Dexamethasone Changes Brain Monoamine Metabolism and Aggravates Ischemic Neuronal Damage in Rats

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Background: Glucocorticoids have been reported to aggravate ischemic brain damage. Because changes in the activities of various neuronal systems are closely related to the outcome of ischemic damage, the authors evaluated the effects of dexamethasone on the monoaminergic systems and ischemic neuronal damage.

Methods: The right middle cerebral artery was occluded for 2 h, and the tissue concentrations of monoamines and their metabolites were determined in the cerebral cortex and the striatum of rats. The turnover of 5-hydroxytryptamine was compared in animals injected with saline and those injected with dexamethasone twice (2 mg/kg in each injection) by evaluating the probenecid-induced accumulation of 5-hydroxyindoleacetic acid. The turnovers of norepinephrine and dopamine were estimated from the α-methyl-p-tyrosine-induced depletion of norepinephrine and dopamine, respectively. The effect of dexamethasone on the infarct volume was evaluated by triphenyltetrazolium chloride stain in rats subjected to 2 h of occlusion.

Results: Dexamethasone did not affect the cortical 5-hydroxytryptamine or 5-hydroxyindoleacetic acid contents. However, it suppressed the turnover of the cortical 5-hydroxytryptamine on both sides. Dexamethasone reduced the turnover of the striatal 5-hydroxytryptamine and facilitated the dopamine turnover. In rats subjected to 2 h of occlusion and 2 h of reperfusion, the infarct volume was 10.5 times greater in the group that received dexamethasone than in the animals that received saline.

Conclusions: Dexamethasone suppresses the inhibitory serotonergic system and facilitates the excitatory dopaminergic system in the rat telencephalon. This may be a mechanism by which dexamethasone aggravates ischemic neuronal injury. (Key words: Cerebral ischemia; glucocorticoids.)

The administration of glucocorticoids has been reported to aggravate ischemic brain damage. Although the mechanism underlying this aggravation is unclear, glucocorticoid-induced hyperglycemia is presumed to be related to it. The plasma concentration of corticosterone (an endogenous glucocorticoid) has been shown to be elevated in cerebral ischemia and in some stress conditions. This increase is also thought to occur with the deleterious effect, because inhibition of the synthesis of endogenous glucocorticoids improved the histologic outcome. Conversely, glucocorticoid treatment has been reported to provoke mental disorders such as depression, and the pathogenesis of depression is closely related to changes in the serotonergic system in the central nervous system. Furthermore, long-term treatment with glucocorticoids has been shown to increase the turnover rate of the central dopaminergic system.

In ischemia, various kinds of neurotransmitters (excitatory and inhibitory) are released from nerve endings. Neurotransmitters such as glutamate and aspartate excite the synaptic membrane, leading to irreversible neuronal injury, whereas inhibitory neurotransmitters or neuromodulators are thought to inhibit the postsynaptic neuron and thus to provide benefit. With respect to the monoaminergic system, a protective effect by the serotonergic system and neurotoxicity by the dopaminergic system have been shown. In the current study, we evaluated the effect of dexamethasone, a pure glucocorticoid, on turnover rates of monoaminergic systems and the static levels of neurotransmitters and their metabolites in the brain. We used an animal model of the middle cerebral artery (MCA) occlusion in rats and evaluated these effects in relation to morphologic outcome.
Materials and Methods

This study was approved by the Committee on Animal Experimentation at Ehime University School of Medicine, Ehime, Japan. Male Wistar rats (Charles River, Yokohama, Japan) that weighed approximately 280 g at the time of operation were housed in groups in a temperature-controlled room at 23 ± 2°C and maintained in an alternating 12-h light and 12-h dark cycle (lights on at 6:00 AM). Animals were deprived of food for at least 6 h before ischemia was induced, because hyperglycemia can influence ischemic brain damage.13-16 In experiment one, 60 rats were used to evaluate brain monoamine turnover. In experiment two, 24 rats were used to evaluate infarct size by triphenyltetrazolium chloride stain. In experiment three, 12 rats were used for the histologic experiment. All animals were subjected to MCA occlusion, and values on the contralateral side were used for those of the intact tissue, because we confirmed in our previous study that there were no differences between the values on the contralateral side in the ischemic group and those of the sham-operated group.17

Cerebral Ischemia (Middle Cerebral Artery Occlusion)

We used the MCA occlusion model described by Kozumi et al.18 Rats were anesthetized with 2% halothane in a gas mixture with 49% oxygen in nitrous oxide (N₂O), and they breathed spontaneously. With the rats in the supine position, the skin was incised along the median line of the neck, and the right carotid artery was exposed. A thermocouple needle probe (TN-800; Unique Medical Corp., Tokyo, Japan) was inserted into the temporal muscle. The body temperature was maintained carefully at 37 ± 0.2°C using a heating lamp during surgery.19 The root of the right MCA was occluded with the insertion of a 4-0 nylon thread from the bifurcation of the internal and external carotid arteries. The 8-mm-tip portion of this thread was coated with silicone to render its diameter 0.30 to 0.34 mm. The tip of the thread was placed 16.5 mm distal from the bifurcation. After all surgical incisions were sutured, the animals were allowed to recover from anesthesia. The animals were brought to their cages in a room maintained at constant temperature and allowed access to water ad libitum. When they recovered from anesthesia, all rats showed paralysis of the contralateral limbs.

Experiment 1

Brain Monoamine Turnover. The 5-hydroxytryptamine (5-HT) turnover was estimated by assessing the probenecid-induced accumulation of 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of 5-HT, because probenecid blocks the transfer of 5-HIAA from the brain to plasma.20 The turnover of norepinephrine and dopamine was estimated by assessing the depletion of norepinephrine and dopamine induced by a-methyl-p-tyrosine (a-MT), because a-MT blocks the synthesis of catecholamines.21 In this experiment, we evaluated the effects of dexamethasone on the accumulation and the depletion for 40 min and 80 min, respectively. The 60 rats were divided evenly into the following 10 groups: group 1, saline-injected animals; group 2, dexamethasone-treated animals; group 3, saline-treated animals injected with probenecid 40 min before decapitation; group 4, dexamethasone-treated animals injected with probenecid 40 min before decapitation; group 5, saline-treated animals injected with probenecid 80 min before decapitation; group 6, dexamethasone-treated animals injected with probenecid 80 min before decapitation; group 7, saline animals injected with a-MT 40 min before decapitation; group 8, dexamethasone-treated animals injected with a-MT 40 min before decapitation; group 9, saline-treated animals injected with a-MT 80 min before decapitation; and group 10, dexamethasone-treated animals injected with a-MT 80 min before decapitation.

Determination of Brain Levels of Monoamines and Their Metabolites. Two hours after the MCA was occluded, each rat was decapitated and their brains were rapidly removed, rinsed in saline, and placed on ice. Along the coronal planes at the optic chiasma and caudal edge of the mamillary body, the brains were cut from the rostral and caudal portions. On both sides, the cerebral cortex to dorsal from the sulcus rhinalis between these cut planes and whole striatum were dissected using dental tweezers, and cerebral infarction was marked on the ischemic side. Each tissue sample was homogenized in 1 ml of 0.4 M perchloric acid containing 0.1% L-cysteine. After being centrifuged and filtered through a membrane filter (0.2 μm), 10 μl of the filtrate was applied to a high-performance liquid chromatography system with electrochemical detection to determine the concentrations of 5-HT, 5-HIAA, dopamine, 3,4-dihydroxyphenylacetic acid, and homovanillic acid, according to the method of Magnusson et al.,22 with a slight modification.17 The high-performance liquid chromatography system consisted of a pump equipped with a damper (EP-300, Eicom, Kyoto, Japan), an electrochem-
lical detector (ECD-300, Eicom) with a graphite working electrode operated at 750 mV versus a silver-silver chloride reference electrode (RE-100, Eicom), and a reverse-phase column (MA-5ODS, 2.1 × 150 mm ID; Eicom). The mobile phase consisted of 0.1 m citrate and 0.1 m sodium acetate buffer (pH 3.3) containing 15% methanol, 1 m sodium 1-octanesulfonate, and 10 μm disodium ethylenediaminetetraacetic acid. Norepinephrine in the remaining supernatant was absorbed onto 10 mg alumina at pH 8.2 and eluted with 200 μl of 0.1 m HCl after washing with redistilled water. After centrifugation, a 10-μl portion was applied to the high-performance liquid chromatography system.

Experiment 2

**Measurement of the Infarct Volume.** In this experiment, 24 rats were evenly assigned to four groups: saline- or dexamethasone-injected animals that were subjected to 2 h of MCA occlusion, and saline- or dexamethasone-injected animals that underwent 2 h of MCA occlusion and 2 h of reperfusion. The right MCA was occluded according to the previously described method after injection with 50 units heparin into the tail vein. After the skin was sutured, the animals were allowed to recover from anesthesia. Animals that underwent reperfusion were anesthetized again and blood flow was resumed 2 h after MCA occlusion by pulling the silicone-coated nylon thread by 5 mm. Again the animals were allowed to recover from anesthesia.

After decapitation at the appropriate time, the rat brains were removed and rinsed in saline. Brain slices, 2-mm thick, between the coronal planes at the optic chiasma and caudal edge of the mamillary body were incubated for 30 min with 2% triphenyltetrazolium chloride in 0.1 m phosphate buffer (pH 7.4) at 37°C. Triphenyltetrazolium chloride is reduced by dehydrogenase enzymes, which exist in viable cells, and results in a formazan precipitate, thereby turning tissue deep red. In contrast, nonviable cells in the infarcted area are pale gray with this procedure. The tissue was stored overnight in 10% formaldehyde. The infarct size was determined using computer-aided planimetry with a Macintosh computer and National Institutes of Health Image Analysis software (version 1.52; Bethesda, MD).4,25

Experiment 3

**Histologic Examination.** The 12 rats were divided into two groups: saline-injected animals and dexamethasone-injected animals. The right MCA was occluded according to the previously described method after injection with 50 units heparin. The animals were allowed to recover from anesthesia, and blood flow was resumed by pulling the silicone-coated nylon thread 40 min after the occlusion under anesthesia. Seventy-two hours later, the animals were anesthetized with an intraperitoneal injection of sodium pentobarbital. The brains were perfused with heparin-prepared saline and fixed with 10% buffered formalin. After dehydration with graded concentrations of alcohol solutions, the brains were embedded in paraffin. Brain slices, 5-μm thick, were stained with hematoxylin and eosin. The numbers of preserved neurons in a constant area (2 × 2 mm) in the cerebral cortex and the striatum were counted in the same level of coronal sections. The number of neurons on the ischemic side of each group were compared.

**Blood Analysis.** The plasma concentrations of glucose and blood gases were measured in the 12 animals subjected to the histologic experiment. Immediately after the MCA occlusion, a 0.5-ml blood sample was collected through the left femoral artery and analyzed according to routine laboratory procedures (blood glucose testing system by electrode, MPG01, Dainin, Osaka, Japan; and blood gas analyzing system, ABL505, Radiometer, Copenhagen, Denmark).

**Drugs and Chemicals**

Dexamethasone, probenecid, and α-MT hydrochloride methyl ester were purchased from Sigma Chemical Company (St. Louis, MO). Triphenyltetrazolium chloride in water was obtained from Research Organs (Cleveland, OH). All chemicals used were of guaranteed reagent grade. Probenecid and dexamethasone were emulsified with 0.5% polysorbate 80. The α-MT hydrochloride methyl ester was dissolved in saline. The doses of all drugs are expressed as the weights of free bases. Dexamethasone was injected twice intraperitoneally 60 min before the MCA was occluded (2 mg/kg) and immediately after the MCA was occluded (2 mg/kg).24 Probenecid (200 mg/kg) or α-MT (250 mg/kg) was injected intraperitoneally.

**Statistical Analysis**

Monoamine turnover, the infarct volume, and histologic data were evaluated by two-way analysis of variance, and *post hoc* comparisons were performed using the Sheffe test by comparing each value in the dexamethasone group with that in the corresponding saline group. The plasma concentration of glucose and the arterial blood gas analysis data were evaluated by analysis of variance with Bonferroni's correction.
Results

Figure 1A shows the tissue concentration of 5-HT in the cerebral cortex in rats subjected to MCA occlusion for 2 h. There was no significant difference in the concentration of 5-HT between the nonischemic and the ischemic sides in the saline-injected animals. Dexamethasone treatment did not significantly affect the concentrations of 5-HT on either side. Figure 1B shows the tissue concentration of 5-HIAA in the cerebral cortex in MCA-occluded rats and the effect of probenecid on it. Among the groups that did not receive probenecid treatment, the concentration of 5-HIAA did not differ significantly between the saline and dexamethasone groups, indicating that the dexamethasone treatment did not affect the static 5-HIAA concentration on either the nonischemic or ischemic sides. On the nonischemic side, the injection of probenecid accumulated 5-HIAA to 146% of the static value for 80 min in the saline group. Similarly, on the ischemic side, the probenecid treatment resulted in an accumulation of 5-HIAA to 195%. The dexamethasone treatment markedly inhibited the probenecid-induced accumulation of 5-HIAA on the nonischemic and ischemic sides, indicating suppression of the 5HT turnover by dexamethasone. Figure 1C shows the tissue concentration of norepinephrine in the cerebral cortex and the effect of α-MT on it. In the saline-injected group without α-MT treatment, 2 h of MCA occlusion markedly reduced the concentration of norepinephrine, the value being 50% of that on the nonischemic side. The dexamethasone treatment did not affect these static values on either side. The α-MT-induced depletion of the cortical norepinephrine on the nonischemic side was marked in the dexamethasone-treated rats compared with that in the saline group, indicating the facilitation of the norepinephrine turnover by dexamethasone. However, the norepinephrine concentration on the ischemic side was not changed.

Figure 2A shows the concentration of striatal 5-HT. In the saline-treated group, the concentration of 5-HT on the ischemic side was reduced by MCA occlusion. The dexamethasone treatment increased the concentration on the nonischemic side to 129% of that in the saline-treated group. The concentration of 5-HT on the ischemic side was also elevated to 230% by the dexametha-
Fig. 2. The effect of dexamethasone on the brain contents of monoamines and their metabolites in the striatum in middle cerebral artery–occluded rats. Dexamethasone was injected twice in 60 min before the occlusion and immediately after the occlusion (2 mg/kg each time), and the animals were decapitated 2 h after the start of the occlusion. (A) The concentration of 5-hydroxytryptamine and the effect of dexamethasone. (B) The concentration of 5-hydroxyindoleacetic acid and the probenecid-induced accumulation of 5-hydroxyindoleacetic acid. Probenecid (200 mg/kg) was injected 40 or 80 min before decapitation. (C) The concentration of dopamine and the α-MT–induced depletion of dopamine. The α-MT (250 mg/kg) was injected 40 or 80 min before decapitation. (D) The concentrations of 3,4-dihydroxyphenylacetic acid and homovanillic acid and the effect of dexamethasone. C = contralateral (nonischemic) side; I = ipsilateral (ischemic) side; Sal = saline-injected animals; Dex = dexamethasone-injected animals; Pro = probenecid; α-MT = α-methyl-p-tyrosine. Each value represents the mean ± SD of six animals. *P < 0.05, **P < 0.01 as compared with the corresponding saline-injected group value.
movanillic acid on the nonischemic side. Middle cerebral artery occlusion produced increases in the concentrations of these metabolites on the ischemic side in the saline-injected and dexamethasone-injected animals, and the extent of the increase was larger in the dexamethasone group.

The cerebral infarction was not observed by triphenyltetrazolium chloride stain immediately after 2 h of MCA occlusion in the saline- and dexamethasone-treated groups (fig. 3). However, when the brains were reperfused for 2 h after 2 h of occlusion, the infarct volume on the ischemic side in the saline group was $1.1 \times 10^{-8}$ m$^3$. Treatment with dexamethasone markedly increased the infarct volume, with a value of $11.5 \times 10^{-8}$ m$^3$.

Figure 4 shows the numbers of preserved neurons in the cerebral cortex and the striatum. There was no difference in the number of preserved neurons on the contralateral side between the dexamethasone- and the saline-treated groups. On the ischemic side in the saline group, 40 min of MCA occlusion damaged the striatum and the cerebral cortex. Treatment with dexamethasone markedly aggravated the damage at both regions.

Animals in the saline- and dexamethasone-treated groups recovered well from halothane anesthesia after it was stopped. No seizures were noted in either group in the 72 h between ischemia and death, and there were no differences between the two groups in their food and water intake. As shown in table 1, there were no differences between the two groups in the blood analysis immediately after the occlusion.

**Discussion**

In the current study, we observed dexamethasone-induced decreases in the activity of the rat serotonergic system in the cerebral cortex and the striatum, and an increase in the activity of the dopaminergic system in the striatum. These phenomena occurred on the nonisch-
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Table 1. Physiologic Variables of Saline and Dexamethasone-treated Rats with MCA Occlusion

<table>
<thead>
<tr>
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<th>Saline</th>
<th>Dexamethasone</th>
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<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>191 ± 30</td>
<td>191 ± 31</td>
</tr>
<tr>
<td>pH</td>
<td>7.394 ± 0.055</td>
<td>7.406 ± 0.042</td>
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<tr>
<td>PaO₂ (mmHg)</td>
<td>42 ± 6</td>
<td>40 ± 4</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>143 ± 18</td>
<td>160 ± 19</td>
</tr>
<tr>
<td>HCO₃⁻ (mmol/l)</td>
<td>29.0 ± 1.4</td>
<td>26.8 ± 1.9</td>
</tr>
<tr>
<td>Base excess (mEq/l)</td>
<td>2.1 ± 2.2</td>
<td>2.3 ± 2.9</td>
</tr>
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Dexamethasone was administered twice, 60 min before occlusion and immediately after MCA occlusion (2 mg/kg each time). After the MCA occlusion, 0.5 ml of blood was collected through the femoral artery and analyzed. Each value represents the mean ± SD of five animals.

The increase in the concentration of 5-HT in the synaptic cleft with the blockade of the reuptake mechanism has been shown to be neuroprotective against ischemia, whereas the depletion of 5-HT from the neuron has been reported to aggravate ischemic neuronal injury. Furthermore, 5-HT₁A agonists can reduce the neuronal injury by inhibiting the release of glutamate in vitro. Therefore, the exacerbation of the histologic outcome by dexamethasone observed in the current histologic experiment may be caused in part by the inhibition of serotonergic activity.

Concerning the effect of dexamethasone on the turnover of brain norepinephrine and dopamine, we applied the enzyme inhibition technique using α-MT, which inhibits the hydroxylation step from tyrosine to dopa, the rate-limiting step in the synthesis of catecholamines. In the current study, the turnover of the cortical norepinephrine on the nonischemic side was diminished by the administration of dexamethasone, although that on the ischemic side was obscured because of the small extent of the decrease by α-MT. In the striatum, the extent of dopamine depletion by α-MT was larger on both sides in the dexamethasone group than in the control group. In addition, the metabolites of dopamine were increased in the dexamethasone-treated group. These findings illustrate the activation of the dopaminergic system by dexamethasone. Because dopamine exerts harmful effects on neurons in ischemia by enhancing the hydrogen peroxide production in its degenerating process by monoamine oxidase, dexamethasone may aggravate the neuronal damage by increasing the dopaminergic activity and by inhibiting the serotonergic activity.

Cerebral infarction was elucidated clearly by tripheny1tetrabrom chloride stain 2 h after reperfusion and 2 h of MCA occlusion, but not immediately after 2 h of MCA occlusion. Because triphenyltetrabrom chloride is reduced by dehydrogenase enzymes in viable cells, the enzymatic function seems to be abolished within 2 h of reperfusion. Conversely, 40 min of MCA occlusion produced a moderate damage in the telecepharon after 72 h, and the degree of the morphologic damage is consistent with that in a previous study that showed delayed damage by transient MCA occlusion. These findings indicate that the functional damage of the neuron precedes the morphologic change after transient ischemia. Changes in the turnover of the monoaminergic systems by dexamethasone may exert deleterious influences on both aspects.

Glucocorticoids increase the plasma concentration of
glucose, and hyperglycemia exacerbates ischemic neuronal injury. This aggravation is speculated to be a result of the intraneuronal lactate acidosis associated with enhanced anaerobic glucose metabolism. However, in the current study, the plasma concentration of glucose did not differ between the saline- and the dexamethasone- treated group. Therefore, it is not likely that hyperglycemia affected the infant size and the histologic outcome, although the current value represents a state immediately after occlusion. Another element specific to dexamethasone besides hyperglycemia seems to have provided the deleterious effect. The inhibition of glucose uptake, protein synthesis, and cell division are conceivable as mechanisms of the acute effect of dexamethasone, whereas chronic administration may harm neurons by modifying nucleic acids through steroid receptors in the nucleus. In our previous studies, dexamethasone facilitated the release of glutamate in ischemia and enhanced the intracellular Ca²⁺ increase in the gerbil hippocampus, both of which are regarded as crucial factors in the development of ischemic neuronal damage. Because the cerebral cortex and the striatum are innervated by monoaminergic neurons and glutamate fibers, changes in the monoaminergic systems that we elucidated in the current study may be involved in the outcome.

In conclusion, we observed the suppression of the inhibitory serotonergic system and the facilitation of the excitatory dopaminergic system after dexamethasone treatment. These changes in monoaminergic systems may be a mechanism by which dexamethasone aggravates ischemic neuronal injury.

References

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