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Dexamethasone reduces energy utilization in ischemic gerbil brain

Naoto Adachi *, Chikara Namba, Takumi Nagaro, Tatsuru Arai

Department of Anesthesiology and Resuscitology, Ehime University School of Medicine, Shitsukawa, Shigenobu-cho, Onsen, Ehime 791-0295, Japan

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Abstract

Glucocorticoids have been reported to aggravate ischemic neuronal damage. Because energy failure is a crucial factor in the development of ischemic neuronal injury, the effects of dexamethasone on histologic outcome and energy metabolism were investigated in gerbil brain. Dexamethasone (3 μg, i.c.v.) was administered 1 h prior to ischemia, and its effect on delayed neuronal death caused by 2 min of bilateral common carotid artery occlusion was observed in hippocampal CA1 pyramidal neurons. The brain concentration of ATP after various durations of decapitation ischemia was determined, and the effect of dexamethasone (3 μg, i.c.v.) was examined. Na^+,K^+-activated adenosine triphosphatase (Na^+,K^+-ATPase) activity was evaluated after the administration of the agent. Forebrain ischemia for 2 min produced neuronal damage in animals pretreated with dexamethasone, although neuronal damage was not observed in vehicle-injected animals. Decapitation ischemia for 0.5 and 1 min reduced the brain ATP concentration to 44% and 15% of the basal level, respectively. Dexamethasone attenuated the ischemia-induced reduction in ATP, and the values were 58% and 25% of the basal level, respectively. Na^+,K^+-ATPase activity at pH 6.7 was suppressed to 47% by dexamethasone treatment (3 μg, i.c.v.), whereas the activity at pH 7.4 was not influenced by the agent. The results show that a contributing factor to the aggravation of ischemic neuronal damage may be a disturbance in Na^+,K^+-ATPase despite adequate levels of ATP.

Keywords: ATP; Cerebral ischemia; Dexamethasone; (Gerbil); Glucocorticoid

1. Introduction

Administration of glucocorticoids in cardiopulmonary resuscitation has been reported to neither provide a beneficial action nor improve neurological recovery following cardiac arrest in retrospective human studies (Grafton and Longstreth, 1988; Jastremski et al., 1989). Furthermore, their detrimental effects on ischemic neuronal damage and glutamate toxicity have been demonstrated in various animal studies (Sapolsky and Pulsinelli, 1985; Koide et al., 1986; Adachi et al., 1998; Chen et al., 1998; Tsubota et al., 1999). This deleterious effect of glucocorticoids has been shown to be attenuated by various forms of energy supplementation (Sapolsky, 1986). Therefore, the damaging potential of glucocorticoids may be linked to an impairment of local energy metabolism. Since the principal high-energy intermediate or carrier compound in the brain is ATP, we investigated the effect of dexamethasone on the ischemia-induced reduction in ATP levels and on the activity of Na^+,K^+-activated adenosine triphosphatase (Na^+,K^+-ATPase) in gerbil brain.

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. All animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by Ehime University School of Medicine. Male Mongolian gerbils weighing 60–80 g (Seiwa Experimental Animals, Fukuoka, Japan) were housed in groups in a room controlled at 23 ± 1 °C and maintained under an alternating 12-h light/12-h dark cycle (lights on at 6:00 am). Animals were deprived of food at least 6 h before the start of experiments because of the influence of the plasma concentration of glucose (Siemkowicz and Hansen, 1978).

In experiment 1, the effect of pre-ischemic treatment with dexamethasone on histologic outcome was examined

* Corresponding author. Tel.: +81-89-960-5383; fax: +81-89-960-5386.

E-mail address: nadachi@m.ehime-u.ac.jp (N. Adachi).

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by assessing delayed neuronal death. In experiment 2, the effect of dexamethasone on energy metabolism was investigated by measuring the brain concentrations of adenylates. In experiment 3, Na^+,K^+-ATPase activity was determined, and the effect of dexamethasone was examined.

2.2. Experiment 1: histologic outcome

In this experiment, 28 gerbils were divided into six groups: saline, cyclodextrin (vehicle), or dexamethasone-injected non-ischemic groups and saline, cyclodextrin, or dexamethasone-injected ischemic groups. The animals were anesthetized and maintained with 2% halothane under balanced 50% oxygen and 50% nitrous oxide. The experiment was performed under spontaneous ventilation. Both common carotid arteries were exposed, and silk threads (4.0) were looped around these arteries. After an animal was placed in a stereotaxic apparatus in a prone position, the skull was exposed and a small burr hole was drilled in the right hemisphere, 0.5 mm posterior and 2.5 mm lateral to the bregma, for drug administration. Another burr hole was drilled in the left hemisphere, 2.0 mm anterior and 2.0 mm lateral to the bregma, for insertion of a thermocouple needle probe.

Saline (10 μl), 50 μg of 2-hydroxypropyl-β-cyclodextrin (Sigma, St. Louis, MO, USA), or 3 μg of dexamethasone–water soluble (cyclodextrin-encapsulated dexamethasone; Sigma) was administered into the right lateral ventricle through the burr hole via a 27-gauge needle at a depth of 2.0 mm below the brain surface. A thermocouple needle probe was inserted through the burr hole, and its tip was positioned about 2.0 mm below the brain surface. One hour after the administration, transient forebrain ischemia for 2 min was achieved by pulling the threads with 8-g weights, while maintaining the brain and rectal temperatures at 37.5 ± 0.2 °C. After the threads were cut to restore blood flow, the brain and rectal temperatures were further maintained at normothermia for 30 min under anesthesia. The skin incision was sutured and the anesthesia was stopped.

Seven days after ischemia, the animals were anesthetized with an intraperitoneal injection of sodium pentobarbital. The brains were perfused with heparinized saline and fixed with buffered formalin. After dehydration with graded concentrations of alcohol, the brains were embedded in paraffin. Brain slices, 5-μm thick, were stained with hematoxylin and eosin, and the number of uninjured neurons in the right hippocampal CA1 field per 1.0-mm length of stratum pyramidale was counted in the same level of the coronal section (1.5 mm posterior to the bregma) in a single blinded manner.

To examine the effect of dexamethasone or its vehicle on non-ischemic brains, gerbils were administered saline, 2-hydroxypropyl-β-cyclodextrin, or dexamethasone, according to the procedure mentioned above. Then, the brains were examined after 7 days.

2.3. Experiment 2: brain concentrations of adenylates

In this experiment, 50 gerbils were prepared and then assigned to five saline groups and five dexamethasone groups (five animals in each) to determine the brain concentrations of ATP, ADP, and AMP after various durations of decapitation ischemia. After halothane anesthesia, animals were injected with saline or dexamethasone (3 μg, i.c.v.) by the method mentioned above and anesthesia was stopped. One hour after saline/drug administration, the animal was decapitated, and the head was frozen in liquid nitrogen after 0, 0.5, 1, 2, or 3 min. During decapitation ischemia, the temperature of the temporal muscle was maintained at 37.5 ± 0.3 °C with a heating lamp.

On dry ice, the frozen brain was removed from the skull, weighed, and quickly homogenized in 4 ml ice-cold 0.4 M perchloric acid. After centrifugation at 20,000 × *g* for 30 min, the supernatant was injected into a high-performance liquid chromatography (HPLC) system to determine the brain concentrations of ATP, ADP, and AMP (Adachi et al., 2000). The HPLC system consisted of a pump (L-7100; Hitachi, Tokyo, Japan) used to deliver the mobile-phase, a model L-7250 sample injector (Hitachi) with a 100-μl sampling loop, two separation columns (GL-W510-S, 7.8 × 300-mm inside diameter; Hitachi), and a UV detector (L-7400; Hitachi). The mobile phase was 0.2 M NaH_2PO_4, with a pH of 3.5 adjusted with 0.2 M H_3PO_4, and the flow rate was 0.6 ml/min. The absorption intensity (peak height) was measured at a wavelength of 270 nm.

2.4. Experiment 3: Na^+,K^+-ATPase activity

Six gerbils were prepared and then assigned to the saline and dexamethasone groups (three animals in each) to evaluate the effect of dexamethasone on the activity of Na^+,K^+-ATPase. After anesthesia, each animal was injected with saline or dexamethasone (3 μg, i.c.v.), and then anesthesia was stopped. One hour after saline/drug administration, the animal was decapitated. The brains were rapidly dissected and quickly homogenized in 10 ml of ice-cold 0.32 M sucrose containing 1 mM ethylenediaminetetraacetic acid and 100 mM Tris–HCl (pH 7.4), using a Teflon-glass homogenizer. The homogenate was centrifuged first at 900 × *g* for 10 min, followed by centrifugation at 12,000 × *g* for 20 min. The supernatant has been reported to contain microsomal membrane particles and a soluble cytosol fraction (Svoboda and Mosinger, 1981). Fifty microliters of the supernatant was added to each of two test tubes containing 900 μl of either 120 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, 0.5 mM ouabain, 100 mM Tris–HCl (pH 7.4) or the same medium without ouabain.

The medium was pre-incubated at 37 °C for 10 min, and the reaction was started by adding 50 μl of 50 mM ATP in buffer to each tube to give a final ATP concentra-

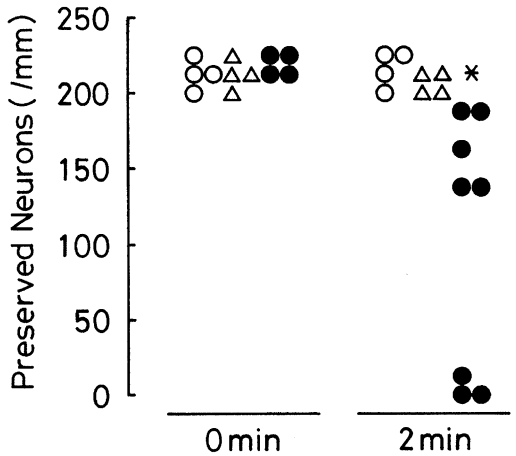

Fig. 1. Effects of dexamethasone (3 μg, i.c.v.) or its vehicle (2-hydroxypropyl-β-cyclodextrin, 50 μg, i.c.v.) on non-ischemic (0 min) and ischemic (2 min) gerbil brains. Hippocampal CA1 pyramidal neurons were examined after 7 days, and the number of pyramidal cells (ordinate) was determined. Values obtained from individual animals are shown; saline-injected animals (○), vehicle-injected animals (△), and dexamethasone-injected animals (●). $^{*}P < 0.01$ compared with each corresponding vehicle-injected group.

tion of 2.5 mM. After 10 min, the reaction was stopped by the addition of 50 μl 60% perchloric acid. The pH was immediately increased to 7.0 with 60 μl 8 M NaOH to prevent the spontaneous degeneration of ATP in acid. The suspensions were centrifuged at 3,000 × *g* for 15 min. The supernatant was injected into the HPLC system to determine the ADP amount according to the method described above. The remaining precipitate was used for protein assay, using bovine serum albumin as the standard (Peterson, 1977). The activity of Na^+,K^+-ATPase was determined as the difference in enzyme activity measured in the absence and the presence of ouabain, and expressed as the ADP produced per hour per mg of protein. To evaluate the effect of dexamethasone on the Na^+,K^+-ATPase activity in acidic buffers, an identical procedure was performed using an incubation medium adjusted to a pH of either 6.7 or 6.3. Two values for each pH were obtained from each homogenate.

Another set of 15 gerbils was assigned to one saline and four dexamethasone groups (three animals in each). After anesthesia, each animal was injected with saline or dexamethasone (0.3, 1, 3, or 10 μg, i.c.v.). The activity of Na^+,K^+-ATPase at pH 6.7 was determined by the method mentioned above. Two values were obtained for each homogenate.

2.5. Statistical analysis

The histologic data were evaluated with the Kruskal–Wallis test followed by the Mann–Whitney test. The biochemical data were analyzed by analysis of variance followed by the Bonferroni test to compare the corresponding saline group.

3. Results

3.1. Histologic outcome

The administration of either dexamethasone or its vehicle (2-hydroxypropyl-β-cyclodextrin) did not affect the number of neurons when ischemia was not induced (Fig. 1). Transient forebrain ischemia for 2 min did not damage the neurons after 7 days in either saline or vehicle-injected animals. However, 2-min ischemia reduced the number of preserved neurons in animals pretreated with dexamethasone.

3.2. Brain concentrations of adenylates

There were no differences between the saline and dexamethasone groups in the values of ATP, ADP, and AMP when the brains were frozen immediately after decapitation (Fig. 2). In the saline group, decapitation ischemia for 0.5 and 1 min produced a severe decrease in the brain ATP content, which was 44% and 15% of that in brains frozen immediately after decapitation, respectively. Pretreatment with dexamethasone suppressed the magnitude of the de-

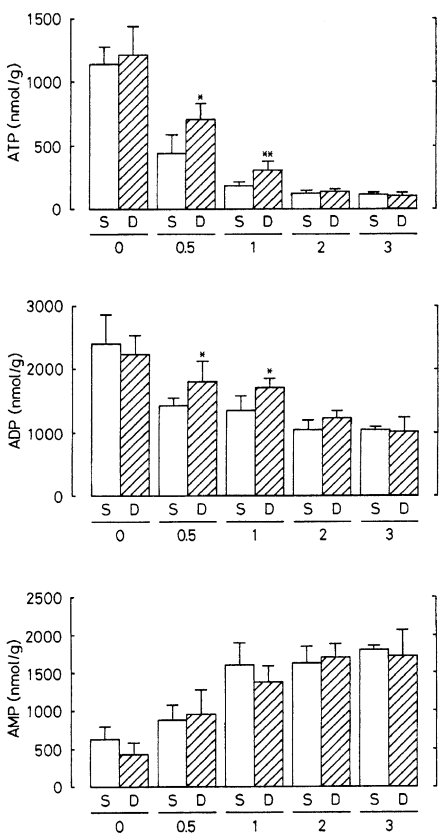

Fig. 2. Effects of dexamethasone (3 μg, i.c.v.) on ischemic changes in the brain concentrations of ATP, ADP, and AMP. Each value represents the mean ± S.D. from five animals. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with each corresponding saline-injected group. S: Saline-injected group and D: dexamethasone-injected group.

crease, and the values were 58% and 28% of the basal level, respectively. However, no differences were found after either 2 or 3 min of ischemia between the two groups, although the long duration of ischemia further decreased the ATP content in both groups.

Similar to the decrease in the ATP level, the brain ADP content decreased after decapitation ischemia, although the magnitude of the decrease was smaller than that of the decrease in ATP. The decrease in the ADP level was suppressed by dexamethasone treatment, and the effect was significant after ischemia for 0.5 and 1 min. In contrast, the brain AMP content increased after ischemia, and the magnitude of the increase tended to be smaller in the dexamethasone groups than in the saline groups.

3.3. Na^+,K^+-ATPase activity

The activity of Na^+,K^+-ATPase at pH 7.4 was 2.6 μmol ADP/h/mg protein in saline-injected gerbils (Fig. 3A). Administration of dexamethasone did not affect the enzyme activity (2.5 μmol ADP/h/mg protein). When the pH of the medium was 6.7, the activity in the saline group was suppressed to 57% of that at pH 7.4. The Na^+,K^+-ATPase activity in the dexamethasone group was further decreased at pH 6.7, and the value was 47% of that in the corresponding saline group. When the pH of the medium was 6.3, the activity was reduced markedly in both groups, and no marked difference was found between the groups.

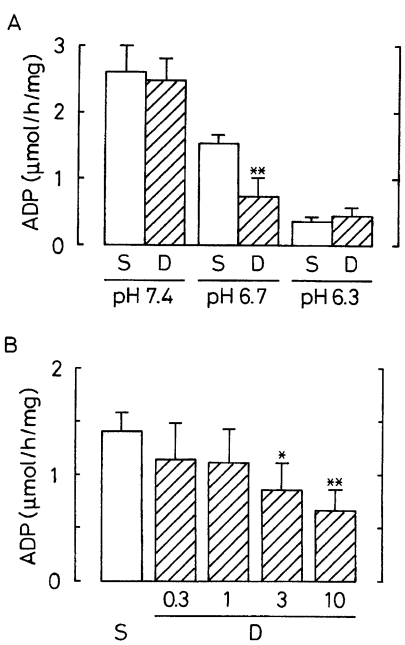

Fig. 3. Effects of dexamethasone (i.c.v.) on the activity of Na^+,K^+-activated adenosine triphosphatase in the brain. Changes in enzyme activity induced by dexamethasone (3 μg) at pH 7.4, 6.7, and 6.3 (A) and changes in activity induced by dexamethasone (0.3, 1, 3, and 10 μg) at pH 6.7 (B). Activity was determined as the difference in enzyme activity measured in the absence and the presence of ouabain, and expressed as the ADP produced per hour per mg of protein. Each value represents the mean ± S.D. of six measurements. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the corresponding saline-injected group. S: Saline-injected animals and D: dexamethasone-injected animals.

The activity of Na^+,K^+-ATPase at pH 6.7 gradually decreased as the dose of dexamethasone increased, and effects were significant at doses higher than 3 μg (Fig. 3B).

4. Discussion

In the present study, dexamethasone aggravated delayed neuronal death caused by 2 min of ischemia in gerbils. The drug also attenuated an ischemic decrease in brain ATP concentration and suppressed Na^+,K^+-ATPase activity in an acidic buffer.

Several reports, and the results of this study, attest to the damaging properties of glucocorticoids in various models of cerebral ischemia (Sapolsky and Pulsinelli, 1985; Koide et al., 1986; Adachi et al., 1998; Tsubota et al., 1999). Because glucocorticoids increase the plasma concentration of glucose, the aggravation has been speculated to be a result of intracellular lactic acidosis associated with hyperglycemia (Nedergaard et al., 1991). However, we confirmed in a previous study that physiologic variables, such as the plasma glucose concentration and arterial blood gas tension, were not altered by the treatment used in the current study (Adachi et al., 1998). Therefore, another element besides hyperglycemia may exert a deleterious effect.

Na^+,K^+-ATPase acts on the hydrolysis step by which ATP supplies free energy. Therefore, it is conceivable that the suppression of Na^+,K^+-ATPase activity is responsible for the aggravation of ischemic neuronal damage, by disturbing the energy supply. However, the magnitude of the suppression of enzyme activity found in the present study might not be sufficient to induce neuronal damage by hampering the supply of energy. When the intracellular Na^+ concentration is increased by blockade of Na^+,K^+-ATPase, the Na^+–Ca^{2+} exchange carrier reverses its resting mode of operation, which leads to an increase in intracellular Ca^{2+} concentration. A marked influx of Ca^{2+} into neurons during ischemia has been shown to provoke catastrophic enzymatic process, leading to irreversible neuronal injury (Mitani et al., 1994). Therefore, the disturbance in the Na^+,K^+-ATPase activity may contribute to the aggravation by increasing the intracellular Ca^{2+} concentration. This is in agreement with our previous study that showed a facilitation of the hypoxic increase in intracellular Ca^{2+} concentration in dexamethasone-treated brain slices (Adachi et al., 1998). Because the intracellular pH decreases in the early stage of ischemia due to anaerobic metabolism, the reduced sodium pump activity in acidosis may be a contributing factor in the development of ischemic neuronal damage. Thus, the agent may have aggravated ischemic damage in animals subjected to even a short duration of ischemia.

In contrast, glucocorticoids have been shown to provide protection against hypoxic or ischemic insult in the immature brain while preserving the brain ATP content (Barks et al., 1991; Tuor et al., 1995, 1997). Although these findings are inconsistent with our results, the contradictory effects of glucocorticoids may be explained by differences in cerebral energy metabolism between the mature and immature brain. Studies of adult animals have demonstrated that local cerebral glucose utilization is decreased by glucocorticoids (Kadekaro et al., 1988; Doyle et al., 1994). In contrast, glucose utilization during ischemia has been shown to be elevated by dexamethasone in the immature rat (Tuor et al., 1997). Therefore, although glucocorticoids keep the brain ATP concentration relatively high during ischemia in both immature and mature brains, the mechanism underlying the preservation of ATP levels may differ between immature and mature brains. It seems that the ATP level in the adult brain is maintained by a disturbance in energy metabolism, whereas that in the immature brain is maintained by an improvement in anaerobic glycolysis.

With respect to the effect of dexamethasone on the energy metabolism of glia, dexamethasone has been reported to enhance the consumption of ATP in cultured astrocytes when they are exposed to hypoxia or glucose deprivation (Tombaugh and Sapolsky, 1992). Because ATP utilization has actually been shown to be increased in cultured astrocytes following dexamethasone administration, the decrease in ATP utilization may occur primarily in other cells such as neurons and could be even greater than that observed in whole brain homogenates. The utilization of ATP in ischemia was suppressed by dexamethasone, and the drug reduced Na^+,K^+-ATPase activity in acidosis. Although the mechanisms responsible for the aggravation of ischemic neuronal damage by glucocorticoids must still be clarified, the suppression of Na^+,K^+-ATPase activity during ischemia may be partly involved in the mechanism underlying the aggravation.

Acknowledgements

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