Effects of Nonpeptide V1 Vasopressin Receptor Antagonist SR-49059 on Infarction Volume and Recovery of Function in a Focal Embolic Stroke Model

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Background and Purpose—Cerebral edema develops very early after the onset of focal cerebral ischemia and may be a major factor in early disability after an acute ischemic stroke. There have been very limited studies on the usefulness of antiedemic agents as neuroprotective agents in the setting of focal cerebral ischemia. We tested the neuroprotective effects of a new potent nonpeptide vasopressin receptor V1 antagonist, SR-49059, in a focal embolic stroke model in rats.

Methods—Focal ischemic injury was induced by embolizing a preformed clot into the middle cerebral artery (MCA). Infarction volume was measured at 48 hours after the MCA occlusion. Neurological deficits, ischemic brain edema, seizure activity, and mortality and hemorrhage rates were also documented.

Results—Treatment with SR-49059 (2 mg/kg), initiated immediately after MCA occlusion, significantly reduced infarction volume (P <0.05) measured at 48 hours after the arterial occlusion. In animals in which the treatment was delayed for 1 hour after MCA occlusion, infarction volume was also reduced significantly (P <0.05). Infarction volume in the rats that received the drug at 3 or 6 hours after MCA occlusion was not different from that in the vehicle-treated group. Treatment with SR-49059, when started early after the arterial occlusion, also reduced neurological deficits and ischemic brain edema. Injection of drug at a higher dose (30 mg/kg) also reduced infarction volume and improved functional recovery but was not superior to the lower dose (2 mg/kg) when the drug was administrated at 1 hour after MCA occlusion.

Conclusions—Our data show that the selective vasopressin receptor antagonist SR-49059 is a potent neuroprotective agent when used early after onset of arterial occlusion in an embolic focal ischemia model in rats. Further studies are needed in stroke models to better understand its neuroprotective properties when used alone or in combination with thrombolysis. (Stroke. 2002;33:3033-3037.)

Key Words: arterial occlusive diseases ■ brain edema ■ ischemia ■ rats

Arterial occlusion in the cerebral circulation leads very quickly to irreversible neuronal injury. Early in the course of ischemic insult, a sudden loss in the availability of high-energy compounds and a drastic change in the extracellular biochemical milieu are the major factors responsible for damaging effects of the insult. Later, as the blood flow is restored from opening of collateral blood vessels or reperfusion, secondary processes, including activation of apoptotic mechanisms, inflammation, and secondary surges in the extracellular concentrations of excitatory amino acids, result in further neuronal injury. During this time, approximately hours to days after the insult, there is progressive swelling (edema) in the region of the damaged tissue.1-3 This is especially prominent in large-volume infarctions and can have devastating effects when the cranial cavity does not allow for expansion to occur.

Edema commences soon after the onset of ischemia and reaches a peak approximately 2 days later. Water content seems to recover to normal by the end of the first and during the second week, a time course that matches well with edema-related neurological symptomatology.4-6 Data from the embolic stroke model used in the present study are also consistent with these observations (C.X. Wang, MD, et al, unpublished data, 2002). Progressive swelling of the ischemic injured brain leads to displacement and damage to the surrounding neuronal tissue and is a major cause of secondary tissue damage. A number of antiedemic agents have been tested in the setting of acute focal cerebral ischemia with varying effects on the volume of infarction and recovery of function.7-9

Arginine vasopressin (AVP) is a neuropeptide that is synthesized in the hypothalamus and transported via the axonal fiber system to the neurohypophysis, which releases the hormone into the blood stream.10 Evidence suggests that centrally released AVP plays an important role in brain
capillary water permeability, brain ionic homeostasis, and the regulation of cerebrospinal fluid production. AVP receptor V₁ has been reported in the brain, and recent experimental evidence also indicates a causative role for centrally released AVP in ischemic brain edema. Treatment with the AVP receptor antagonist OPC-21268 prevented vasogenic edema in the brain induced by cold injury. A new potent AVP antagonist, SR-49059, has been characterized recently. SR-49059 selectively binds V₁ receptor in both animals and humans. This compound inhibits AVP-induced vascular smooth cell contraction and blood pressure elevation for at least 8 hours and also inhibits AVP-induced human platelet aggregation in a concentration-dependent manner. A study also demonstrated that the compound can bind to brain regions devoid of blood-brain barrier but not in the parenchyma when injected peripherally in intact animals. It is known that if the blood-brain barrier is breached after ischemic injury, this may help the entrance of the compound into the brain. In this article we report our findings that SR-49059 acts as a neuroprotective agent in a focal embolic stroke model.

Materials and Methods
Male Wistar rats, weighing 300 to 350 g, were purchased from Charles River, St Constant, Canada. The rats were housed in a 12-hour light/dark cycle and had free access to water and food. Animal care and the general protocols for animal use were approved by the Animal Ethics Committee of the University of Alberta.

Cerebral Focal Ischemia Model
Focal cerebral ischemia was induced by embolizing a preformed clot into the middle cerebral artery (MCA) as reported previously. In brief, the rats were initially anesthetized with 3.0% halothane and then maintained with 1.5% halothane in a mixture of O₂ and NO₂ during surgery. Body temperature was maintained at 37°C with a heating pad for the duration of surgery and the immediate postoperative period until the animal recovered fully from anesthesia. A longitudinal incision of 1.5 cm in length was made in the midline of the ventral cervical skin. The right common carotid artery, right internal carotid artery, and right external carotid artery were exposed. The distal portion of the external carotid artery was ligated and cut. A modified PE-10 catheter, filled with bovine thrombin (Thrombostat, TM Warner-Lambert Co), was introduced into the lumen of the right external carotid artery via a small puncture. Ten microliters of blood was withdrawn into the catheter and retained for 15 minutes to allow formation of a clot. Once the clot formed, the catheter was advanced 17 mm in the internal carotid artery until its tip was 2 mm away from the origin of the MCA. The preformed clot in the catheter was then injected, and the catheter was removed. The wound was closed, and the animal was returned to its cage. The dynamic changes of the microvessel occlusion in this model have been characterized. In our previous experiments we did not observe any significant changes in physiological parameters, including arterial pulse rate, mean arterial pressure, and arterial blood oxygen saturation, during and after MCA occlusion. To allow for better postoperative recovery, we chose not to monitor these physiological parameters in the present study because additional surgical procedures are needed for this monitoring. Brain temperature was monitored in 5 rats before, during, and after MCA occlusion. The temperature in the brain was approximately 0.5°C lower than in the rectum, and the brain temperature did not change during and until 40 minutes after MCA occlusion compared with that observed before the arterial occlusion.

Quantification of Brain Infarct Volume and Edema
The quantification of infarct volume has been detailed previously. Briefly, 48 hours after MCA occlusion, rats received intracardiac perfusion of 100 mL normal saline under deep anesthesia by injection of overdose thiopental (100 mg/kg). The brains were removed from the skull and cooled in ice-cold saline for approximately 5 minutes. For morphometric study, 2-mm-thick coronal sections were cut with the use of a rat brain matrix. A total of 8 coronal sections were collected, and the sections were stained with a 2% 3,3′-triphenyltetrazolium chloride (TTC) solution. The infarct appears pale white on a background of red “normal” brain. The stained brain sections were placed directly on the scanning screen of a color flatbed scanner (Scanjet 4p, Hewlett-Packard). The images were analyzed by a person who was unaware of the treatments, using a commercial image processing software program (PhotoShop, version 4.0, Adobe system). The total volume of each hemisphere and infarction was determined by integration of the distance of the 8 sections. The infarct volume was calculated with the following formula: infarct volume = (volume of left hemisphere – (volume of right hemisphere – measured infarct volume))/volume of left hemisphere. Brain swelling was determined with the following formula: swelling (edema) = (volume of right hemisphere – volume of left hemisphere)/volume of left hemisphere. The infarction volume and brain swelling were expressed as percentages.

Behavioral Tests
Neurological deficits and seizure activities were recorded at 2 and 48 hours after embolization. Neurological deficits were determined with a modification of the scoring system of Bederson et al., as follows: 0, no observable deficit; 1, forelimb flexion; 2, forelimb flexion plus decreased resistance to lateral push; 3, unidirectional circling; and 4, unidirectional circling plus decreased level of consciousness. Seizure activity was scored with a modification of the scoring system of Racine, as follows: 0, no seizure was observed; 1, rhythmic mouth and facial movement; 2, rhythmic head nodding; 3, forelimb clonus; 4, rearing and bilateral forelimb clonus; and 5, rearing and falling.

Therapeutic Regimen
The rats were divided into 2 major series of experiments. In the first series, SR-49059 was injected immediately, 1, 3, or 6 hours after the MCA occlusion or at 1, 3, and 6 hours after the arterial occlusion. SR-49059 was used in a dose of 2 mg/kg on the basis of information obtained from the literature and our preliminary study. SR-49059 was dissolved in 10% dimethyl sulfoxide and injected intraperitoneally in all experiments. In the control group, 10% dimethyl sulfoxide was injected intraperitoneally immediately after MCA occlusion. In the second series of experiments, we used 2 doses of SR-49059 (2 and 30 mg/kg) and compared results with those in vehicle-treated animals. The drug was administered at 1 hour after the onset of MCA occlusion in the second series of experiments. SR-49059 was a generous gift from Sanofi-Synthelabo (Toulouse, France).

Statistical Analysis
The differences in infarction volume were analyzed with 1-way ANOVA followed by the Tukey test. Neurological deficit scores were reported as medians and interquartile ranges (25th to 75th percentiles). The neurological scores were analyzed with the Kruskal-Wallis test when ≥2 groups were analyzed and with the Mann-Whitney test when 2 groups were compared. The rates of mortality, hemorrhage, and seizure occurrence after different treatments were compared with the χ² test. Correlation between ischemic brain edema and infarction volume was analyzed with linear regression. Differences were considered significant when P < 0.05.

Results
First Series of Experiments
In the first series, the neuroprotective effect of SR-49059 was examined when the drug (2 mg/kg) was administered at different times after the embolization.
Infarction Volume
Embolicization of a preformed clot resulted in an infarction in the ipsilateral hemisphere, mainly located in the MCA irrigated region. In the control group, the infarction volume was $36.4 \pm 3.9\%$ (mean $\pm$ SEM), measured at 48 hours after the embolization (Figure 1). Compared with the control group, administration of SR-49059 immediately after the embolization significantly reduced infarction volume by 53% ($P<0.05$). Administration of SR-49059 at 1 hour after the embolization also significantly reduced the infarction volume compared with the control group ($P<0.05$). However, the infarction volume in the groups that received SR-49059 at 3 or 6 hours after the embolization did not differ significantly from the control group.

Ischemic Brain Swelling (Edema)
The brain swelling in the control group was $6.3 \pm 1.7\%$ at 48 hours after the ischemic injury (Figure 2). Administration of SR-49059 immediately after the embolization significantly reduced brain swelling by 87% compared with the control group ($P<0.05$). Administration of SR-49059 at 1, 3, or 6 hours after the embolization did not reduce the brain swelling significantly compared with the control animals.

Correlation between infarction volume and brain swelling was compared in transformed data with linear regression analysis. The mean infarction volume in the different groups was significantly correlated with the mean value of the brain swelling ($r=0.87; P<0.001$). When the values collected from individual animals were used for the analysis, the infarction volume was also significantly correlated with the brain swelling ($r=0.51, P<0.001$).

Behavioral Tests
Changes of neurological deficits in different groups are shown in Table 1. At 2 hours after embolization, all animals showed significant motor deficits, with average scores of 3 in all 5 groups. At 48 hours after embolization, the differences in the neurological deficits were not significant among these 5 groups. In the control group, neurological deficits did not change significantly at 48 hours compared with the scores at 2 hours after embolization. In the rats that received SR-49059 immediately after ischemia, the neurological deficits were significantly improved at 48 hours compared with the scores at 2 hours after embolization. In the rats that received SR-49059 immediately or 1 hour after ischemia, the neurological deficits were significantly improved at 48 hours compared with the scores at 2 hours after embolization ($P<0.05$). However, neurological deficits did not change significantly at 48 hours in the rats that received SR-49059 at 3 or 6 hours compared with the scores at 2 hours after embolization.

At 2 hours after embolization, seizure activity was observed in 2 rats in the control group. No seizure activity was seen in the rats that received SR-49059 immediately after embolization. Seizures occurred in 1, 2, and 3 rats that received the drug 1, 3,
TABLE 2. Percentage of Infarction Volume*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control (n = 10)</th>
<th>2 mg/kg (n = 8)</th>
<th>30 mg/kg (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>36.4</td>
<td>18.2 ‡</td>
<td>21.1 ‡</td>
</tr>
<tr>
<td>SEM</td>
<td>3.9</td>
<td>2.2</td>
<td>4.3</td>
</tr>
</tbody>
</table>

*The infarction was measured at 48 h after ischemic injury in the TTC-stained brain sections.
†SR-49059 was administered at 1 h after injury. The control group received 10% dimethyl sulfoxide.
‡Significantly different from the control group.

Second Series of Experiments

The second series examines whether a higher dose of SR-49059 can give a better protective effect in the ischemic injured brain when administered at 1 hour after embolization. Higher doses of the drug (30 mg/kg) significantly reduced infarction volume by 42% at 48 hours after embolization compared with the control group (P < 0.05) (Table 2). This treatment also significantly reduced neurological deficits at 48 hours after embolization (P < 0.05) compared with the control group (Table 3). However, brain swelling in the rats that received this treatment did not differ significantly from that in the controls at 48 hours after embolization. When these parameters were compared between the 2 drug-treated groups (2 versus 30 mg/kg), no significant changes were observed. Nevertheless, no observable side effects were found in any dose used in this study.

Discussion

The major finding of the present study is that application of the nonpeptide AVP receptor antagonist SR-49059 produced significant neuroprotective actions and reduced ischemic brain edema in an embolic model of stroke in rats. Administration of SR-49059 immediately or 1 hour (2 mg/kg) after MCA occlusion significantly reduced infarction volume and improved neurological deficits. However, the infarction volume in the groups that received SR-49059 at 3 or 6 hours (2 mg/kg) after MCA occlusion did not reveal significant neuroprotection. Results also showed that a higher dose of this compound (30 mg/kg) reduced infarction volume and improved functional recovery, although it was not superior to the lower dose (2 mg/kg) when it was administered at 1 hour after MCA occlusion. Additionally, when administered at 3 hours after MCA occlusion, higher doses of this compound did not have neuroprotective actions in the present model (data not shown). The mechanisms for the neuroprotection of SR-49059 in ischemic brain injury may be due to (1) reduction of ischemic brain edema (also see following section) since edema has been thought to exaggerate ischemic injury by reducing the already suppressed local blood flow and to cause increasing intracranial pressure and vascular compression and (2) the ability of the drug to protect the ischemic injured brain via unknown mechanisms because it could reduce the infarction volume even without reducing brain edema when administered at 1 hour after the arterial occlusion (both lower and higher doses).

In the present study we also found that administration of SR-49059 immediately after MCA occlusion significantly reduced brain edema, but not when SR-49059 was injected in the later time points. Cerebral edema occurs very commonly during the acute phase of large cerebral infarction. Our unpublished results showed that in the model used in the present study ischemic brain edema occurred at 1 hour and reached a maximal level at 48 hours after MCA occlusion (C.X. Wang, MD, et al, unpublished data, 2002). Edema still existed in the ischemic brain at 72 hours after MCA occlusion, the last time point in the study. Ischemic edema can be divided into the 2 major categories of cytotoxic and vasogenic forms. Vasogenic edema is the most prominent form of brain edema observed in ischemic stroke. Vasogenic edema is initially observed at 4 to 6 hours and reaches maximal levels at 2 to 4 days after ischemia. We believe that SR-49059 mainly inhibited vasogenic edema, although it cannot be ruled out that it may also reduce cytotoxic edema. Furthermore, the data also showed that ischemic brain edema and infarction correlated well in this ischemic injury model. This suggests that the ischemic edema may contribute to the severity of the infarction in the present model.

Before our study, a few antiedemic agents were tested in the setting of acute brain ischemia with varying degree of success. Dexamethasone can reduce cytotoxic edema in both global and focal ischemic stroke if it is administered before injury, but the results are variable if it is administered after injury. The antiedemic actions of dexamethasone are not fully understood, but such medications may act by preservation of the chemical.
and structural integrity of cellular elements in the tissue at risk for ischemic injury. Glycerol has been used for the alleviation of brain edema in patients with acute cerebral infarction. It has been shown that glycerol improves the neurological status in patients with cerebral infarction by enhancing regional cerebral blood flow in the ischemic brain secondary to reduction of focal cerebral edema. Recently, it has been shown that several compounds that can inhibit postischemic edema also reduce ischemic infarction. These compounds include NS-7, a novel N²/Ca²⁺ channel blocker; mamillo³, S-0139, an endothelin type A receptor antagonist; MDL 72527, a polyamine oxidase inhibitor; and human albumin. Taken together, these studies demonstrate that postischemic edema plays an important role in cell death, and improvement of edema can be essential for the survival and functional recovery of ischemic injured neurons.

In summary, the present study shows for the first time that a new AVP receptor antagonist, SR-49059, has neuroprotective effects in ischemic brain injury when injected early after occlusion of the MCA. Before it can be used clinically for treatment of ischemic injury, more studies are needed to answer questions such as the following: What is the optimal dose of the drug for neuroprotection in ischemic injury? How will this compound behave when combined with a thrombolytic agent in ischemic brain injury? The mechanisms of this compound in the protection of ischemic brain injury also need to be clarified. The present results strongly indicate that this compound can protect the ischemic injured brain not only by reducing ischemic edema but also through presently unknown mechanisms.

Acknowledgment
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References
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