Potential Protection by a Specific Kappa-opiate Agonist U-50488H Against Membrane Failure in Acute Ischemic Brain

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Abstract

The effects of a novel opioid κ-receptor agonist U-50488H on Na⁺-K⁺-adenosine triphosphatase (ATPase) activity and regional cerebral blood flow (rCBF) were studied in the acute ischemic brain of rats after middle cerebral artery (MCA) occlusion. Administration of U-50488H 15 minutes prior to MCA occlusion attenuated ischemic reduction in Na⁺-K⁺-ATPase activity 15 minutes after MCA occlusion. The effect was statistically significant at a dosage of 30 mg/kg, but not at lower doses (0.3 and 3 mg/kg). There was no effect on rCBF before MCA occlusion, and the decreased flow after occlusion was enhanced with a significant fall in systemic blood pressure at a dosage of 30 mg/kg. These results indicate that U-50488H has therapeutic potential in cerebral ischemia by mechanisms other than improvement in CBF.

Key words: opioid agonist, opioid receptor, Na⁺-K⁺-ATPase, cerebral ischemia

Introduction

Opioids acting in the central nervous system may affect the pathophysiology of cerebral ischemia. At present, the opioid receptors are classified into three types, μ, δ, and κ. The author previously suggested that β-endorphin, an endogenous μ-agonist, potentiates ischemic cerebral damage, consistent with the proposed therapeutic effect of μ-antagonist naloxone in stroke. In contrast, activation of κ-receptors may alleviate ischemic damage. Administration of dynorphin, an endogenous κ-agonist, markedly prolonged the survival of cats with focal cerebral ischemia. Our recent study also suggested that activation of μ- or κ-receptors causes opposite effects on the membrane function in ischemic brain. These results encouraged the investigation of κ-agonist as a possible therapeutic agent for cerebral ischemia.

U-50488H is a novel κ-agonist with greater selectivity than other κ-agonists such as ketazocine, ethylketocyclazocine, and dynorphin. This study investigated the effects of various doses of U-50488H on cerebral Na⁺-K⁺-adenosine triphosphatase (ATPase) activity, systemic blood pressure, and regional cerebral blood flow (rCBF) in rats after occlusion of the middle cerebral artery (MCA).

Materials and Methods

Male Wistar rats, weighing between 250 and 300 gm, were anesthetized with ketamine hydrochloride (150 mg/kg) and underwent tracheostomy. Systemic blood pressure was monitored from the tail by the photoelectric oscillometric method using a high response sensor (model UR-5000; Ueda Electric Works, Tokyo). The rats were placed in the lateral position. Subtemporal craniectomy exposed the MCA. A small burr hole was made in the ipsilateral skull for a bipolar electrode, which generated hydrogen gas by electrolysis of the brain tissue. The hydrogen clearance was monitored using a flow meter (model DHM-3001; MT Engineering, Tokyo). The electrode was implanted into the parietal cortex to about 3 mm from the brain surface. The rCBF was calculated from the hydrogen clearance curve.

After basal rCBF was determined, the rats were given a single intraperitoneal injection of 0.5 ml saline or U-50488H in saline (0.3, 3, and 30 mg/kg). The rCBF was measured 15 minutes later and then the unilateral MCA was coagulated between the

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rhinocortical and lenticulostriate arteries with bipolar forceps. The rCBF was measured after 15 minutes of MCA occlusion. The brain was then rapidly removed, separated into ischemic and non-ischemic hemispheres, and kept frozen at −70°C for assay of Na⁺-K⁺-ATPase activity.

The activity of Na⁺-K⁺-ATPase was determined by a modified Fiske and Subbarow method. Each hemisphere was homogenized in 10 ml of 25 mM Tris-HCl buffer containing 0.32 M sucrose (pH 7.4). 200 μl of homogenate was incubated at 37°C in a total 1 ml of this same buffer containing 100 mM NaCl, 30 mM KCl, 25 mM MgCl₂, and 3 mM 2Na adenosine triphosphate (ATP). Reaction was started by addition of ATP and terminated by addition of sodium dodecyl sulfate. Inorganic phosphorus released from the ATP was stained with ammonium molybdate and 1,2,4-aminonaphtol sulfonic acid and measured by calorimetry. Na⁺-K⁺-ATPase activity was calculated as the difference in inorganic phosphorus between reaction mixtures with and without 1 mM ouabain. Protein in the homogenate was measured by the method of Lowry et al. Na⁺-K⁺-ATPase activity was expressed as μmole Pi/mg protein/10 min.

Changes in mean arterial blood pressure (MABP), rCBF, and Na⁺-K⁺-ATPase activity were analyzed by the paired t-test in each group and with the unpaired t-test between groups.

Results

Table 1 lists the measurements of Na⁺-K⁺-ATPase activity. The control group receiving saline only demonstrated lower activity in the ischemic hemisphere than in the contralateral non-ischemic hemisphere (p < 0.1). The group receiving 0.3 mg/kg U-50488H also showed reduced activity in the ischemic hemisphere (p < 0.1). However, groups receiving 3 or 30 mg/kg U-50488H showed no reduction in Na⁺-K⁺-ATPase activity in the ischemic hemisphere. Na⁺-K⁺-ATPase activity in the group receiving 30 mg/kg was rather higher in the ischemic hemisphere than in the contralateral hemisphere, although there was no significant difference. To assess the possibility of U-50488H inhibiting the ischemic reduction in Na⁺-K⁺-ATPase activity, ratios of activity on the ischemic side to the contralateral side were evaluated (Fig. 1). The ratio rose as the dosage was increased. 30 mg/kg U-50488H significantly increased the ratio compared to the con-

Table 1 Effects of U-50488H on Na⁺-K⁺-ATPase activity in the brain with unilateral MCA occlusion

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Na⁺-K⁺-ATPase activity (μmole Pi/mg protein/10 min)</th>
<th>A/B (%)⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ischemic hemisphere (A)</td>
<td>Non-ischemic hemisphere (B)</td>
</tr>
<tr>
<td>Saline</td>
<td>5</td>
<td>1.72 ± 0.24*</td>
</tr>
<tr>
<td>U-50488H</td>
<td>4</td>
<td>1.87 ± 0.17*</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.86 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.06 ± 0.11</td>
</tr>
</tbody>
</table>

Values are means ± SEM. *p<0.1: trend toward reduction as compared to values in the corresponding contralateral hemisphere. Comparisons among groups are shown in Fig. 1.
Protection by U-50488H Against Ischemic Membrane Failure

Table 2 Effects of U-50488H on MABP before and after ischemia

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>MABP (mmHg)</th>
<th>B/A (%)</th>
<th>C/A (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment (A)</td>
<td>15 min after treatment (B)</td>
<td>15 min after MCA occlusion (C)</td>
</tr>
<tr>
<td>Saline</td>
<td>123.1 ± 12.5</td>
<td>123.1 ± 11.8</td>
<td>120.0 ± 14.0</td>
</tr>
<tr>
<td>U-50488H</td>
<td>0.3 mg/kg</td>
<td>127.0 ± 14.9</td>
<td>126.0 ± 14.2</td>
</tr>
<tr>
<td></td>
<td>3 mg/kg</td>
<td>122.3 ± 13.9</td>
<td>115.3 ± 9.0</td>
</tr>
<tr>
<td></td>
<td>30 mg/kg</td>
<td>118.0 ± 7.4</td>
<td>98.3 ± 4.4</td>
</tr>
</tbody>
</table>

Values are means ± SEM. *p<0.05: significant difference from each basal value. **p<0.01: comparisons among groups are shown in Fig. 2.

Table 2 shows the MABP. Figure 2 gives percentage change from basal level in each group for easy comparison among groups. The MABP was significantly affected before and after MCA occlusion only by 30 mg/kg U-50488H, when MABP decreased compared to the control value (p < 0.1) before MCA occlusion and was significantly lower than the basal (p < 0.05) and control values (p < 0.01) after MCA occlusion.

rCBF showed no significant change 15 minutes after U-50488H administration before MCA occlusion in all groups compared to both basal and control values (Table 3, Fig. 2). MCA occlusion reduced rCBF to a level significantly lower than the basal level in the control group (p < 0.005) and U-50488H groups (0.3 and 3 mg/kg, p < 0.025; 30 mg/kg, p < 0.01). These rCBF reductions, however, were not significant between groups. Only the rCBF in the group with 30 mg/kg was reduced compared to the control group (p < 0.1).

Discussion

Na⁺-K⁺-ATPase, an integral part of the sodium pump, is vital for the regulation of the ionic and water balance between the inside and outside of the cell, and is closely involved in the pathophysiology of cerebral ischemia. In ischemic brain, energy depletion and peroxidation of phospholipids in the cell membrane inactivate the Na⁺-K⁺-ATPase enzyme, resulting in failure to pump out Na⁺ into the extracellular compartment with consequent retention of extracellular K⁺. Simultaneously, lactic acidosis resulting from insufficient oxidation of pyruvate also raises the intracellular Na⁺ level in exchange for H⁺. This disturbance of the Na⁺-K⁺-equilibrium induces edema and opens voltage-sensitive calcium channels, facilitating ischemic cell damage. Our finding that U-50488H attenuates the ischemic impairment.
of Na\(^+\)-K\(^+\)-ATPase is important for therapy of cerebral ischemia, and may explain the cerebroprotection achieved in earlier studies.\(^{15,16,26,37}\) The effective dose (30 mg/kg) in our study was similar to the dosage which manifested the cerebroprotective effects in other studies.\(^{15,16,26,32,37}\)

Table 3 Effects of U-50488H on rCBF before and after ischemia

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>rCBF (ml/100 gm brain/min)</th>
<th>B/A (%)</th>
<th>C/A (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment (A)</td>
<td>15 min after treatment (B)</td>
<td>15 min after MCA occlusion (C)</td>
</tr>
<tr>
<td>Saline</td>
<td>3</td>
<td>58.7 ± 1.9</td>
<td>56.5 ± 2.3</td>
</tr>
<tr>
<td>U-50488H</td>
<td>3</td>
<td>57.4 ± 1.1</td>
<td>56.0 ± 1.5</td>
</tr>
<tr>
<td>0.3 mg/kg</td>
<td>3</td>
<td>51.1 ± 3.3</td>
<td>44.6 ± 2.9</td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>3</td>
<td>59.3 ± 0.9</td>
<td>52.0 ± 4.6</td>
</tr>
</tbody>
</table>

Values are means ± SEM. *p<0.025, **p<0.01, ***p<0.005: significant difference from each basal value. +Comparisons among groups are shown in Fig. 2.

However, the effect of U-50488H may not be primarily responsible for such cerebroprotection directly but has other beneficial effects, resulting in protection of the cell membrane. U-50488H has a diuretic action, partially antagonized by antidiuretic hormone.\(^{34}\) Silvia et al.\(^{35}\) attributed the cerebroprotective effect to this because the water content was significantly reduced in the ischemic brain of rats treated with this compound, concomitant with increased plasma osmolarity.

Excessive release of excitatory neurotransmitters, probably glutamate, results in neuronal damage due to increased Ca\(^++\) influx, mainly through channels linked with glutamate receptors.\(^{6,24,29}\) Such neurotoxicity under ischemic conditions is blocked by N-methyl-D-aspartate receptor antagonists.\(^{21,27,33}\) Calcium channel blockers such as nicardipine also reduce ischemic brain damage.\(^{1,14}\) Recently, investigators have reported that U-50488H inhibited glutamate release\(^{33}\) and Ca\(^++\) influx in the cerebral synaptosome.\(^{4,40}\) Excess intracellular Ca\(^++\) leads to neural damage by a variety of mechanisms, including breakdown of membrane phospholipids incorporating Na\(^+\)-K\(^+\)-ATPase which is initiated by activation of phospholipase A\(_2\).\(^{5,25,31}\) Prevention of ischemic impairment of Na\(^+\)-K\(^+\)-ATPase by U-50488H may be secondary to balancing the deacylation with energy-absorbing reacylation of phospholipids. The resultant reduction in arachidonic acid release may also preserve Na\(^+\)-K\(^+\)-ATPase, because activity of this enzyme is inhibited by addition of arachidonic acid to incubated rat brain.\(^{5}\)

Our study found that the cerebroprotective action of U-50488H against ischemic impairment of Na\(^+\)-K\(^+\)-ATPase activity was not due to changes in rCBF. rCBF during ischemia was not improved and even decreased at the dosage of 30 mg/kg, probably because of the fall of systemic blood pressure. This result agrees with Hall et al.\(^{40}\) who found no significant alteration in CBF to account for enhanced neurological recovery in a mouse acute head injury model. McIntosh et al.\(^{23}\) reported that increased dynorphin immunoreactivity in traumatic cat brain correlated well with the degree of posttraumatic hypotension. A study of the cardiovascular properties of opioid peptides showed that dynorphin caused a decrease in systemic blood pressure in anesthetized rats.\(^{38}\) There is little doubt that changes in CBF are not involved in the protective action of U-50488H against ischemic brain damage.

A few feasible mechanisms for the protective effect of U-50488H against ischemic impairment of Na\(^+\)-K\(^+\)-ATPase were discussed above, but further studies...
are required to determine whether the effect is primary or secondary. However, this κ-agonist does have a beneficial action on membrane function.

Acknowledgments

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