Quantitative Analysis of Effects of \(\kappa\)-Opioid Agonists on Postischemic Hippocampal \(\text{CA}_1\) Neuronal Necrosis in Gerbils

Edward D. Hall, PhD, and Kay E. Pazara, BA

The ability of the \(\kappa\)-opioid receptor agonists U50488H and U62066E (spiradoline mesylate) compared with the non-\(\kappa\) close structural analogue U54494A to affect postischemic necrosis of the selectively vulnerable hippocampal \(\text{CA}_1\) neurons was examined in male Mongolian gerbils. The gerbils were treated with either saline vehicle or 10 mg/kg i.p. of one of the test drugs 30 minutes before and again 2 hours after a 10-minute period of bilateral carotid artery occlusion or sham occlusion under light methoxyflurane anesthesia. Seven days after ischemia and reperfusion the brains were perfusion-fixed, and hippocampal \(\text{CA}_1\) cells were counted in a blind fashion. In ischemic gerbils that received only vehicle, there was a 78.9% loss of \(\text{CA}_1\) neurons compared with sham-occluded gerbils. In contrast, in U50488H-treated gerbils, mean cell loss was reduced to 33.9% (\(p<0.01\) vs. vehicle-treated group). U62066E was even more effective in reducing postischemic \(\text{CA}_1\) degeneration to only 20.7% (\(p<0.0001\) vs. vehicle-treated group).

However, treatment with the non-\(\kappa\) analogue U54494A did not cause any apparent protection; the gerbils in this group showed an 80.7% loss of \(\text{CA}_1\) neurons. Our results are consistent with the hypothesis that \(\kappa\)-receptor stimulation is associated with improved postischemic neuronal preservation. (Stroke 1988;19:1008-1012)

Following the empirical demonstration of a beneficial effect of the general opiate receptor antagonist naloxone in experimental cerebral ischemia in gerbils,\(^1\) rats,\(^2\) cats,\(^3\) and primates,\(^4,5\) considerable interest has developed concerning the possible role of endogenous opioids in ischemic pathophysiology and neuronal degeneration. Based on the anti-ischemic action of naloxone, an hypothesis has been developed that postischemic neuronal degeneration may in part depend on an excessive activation of endogenous opioid systems, which could be therapeutically antagonized with opiate receptor blocking drugs such as naloxone or naltrexone.

However, some investigators have failed to observe a positive effect of naloxone in their particular ischemia models.\(^6,7\) More recent studies have suggested that pharmacologic stimulation of the \(\kappa\)-opioid receptor subtype can actually ameliorate rather than exacerbate postischemic neuronal degeneration and promote neurologic recovery and survival. The initial study in this regard showed that...

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From the Central Nervous System Diseases Research Unit, The Upjohn Company, Kalamazoo, Michigan.
Address for correspondence: Edward D. Hall, PhD, 7251-209-4, The Upjohn Company, 301 Henrietta Street, Kalamazoo, MI 49001.
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logue of U50488H, and U54494A, a non-κ structural analogue, are compared with those of U50488H.

Materials and Methods

Male Mongolian gerbils weighing 50–70 g were anesthetized with methoxyflurane. A 1–2 cm midline throat incision provided access to both carotid arteries while causing minimal tissue damage. After the arteries were located, they were loosely encircled with silk thread to facilitate occlusion with microaneurysm clamps. Thirty minutes before occlusion, intraperitoneal injections of 0.2-ml volumes of either vehicle (0.9% saline) (18 gerbils) or 10 mg/kg U50488H (8 gerbils), U62066E (9 gerbils), or U54494A (7 gerbils) were administered. Following 10 minutes of bilateral carotid occlusion (BCO) the clamps were removed, reperfusion was verified, and the wounds were closed with silk thread. Two hours after reperfusion the gerbils were injected a second time. Seven sham-occluded gerbils underwent the same anesthesia and surgical procedures without BCO.

One week after BCO, the gerbils were anesthetized again with methoxyflurane and the brains were fixed via cardiac perfusion with a combination of 10% formaldehyde, 10% acetic acid, and 80% methanol (FAM) following a saline flush. Immediately following perfusion, the brains were removed, placed in FAM overnight, and then embedded in paraffin. Five-micrometer-thick cross sections were taken between 1.4 and 3.0 mm posterior to the bregma, stained with cresyl violet, and examined by light microscopy at x 320 magnification. Slide labels were covered with tape to enable blind evaluation. All normal-appearing pyramidal cells in a 315-μm length of the CA1 region of the dorsal hippocampus were counted bilaterally and averaged. Moreover, two sections were examined in each gerbil, and the cell counts were averaged. Vehicle-treated and drug-treated gerbils were compared using a one-way analysis of variance or the χ² test.

Results

All ischemic gerbils (vehicle-treated and drug-treated) survived to the Day 7 histologic assessment. The 10-minute BCO produced a selective decrease in hippocampal CA1 neurons, with other areas of the hippocampus and other brain regions unaffected. This selective vulnerability of the CA1 subfield to a brief period of ischemia is identical to that described earlier by Kirino. Interestingly, in five of 18 (27.8%) vehicle-treated ischemic gerbils, the CA1 cell loss, while apparent on both sides of the brain, was quite asymmetric (i.e., difference of ≥20 cells between the hemispheres), which has not been noted previously in this model. Moreover, no asymmetry was observed in the sham-occluded gerbils.

Figure 1 shows a comparison of 1-week postischemic hippocampal CA1 cell counts in sham-occluded and vehicle- and drug-treated gerbils subjected to a 10-minute BCO. In gerbils that received only vehicle, there was a 78.9% decrease in number of CA1 neurons compared with sham-occluded gerbils. The postischemic cell loss appeared to have proceeded to completion since the cells that remained appeared normal. In the U50488H-treated group, the mean decrease was only 33.9% (p<0.01 vs. vehicle-treated group). U62066E was even more effective as it reduced the cell loss to only 20.7% (p<0.0001 vs. vehicle-treated group). The asymmetry in the CA1 population seen in the vehicle-treated gerbils was observed in only one of the U50488H-treated gerbils and in none of the U62066E-treated gerbils.

Table 1 indicates that 75.0% of the U50488H-treated and 88.9% of the U62066E-treated gerbils had >50% CA1 cell survival compared with only 16.7% of the vehicle-treated gerbils. Figure 2 presents representative photomicrographs of vehicle-treated and U50488H-treated brain sections. In contrast to the protective effects of the selective κ-opioid receptor agonists U50488H and U62066E, the close structural analogue U54494A (compare structures in Figure 1), which lacks the κ-opioid agonist property, failed to protect the CA1 neurons from postischemic degeneration (Figure 1, Table 1).

Discussion

Our results confirm the previous observation that the selective κ-opioid receptor agonist U50488 (H salt in the current study) can significantly protect the selectively vulnerable hippocampal CA1 region from postischemic neuronal degeneration second-ary to a brief nonlethal episode of global brain ischemia. The probability that this anti-ischemic effect is related to U50488H's κ-opioid agonist properties is supported by the slightly greater protective effect of the more potent U50488H analogue U62066E. Furthermore, if the structure of U50488H is altered slightly but to the point of destroying the κ-opioid agonism, then the anti-ischemic action disappears. This is clear in the lack of effect of the non-κ compound U54494A. In a similar fashion, Tang showed that the more κ-active levo-enantiomer of the racemic U50488 is much more effective than the less κ-active dextro-enantiomer in protecting gerbils from postischemic hyperactivity.

The precise mechanism of the apparently κ-opioid receptor-mediated cerebroprotective action of U50488H and U62066E is still uncertain although three distinct possibilities can be gleaned from related cerebral ischemia and trauma studies. First, the original rationale for testing U50488H was the expectation that the diuretic action of this as well as other κ-opioid agonists would antagonize ischemic cerebral edema. Indeed, in a second more recent study, Tang and colleagues have shown that U50488H reduces ischemic edema in Fischer rats subjected to 4 hours of BCO. This reduction in edema occurs with an increase in plasma hyperosmolarity due to water diuresis. Conversely, the
administration of antidiuretic hormone prevents the plasma hyperosmolar action of U50488H as well as the reduction of ischemic cerebral edema. Thus, it is conceivable that the protection of the gerbil CA1 region from degeneration could be in part the result of a κ-opioid-mediated water diuresis and a possible reduction of cellular edema in the peculiarly susceptible CA1 pyramidal neurons. Tang et al. in fact noted greater swelling of the CA1 cells in untreated than in U50488H-treated gerbils 24 hours after ischemia (i.e., prior to actual degeneration), which is consistent with this possibility.

A second candidate mechanism concerns the purported role of an excitatory amino acid-induced calcium influx in the susceptibility of the hippocampal CA1 region to brief periods of ischemia. A recent study strongly supports the involvement of disturbed calcium homeostasis in postischemic damage to the gerbil CA1 region. In this regard, U50488H has been shown to antagonize excitatory amino acid (e.g., kainate, N-methyl-D-aspartate, and quisqualate)-triggered seizures in mice and to decrease potassium-induced calcium uptake by rat brain synaptosomes. However, the likelihood that these effects are related to the anti-ischemic action of U50488H (or U62066E) seems remote since they are also shared by the non-κ analogue U54494A, which was completely ineffective in retarding postischemic CA1 degeneration.

A third potential contributing factor in the antischismic action of κ-opioid agonists has to do with the demonstrated effect of U50488H to significantly attenuate posttraumatic hypoperfusion as shown in injured mouse brain and cat spinal cord. A similar improvement in blood flow maintenance by the κ-opioid agonists after reperfusion of the ischemic gerbil brain could serve to promote postischemic neuronal survival. However, this possible scenario seems of little importance to our current protection of the CA1 region since brain hypoperfusion does not occur in the gerbil brain after only brief periods of ischemia.

**Figure 1.** Comparison of hippocampal CA1 neuronal counts 1 week after 10-minute bilateral carotid artery occlusion in male Mongolian gerbils. U54494A is a non-κ analogue of κ-opioid agonists U50488H and U62066E. Cells were counted at ×320 over 315-μm length of CA1 region.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>18</td>
<td>3.0</td>
</tr>
<tr>
<td>U50488H</td>
<td>8</td>
<td>75.0</td>
</tr>
<tr>
<td>U62066E</td>
<td>9</td>
<td>88.9</td>
</tr>
<tr>
<td>U54494A</td>
<td>7</td>
<td>14.3</td>
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Probability assessed using χ² compared with vehicle.
Thus, of the three mechanisms discussed, the most tenable explanation for the antagonism of delayed postischemic CA, degeneration by U50488H and U62066E appears to be a reduction in cellular edema secondary to a κ-opioid receptor-mediated diuretic action as proposed by Tang and coworkers. This apparent inhibition of cellular edema is probably complex in that other antiedema agents such as the glucocorticoid steroids can actually exacerbate postischemic CA, necrosis. Such an effect has been reported for corticosterone pretreatment of rats subjected to 20 minutes of severe forebrain ischemia. Furthermore, we have observed that high-dose treatment of gerbils with methylprednisolone significantly increases CA, neuronal loss in the currently employed model (E.D. Hall and K.E. Pazara, unpublished observations).

In addition to a complex antiedema effect, a direct protective action of the κ-opioid agonists on the hippocampal CA, region should not be dismissed. A recent report showed that concentrations of the endogenous κ-opioid agonist peptide dynorphin in the gerbil hippocampus, which are higher than in other brain regions except the hypothalamus, decrease by approximately 50% and remain decreased for at least a week after a 5-minute episode of BCO. The decrease in dynorphin precedes the onset of ischemic cell change in the CA, subfield. It is tempting to speculate from this that the partial loss of dynorphin in some way contributes to the degenerative pathophysiology and that administration of an exogenous κ-opioid receptor agonist serves to restore protection in a replacement therapy fashion. Confirmation of this possible
direct effect as well as the indirect diuresis-induced reduction of cellular edema requires further study.

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KEY WORDS • cerebral ischemia • hippocampus • endorphins • gerbils
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E D Hall and K E Pazara

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