⁴¹ Interpreted in combination with the present ISE data showing a fall and recovery of $[Na^+]_e$ post CCI, the elevation of tissue Na^+ content previously reported⁴¹ suggests that intracellular Na^+ must have been elevated post CCI and is likely to contribute to concomitant cytotoxic edema. Moreover, the observed extracellular K^+ accumulation may initiate cortical spreading depolarization (CSD), a common pathology in TBI. CSD usually signifies a labile balance of energy supply and demand, which contributes to its occurrence.^{51–53} Elevated $[K^+]_e$ also will alter the vascular supply of nutrients by triggering local vasoconstriction.⁵⁴ On the other hand, because SR49059 did not alter the recovery of $[K^+]_e$ but significantly reduced ICP, the time course of extracellular K^+ accumulation alone is not sufficient to explain the time course of ICP after injury.

In untreated sham-vehicle-ISE animals, both $[Na^+]_e$ and $[K^+]_e$ levels slowly recovered after CCI. $[K^+]_e$ remained significantly above baseline for 45 min, whereas $[Na^+]_e$ was elevated for 60 min. Multiple mechanisms may participate in the recovery process including ion channels, pumps and exchangers, spatial buffering by astrocytes and their end-foot processes, and bulk flow through the ECF, which drains into the cerebrospinal fluid (CSF).^{47,48,50} It is important to emphasize that post-CCI $[Na^+]_e$ and $[K^+]_e$ alterations were transient in untreated animals and that elevation of ICP occurred over the same time period, whereas the enhanced rate of recovery of $[Na^+]_e$ after SR49059 treatment prevented the devel-

opment of elevated ICP. These observations suggest that early cation changes in brain ECF create favorable conditions for the evolution of edema after injury and that the ionic disturbances need not be maintained. ICP remained elevated for 5 h in untreated CCI despite the recovery of extracellular cations, and our previous work supports ionic dysfunction, brain edema, astrocytic cell swelling, and increased AQP4 expression at the same time point.^{30,40,41,55} Elevated ICP requires an increase in water and osmolytes within the brain, and one can infer that a net flux of water from vascular compartment to intracellular and/or extracellular compartments must have occurred. We posit that the transient shifts in $[Na^+]_e$ and $[K^+]_e$ that were observed may “light the fire” that initiates a deprivation of energy supply, cellular metabolic imbalance, increased production of osmotically active products by metabolism, neurotransmitter release, and alterations in cellular function that give rise to and sustain brain edema. To the extent that such a transient trigger for the evolution of sustained edema is present in patients with comparable TBI,^{45,46} the present results emphasize to need for early intervention to prevent or reverse the development of elevated ICP and its sequelae.

Beneficial effects of V1aR inhibition by SR49059

Selective inhibition of V1aR with SR49059 (CCI-SR49059-ISE group) allowed faster recovery of $[Na^+]_e$, compared with that in vehicle-treated animals (CCI-vehicle-ISE group), whereas the kinetics of $[K^+]_e$ recovery was unaffected. Moreover, SR49059 treatment prevented an increase in ICP following CCI. V1aR expression is significantly increased after traumatic brain injury in the neocortex,^{20,21} and SR49059 application reduces brain edema after injury,³⁰ astrocytic swelling, and AQP4 expression.⁴⁰ Because transmembrane Na^+ flux is coupled to osmotic water flux, SR49059 may reduce water influx into cells after brain injury, in part by balancing the rate of osmotically active Na^+ and K^+ recovery. These actions would give ECF osmolytes and water a greater opportunity to drain by bulk flow through the ECF and CSF, and in turn, minimizing cell swelling and brain edema, and limiting the elevation in ICP.

Further, selective V1aR inhibition also reduces AQP4 expression post-injury.^{40,41,55} AQP4 is the primary water channel in astrocytes, and down regulation of its expression by SR49059 may reduce cell membrane hydraulic conductivity, slow the kinetics of cellular water influx, and reduce cellular edema, favoring the drainage of ECF.^{21,24–26} Previous work demonstrating that vasopressin may induce brain edema^{20,21,56} is supported by multiple studies showing that edema is abolished by blocking the V1aR receptors.^{24–29}

The present results demonstrate that V1aR inhibition accelerates the recovery of $[Na^+]_e$ supporting its potential to blunt the ionic dysfunction that leads to edematous changes. Taken together, our findings suggest that SR49059 and other V1aR inhibitors may have potential as therapeutic agents for treating cellular edema by both speeding post-injury ionic recovery and reducing injury-induced elevation of ICP following CCI.

We previously demonstrated the upregulation of AQP4 in this model.^{30,41,55} AQP4 upregulation will augment water entry into astrocytes following the cellular influx of Na and efflux of K , supporting the idea that the AQP4 increase adds to cellular swelling, cerebral edema and raised ICP. Others support the idea that AQP4 expression increases following similar insults and may provide a key avenue for strategies aimed at reducing its effects.^{57–59} On the other hand, a decrease in AQP4 expression was reported using a CCI model at more chronic time points⁶⁰ but these authors concluded

that AQP4 expression was upregulated during intracellular edema. Similarly, in closed head, weight drop TBI, AQP4 levels are significantly increased.^{61,62} In contrast, in a model of fluid percussion TBI, no AQP4 increase was observed up to seven days post insult.⁶³ It must be noted that in models of purely vasogenic edema, such as intraparenchymal fluid infusion or freeze-injury where there is a direct insult to the blood brain barrier, AQP4 expression facilitates reabsorption and minimizes injury.⁶⁴ So, although there is now a general consensus that following cerebral contusion AQP4 is up regulated,^{65,66} studies aimed at understanding the mechanism and potential neuroprotective properties of pharmacological agents must consider that distinct brain injury models trigger vastly different cellular mechanism that may or may not be related to modulation of AQP4.

A potential limitation of this study is that stereotactic implantation of two ISE probes, a reference electrode and an ICP probe unavoidably causes local injury and thus may contribute to the characteristic hemorrhage that is often a component of this injury as described in the seminal work by Dixon and colleagues.⁶⁷ To minimize the influence of this confounding factor, any animal demonstrating significant intraparenchymal or supradural hemorrhage was excluded from these studies. Further, following ISE implantation in all three experimental groups, a recovery period of more than 45 min was inserted to allow acute effects of tissue injury to largely subside before beginning recording, initiating CCI and drug treatment. Nevertheless, recovery from ISE implantation may have continued over a longer time course. Although core body normothermia was vigorously maintained, in the present study we did not measure cranial temperature, a variable that may affect outcome post injury. Future studies in transgenic mice and cell culture injury models are likely to be needed to more directly address the cellular and molecular mechanism linking selective V1aR inhibition to the time course of recovery of extracellular Na⁺ and K⁺ and their effect on ICP after TBI.

Conclusion

Large extracellular ionic changes rapidly occur after focal brain injury induced by a controlled cortical impact to the parietal cortex of rats. Extracellular Na⁺ decreases by ~35–40% and extracellular K⁺ increases ~150–300% within 5 min of injury, and both return slowly to normal values. Selectively inhibiting the V1aR with SR49059 allowed faster recovery of extracellular Na⁺ concentration, whereas the time course of recovery of K⁺ was unaffected. Additionally, SR49059 treatment prevented the increase in ICP following CCI. Cellular edema may result, in part, from unbalanced influx and efflux of cations. By balancing the rate of osmotically active Na⁺ and K⁺ recovery, SR49059 may reduce water influx into cells after brain injury and minimize elevation of ICP. These findings suggest SR49059 and V1aR inhibitors as potential therapeutic tools for treating cellular brain edema.

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Author Disclosure Statement

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