

Short Communication

Baclofen Does Not Protect Against Cerebral Ischemia in Rats

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Presynaptic release of glutamate into the extracellular compartment and activation of receptor-operated calcium channels may contribute to ischemic neuronal damage. We evaluated the effect of baclofen, a selective inhibitor of presynaptic glutamate release, on mortality, working memory, and light microscopic hippocampal and cortical damage in the four-vessel occlusion model of cerebral ischemia using 64 male Wistar rats. Baclofen (10 mg/kg i.p.) given 1 hour before and 30–60 minutes after 20 minutes of global ischemia did not lessen mortality, prevent ischemic cellular damage, or significantly improve working memory compared with no treatment. We conclude that preischemic and postischemic administration of baclofen does not protect neurons from ischemic injury. (*Stroke* 1990;21:138–140)

Glutamate may play a pivotal role in the early stages of ischemic cellular injury. Excessive release of this excitatory amino acid from a neurotransmitter pool within presynaptic terminals has been documented after ischemia.^{1–5} Ischemic cellular injury can be prevented by severing glutamatergic afferents to selectively vulnerable CA1 neurons⁶ and by intraventricular and systemic administration of glutamate release inhibitors and glutamate receptor antagonists.^{7–9} Tissue culture studies^{10,11} have documented that the damaging effect of glutamate occurs in the presence of calcium, which enters neurons through receptor-operated channels activated by glutamate.

Baclofen, β -(*p*-chlorophenyl)- γ -aminobutyric acid, is a racemic mixture of D and L isomers and is used clinically to reduce spasticity. This agent, initially synthesized as an analogue of the inhibitory neurotransmitter γ -aminobutyric acid (GABA), may inhibit the release of excitatory amino acid neurotransmitters at concentrations lower than those needed to influence GABA receptors or GABA release. Baclofen depresses afferent depolarization in the spinal cord by inhibiting transmitter release and has been shown to depress the electrically evoked release

of glutamate and aspartate from slices of cerebral cortex from guinea pigs.¹² Baclofen readily crosses the blood–brain barrier in doses associated with relatively minor side effects.¹³

As part of a series of experiments evaluating potential neuronal protective agents in an animal model of global cerebral ischemia, we evaluated the effect of baclofen on three outcome measures (mortality, histologic damage, and memory) in rats.

Materials and Methods

To determine the dose–response curve for baclofen's effect on brain glutamate levels in normal animals, 0, 5, 10, or 20 mg/kg baclofen dissolved in 1.5 ml normal saline were given intraperitoneally to groups of three rats each 1 hour before in situ freeze-fixation. These results have been published in part.¹⁴ Baclofen at 5 mg/kg reduced glutamate release in the cortex and medial hippocampus compared with vehicle-treated controls. Similar results in the cortex and hippocampus were seen with 10 mg/kg baclofen, but no greater effect occurred with 20 mg/kg, and this dose was associated with decreased responsiveness and bradypnea. Therefore, a dose of 10 mg/kg i.p. baclofen given 1 hour before and 30–60 minutes after ischemia was chosen for further studies.

Sixty-four 250–300-g male Wistar rats that received no treatment or were treated with baclofen as described above were rendered globally ischemic for 20 minutes by a modification of the Pulsinelli method,^{15,16} bilateral vertebral artery cauterization followed 24 hours later by 20-minute occlusion of both common carotid arteries and tight ligation of the

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TABLE 1. Histologic Score for Ischemic Damage in Eight Brain Regions of Baclofen-Treated and Untreated Rats After 20 Minutes of Global Ischemia

Rat	Region							
	Cortex	Subiculum	Medial CA1	Lateral CA1	Lateral CA3	Ventral CA3	CA4	Dentate
Untreated								
A	2	4	4	3	3	3	3	3
B	2.5	4	4	4	(torn)	4	4	4
C	2	4	4	3.5	2	2	1	1
D	1	2	3	2	1	0	0	1
E	1	4	3.5	3.5	1	1	1	1
F	1	4	3.5	3.5	1	1	1	1
Baclofen-treated								
A	3	4	4	3	2	2	2	2
B	3	4	4	4	3	2	2	2
C	2	1	1	3	1	3	3	2
D	1	4	4	2	1	0	0	1
E	1	3	2.5	2	1	1	1	1
F	1.5	2	3	2	1	1	1	1

In all brains, both hemispheres were examined. If side-to-side differences were present, score reported was mean of right and left scores.

cervical muscles providing collateral blood flow to the brain. Rats were used for further study only if they lost their righting reflex and had no electrical activity on electroencephalography (EEG) for the entire 20 minutes. With this technique, rats do not require mechanical ventilation or anesthesia during ischemia or reperfusion. The rats were fasted for 24 hours before ischemia, and physiologic variables that might affect outcome (i.e., blood pressure, arterial blood gases, body and head temperature, plasma glucose concentration, hematocrit, and EEG) were monitored during ischemia. There were no differences in these variables between untreated and baclofen-treated rats.

Seventy-two hours after the 20 minutes of ischemia, while under light ether anesthesia, six untreated and six baclofen-treated rats were infused with phosphate-buffered 10% formalin and decapitated. The brains were removed, imbedded in paraffin, sectioned, and stained with hematoxylin and eosin. Ischemic cell changes were quantified by light microscopy in the entire parietal cortex and the entire hippocampus of both hemispheres on a single coronal section. For data analysis, the entire hippocampus was divided into seven regions (subiculum, medial CA1, lateral CA1, lateral CA3, ventral CA3, CA4, and dentate), but the entire parietal cortex was graded as a single region. Each region was given a score from 0 to 4 based on the percentage of cells with shrunken eosinophilic cytoplasm and pyknotic nuclei (0, no abnormal cells; 1, 1–25%; 2, 26–50%; 3, 51–75%; 4, 76–100% abnormal cells). Previous examination of nonischemic brains fixed in the same manner allowed recognition and elimination of fixation-related changes such as dark-cell artifacts. The scores for each region in the baclofen-treated

and untreated rats were compared using the Mann-Whitney *U* test for nonparametric samples.

Six baclofen-treated rats were tested on an eight-arm radial maze as described¹⁶ and compared with four untreated sham-operated rats (controls) and with six untreated rats. Testing was begun 1 month after ischemia. All rats were given one trial a day for 50 days, but the first 15 trials were considered a conditioning period. Five of the eight arms of the maze were baited, and the number of working errors (choosing the same arm twice) was recorded. Previous studies^{16,17} have demonstrated that this is the most sensitive test of memory dysfunction in this model. The data were analyzed using two-way repeated-measures analysis of variance.

Results

Thirteen of 24 (54%) baclofen-treated rats died ≤ 72 hours after 20 minutes of ischemia compared with 17 of 40 (43%) untreated rats. This difference was not significant.

No difference in mean histologic score was found in any of the eight regions when the baclofen-treated and untreated groups were compared (Table 1).

The untreated group made significantly more working errors than the control group ($F=23.6$, $p<0.005$). The baclofen-treated group did not perform significantly better than the untreated group (Figure 1).

Discussion

Our study demonstrates that systemically administered baclofen did not lessen mortality, did not prevent ischemic cellular damage, and did not significantly improve functional outcome compared with no treatment. These negative results contrast with those of our previous studies of nicardipine¹⁶ and those of current studies of a glutamate receptor

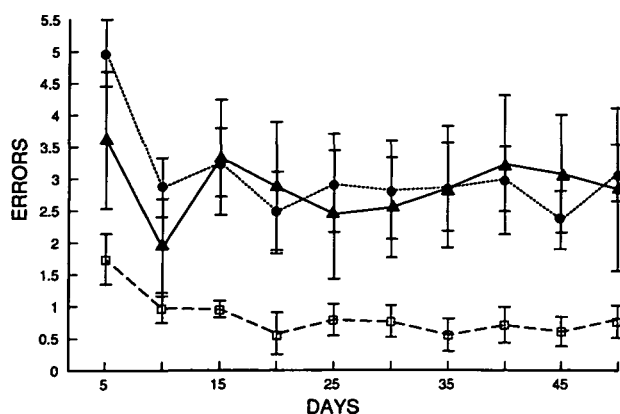


FIGURE 1. Graph of mean \pm SEM working errors in untreated ischemic (\bullet , $n=6$), baclofen-treated ischemic (\blacktriangle , $n=6$), and untreated sham-operated control (\square , $n=4$) rats. Untreated ischemic rats made significantly more errors than did controls. Baclofen-treated rats performed no better than untreated ischemic rats.

antagonist,¹⁸ which indicated improved outcome using the same model of ischemia.

Do our results mean that the glutamate hypothesis of ischemic cellular injury is incorrect? The importance of glutamate in the genesis of ischemic injury was strongly supported by early studies demonstrating that surgical section of glutamatergic afferents to CA1 resulted in striking protection of CA1 neurons from subsequent ischemic insult.⁶ Recently, similar results were found using adenosine, an inhibitor of glutamate release.⁹ Since baclofen prevents glutamate release in normal animals, resulting in elevation of whole-brain glutamate levels, this drug should also protect against ischemic damage. Our negative results are especially disturbing in the medial CA1, where baclofen inhibited glutamate release immediately after ischemia,¹⁴ but where severe ischemic damage nevertheless took place. Recently, Sternau et al¹⁹ found significant neuronal protection in ischemic gerbil hippocampi after pretreatment with baclofen. However, higher doses of baclofen (25 mg/kg) were administered in that study in an effort to produce a GABA-agonist effect.

While our results do not disprove the glutamate hypothesis, it is likely that either higher doses of baclofen or a more potent inhibitor of glutamate release must be used. Alternatively, baclofen therapy might be supplemented by glutamate receptor antagonists or by blockade of intracellular events resulting from glutamate release.

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