(S')-Emopamil Protects Against Global Ischemic Brain Injury in Rats

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(S')-Emopamil is a novel calcium channel blocker of the phenylalkylamine class, with potent serotonin S2 antagonist activity. We investigated the effect of (S')-emopamil on the histopathologic consequences of global brain ischemia in anesthetized rats. Pretreated rats (n=15) received 20 mg/kg i.p. (S')-emopamil 30 minutes before and 2 hours following 10 minutes of bilateral common carotid artery occlusion plus arterial hypotension (50 mm Hg). Quantitative cell counts following 3 days' survival revealed a marked loss of pyramidal neurons in all subsectors of the hippocampal CA1 area of untreated ischemic rats (n=15). In contrast, in (S')-emopamil pretreated rats numbers of normal neurons were significantly higher, by 2.4-, 1.9-, and 1.8-fold, respectively, in the medial, middle, and lateral subsectors of the CA1 area. For example, normal neuron counts in the medial CA1 subsector were 34±9 (mean±SEM) in untreated ischemic rats compared with 82±13 in (S')-emopamil pretreated rats (control nonischemic value [n=5] 157±2). By semiquantitative grading, (S')-emopamil also decreased ischemic changes in the cerebral cortex. No significant effect of (S')-emopamil on ischemic injury was detected in rats treated beginning 30 minutes after the ischemic insult (n=10). Thus, pretreatment with (S')-emopamil is beneficial in decreasing the severity of neuronal injury in global brain ischemia. (Stroke 1990;21:1734-1739)

As ischemic cerebrovascular disease is a major source of death and disability throughout the civilized world, the development of therapeutic means of protecting the brain from ischemic damage represents a clinical challenge of great importance.1 Calcium channel blockers have attracted wide attention as possible therapeutic agents in brain ischemia, but conflicting studies continue to appear.2,3 (S')-Emopamil is a novel compound of the phenylalkylamine class of calcium channel blockers having exceptionally high blood-brain barrier penetrability; it is also a potent antagonist of serotonin S2 receptors. In a previous investigation, we reported that (S')-emopamil was capable of reducing infarct size following middle cerebral artery (MCA) occlusion in rats, even when administered 1 hour after occlusion.4 We designed the present study to assess the efficacy of (S')-emopamil in the setting of transient global cerebral ischemia in rats. We have presented a preliminary report of these findings.5

Materials and Methods

(S')-Emopamil [(2S)-2-isopropyl-5-(methylphenethylamino)-2-phenyl-valeronitrile hydrochloride] was kindly provided by Knoll A.G. (Ludwigshafen, F.R.G.). It was dissolved in distilled water (6 mg/ml) prior to use.

Male Wistar rats weighing 250–325 g were used in these studies following an overnight fast. Anesthesia was induced with 3.5% halothane, 70% nitrous oxide, and a balance of oxygen. Femoral arteries were catheterized to permit blood pressure measurement and arterial blood sampling. The rats were then intubated and mechanically ventilated on 0.5–1% halothane, 70% nitrous oxide, and a balance of oxygen so as to maintain Pco2 and PaO2 in the normal ranges. Through a midline pretracheal incision, the two common carotid arteries were catheterized to permit blood pressure measurement and arterial blood sampling. The rats were then intubated and mechanically ventilated on 0.5–1% halothane, 70% nitrous oxide, and a balance of oxygen so as to maintain Paco2 and PaO2 in the normal ranges. Through a midline pretracheal incision, the two common carotid arteries were catheterized and gently separated from the surrounding nerves. Ligatures consisting of Silastic tubing were passed around each artery. Rectal temperature was measured continually and maintained at 37–37.5°C throughout the experiment. As we have previously shown brain temperature to be a critical determinant of outcome following global ischemia,6 the temperature of the head was separately monitored with a
thermocouple implanted in the temporalis muscle and was maintained throughout the experiment at 36.5–37.5°C by means of a small warming lamp placed above the animal's head. Following completion of preparatory steps, halothane was discontinued and the rats were maintained on 70% nitrous oxide in oxygen and were immobilized with 0.75 mg/kg intravenous pancuronium bromide. A 45-minute stabilization period was permitted prior to the ischemic insult.

To produce high-grade forebrain ischemia, 10 minutes of bilateral common carotid artery occlusion was combined with reduction of mean arterial blood pressure (MABP) to 50 mm Hg by withdrawal of blood into a heparinized syringe. At the end of the ischemic period, the carotid ligatures were loosened and removed and the shed blood was reinfused to restore normotension. All incisions were closed, and the rats were awakened and placed into individual cages with free access to food and water. Arterial blood gases were monitored before, during, and after ischemia.

Two separate (S)-emopamil treatment series were considered. In the pretreatment series, treated rats (n=15) received an intraperitoneal injection of (S)-emopamil, 20 mg/kg body wt, 30 minutes prior to the ischemic insult; a second dose of (S)-emopamil similar to the first was given 2.5 hours later. Untreated ischemic rats of this series (n=15) received an intraperitoneal injection of distilled water vehicle, of the same volume as the drug itself. In the posttreatment series, treated rats (n=10) received their first dose of (S)-emopamil (10 mg/kg body wt) 30 minutes following the ischemic insult; three additional 10 mg/kg doses were administered 1, 2, and 3 hours later to avoid the production of hypotension. Untreated ischemic rats of this series (n=10) received comparable intraperitoneal injections of distilled water vehicle. Comparably anesthetized rats not subjected to cerebral ischemia served as normal controls (n=5).

In preparation for histopathologic evaluation, 3 days following the ischemic insult the rats were tracheostomized under 2% halothane anesthesia, immobilized with 5 mg i.p. d-tubocurarine, and mechanically ventilated. The heart was then exposed via a median sternotomy, and the ascending aorta was catheterized through the left ventricle. The brain was perfused for 1 minute with a physiological saline solution, followed by perfusion with a mixture of 40% formaldehyde, glacial acetic acid, and methanol, 1:1:8 by volume (FAM), which was continued for 19 minutes under a constant pressure of 110–120 mm Hg. The rats were then decapitated, and the heads were immersed in FAM overnight at 4°C. The brains were then removed from the skulls, and coronal blocks were embedded in paraffin. Brain sections, 10 μm thick, were prepared at 250-μμm intervals. These sections were stained with hematoxylin and eosin. The frontoparietal neocortex, striatum, and hippocampus were examined by an observer blinded to the experimental conditions. In the hippocampal CA1 area, ischemic neuronal injury was assessed quantitatively by direct counting of the normal pyramidal neurons per 1,000 μm length within the medial, middle, and lateral subsectors. In the neocortex and striatum, neuronal damage was estimated semiquantitatively on a four-point scale: 0, no affected neurons; 1, few affected neurons; 2, moderate number of affected neurons; 3, majority of neurons affected.

A one-tailed Mann-Whitney U test was used for statistical analysis of differences in the number of normal neurons in the various subsectors of the hippocampal CA1 area and for comparison of semiquantitative neuronal injury grades in the neocortex and striatum of treated versus untreated rats; p<0.05 was considered to be significant.

**Results**

Table 1 summarizes arterial blood gases and the plasma glucose level measured just prior to the ischemic insult, as well as MABP determined 15 minutes prior to and 20 minutes after ischemia. No significant intergroup differences were noted in these variables, although there was a tendency for a higher plasma glucose level and a lower MABP following (S)-emopamil pretreatment compared with untreated controls. In (S)-emopamil–pretreated rats, mean±SD baseline MABP prior to drug administration was 125±8 mm Hg. (S)-Emopamil pretreatment was followed by a maximal decrease in MABP averaging 29 mm Hg (to 94±19 mm Hg), but by the time the ischemic insult was begun, MABP had partially recovered (to 103±12 mm Hg).

Six untreated ischemic rats died prior to histopathologic analysis; of these, four developed apnea...
TABLE 2. Normal Neuron Counts in Subsectors of Hippocampal CA1 Area

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Subsector</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Medial</td>
<td>Middle</td>
<td>Lateral</td>
</tr>
<tr>
<td>Nonischemic controls</td>
<td>5</td>
<td></td>
<td>157.0±1.9</td>
<td>161.8±8.0</td>
<td>167.2±4.7</td>
</tr>
<tr>
<td>Pretreatment series</td>
<td></td>
<td>Untreated</td>
<td>34.4±8.9</td>
<td>44.0±10.2</td>
<td>53.0±11.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treated</td>
<td>81.5±13.3*</td>
<td>82.7±11.9†</td>
<td>93.9±12.5‡</td>
</tr>
<tr>
<td>Posttreatment series</td>
<td>10</td>
<td>Untreated</td>
<td>39.4±16.3</td>
<td>37.9±15.8</td>
<td>42.4±16.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treated</td>
<td>14.2±4.5</td>
<td>23.3±12.0</td>
<td>29.6±9.3</td>
</tr>
</tbody>
</table>

Values are mean±SEM normal-appearing pyramidal neurons per 1,000-μm linear extent of microscopic field.

*†‡p<0.007, 0.012, 0.028, respectively, different from untreated group by one-tailed Mann-Whitney U test.

on the second postischemic day and one died on the first day of apparent heart failure. Among the (S)-emopamil-treated rats, three of the pretreatment series died, two on the second day in association with respiratory distress and one of hypotension at the end of the experiment. One (S)-emopamil-posttreated rat also died. These animals were excluded from the data analysis.

Histopathologic examination revealed extensive neuronal necrosis in all three subsectors of the hippocampal CA1 area in untreated ischemic rats (Table 2, Figures 1 and 2). Normal neuron counts were reduced to 22–32% of the nonischemic control values (Table 2). Relative to normal neuron counts in the untreated ischemic rats, (S)-emopamil-pretreated rats showed mean increases of 2.4-fold ($p=0.007$), 1.9-fold ($p=0.012$), and 1.8-fold ($p=0.028$), respectively, in the medial, middle, and lateral subsectors (Table 2). In the pretreated rats, ischemic neurons were scattered among normal-appearing neurons (Figure 2). In contrast, (S)-emo-

![Figure 1](http://stroke.ahajournals.org/)

**Figure 1.** Photomicrographs of paraffin-embedded brain sections, stained with hematoxylin and eosin, from untreated (A and B) and (S)-emopamil-pretreated (C and D) rats. A: CA1 hippocampal necrosis is evident. Arrowheads mark boundary between normal and ischemic neurons. B: Higher magnification of CA1 sector, showing ischemic neurons. C: Normal-appearing neurons are present in CA1 sector. D: Higher magnification of CA1 sector, showing two ischemic neurons (arrowheads) in field of otherwise normal-appearing neurons. Magnifications: ×120 in A and C, ×1104 in B and D.
FIGURE 2. Scatterplots of normal neuron counts in three subsectors of hippocampal CA1 area of rats. Left: Pretreatment series. Right: Posttreatment series. Symbols represent individual animals, bars denote mean values. ○, treated with (S)-emopamil; ●, untreated. Statistical comparisons shown in Table 2.

Discussion

In our present study, we used a model of bihemispheric forebrain ischemia that consistently leads to neuropathologic alterations of pyramidal neurons within the hippocampal CA1 area—a region highly vulnerable to transient ischemia. Cranial temperature was maintained within a narrow normothermic range because our previous studies have shown this to be essential for a reproducible pathologic outcome.

The salient finding of our present study is that pretreatment with (S)-emopamil increased the number of surviving neurons in the hippocampal CA1 area by 1.8–2.4-fold compared with the number surviving in the absence of drug therapy. The effect is robust in that it was observed in all three subsectors of the CA1 area and occurred despite the presence of mild transient preischemic hypotension that complicated (S)-emopamil pretreatment (Table 1). By contrast, (S)-emopamil failed to protect hippocampal neurons when the initiation of treatment was delayed to 30 minutes following the ischemic insult; a similar result has been observed in other studies of calcium channel blockers.

We also observed an improvement in histologic grade of neocortical neurons with (S)-emopamil pre-
treatment as well as an improvement in striatal histologic grade with posttreatment. It should be emphasized, however, that a 10-minute ischemic insult fails to give rise to a consistently severe degree of ischemic injury in these structures (Figure 3). Thus, confidence as to the ability of this agent to protect striatal or neocortical neurons should await studies employing longer periods of forebrain ischemia.

In a previous study of permanent MCA occlusion in rats, Nakayama et al found that (S)-emopamil reduced cortical infarct volume by >50%, irrespective of whether treatment was begun 30 minutes prior to, immediately after, or even 1 hour following MCA occlusion.

Previous studies of global cerebral ischemia have also pointed to an ameliorative effect of emopamil on restoration of high energy phosphate levels, improvement of postischemic blood flow, and reduction of cell death. In gerbils subjected to 7 minutes of bilateral carotid occlusion, (S)-emopamil increased the number of surviving neurons in the hippocampal CA1 area by approximately 75%. In a rat model of forebrain ischemia similar to that of the present study but without separate control of cranial temperature, pretreatment with 2 mg/kg (S)-emopamil reduced neuronal injury by approximately one half, but higher doses afforded less protection. Finally, in the isolated rat brain preparation perfused at constant pressure and subjected to simulated ischemia, emopamil increased perfusion rate and improved postischemic cortical levels of high energy phosphate compounds. These results were not observed in preparations perfused at constant volume, suggesting a possible vascular mechanism of action.

(S)-Emopamil possesses an unusually high blood-brain barrier permeability, having a brain uptake index (relative to water) of 110, compared with 41 for verapamil. Thus, (S)-emopamil offers excellent cerebral availability, which is undoubtedly important to its efficacy.

(S)-Emopamil is known from other studies to be not only a calcium channel antagonist, but also a potent stereoselective antagonist of the serotonin S2 receptor. Serotonin is a potent vasoconstrictor, and the S2 receptor subtype is thought to mediate serotonin-induced vasoconstriction. Pial and parenchymal vessels of the neocortex receive serotonergic innervation, and the rat neocortex is rich in serotonin S2 receptors. Dietrich et al have recently shown that serotonin modulates remote hemodynamic effects observed following cerebral infarction. Thus, serotonin may facilitate cerebral ischemic injury, and we cannot exclude a serotonergic mechanism in (S)-emopamil’s cerebroprotective effect. In a recent abstract, Fujikura et al demonstrated a marked protective effect of the serotonin S2 antagonist naftidrofuryl on hippocampal CA1 neuronal injury in gerbils with transient bilateral carotid occlusion.

Globus et al have recently shown that (S)-emopamil tends to diminish, by approximately one third, the massive release of dopamine into the brain extracellular space that occurs during and immediately following ischemia—an effect possibly attributable to either its calcium antagonistic or its serotonin-blocking properties.

In summary, we have shown that (S)-emopamil pretreatment substantially protects hippocampal neurons from ischemic injury. These findings suggest a possible role for this agent in the prevention of...
ischemic neuronal injury in those clinical situations (e.g., cardiac bypass procedures) in which a global decline in cerebral perfusion can be anticipated.

Acknowledgment

We are grateful to Helen Valkowitz for preparing the typescript.

References


Key Words • calcium channel blockers • neuroprotection • rats
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Stroke. 1990;21:1734-1739
doi: 10.1161/01.STR.21.12.1734

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