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Short communication

Dexamethasone augments ischemia-induced extracellular accumulation of glutamate in gerbil hippocampus

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Abstract

Glucocorticoids exacerbate neuronal damage due to hypoxia, ischemia, seizure and hypoglycemia. Because the release of glutamate is closely involved in neuronal damage, the effects of dexamethasone on the ischemia-induced accumulation of extracellular amino acids (aspartate, glutamate, and glycine) were investigated in the gerbil hippocampal CA1 region by a microdialysis-high-performance liquid chromatography procedure in vivo. There were no differences in the extracellular concentrations of amino acids before ischemia between the control group and the dexamethasone (3 μg, i.c.v.)-injected group. The concentration of glutamate reached 246% of that before ischemia within 2.5 min of transient forebrain ischemia. Dexamethasone augmented the increase in glutamate to 508% of that before ischemia. This finding suggests that glucocorticoids aggravate ischemic neuronal damage by causing glutamate to accumulate in the extracellular space. © 1998 Elsevier Science B.V.

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1. Introduction

There are many reports that glucocorticoids exacerbate neuronal damage due to hypoxia, seizure and hypoglycemia (reviewed by Sapolsky, 1990). We have also reported that dexamethasone aggravates ischemia-induced neuronal death in the gerbil hippocampus (Adachi et al., 1998). In cerebral ischemia, energy failure induces the release of various neurotransmitters such as glutamate and aspartate (Benveniste et al., 1984; Adachi et al., 1991), which provokes the catastrophic enzymatic process leading to irreversible neuronal damage (Mitani et al., 1994). Furthermore, glucocorticoids have been shown to enhance stress- and seizure-induced accumulation of excitatory amino acids in the extracellular space (Stein-Behrens et al., 1992; Moghaddam et al., 1994). In the present study, we investigated the effect of dexamethasone on the ischemia-induced accumulation of amino acids in the gerbil hippocampal CA1 region, using an in vivo animal model of transient forebrain ischemia.

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2. Materials and methods

2.1. Animals

This study was approved by the Committee on Animal Experimentation at Ehime University School of Medicine, Ehime, Japan. Male Mongolian gerbils weighing 60–80 g (Seiwa Experimental Animals, Fukuoka, Japan) were housed in groups in a room controlled at 23 ± 1°C with 12-h light/12-h dark cycle (light on at 6:00 am). Food and water were provided ad libitum. The animals were deprived of food for at least 6 h before ischemia to prevent the influence of hyperglycemia on ischemic brain damage. The experiment was performed under spontaneous ventilation.

2.2. Microdialysis

Ten gerbils were prepared and evenly assigned to two groups. We used an animal model of transient forebrain ischemia described previously (Amakawa et al., 1996). In brief, the animals were anesthetized with 2% halothane, and both common carotid arteries were exposed. After the animal was placed in a stereotaxic apparatus in the prone

position, the skull was exposed, and a small burr hole was drilled in the left hemisphere 2 mm anterior and 2 mm lateral to the bregma for the insertion of a thermocouple needle probe. Another burr hole was drilled in the right hemisphere 2 mm posterior and 2 mm lateral to the bregma to insert microdialysis probe, and a third burr hole was drilled in the left hemisphere 0.5 mm posterior and 2.5 mm lateral to the bregma for drug administration. Brain and rectal temperatures were carefully maintained at 37.5 $\pm$ 0.2°C during the experimental period with a heating lamp. The dialysis probe was I-shaped and made of cellulose membrane (1 mm long, 0.22 mm outside diameter, molecular weight cutoff at 50 000, Eicom, A-I-8-01, Kyoto, Japan), and was stereotaxically inserted. Ringer's solution was perfused at a rate of 2 μl/min and brain perfusates were collected every 3 min into microtubes. After a stabilization period of 1 h, 3 μg of water soluble-dexamethasone (dissolved in 10 μl saline) was administered to the animals i.c.v. via a 27-gauge needle (control animals were given saline).

Transient forebrain ischemia was achieved 30 min after the drug administration. The blood flow was restored after 2.5 min of ischemia. Collected perfusates were immediately frozen and stored at -80°C until analysis.

2.3. Amino acid analysis

Amino acid concentrations were determined by a high-performance liquid chromatography (HPLC) system which consisted of one pump (L-7100, Hitachi, Tokyo, Japan) serving to deliver mobile-phase, three reagent delivery pumps (L-7100, Hitachi), a model AS-2000 sample injector (Hitachi) with a 100-μl sampling loop, a cation-exchange separation column (150 mm $\times$ 4.0 mm inside diameter), a thermostatic reactor (L-7300, Hitachi), and a fluorescence detector (L-7400, Hitachi). Mobile-phase was 835 Buffer Solution PF-1 (Wako), and the flow rate was 0.4 ml/min. Brain microdialysates were directly injected into the HPLC. The eluate from the separation column was subjected to reaction with the mixture of reagent 1 (1 M NaOH, 3 ml/min) and reagent 2 (0.35 M boric acid containing 0.1 mM sodium hypochlorite and 4 ml Brij 35 solution, 0.3 ml/min) in a coil of Teflon tube at 37°C, then reagent 3 (0.35 M boric acid containing 6 mM *o*-phthalaldehyde, 29 mM 2-mercaptoethanol, and 4 ml Brij 35 solution, 0.3 ml/min) was added to the mixture in another Teflon tube. The fluorescence intensity (peak height) was measured at an excitation wavelength of 340 nm and an emission wavelength of 450 nm.

An in vitro recovery test was performed by inserting the dialysis probe into Ringer's solution containing 10 μM of each amino acid at 37°C. The recovery rates of aspartate, glutamate and glycine in the perfusate were 7.7% $\pm$ 0.9%, 7.6% $\pm$ 0.9%, and 9.3% $\pm$ 1.3% (mean $\pm$ S.D., $n = 4$), respectively, when the probe was perfused at 2 μl/min.

2.4. Drugs and chemicals

Water soluble-dexamethasone was purchased from Sigma (St. Louis, MO, USA). Halothane was obtained from Takeda (Osaka, Japan). Other chemicals were all of reagent grade. The dose of water soluble-dexamethasone was expressed as the weight of the free base.

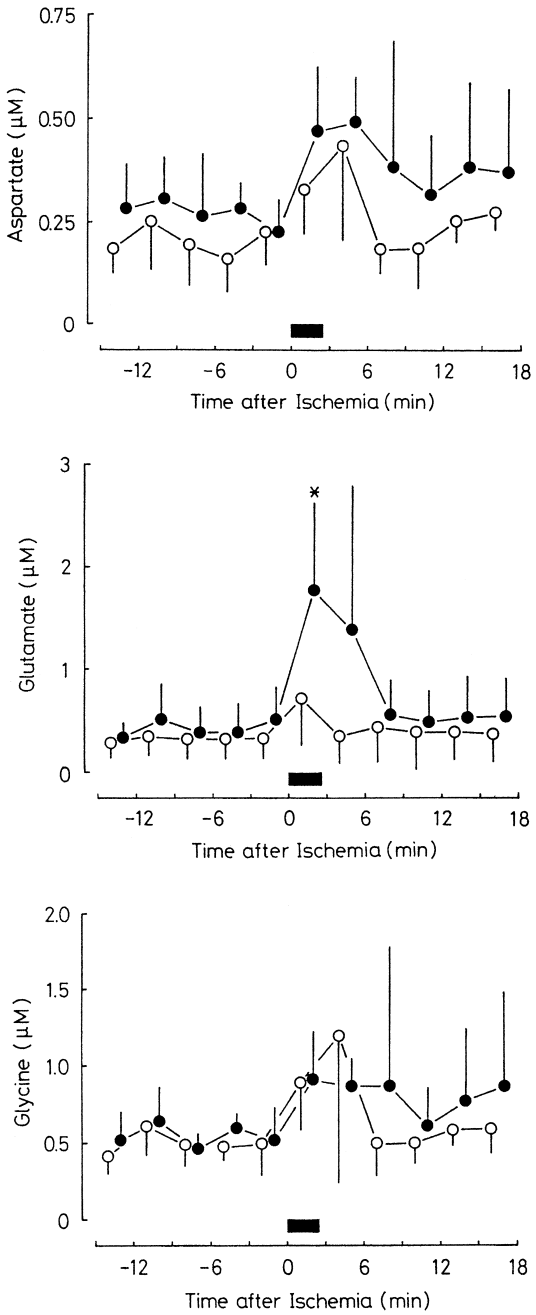

Fig. 1. Effects of dexamethasone on the increase in the amino acids induced by transient forebrain ischemia in hippocampal CA1 region. Control group (○), dexamethasone (3 μg)-treated group (●). Each value represents the mean $\pm$ S.D. of five animals. $* P < 0.05$ compared with the respective values in the control group. Rectangles represent the duration of transient forebrain ischemia produced by occlusion of bilateral carotid arteries.

2.5. Data analysis

The data were evaluated with repeated measures two-way analysis of variance, followed by Scheffés test to compare each value with the corresponding value in the control group.

3. Results

Changes in the concentrations of amino acids in dialysates in the hippocampal CA1 region are shown in Fig. 1. There were no significant differences in the values of amino acids before ischemia between the control and the dexamethasone groups. In the control group, transient forebrain ischemia produced increases in amino acids immediately after the start of ischemia, and the levels of aspartate, glutamate, and glycine reached 202%, 246%, and 209% of their preischemic values, respectively. In gerbils treated with dexamethasone, the peak values of each amino acid reached 187%, 508%, and 188% of their preischemic values, respectively. The extent of the increase in glutamate in the dexamethasone group was significantly larger than that in the control group ($t = 0–3$ min).

4. Discussion

In the present study, we observed the facilitated increase in the extracellular concentration of glutamate by dexamethasone during ischemia.

Glucocorticoids have been shown to endanger hippocampal neurons and exacerbate damage caused by various injuries such as stress, seizure, hypoxia, ischemia, and hypoglycemia by facilitating the glutamate/Ca^{2+} cascade (Sapolsky, 1990; Stein-Behrens et al., 1992; Moghaddam et al., 1994). We have also demonstrated that dexamethasone facilitates the onset of anoxic depolarization and exacerbates the increase in the intracellular Ca^{2+} concentration (Adachi et al., 1998). In contrast, the neurotoxic effect of glucocorticoids has been shown to be prevented by the blockade of the NMDA receptor (Armanini et al., 1990). Thus, the aggravation of ischemic neuronal damage by glucocorticoids seems to be closely related to the increase in the extracellular concentration of glutamate. The facilitation of the increase in extracellular glutamate by dexamethasone during ischemia that we observed in the present study seems to be in good agreement with these findings.

Two mechanisms are conceivably responsible for the facilitation of the ischemia-induced increase in extracellular glutamate. One is the facilitation of glutamate release from neurons, and the other is the inhibition of uptake into the glia. Membrane depolarization in ischemia triggers the release of glutamate from synaptic vesicles in a Ca^{2+}-dependent exocytotic manner (Drejer et al., 1985; Kauppinen et al., 1988), and energy failure causes Ca^{2+}-independent leakage of glutamate (Drejer et al., 1985; Dagani and Erecsinka, 1987). Since glucocorticoids facilitate anoxic depolarization (Adachi et al., 1998), most likely by inhibiting the intracellular energy store (Kadekaro et al., 1988; Virgin et al., 1991), glucocorticoids may facilitate the release of glutamate during ischemia. On the other hand, glucocorticoids have been shown to inhibit the uptake of glutamate into the glia by inhibiting glucose uptake and utilization (Kadekaro et al., 1988; Armanini et al., 1990; Virgin et al., 1991). Therefore, both the facilitation of the release and the inhibition of the uptake seem to take part in the accumulation of glutamate due to dexamethasone.

In the present experiment, the enhanced accumulation of aspartate due to dexamethasone was not observed, whereas the increase in the extracellular concentration of glutamate was facilitated. A similar difference between glutamate and aspartate increases has been reported previously. At a low level of glucocorticoid, kainic acid induces an increase in glutamate but not aspartate, whereas at a high level of glucocorticoid, both glutamate and aspartate increases (Stein-Behrens et al., 1992, 1994).

In this study, we observed the facilitation of the accumulation of glutamate in the extracellular space by dexamethasone during ischemia in the gerbil hippocampal CA1 region. This finding may be involved in the development of ischemic neuronal damage by glucocorticoids.

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