The Kappa Opioid Agonist Niravoline Decreases Brain Edema in the Mouse Middle Cerebral Artery Occlusion Model of Stroke

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ABSTRACT
The effect of niravoline (RU 51599), a kappa opioid receptor agonist with water diuretic properties, was assessed on the resorption of postischemic cerebral edema in the conscious mouse in comparison with U 50488, another kappa opioid receptor agonist, and mannitol. Ischemia was obtained by permanent occlusion of the right middle cerebral artery. Twenty-four hours after occlusion, at a time when brain water content is submaximal, blood samples were collected to measure serum osmolality, and brains were removed to measure the brain water content of two samples of frontoparietal cortical tissue corresponding to the core and the periphery of ischemia. When administered from 3 to 30 mg/kg as a single i.p. injection 20 h after occlusion, niravoline significantly reduced the brain cortical water increase by 27% up to 48% in the periphery of the ischemic tissue. At these same doses, it increased the serum osmolality to the same extent in ischemic as in nonischemic mice: 4 to 10 mOsm/kg. U 50488 generally showed a similar activity. In contrast, mannitol (1 or 2 g/kg i.p. 23 h after occlusion) increased serum osmolality but did not decrease brain water content. In conclusion, kappa opiate agonists could be an alternative to hyperosmotic agents in the treatment of cerebral edema of the focal ischemia type, the use of which is limited to the early phase of cerebral edema.

Many compounds, belonging to different pharmacological classes and endowed with neuroprotective activities, have been studied on the MCAO model, but most studies deal with the infarct volume rather than brain edema. Nevertheless, brain edema is a major threat for stroke victims, and at present there is little possibility of decreasing brain edema after stroke in humans. The clinical efficacy of mannitol and glycerol has not yet been established, and the administration of such hyperosmolar agents may cause adverse effects, such as rebound of intracranial pressure or increase in cerebral volume (Larsson and Marinovich, 1976; Goluboff et al., 1964; Shenkin et al., 1962) with increase in secondary edema, which restrict the use of these agents at an early stage of stroke, before the breakdown of BBB.

The stimulation of kappa opioid receptors leads to a reduction of cerebral edema after global ischemia (Silvia et al., 1987). This effect is attributed to a decrease in AVP release and subsequent water diuresis (Leander, 1983), which resulted in an elevation of blood osmolality. This antiedema activity was attributed to the creation, between both sides of the BBB, of an osmotic gradient facilitating the movement of water from the edematous parenchyma to the hyperosmolar blood. In agreement with this interpretation, Dickinson and Betz (1992) have shown an attenuated development of edema after focal ischemia in the Brattleboro rat lacking AVP and showing an increased serum osmolality.

The purpose of the present study was to investigate the effect of the kappa opioid receptor agonist niravoline on both serum osmolality and cortical water content after focal cerebral ischemia achieved by permanent occlusion of a MCA in the mouse; indeed, this model, and its equivalent in the rat, has become the reference model of thromboembolic stroke (Millikan, 1992; Hunter et al., 1995). The marked water diuretic activity of niravoline (Hamon et al., 1994) makes it a likely compound for increasing blood osmolality and therefore to show antiedema activity in the brain after focal cerebral ischemia. The kappa opioid receptor agonist U 50488 (Silvia et al., 1987) and mannitol were used as reference compounds. Moreover, because hypothermia reduces damage to the brain and especially edema (Minamisawa et al., 1990), the body temperature of the animals was controlled and maintained within physiological range throughout the experiments.

**Methods**

**Animals**
The experiments were carried out on male Swiss CD 1 mice (Charles River, Saint Aubin-les-Elbeuf, France) weighing 27 to 41 g.

**ABBREVIATIONS:** AVP, arginine vasopressin; BBB, blood-brain barrier; MCA, middle cerebral artery; MCAO, middle cerebral artery occlusion; mOsm/kg, milliosmole/kg.
The mice had free access to food and water before the start of the experiment.

Compounds

The compounds were administered as a single i.p. injection with a volume of 10 ml/kg. Niravoline [N-methyl-2-(3-nitrophenyl)-N-[(1S,2S)-2-(1-pyridinyl)-1-indany] acetamide monohydrachloride; RU 51599] (Clémente et al., 1989) and U 50488 (Vonvoigtlander et al., 1983), synthesized by Roussel-Uclaf as hydrochloride salt, were dissolved in distilled water. Mannitol (Fluka Chemie AG, Buchs, Switzerland) was dissolved in distilled water (serum osmolality was calculated after Mishima and Hossmann, 1992). The compounds were administered as a single i.p. injection with a volume of 10 ml/kg. Niravoline and U 50488; 3 min, 10 min, 30 min, 1 h, 3 h and 6 h for niravoline, U 50488 and mannitol on brain water content and serum osmolality measured at time t after onset of ischemia.

Time course of serum osmolality after administration of compounds in nonischemic mice. Groups of three or four mice were kept in the same box at laboratory temperature and deprived of water and food. The time lapse between compound administration and blood sampling was as follows: 30 min, 1 h, 2 h, 4 h and 6 h for niravoline and U 50488; 3 min, 10 min, 30 min, 1 h, 3 h and 6 h for mannitol. A vehicle group was included in each experiment. Niravoline and U 50488 were injected at the doses of 3, 10 and 30 mg/kg and mannitol was injected at the doses of 1 and 2 g/kg.

Time course of brain water content after focal ischemia. The time course of brain water content after focal ischemia was measured from 10 experiments without any treatment. Different times were established between MCAO or sham occlusion and measurements: 0 (immediately after occlusion; focal ischemia group only), 6 h, 24 h, 2 days, 3 days, 4 days, 7 days, 14 days and 28 days. In each experiment, 10 mice underwent focal ischemia and five were prepared as sham-operated. Between surgery and measurements, mice had free access to food and water.

Effect of niravoline, U50488 and mannitol on brain water content and serum osmolality in focal ischemia. Niravoline and U 50488 were administered i.p. at the doses of 1, 3, 10 and 30 mg/kg 20 h after occlusion (= 4 h before measurements) and mannitol was administered at the doses of 1 and 2 g/kg 23 h after occlusion (= 1 h before measurements); the time and dose administration schedules were constructed from data of the preliminary studies showing a peak of osmolality in nonischemic mice. When the compounds were administered, the mice had fully recovered from anesthesia. A group of ischemic control animals and a group of sham-operated animals were included in each experiment. Between surgery and measurements, mice had free access to food and water.

Techniques

Ischemia. Anesthesia was induced with chloral hydrate dissolved in distilled water at a dose of 400 mg/kg i.p. Each mouse was placed on its left side and maintained in this position by an in-house device. Under an operating microscope (M 690 Leica), a 3-mm vertical skin incision was made 2 mm behind the right orbit and just under the line joining the inferior part of the orbit and the base of the external ear. The temporal muscle was deflected with a forceps and, by use of a dental drill (12 220 Techdent), a small craniotomy was made at the point of the midline where the zygoma fuses to the temporal bone; the dura was incised and deflected and the distal part of the right MCA was exposed. Then, the artery was occluded just upstream to the main bifurcation by bipolar electrocoagulation (microcoagulator tb 20 Aesculap) with fine forceps (G 693 Aesculap whose tips had been sharpened to 150 μm). Mice with atypical MCA or hemorrhage subsequent to coagulation were excluded from the study. In sham animals, the MCA was exposed but not occluded; only a small coagulation of parenchyma adjacent to the artery was performed to mimic occlusion. The tissues were replaced and the skin was sutured. The mice recovered from anesthesia between 0.5 and 1 h after surgery.

Measurement of edema. Brain water content was obtained by the wet and dry method (weight difference between wet and dry samples). Because the occlusion of the distal part of MCA in the mouse leads to damage restricted to the frontoparietal cortex (Nowicki et al., 1991; Backhaus et al., 1992), brain samples were limited to this area. Twenty-four hours after artery occlusion, the brains were removed and placed on a cooled (approximately 0°C) metallic plate. On the right side (ipsilateral to occlusion), the cortical mantle was dissected and two sections of frontoparietal cortex of 30 to 50 mg each were taken: the first sample, corresponding to the core of ischemic tissue, was obtained from a section of 5-mm diameter from the cortex underlying the initial portion of the MCA; the second, corresponding to the periphery of ischemia, was sampled around the former with a 8-mm diameter section (fig. 1). The sections were put into preweighed borosilicated glass flasks (1538 45 Brand). The closed flasks were immediately weighted to obtain the wet tissue weight (wW). Then, the open flasks were placed in an oven at 100°C (Memmert U 30). Twenty-four hours later, the flasks were closed and weighed 1 h after removal from the oven to obtain the dry tissue weight (dW). The percentage of water was calculated according to the formula: (wW – dW)/wW × 100. The percentage of decrease in edema was calculated taking the difference between brain water contents of sham-operated and ischemia controls animals as 100%.

Serum osmolality. Mice were anesthetized with 4% isoflurane. After decapitation, the blood was collected in 1.5-ml polypropylene tubes. After centrifugation the serum was collected and osmolality was measured on a 20-μl volume (Fiske one-ten osmometer). When osmolality measurement was delayed, the sera were stored at 4°C until assay.

Body temperature. In all ischemia experiments, rectal temperature was measured by a rectal probe (Ret-3 Harvard Apparatus) and the ipsilateral temporal muscle temperature, which provides a reliable estimate of brain temperature in the course of an ischemic insult (Busto et al., 1987), was measured with a needle thermoprobe (MT-29/2 Harvard Apparatus); the two probes were connected to a thermometer (Bat-12 Sensortek). Rectal temperature was measured at a depth of 20 mm: this length was extrapolated from data as required for an accurate measurement of body temperature in the rat (Miyasawa and Hossmann, 1992).

After surgery, the mice were returned to their common box which

Fig. 1. Lateral view of the right hemisphere showing MCA distribution, occlusion site and localization of the two sites at which samples of cortex (core and periphery) were taken.
was placed in a hot water bath with thermostatic control. The box temperature was kept between 31 and 33°C for 24 h. After anesthesia (before surgery) and at various times during the recovery period (immediately and 1 h, 20 h and 24 h for niravoline and U 50 488; immediately and 1 h, 23 h and 24 h for mannitol), rectal temperature was monitored and maintained within normal limits by adjusting the temperature of the bath.

**Statistical analysis.** A two-way analysis of variance followed by a pairwise comparison (Tukey’s test) was performed to assess: 1) the effects of compounds vs. vehicle controls in nonischemic mice osmolality; 2, the effect of MCA occlusion vs. sham operation on core and periphery brain water contents; 3, the differences in both rectal and temporal muscle temperatures between the treated groups. Data of brain water content and serum osmolality in ischemic mice was analyzed with the nonparametric Mann-Whitney U test. For each test, the significant differences were represented by: * for .01 ≤ P < .05; ** for .001 ≤ P < .01; *** for P < .001.

**Results**

**Time Course of Serum Osmolality in Nonischemic Mice (fig. 2)**

**Niravoline.** Niravoline increased serum osmolality at the doses of 10 and 30 mg/kg with a maximum increase (3–4%) occurring 2 to 4 h after injection.

**U 50488.** Serum osmolality was increased at the doses of 10 and 30 mg/kg; it was increased at 2 h after 10 mg/kg and from 2 to 6 h after 30 mg/kg injection. The maximum increase (3–5%) occurred 2 h after injection.

**Mannitol.** At the dose of 1 g/kg, serum osmolality had a tendency to increase. At the dose of 2 g/kg, serum osmolality was significantly increased for 1 h, with a maximum reached rapidly, 10 min after injection (+3.9%).

In view of these results, and in the subsequent MCA occlusion studies, the two kappa agonists on the one hand, and mannitol on the other hand, were administered 4 h and 1 h before measurement, respectively.

**Time Course of Cerebral Edema in Focal Ischemia (fig. 3)**

Occlusion of the right MCA in the mouse was followed by a very good survival rate at 4 weeks (88 of 90 mice). In the sham-operated animals, the brain water content was between 78.61 ± 0.15% and 79.63 ± 0.24% and did not change with time either in the core or the periphery. In the MCA-occluded groups, the core water content was increased at 6 h (82.06 ± 0.68%) and progressively increased thereafter, reaching a maximum on the second day (84.92 ± 0.65%). Thus, the rate of water increase in the core was 0.141 ml/g dry wt/h during the first 6-h period and 0.043 ml/g dry wt/h from 6 to 24 h. On the third day, the core water content started to decrease, although it was still higher than preoperative values on the seventh day (80.51 ± 0.44%). In the periphery of the ischemic tissue, the increase in brain water content showed a parallel time course with a significant increase from 6 h (80.54 ± 0.35%) to 4 days (80.97 ± 0.35%) with a maximum reached on the second day (81.81 ± 0.49%); the rate of water increase was 0.038 ml/g dry wt/h during the first 6-h period and 0.012 ml/g dry wt/h from 6 to 24 h. These data show that both the rate of water increase and the brain water content were about three times lower in the periphery than in the core. Given these results, it was decided for the subsequent studies to measure brain water content 24 h after occlusion when edema is submaximal.

**Effect of Niravoline and U 50488 on Cerebral Edema and Serum Osmolality in Ischemia**

When administered at 3, 10 or 30 mg/kg, niravoline and U 50488 reduced cortical brain water content in the periphery of ischemic tissue and increased the serum osmolality by 4 to 10 mOsm/kg (fig. 4). At the dose of 30 mg/kg, the effect on water content was close to being significant for niravoline (P < .01). The maximum decrease in brain water content in the periphery of ischemic tissue was obtained at the dose of
10 mg/kg for niravoline (−48%) and at the dose of 30 mg/kg for U 50488 (−43%). In the core, the brain water content showed a trend toward decrease (−9 to −21%), and the only significant decrease was observed for the dose of 3 mg/kg of U 50488 (−20% P < .01; results not shown). The doses of 1 mg/kg were found to be inactive for both compounds.

Warming the mice during surgery and recovery allowed rectal and temporal muscle temperatures to be maintained within physiological range; extreme mean rectal and temporal muscle temperatures were, respectively, 36.7 ± 0.1 to 37.9 ± 0.1°C and 36.7 ± 0.1 to 37.4 ± 0.1°C. No difference was found between ischemia control and treated groups.

Effect of Mannitol on Cerebral Edema and Serum Osmolality in Ischemia (table 1)

Mannitol (1 and 2 g/kg), administered 23 h after onset of ischemia, increased serum osmolality (1.2 and 3.3% respectively, compared with ischemia controls) but did not significantly modify the cortical brain water content in either the periphery or the core. Rectal and temporal muscle temperatures of the different groups did not differ (results not shown).

Discussion

Time course of brain edema. Negligible postoperative mortality has allowed us to measure brain water content for 4 weeks after onset of ischemia. Up to now, the time course of brain edema after permanent focal cerebral ischemia had been studied on one brain sample only (Hatashita and Hoff, 1990); we decided to define two zones of ischemic tissue, one corresponding to the core, which undergoes infarction within 3 to 4 h, and the second we called periphery (Obrenovitch, 1995). The size and location of the core sample were chosen to represent brain tissue that was always infarcted: they were extrapolated from the cortical infarct surface (31.8 ± 1.3 mm², mean ± S.E.M. of 24 mice) found in ischemia control mice in a parallel study with tryphenyl tetrazolium chloride and the same model. A ring sample of 30 mm² was chosen around the core sample to represent the less densely ischemic tissue perfused by collaterals. The data of the present study demonstrate that the permanent occlusion of a MCA in the mouse leads to an increase in water content about three times greater in the core than in the periphery of ischemic tissue; it increased during the first 2 days before returning close to preoperative values 7 days (periphery) and 14 days (core) after occlusion. This result is in accordance with the finding

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<th>Table 1 Effect of mannitol in ischemic mice*</th>
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<td>Dose (g/kg)</td>
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<td>a. Brain water content (%)</td>
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<td>b. Serum osmolality (mOsm/kg)</td>
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* Mannitol was administered i.p. 23 h after onset of ischemia and measurements made at time 24 h. Brain water was measured at 24 h in the core (C) and the periphery (P) of the ischemic tissue. Mean ± SEM of 11 to 14 animals per group. Mann-Whitney U test vs. ischemia controls: * P < .05; *** P < .001.
that the amount of edema fluid is all the greater as the cerebral blood flow is lower (Martz et al., 1990).

The kinetic profile of ischemic brain edema in the mouse was similar to that found after occlusion of a MCA in the rat (Hatashita and Hoff, 1990) and in the cat (O’Brien et al., 1974); moreover, the rate of increase in water in the ischemic core for the first 6 h of ischemia (0.14 ml/g dry wt/h) was the same as the rate found after a proximal occlusion of the MCA in the rat (0.15 ml/g dry wt/h; Betz et al., 1994). Because the formation of ischemic brain edema depends primarily on the degree and duration of ischemia and ultimately on the infarct size (Astrup et al., 1981), the amount of edema found in this study is in accordance with the presence (in a parallel study with the same model) of a marked infarct size (29.6 ± 3.1 mm³ mean ± S.E.M. of 16 mice) which squares with 19% of the ipsilateral hemisphere volume. This also corresponds to findings from others showing the presence of a substantial cortical infarct after focal cerebral ischemia in the mouse (Novicki et al., 1991; Backhauß et al., 1992).

**Effect of niravoline on brain edema.** The purpose of the study was to evaluate the effectiveness of niravoline as an antiedema agent after permanent MCAO in the mouse. In this regard, a single i.p. injection of niravoline 20 h after the beginning of ischemia, that is at a time when edema fluid continues to accumulate in the ischemic tissue, decreased the amount of brain edema 4 h later. This effect was patent in the periphery of ischemic tissue, whereas no decrease was observed in the core. Although the protection with a kappa opioid agonist has already been shown in cerebral edema after global ischemia (Silvia et al., 1987), this is the first demonstration of the efficacy of such a compound in focal ischemia. Brain edema is a common histopathologic response to focal cerebral ischemia. In addition to the severity of ischemia, brain accumulation of water can be modulated by various systemic parameters; once the barrier is open, hydrostatic pressure becomes an additional important driving force (Cole et al., 1990; Valtysson et al., 1992) which favors the movement of solutes and water into the brain and modulates the amount of brain edema. Finally glycemia and the associated brain lactic acid level can modulate the severity of ischemia (Marie and Bralet, 1991; Courten-Myers et al., 1994). The neuroprotective effect of kappa opioid agonists has been well established in focal cerebral ischemia (Birch et al., 1991; Mackay et al., 1993; Baskin et al., 1994); however, the decrease in brain edema observed in the present study with niravoline cannot be caused by a decrease of lesion size because the time of injection (20 h after onset of ischemia) is far beyond the end of the therapeutic window, which is about 3 h in rodents (Slivka et al., 1995); consequently, the ischemic tissue at that time is irreversibly damaged (Garcia et al., 1995). Furthermore, pharmacological properties of niravoline and especially the absence of hypoglycemic and of appreciable hemodynamic effects (Hamon et al., 1994) permit the exclusion of a major systemic effect of niravoline in the observed reduction of brain edema.

Two possible mechanisms of action can be used to explain the decrease in brain water content observed in this study with niravoline: either a decrease in brain capillary permeability or an enhanced osmotic gradient across the BBB. Both putative mechanisms are thought to originate from the stimulation of kappa opioid receptors and subsequent decrease of AVP release. Indeed, kappa opioid agonists induce inhibition of AVP secretion by acting at receptors within the BBB, possibly in the paraventricular and/or supraoptic nuclei (Oiso et al., 1988; Brooks et al., 1993). Centrally released AVP has been implicated in the regulation of brain fluid balance under both physiological and pathological conditions; intraventricular administration of AVP increased brain capillary permeability (Raichle and Grubb, 1978) and brain water content (Dóczy et al., 1982). Elevated AVP levels are reported with raised intracranial pressure from various causes including stroke and subarachnoid hemorrhage (Mather et al., 1981; Sorensen et al., 1984). Finally, AVP receptor antagonists are known to reduce brain edema after cold injury or hemorrhage (Nagao et al., 1994; Rosenberg et al., 1992). Because the selective kappa opioid agonist niravoline induces a decrease in AVP plasma levels (Hamon et al., 1994), a decreased brain capillary permeability may well have participated in the observed decrease in edema. However, in our study, the injection of niravoline was delayed 20 h after artery occlusion, that is only 4 h before measurements at a time when edema is submaximal; so it is unlikely that a decrease in brain capillary permeability may account for the totality of the antiedema effect. In addition to a putative effect on brain capillary permeability, a decrease in AVP levels is also known to induce a water diuretic effect and an increase of blood osmolality (Silvia et al., 1987). In this latter study, the dose-related increase in urinary output was accompanied by a marked decrease in urine osmolality with no modification of electrolyte excretion, and these were blocked by naloxone, which indicates that the effects are mediated by opioid receptors. In the present study, niravoline decreased ischemic focal cerebral edema at doses (3, 10 and 30 mg/kg) which also increased AVP levels, had no effect on brain water content. These results suggest that a hyperosmotic mechanism was at the origin of the antiedema effect. Blood hyperosmolality is believed to enable the excess brain water to reach the blood compartment and subsequently to decrease brain edema. Conversely, blood-to-brain Na⁺ transport, which is fueled by the remaining capillary Na⁺/K⁺-ATPase activity (Betz et al., 1994), is limited by energetic failure and consequently is not likely to mitigate the brain-to-blood Na⁺ movement.

The blood osmotic effect of mannitol was similar to that produced by niravoline and U 50488. However, beyond its diuretic effect, blood hyperosmolality observed after mannitol is caused by its own presence in the blood compartment. For the first hours after a MCA occlusion, BBB is intact and edema is mainly intracellular, but after 12 h of focal cerebral ischemia, the vasogenic component of edema grows with an increasing BBB permeability to serum protein or smaller molecules (Hatashita and Hoff, 1990). In these conditions, when injected 23 h after onset of ischemia, mannitol could leak into the extravascular compartment, with no resulting osmotic gradient across BBB despite the blood osmolality increase. This would explain its absence of effect in our experimental conditions and also its contraindication clinically in constituted edema. Therefore, our data indicate that hyperosmolality per se is not sufficient for reduction of edema because mannitol is ineffective. We cannot, however, completely exclude the need for a longer interval of time (50 min in our experiments) between the peak of increased plasma...
osmolality and the measure of brain water content to demonstrate the activity of mannitol.

In conclusion, these results show that the kappa opioid agonist naloxone reduces brain edema when injected in conscious mice, 20 h after onset of ischemia, once edema of a vasogenic type is developing. A similar protective effect was observed with the kappa opioid receptor agonist U 50488. These effects are thought to arise from the increase in serum osmolality subsequent to water diuresis leading to the creation of an osmotic gradient across the BBB that would facilitate edema resorption. On the other hand, the delayed increase of BBB permeability with subsequent leakage of mannitol in the extravascular space could explain the inefficacy of this hyperosmolar agent in constituted edema. The use of kappa opiate agonists in the treatment of cerebral edema of the focal ischemia type could be an alternative to hyperosmotic agents, the use of which is limited to the early phase of cerebral edema when the BBB is still intact.

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References


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