Protective effect of the V1a receptor antagonist SR49059 on brain edema formation following middle cerebral artery occlusion in the rat

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Summary

There exists no pharmacological treatment for fulminating brain edema. Since evidence indicates that brain aquaporin-4 (AQP4) water channels are modulated by vasopressin V1a receptors, we examined the edema-reducing properties of the selective V1a receptor antagonist, SR49059, following middle cerebral artery occlusion (MCAO).

Male Sprague-Dawley rats were randomly assigned to sham procedure, vehicle, or SR49059 infusion at different dosages (each $n = 6$, 480 µL/hr, 640 µL/hr, 720 µL/hr) and starting 60 minutes before or after MCAO. After a 2-hour period of ischemia and 2 hours of reperfusion, the animals were sacrificed for assessment of brain water content, sodium, and potassium concentration. Statistics were performed using an ANOVA followed by a Tukey post hoc analysis.

SR049059 treatment reduced brain water content in the infarcted area given at 640 µL/hr ($p = 0.036$), 720 µL/hr 60 minutes before ($p = 0.002$) or 60 minutes after ($p = 0.005$) MCAO. The consecutive sodium shift into the brain was prevented ($p = 0.001$), while the potassium loss was inhibited only by pre-treatment ($p = 0.003$).

These findings imply that in ischemia-induced brain edema, the selective V1a receptor-antagonist SR49059 inhibits brain edema and the subsequent sodium shift into brain. This substance offers a new avenue in brain edema treatment and prompts further study into AQP4 modulation.

Keywords: AQP water channel; vasopressin; middle cerebral artery occlusion; brain edema.

Introduction

Brain edema following many types of brain insult causes an increase in intracranial pressure, thereby contributing to the high rate of secondary complications and consecutive mortality found in these patients. Aquaporins (AQP) are a family of water-selective transporting proteins and the subtype, aquaporin-4 (AQP4), is abundant in astrocytes and ependymal cells, having a highly polarized distribution in glial membranes facing capillaries and the pia mater [1, 15]. Many authors have described the important role of AQP4 in water homeostasis during traumatic and ischemic brain edema development [13, 14], although controversial results have been obtained.

Recent evidence suggests that AQP4 may be regulated by arginine vasopressin. In vitro, the AQP4-mediated water flux is facilitated by vasopressin V1a receptor agonists [12], and the non-peptide V1 receptor antagonist OPC-21268 significantly reduces brain edema after cold injury [3]. Therefore, we assessed the efficacy of different dosages of the selective vasopressin V1a antagonist SR49059 on brain edema development when given intravenously before middle cerebral artery occlusion (MCAO). Specifically, we measured water content, sodium, and potassium concentrations in the ischemic and non-ischemic hemispheres. We determined whether treatment started after MCAO was as effective as treatment started before induction of ischemia.

Materials and methods

Animals and surgical procedure

The studies were conducted under approval of the Institutional Animal Care and Use Committee using National Institutes of Health guidelines. Experiments were carried out on 330 to 400 g adult male Sprague-Dawley rats (Harlan, Indianapolis, IN). Rats were housed at $22 \pm 1\,°C$ with 60% humidity, 12-hour light/12-hour dark cycles, and pellet food and water ad libitum. Surgery was performed after intubation under halothane anesthesia and controlled ventilation (1.3% halothane in 70% nitrous oxide and 30% oxygen). Rectal temperature was maintained at $36.5 \pm 0.5\,°C$ using a heat lamp. The left femoral artery and vein were cannulated with polyethylene tubing (P.E. 50, Becton Dickinson and Company, Sparks, MD) for continuous monitoring of mean arterial blood pressure (MABP), blood sampling, and drug infusion. Adequate ventilation was verified by arterial blood gas measurement after 1 hour of anesthesia.

Cerebral blood flow (CBF) to the territory of the right middle cerebral artery was continuously monitored by Laser Doppler Flow-

metry (LaserFlo Vasamedics Inc., St Paul, MN) through a burr hole located 1 mm posterior and 5 mm lateral to bregma leaving the dura intact. Animals were placed in a supine position over the laser Doppler probe, and CBF as well as MABP were recorded continuously using a data acquisition system (ADInstruments, Colorado Springs, CO).

MCAO was induced using a slightly modified version of the intraluminal suture method described elsewhere [2]. Through a midline neck incision, the bifurcation of the right common carotid artery was exposed, and branches of the external carotid artery (ECA) and internal carotid artery (ICA) including the occipital, lingual, and maxillary arteries were microsurgically separated and coagulated. The ECA was ligated with a 4-0 silk suture, and after temporary occlusion of the ICA and common carotid artery with vascular mini-clips, a 4-0 monofilament nylon suture (4-0 SN-644 MONOSOF nylon Polyamide) with a silicon tip of 0.30 to 0.35 mm diameter was inserted through the ECA stump and secured with a suture. The clips were removed and the filament was advanced through the ICA into the circle of Willis while occluding the pterygopalatine artery with a forceps. A CBF reduction between 70 and 80% to the baseline was observed when the suture was advanced at a distance of 22 to 24 mm from the carotid bifurcation, thereby verifying proper MCAO. Two hours after occlusion, the middle cerebral artery territory was perfused by withdrawing the suture into the ECA stump, confirmed by an increase in CBF.

Study protocol and drug preparation

The objective of these experiments was to assess the effect of intravenous infusion of SR49059 on brain edema at different concentrations (83 mmol at 720 μL/hr, n = 6; 73 mmol at 640 μL/hr, n = 6; 56 mmol at 480 μL/hr, n = 6) and started at different time points (60 minutes pre-ischemia, n = 6; 60 minutes post-ischemia, n = 6) following MCAO. The animals were randomly assigned to sham procedure (n = 4), vehicle infusion (n = 18), or intravenous SR49059 at different doses and time points after MCAO. SR49059 (Sanofi Recherche, Montpellier, France) was dissolved in 1% dimethyl sulfoxide as vehicle solution (Sigma-Aldrich, St Louis, MO). The drug was intravenously administered using a continuous infusion pump (sp210w syringe pump, KD Scientific, Holliston, MA). At the end of the experiments, the animals were sacrificed by an overdose of halothane, decapitated, and the brains removed.

Tissue processing

Cerebral tissue was immediately cut into 4 consecutive 4 mm coronal sections excluding the most rostral and caudal sections from further analysis. After division into the right and left hemispheres along the anatomic midline, the 4 regional samples obtained were processed for water content measured by the wet/dry weight method. The wet weight of each sample was measured using an electronic analytical balance before drying the sample at 95 °C for 5 days and reweighing to obtain the dry weight. The water content of each sample is given as percentage of total tissue weight. For measurement of brain sodium and potassium concentrations, the dried samples were ashed in a furnace for 24 hours at 400 °C. The ash was then extracted with distilled water, and the concentrations of sodium and potassium were determined using a flame photometer (943 nm; Instrument Laboratory, San Jose, CA) with caesium as an internal standard.

Statistical analysis

SPSS software (SigmaStat, Chicago, IL) was used for statistical analysis. The data were analyzed by a randomized one-way

Table 1. *Effects of different doses of SR49059 on brain water, sodium, and potassium after middle cerebral artery occlusion**

Groups	% Tissue water	Tissue sodium mEq/kg dry wt	Tissue potassium mEq/kg dry wt
Vehicle	2.3 ± 0.0	116.1 ± 8.8	−43.2 ± 6.0
SR49059 480 μL/hr	1.8 ± 0.4	69.2 ± 19.0	−26.0 ± 12.6**
SR49059 640 μL/hr	1.2 ± 0.3**	39.9 ± 13.5**	−27.5 ± 6.3
SR49059 720 μL/hr	0.8 ± 0.2**	36.7 ± 6.2**	2.1 ± 11.3**

* Data shown are average ± SEM, ** $p < 0.05$ as compared with vehicle.

ANOVA for group variations followed by a Tukey post hoc analysis. Statistical significance was accepted at $p < 0.05$.

Results

The injury-induced mortality was 14% following MCAO. MABP and arterial blood gases were kept within physiological limits throughout the experimental procedure, requiring few adjustments in halothane concentration and ventilation parameters.

The comparison of different doses of SR49059 by ANOVA produced a significant group effect for the reduction of brain water content in the ischemic area ($F_{3,66} = 9.34$, $p < 0.001$), the reduction of sodium accumulation ($F_{3,66} = 10.42$, $p < 0.001$) and the reduction of potassium loss ($F_{3,66} = 4.40$, $p \leq 0.001$) (Table 1). Tukey post hoc analysis indicated that the effect of SR49059 treatment on brain water content, as compared to vehicle infusion, was more effective at an infusion rate of 720 μL/hr ($p \leq 0.002$), than at 640 μL/hr ($p \leq 0.036$) or 480 μL/hr ($p \leq 0.870$) when started 60 minutes before MCAO. Accordingly, the Tukey post hoc analysis for the effect of different doses of SR49059 on sodium accumulation and potassium loss confirmed the protective effect of the 720 μL/hr infusion rate.

In order to test a clinically relevant time point for SR49059 application, we compared the infusion rate of 720 μL/hr started either 60 minutes before or 60 minutes after MCAO to a vehicle infusion (Table 2). Comparison by ANOVA of SR49059 infusion started before or after MCAO produced a significant group effect for the reduction of brain water content in the ischemic area ($F_{3,66} = 8.30$, $p < 0.001$), the reduction of sodium accumulation ($F_{3,66} = 11.53$, $p < 0.001$) and the reduction of potassium loss ($F_{3,66} = 5.09$, $p \leq 0.001$).

Table 2. *Effect of SR49059 on brain water, sodium, and potassium at different times after middle cerebral artery occlusion**

Groups	% Tissue water	Tissue sodium mEq/kg dry wt	Tissue potassium mEq/kg dry wt
Vehicle (n = 18)	2.2 ± 0.1	111.3 ± 6.5	−43.2 ± 6.0
SR49059 60 min before (n = 6)	0.9 ± 0.2**	38.2 ± 8.0**	3.6 ± 21.2**
SR49059 60 min after (n = 6)	1.1 ± 0.3**	39.9 ± 8.3**	−14.5 ± 12.6

* Data shown are average ± SEM, ** $p < 0.05$ as compared with vehicle.

The effects of SR49059 treatment according to the Tukey post hoc analysis follow. 1) Brain water content compared to vehicle infusion: treatment was equally effective when started 60 minutes before ($p < 0.001$) or 60 minutes after ($p < 0.003$) MCAO. 2) Brain sodium accumulation compared to vehicle infusion: treatment was equally effective when started 60 minutes before ($p < 0.001$) or 60 minutes after ($p < 0.001$) MCAO. 3) Brain potassium loss compared to vehicle infusion: treatment was only effective when started 60 minutes before ($p < 0.003$) but not 60 minutes after ($p < 0.385$) MCAO.

Discussion

To the best of our knowledge, this study is the first to demonstrate that brain edema resulting from MCAO is reduced by treatment with SR49059, the selective vasopressin V1a receptor antagonist. SR49059 was administered intravenously for 5 hours starting 1 hour before occlusion, testing with different concentrations of the drug. Afterwards, the most effective concentration was used starting before or after MCAO. We demonstrated that SR49059 caused a significant dose-dependent reduction in brain water content and subsequent electrolyte shift, and was still effective when given 1 hour after onset of ischemia. The most significant effect in the ischemic area was observed using the highest dose of 83 mmol SR49059, starting either 1 hour before or after MCAO. Similar results were obtained regarding brain sodium and potassium shift after ischemic injury. SR49059 treatment was able to reduce sodium uptake and increase potassium levels in a dose-dependent manner. These findings are consistent with the generally accepted opinion that water and sodium tend to coexist and transfer together through the plasma membrane under physiological and pathological conditions [6, 10, 17].

Water can cross cell membranes through different pathways: specific water channels (aquaporins), the lipid bilayer [5], or through ion-water cotransport proteins [19, 20]. Because of its specific anatomical and cellular localization in the central nervous system, AQP4 has been suggested to play a role in cerebral water balance. According to this hypothesis, AQP4-deficient mice developed less brain edema after acute water intoxication and MCAO [11]. Regarding the function and regulation of AQP4 following injury, there exist conflicting results. In different models inducing neuronal degeneration, AQP4 mRNA was up-regulated following blood-brain barrier disruption [16]. In a combined head injury model, AQP4 immunostaining was negative and the AQP4 mRNA down-regulated in areas with impaired blood-brain barrier [7]. Following cortical impact injury, hemispheric ipsilateral AQP4 was progressively down-regulated within the first 48 hours [8]. Similar results were found following ischemia [13] and hypoxia [18], all known to produce a predominately cytotoxic edema.

Evidence indicates that AQP4 is regulated by vasopressin, a neuropeptide endogenous to the brain [9] that includes a vasopressin-containing fiber system [4]. In vitro experiments on rat neocortical slices suggest that there exists a tonic, vasopressin V1a receptor-mediated facilitation of water permeability [12]. V1a receptors are coupled via G-proteins to phospholipase C, resulting in an IP3-dependent Ca^{2+} release from internal stores. V1a receptor stimulation also causes activation of protein kinase C, and evidence suggests that vasopressin exerts part of its facilitatory effect through this signaling pathway [12]. One possible explanation for the protective effects on brain edema development following ischemic injury found in this study may be the vasopressin-dependent inhibition of AQP4 activity or expression through V1a receptors. However, additional studies are necessary to clarify the precise biochemical pathway by which the selective V1a antagonist SR49059 prevents brain edema development after MCAO.

Acknowledgments

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