OPEN

Changes in Energy Levels by Dexamethasone in Ischemic Hearts and Brains in Male Mice

Toshihiro Yorozuya, MD, Chikara Namba, MD, PhD, Naoto Adachi, MD, PhD, Kazuo Nakanishi, MD, Kentaro Dote, MD, and Takumi Nagaro, MD

Background: Glucocorticoids have been shown to alleviate ischemia-induced myocardial injury, while aggravating neuronal damage caused by ischemia. As energy failure is a predominant factor in cellular viability, we examined the effects of glucocorticoids on energy utilization in the mouse heart and brain.

Methods: Seventy-two male ddY mice were assigned to 1 of 3 groups: saline (S), dexamethasone (a glucocorticoid without mineralocorticoid activity, 5 mg/kg) (D), and metyrapone (a potent inhibitor of the synthesis of glucocorticoids, 100 mg/kg) (M) groups (n = 24 in each). Three hours after intraperitoneal administration, all animals were decapitated, and the heads were frozen in liquid nitrogen after 0, 0.5, 1, or 2 minutes (n = 6 in each). The hearts were immediately removed and frozen in liquid nitrogen after 0, 5, 10, or 20 minutes of incubation at 37°C (n = 6 in each). The concentrations of adenylates and monoamines were determined by high-performance liquid chromatography.

Results: In the heart, the adenosine 5′-triphosphate (ATP) concentration did not differ among the 3 groups at 0 minute of ischemia (3 h of S, D, or M treatment). Ischemia for 5 minutes decreased the ATP content to 21% of the basal level in the S group. The ATP decrease was suppressed by either the D or M treatment, such that after 5 minutes ATP levels were 63% and 64% of each basal level, respectively. In the brain, the ATP level in the M group was 62% of that in the S group at 0 minute of ischemia, and the 5′-monophosphate (AMP) level was 276% of that in the S group. Brain dopamine metabolism was facilitated by dexamethasone, and suppressed by metyrapone.

Conclusions: The relationship between effects of glucocorticoids on ischemia-induced changes in energy levels and cellular viability was not clearly elucidated.

Key Words: adenosine 5′-triphosphate, brain, dexamethasone, dopamine, heart, ischemia, mice

(*J Neurosurg Anesthesiol* 2015;27:295–303)

The cardioprotective effects of glucocorticoids in the acute setting of ischemia/reperfusion have been experimentally demonstrated in animals[1–7] and humans.[8] Although the mechanisms underlying glucocorticoid cardioprotection are not known with certainty, glucocorticoids are reported to preserve cellular function in ischemia thereby prolonging the period of myocardial viability. In previous studies, glucocorticoids were reported to improve lactate imbalance and prevent the leakage of intracellular enzymes caused by myocardial ischemia.[6,7]

At the same time, deleterious effects of glucocorticoids on ischemia-induced neuronal damage have been shown in various animal models, despite the ability of glucocorticoids to alleviate cerebral edema caused by traumatic brain injury and hemorrhage.[9–13] Further, steroids are not indicated for traumatic brain injury in humans, as they do not improve clinical outcomes.[14]

Because adenosine 5′-triphosphate (ATP) provides energy to maintain membrane functional integrity in both the heart and brain, and ATP depletion precedes loss of cellular viability, the different effects of the agents in ATP utilization in the heart and brain during ischemia could well explain differences in ischemia-induced organ injury. In the present study, therefore, we investigated the effect of dexamethasone, a glucocorticoid without mineralocorticoid activity, on ischemia-induced reduction of ATP in the mouse heart and brain. In addition, we examined the effect of metyrapone, a potent inhibitor of the synthesis of glucocorticoids, to assess the role of endogenous glucocorticoids. We hypothesized that ATP levels would be diminished in neuronal tissue subjected to dexamethasone and improved in cardiac tissue subjected

Received for publication July 25, 2014; accepted November 18, 2014.
From the Department of Anesthesia and Perioperative Medicine, Ehime University Graduate School of Medicine, Ehime, Japan.
Present address: Chikara Namba, MD, PhD, Division of Anesthesia, Mizushima Chuo Hospital, Okayama 712-8064, Japan.
Present address: Naoto Adachi, MD, PhD, Mabuchi Clinic, Kyoto 600-8357, Japan.
Supported, in part, by the Department of Anesthesia and Perioperative Medicine, Ehime University Graduate School of Medicine.
The authors have no conflicts of interest to disclose.
Reprints: Toshihiro Yorozuya, MD, Department of Anesthesia and Perioperative Medicine, Ehime University Graduate School of Medicine, Shitsukawa, To-on, Ehime 791-0295, Japan (e-mail: yorozuya@m.ehime-u.ac.jp).

to dexamethasone. In contrast, ATP levels would be improved in neuronal tissue subjected to metyrapone and diminished in cardiac tissue subjected to metyrapone.

MATERIALS AND METHODS

This study was approved by the Committee on Animal Experimentation at Ehime University Graduate School of Medicine, Ehime, Japan. All animals were cared for in compliance with the Principles of Laboratory Animal Care formulated by Ehime University Graduate School of Medicine. Male ddY mice at 10 weeks of age, weighing about 40 g (Japan SLC, Shizuoka, Japan), were housed in an acrylic cage (5 mice in each cage) (CLEA Japan Inc., Tokyo, Japan) with paper bedding (Japan SLC) at 23 ± 1°C, and maintained in an alternating 12-hour light/12-hour dark cycle (lights on at 06:00 h). A certified diet (MF; Oriental Yeast Co. Ltd., Tokyo, Japan) and water filtrated with activated carbon were provided ad libitum. The mice were deprived of food for at least 6 hours preceding the start of experiments to eliminate the influence of glucocorticoids on the plasma concentration of glucose.

Seventy-two mice were prepared and then randomly assigned to 4 saline groups, 4 dexamethasone groups, and 4 metyrapone groups (6 animals in each). Saline, dexamethasone (5 mg/kg), or metyrapone (100 mg/kg) was administered intraperitoneally, and mice were decapitated after 3 hours. Previous studies reported that these agents did not affect plasma concentrations of glucose and electrolytes 3 hours after treatments.[11,13,15]

The heart was removed and incubated in a hypoxic phosphate buffer solution equilibrated with nitrogen (pH 7.4, 37°C). The partial pressure of oxygen in the hypoxic phosphate buffer was kept between 29.9 and 41.5 mm Hg. Then, the heart was frozen in liquid nitrogen after 5, 10, or 20 minutes of incubation. In the nonischemic (0 min) groups, the heart was frozen immediately after its removal, and body temperature was quickly measured in the sternocleidomastoid muscle on the side to the sternal origin of the body by a thermocouple needle probe (DTE-10 N; Inter Medical, Aichi, Japan).

After decapitation, the head was frozen in liquid nitrogen after 0, 0.5, 1, or 2 minutes. The thermocouple needle probe was inserted into the sternocleidomastoid muscle on the side to its insertion of the skull, and head temperature was kept approximately 37.5°C with a heating lamp during decapitation ischemia in the 0.5-, 1-, and 2-minute groups. The temperature of the sternocleidomastoid muscle was recorded before freezing the head after 0.5, 1, and 2 minutes. The brain was removed from the frozen skull on dry ice to prevent the degeneration of adenylates.

We monitored temperature in the sternocleidomastoid muscle as a surrogate for temporal muscle temperature, as hypothermia is well known to provide benefits on ischemia-induced organ injury, and temporal muscle temperature well reflects brain temperature.[16,17] However, we could measure neither brain nor temporal muscle temperature in the 0-minute groups, because the head had been frozen immediately after decapitation. We measured temperature in the sternocleidomastoid muscle near its insertion on the skull and its sternal origin as a surrogate for brain and body temperatures, respectively, immediately after decapitation in the 0-minute groups, although there are no reports that showed the relationship between sternocleidomastoid muscle temperature and temporal muscle and body temperatures.

The frozen heart and brain were weighed and quickly homogenized with ice-cold perchloric acid (3 mL of 0.4 mol/L). After centrifugation at 20,000*g* for 30 minutes, the supernatant was injected into a high-performance liquid chromatography (HPLC) system to determine the tissue concentrations of ATP, adenosine 5′-diphosphate (ADP), and adenosine 5′-monophosphate (AMP).[12] 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, a separation column (GL-W510-S, 7.8 × 300 mm inside diameter; Hitachi), and an ultraviolet detector (L-7400; Hitachi). The mobile phase was 0.2 mol/L NaH_2PO_4, with an adjusted pH of 3.5 with 0.2 mol/L H_3PO_4, and the flow rate was 0.5 mL/min. The absorption intensity (peak height) was measured at a wavelength of 270 nm.

The concentrations of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-hydroxytryptamine, 5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) in the brain homogenates were determined using an HPLC system with electrochemical detection (Eicom, Kyoto, Japan).[18] The HPLC system consisted of a pump equipped with a damper (EP-300; Eicom), an electrochemical 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 inside diameter; Eicom). The mobile phase consisted of 0.1 mol/L citrate and 0.1 mol/L sodium acetate buffer (pH 3.3) containing 15% methanol, 1 mmol/L sodium 1-octanesulfonate, and 10 μmol/L disodium ethylenediaminetetraacetic acid.

Although agents were not injected in a blinded manner, the sampling of organs and the measurements of adenylates and monoamines were performed by investigators who were unaware of the particular treatment group.

The data were analyzed using 2-way analysis of variance to evaluate interactions between treatments and time points. Then, the data on the 3 treatment groups at each time point were analyzed by 1-way analysis of variance, and post hoc comparisons were performed with the Scheffé test (StatView for Windows, ver. 5.0; SAS Institute Inc., Cary, NC). $P < 0.05$ was considered statistically significant. Confidence intervals were calculated with SPSS Statistics, ver. 11.0.1 J (SPSS Inc., Chicago, IL).

RESULTS

Body temperature measured in the sternocleidomastoid muscle, on the side of the sternal origin of the body, immediately after decapitation in the saline, dex-

TABLE 1. Changes in Temperature in the Sternocleidomastoid Muscle During Ischemia

	Duration of Ischemia (min)		
	0.5	**1**	**2**
Saline (95% CI)	37.4 ± 0.5 (36.9-37.9)	37.4 ± 0.8 (36.5-38.2)	37.8 ± 0.4 (37.4-38.2)
Dexamethasone (95% CI)	37.0 ± 0.7 (36.2-37.8)	37.5 ± 0.6 (36.8-38.2)	37.5 ± 0.5 (37.0-38.0)
Metyrapone (95% CI)	36.6 ± 0.6 (36.0-37.2)	36.6 ± 0.5 (36.1-37.1)	37.1 ± 0.7 (36.3-37.8)
P	0.12	0.07	0.10
Power	0.41	0.50	0.45

Temperature in the sternocleidomastoid muscle, on the side to its insertion of the skull, was measured as a surrogate for temporalis muscle temperature immediately before freezing. Each value represents the mean ± SD of 6 animals.

P and power values were obtained at each time point by analysis of variance. *P* values for body temperature by 2-way analysis of variance were $P < 0.01$ (treatments), $P = 0.09$ (time points), and $P = 0.80$ (interaction).

CI indicates confidence intervals.

amethasone, and metyrapone groups was 37.8 ± 0.4, 37.6 ± 0.4, and 36.9 ± 0.6°C (mean ± SD, n = 6), respectively. Body temperature in the metyrapone group was significantly lower than that in the saline group ($P < 0.05$). No significant differences were observed between the dexamethasone and saline groups ($P = 0.76$), and between the dexamethasone and metyrapone groups ($P = 0.11$). Consistent with our attempt to maintain head temperature at 37.5°C with a heating lamp after decapitation, no statistical differences were observed in temperatures measured in the sternocleidomastoid muscle, on the side to its insertion of the skull, among the 3 groups at each time point between 0.5 and 2 minutes of ischemia, although the results were not sufficiently powered with an n = 6 per experimental group (Table 1).

There were no differences in the ATP concentration in the heart among the 3 groups when the hearts were frozen immediately after removal (Fig. 1, Table 2). However, the power value was not sufficient. In the saline group, ischemia for 5 minutes decreased the ATP content to 21% of the basal level. However, the extent of the ATP decrease caused by 5 minutes of ischemia was significantly suppressed in the dexamethasone and metyrapone groups. The ATP levels were 63% and 64% of each basal level, respectively. Longer duration of ischemia further lowered the ATP content in each group and no differences were found among the 3 groups after 10 or 20 minutes of ischemia.

Similar to the decrease in ATP levels, the heart ADP content decreased during ischemia. Pretreatment with dexamethasone suppressed the extent of the ADP decrease, and the effect was significant after 5 minutes of ischemia. In the metyrapone group, the ADP level was significantly higher at any duration of ischemia than that in the saline and dexamethasone groups. In the dexamethasone and metyrapone groups, the heart AMP content at 5 minutes of ischemia was significantly lower than that in the saline group ($P < 0.01$).

TABLE 2. Changes in the Concentrations of ATP, ADP, and AMP in the Heart

	Duration of Ischemia (min)			
	0	**5**	**10**	**20**
ATP (nmol/g)				
Saline (95% CI)	1568 ± 472 (1073-2064)	332 ± 127 (199-465)	304 ± 124 (174-435)	167 ± 17 (148-185)
Dexamethasone (95% CI)	1724 ± 259 (1452-1996)	1084 ± 294 (775-1393)	462 ± 210 (242-682)	163 ± 23 (139-187)
Metyrapone (95% CI)	1775 ± 414 (1340-2210)	1144 ± 283 (847-1441)	509 ± 256 (241-778)	155 ± 21 (133-177)
P	0.64	< 0.01	0.22	0.63
Power	0.11	1.00	0.29	0.11
ADP (nmol/g)				
Saline (95% CI)	4287 ± 768 (3481-5093)	2015 ± 337 (1661-2368)	1836 ± 394 (1422-2249)	1354 ± 69 (1281-1427)
Dexamethasone (95% CI)	4390 ± 138 (4246-4535)	3226 ± 330 (2880-3573)	2047 ± 767 (1242-2851)	1291 ± 67 (1220-1362)
Metyrapone (95% CI)	7612 ± 1211 (6341-8883)	5810 ± 570 (5212-6407)	3995 ± 1053 (2889-5100)	2277 ± 257 (2007-2547)
P	< 0.01	< 0.01	< 0.01	< 0.01
Power	1.00	1.00	1.00	1.00
AMP (nmol/g)				
Saline (95% CI)	1590 ± 538 (1025-2154)	2539 ± 540 (1973-3105)	1857 ± 186 (1662-2052)	1734 ± 93 (1637-1831)
Dexamethasone (95% CI)	1599 ± 342 (1240-1957)	1287 ± 366 (903-1671)	1732 ± 335 (1380-2083)	1740 ± 84 (1652-1829)
Metyrapone (95% CI)	1286 ± 371 (897-1675)	1269 ± 149 (1112-1426)	1613 ± 298 (1300-1925)	1904 ± 198 (1696-2112)
P	0.37	< 0.01	0.35	0.08
Power	0.19	1.00	0.21	0.49

Each value represents the mean ± SD of 6 animals. *P* and power values were obtained at each time point by analysis of variance. *P*-values for ATP by 2-way analysis of variance were $P < 0.01$ (treatments), $P < 0.01$ (time points), and $P < 0.01$ (interaction). *P*-values for ADP by 2-way analysis of variance were $P < 0.01$ (treatments), $P < 0.01$ (time points), and $P < 0.01$ (interaction). *P*-values for AMP by 2-way analysis of variance were $P < 0.01$ (treatments), $P = 0.04$ (time points), and $P < 0.01$ (interaction).

ADP indicates adenosine 5′-diphosphate; AMP, adenosine 5′-monophosphate; ATP, adenosine 5′-triphosphate; CI, confidence intervals.

TABLE 3. Changes in the Concentrations of ATP, ADP, and AMP in the Brain

	Duration of Ischemia (min)			
	0	**0.5**	**1**	**2**
ATP (nmol/g)				
Saline (95% CI)	1201 ± 153 (1040-1362)	403 ± 100 (299-508)	193 ± 19 (173-214)	124 ± 8 (116-132)
Dexamethasone (95% CI)	1331 ± 170 (1152-1509)	493 ± 45 (445-540)	253 ± 67 (183-323)	160 ± 67 (89-230)
Metyrapone (95% CI)	746 ± 113 (627-865)	302 ± 34 (266-338)	284 ± 136 (142-427)	188 ± 60 (125-251)
P	< 0.01	< 0.01	0.22	0.14
Power	1.00	0.99	0.29	0.38
ADP (nmol/g)				
Saline (95% CI)	1472 ± 57 (1411-1532)	1528 ± 90 (1434-1622)	998 ± 29 (968-1029)	901 ± 29 (871-931)
Dexamethasone (95% CI)	1693 ± 107 (1581-1805)	1577 ± 106 (1466-1688)	1203 ± 86 (1112-1293)	915 ± 20 (894-935)
Metyrapone (95% CI)	1585 ± 149 (1429-1742)	1257 ± 109 (1142-1371)	1117 ± 79 (1034-1199)	927 ± 49 (876-979)
P	0.01	< 0.01	< 0.01	0.44
Power	0.81	1.00	0.99	0.17
AMP (nmol/g)				
Saline (95% CI)	304 ± 89 (210-398)	1119 ± 95 (1020-1219)	1319 ± 43 (1274-1364)	1503 ± 49 (1451-1554)
Dexamethasone (95% CI)	300 ± 94 (200-399)	1007 ± 46 (959-1056)	1309 ± 87 (1218-1400)	1482 ± 63 (1416-1548)
Metyrapone (95% CI)	840 ± 126 (707-973)	1363 ± 81 (1278-1447)	1414 ± 150 (1257-1571)	1477 ± 71 (1402-1552)
P	< 0.01	< 0.01	0.18	0.75
Power	1.00	1.00	0.32	0.09

Each value represents the mean ± SD of 6 animals. *P* and power values were obtained at each time point by analysis of variance. *P*-values for ATP by 2-way analysis of variance were $P < 0.01$ (treatments), $P < 0.01$ (time points), and $P < 0.01$ (interaction). *P*-values for ADP by 2-way analysis of variance were $P < 0.01$ (treatments), $P < 0.01$ (time points), and $P < 0.01$ (interaction). *P*-values for AMP by 2-way analysis of variance were $P < 0.01$ (treatments), $P < 0.01$ (time points), and $P < 0.01$ (interaction).

ADP indicates adenosine 5′-diphosphate; AMP, adenosine 5′-monophosphate; ATP, adenosine 5′-triphosphate; CI, confidence intervals.

Concerning the brain concentrations of adenylates, the ATP levels in the saline and dexamethasone groups at 0.5 minute of ischemia were 34% and 37% of those at the beginning of ischemia, respectively (Fig. 2, Table 3). Suppression of the ATP decrease by dexamethasone was not significant in the brain. The ATP concentration in the metyrapone group was lower than that in the other groups at the start of cerebral ischemia with 62% of the concentration of the saline group. The ADP level in the dexamethasone group was higher than that in the saline group at both 0 and 1 minute of ischemia. The ADP concentration in the metyrapone group was lower than that in the saline and dexamethasone groups at 0.5 minute of ischemia. Conversely, the AMP concentration in the metyrapone group was 276% of that in the saline group at 0 minute and was still marked at 0.5 minute of ische-

TABLE 4. Changes in the Concentrations of Dopamine, DOPAC, and HVA in the Brain

	Duration of Ischemia (min)			
	0	**0.5**	**1**	**2**
Dopamine (ng/g)				
Saline (95% CI)	815 ± 98 (713-918)	790 ± 68 (719-862)	868 ± 147 (713-1023)	795 ± 62 (730-859)
Dexamethasone (95% CI)	759 ± 117 (636-882)	951 ± 120 (825-1077)	830 ± 91 (734-925)	772 ± 59 (710-833)
Metyrapone (95% CI)	817 ± 85 (727-906)	738 ± 78 (656-820)	754 ± 37 (715-792)	736 ± 101 (629-842)
P	0.54	< 0.01	0.18	0.42
Power	0.13	0.94	0.33	0.17
DOPAC (ng/g)				
Saline (95% CI)	113 ± 16 (97-129)	135 ± 24 (110-60)	120 ± 14 (105-135)	103 ± 14 (88-117)
Dexamethasone (95% CI)	125 ± 26 (98-153)	185 ± 29 (155-215)	145 ± 35 (109-182)	96 ± 15 (81-112)
Metyrapone (95% CI)	100 ± 18 (81-119)	85 ± 11 (73-97)	89 ± 10 (79-99)	93 ± 19 (74-113)
P	0.14	< 0.01	< 0.01	0.60
Power	0.37	1.00	0.96	0.12
HVA (ng/g)				
Saline (95% CI)	104 ± 20 (82-125)	229 ± 28 (199-258)	110 ± 18 (90-129)	99 ± 13 (86-113)
Dexamethasone (95% CI)	244 ± 66 (176-313)	325 ± 55 (267-382)	264 ± 75 (185-342)	89 ± 10 (79-100)
Metyrapone (95% CI)	93 ± 6 (87-99)	88 ± 8 (79-96)	86 ± 16 (70-103)	87 ± 17 (70-105)
P	< 0.01	< 0.01	< 0.01	0.29
Power	1.00	1.00	1.00	0.24

Each value represents the mean ± SD of 6 animals. *P* and power values were obtained at each time point by analysis of variance. *P*-values for dopamine by 2-way analysis of variance were $P = 0.03$ (treatments), $P = 0.25$ (time points), and $P = 0.02$ (interaction). *P*-values for DOPAC by 2-way analysis of variance were $P < 0.01$ (treatments), $P < 0.01$ (time points), and $P < 0.01$ (interaction). *P*-values for HVA by 2-way analysis of variance were $P < 0.01$ (treatments), $P < 0.01$ (time points), and $P < 0.01$ (interaction).

CI indicates confidence intervals; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid.

TABLE 5. Changes in the Concentrations of 5-HT and 5-HIAA in the Brain

	Duration of Ischemia (min)			
	0	**0.5**	**1**	**2**
5-HT (ng/g)				
Saline (95% CI)	300 ± 86 (210-389)	348 ± 82 (262-434)	319 ± 59 (257-382)	427 ± 27 (398-455)
Dexamethasone (95% CI)	379 ± 88 (287-471)	339 ± 105 (228-449)	272 ± 114 (152-392)	425 ± 24 (400-450)
Metyrapone (95% CI)	362 ± 38 (322-402)	336 ± 43 (291-381)	313 ± 36 (275-351)	394 ± 99 (291-498)
P	0.18	0.96	0.53	0.59
Power	0.32	0.06	0.14	0.12
5-HIAA (ng/g)				
Saline (95% CI)	226 ± 34 (191-261)	353 ± 35 (316-389)	241 ± 21 (219-262)	235 ± 17 (217-253)
Dexamethasone (95% CI)	388 ± 76 (308-467)	420 ± 85 (331-510)	377 ± 135 (236-518)	227 ± 29 (196-257)
Metyrapone (95% CI)	190 ± 29 (159-220)	175 ± 29 (145-206)	186 ± 29 (155-216)	190 ± 56 (132-249)
P	< 0.01	< 0.01	< 0.01	0.13
Power	1.00	1.00	0.95	0.40

Each value represents the mean ± SD of 6 animals. *P* and power values were obtained at each time point by analysis of variance. *P*-values for 5-HT by 2-way analysis of variance were $P = 0.97$ (treatments), $P < 0.01$ (time points), and $P = 0.42$ (interaction). *P*-values for 5-HIAA by 2-way analysis of variance were $P < 0.01$ (treatments), $P < 0.01$ (time points), and $P < 0.01$ (interaction).

5-HIAA indicates 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine; CI, confidence intervals.

mia. There was no significant difference in the AMP level between the saline and dexamethasone groups.

The dopamine concentration in the brain was almost constant in the saline and metyrapone groups during cerebral ischemia (Fig. 3, Table 4). However, the dopamine level in the dexamethasone group increased at 0.5 minute and returned to the basal level at 1 minute of ischemia. Although the concentration of DOPAC, a metabolite of dopamine, in the saline group was almost constant during ischemia, its level in the dexamethasone group was higher than that in the saline group at 0.5 minute of ischemia. The DOPAC level in the metyrapone group was lower than that in the other 2 groups during the ischemic period. In the saline group, the concentration of HVA, a metabolite of dopamine, increased at 0.5 minute of ischemia and returned to the basal level at 1 minute of ischemia. In the dexamethasone group, the HVA concentration remained high for 1 minute, and its level in the metyrapone group did not change during ischemia.

The 5-HT level did not differ among groups during ischemia (Fig. 4, Table 5). Changes in the concentration of its metabolite, 5-HIAA, were similar to those in the HVA level. The 5-HIAA level at 0 minute was higher in the dexamethasone group than in the other groups, and its level in the metyrapone group remained low during ischemia.

DISCUSSION

The findings in the present study are: (1) in the heart, metyrapone increased ADP at preischemia and during ischemia, and both metyrapone and dexamethasone increased ATP and decreased AMP in the early ischemic period; (2) in the brain, metyrapone decreased ATP and increased AMP at preischemia, and decreased ADP and increased AMP in the early ischemic period; and (3) in the brain, dexamethasone increased metabolites of dopamine and 5-HT before and during ischemia.

There are many studies reporting that glucocorticoids alleviate ischemia-induced myocardial injury. In this study, dexamethasone attenuated an ischemia-induced decrease in the heart ATP concentration. This may be caused by an enhancement of ATP synthesis in the heart or a suppression of ATP consumption in an anaerobic state. In the present study, however, the ATP level at the beginning of ischemia did not differ among the groups, and generation of ATP under ischemic states is unlikely in any of the groups. Therefore, it is highly probable that dexamethasone reduced ATP consumption due to a decrease in the energy requirement in the heart. In a canine study on myocardial ischemia, a pretreatment with methylprednisolone improved the segmental myocardial function and lactate imbalance, indicating the improvement of energy metabolism in an anaerobic state.[7] These findings, taken together with our present results, indicate that glucocorticoids provide benefits in the heart by reducing energy requirements during ischemia, although the power value was not sufficient.

Preservation of ATP in the heart during ischemia was also reported in studies on ischemic preconditioning.[19–21] Preconditioning myocardium with 4 cycles of 5-minute ischemia and reperfusion slowed the rate of ATP depletion during sustained ischemia, and inhibited irreversible injury in the canine heart.[19] Considering preconditioning effects of preischemic administration of glucocorticoids, reduction of energy demand may be a contributing factor in alleviation of ischemia-induced myocardial damage.

There are some controversial reports that glucocorticoids do not protect against ischemia-induced myocardial changes.[22,23] There are differences in the methodology of positive and negative reports, such as timing of drug administration, dosage, duration of ischemia, and animal species. In most studies that failed to show benefits, glucocorticoids were administered after ischemic events.[23] Because postischemic treatments cannot exert an influence on energy metabolism during ischemia, pre-

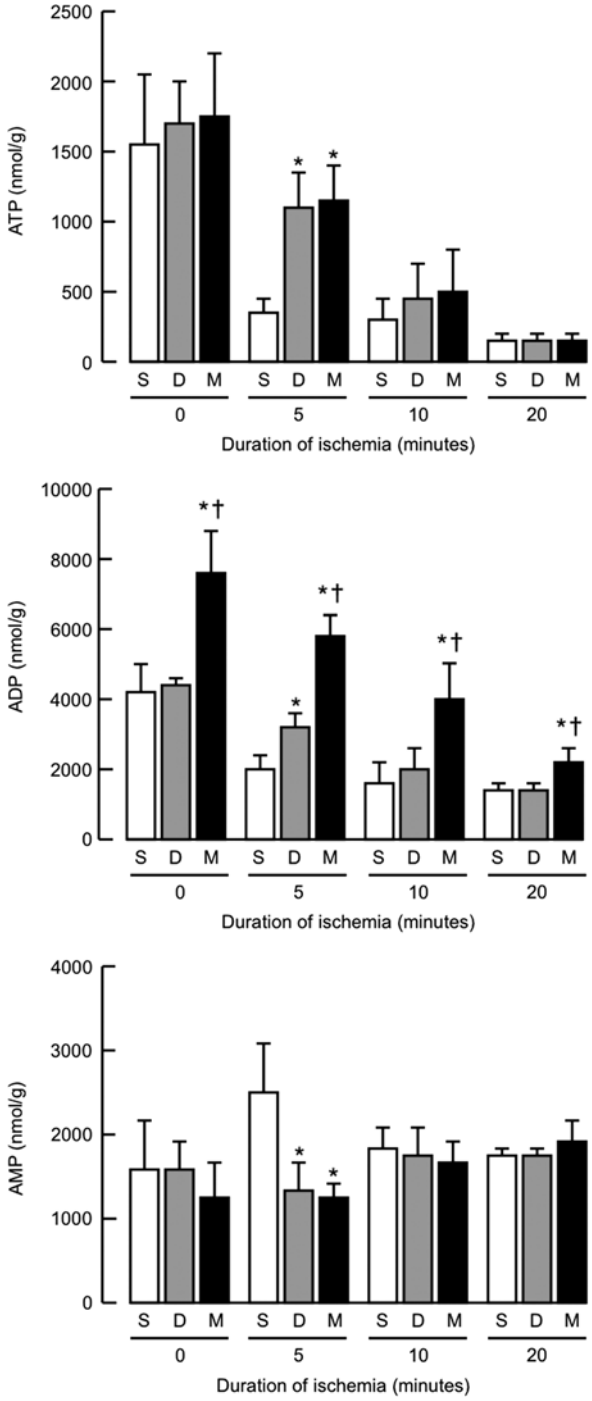

FIGURE 1. Effects of pretreatment with dexamethasone (5 mg/kg, intraperitoneally) and metyrapone (100 mg/kg, intraperitoneally) on ischemia-induced changes in heart concentrations of adenosine 5′-triphosphate (ATP), adenosine 5′-diphosphate (ADP), and adenosine 5′-monophosphate (AMP). Each value represents the mean ± SD of 6 animals. $*P<0.01$ compared with the value in corresponding saline-injected group at each time point. $\dagger P<0.01$ compared with the value in corresponding dexamethasone-injected group at each time point. D indicates dexamethasone-injected group; M, metyrapone-injected group; S, saline-injected group.

ischemic administration seems to be considered. An acute cardioprotective effect of a high-dose methylprednisolone before cardiopulmonary bypass on ischemia-reperfusion injury has also been reported in a clinical study.[24]

In the present study, the brain concentration of dopamine in the dexamethasone group was higher than that in the other 2 groups. Furthermore, the concentrations of its metabolites, DOPAC and HVA, increased during ischemia. Particularly, the HVA concentration in the dexamethasone group was higher at the start of ischemia than that in the control group, suggesting facilitated activity of the central dopaminergic system by dexamethasone before induction of ischemia. Despite the enhancement of neuronal activity, ATP consumption was not facilitated. In our previous studies on ATPase activity, identical doses of dexamethasone suppressed enzyme activity of the sodium pump in acidic conditions and suppressed ATP hydrolysis.[25] Both decreased supply and increased demand of energy may make neurons relatively energy depleted, and thus vulnerable.

Besides energy depletion, an increase in dopamine metabolism is considered to be an important mechanism for dopamine toxicity, because oxidation of dopamine by monoamine oxidase generates reactive oxygen species as well as DOPAC.[26] Furthermore, spontaneous and enzymatic oxidation of the catechol ring forms hydrogen peroxide.[27] Dissimilar to neurotoxicity of the dopaminergic system, the serotonergic system has been reported to provide benefits on ischemia-induced neuronal injury.[28,29] In the present study, the 5-HIAA concentrations in the dexamethasone group were higher than those in the saline group. As glucocorticoids aggravate ischemia-induced neuronal injury, the protective effects of the serotonergic system may be insufficient.

Glucocorticoids have been shown to increase the plasma glucose level, and hyperglycemia is known to exacerbate ischemia-induced neuronal damage due to the intracellular lactic acidosis associated with enhanced anaerobic metabolism. In the current study, agents were administered 3 hours before induction of ischemia, and the treatments did not affect physiological variables, plasma levels of glucose, electrolytes, and arterial blood gas tensions in our previous studies.[11,13,15] Furthermore, effects of dexamethasone were evaluated by isolated hearts, using a glucose-free medium, and isolated brains. Therefore, it is unlikely that the glucose level affected the current results. Likewise, genomic effects by modifying nucleic acids through steroid receptors in the nucleus may not be relevant, as agents were administered 3 hours before induction of ischemia. Rapidly induced enzymes by glucocorticoids that detoxify mediators released during stress are conceivable as mechanisms of acute effects of glucocorticoids.[30,31]

Metyrapone is a potent and rapid inhibitor of the synthesis of glucocorticoids. It blocks the 11β-hydroxylation step by inhibiting cytochrome P450 and reduces the plasma concentration of endogenous glucocorticoids.[32,33] In the present study, the agent attenuated a ischemic decrease in the ATP concentration, as did the

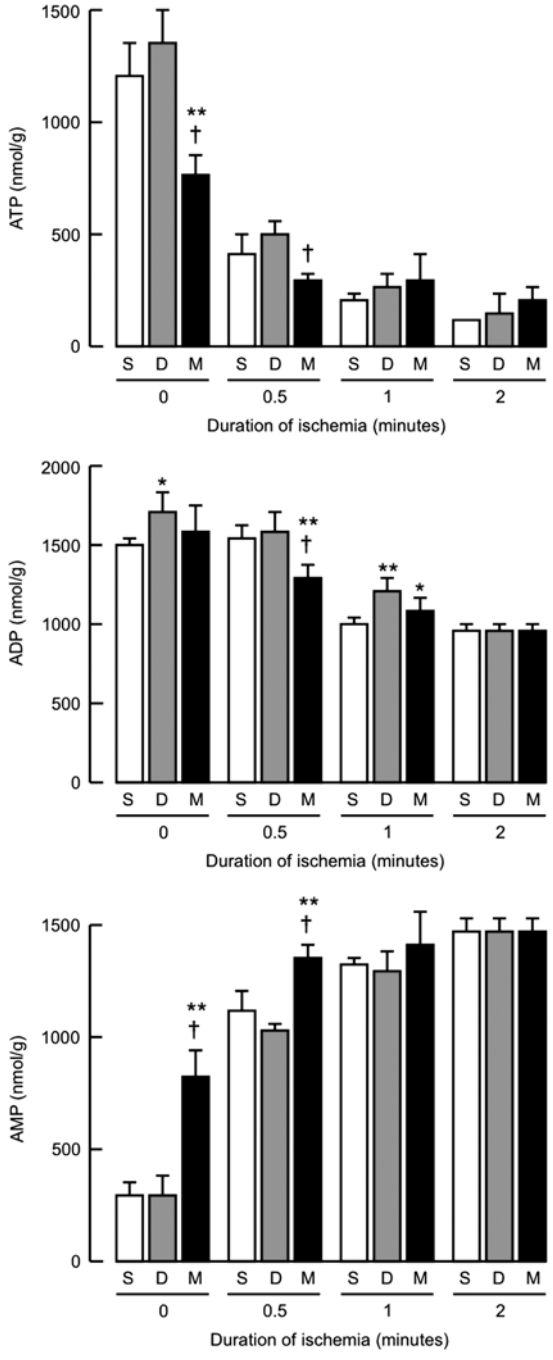

FIGURE 2. Effects of pretreatment with dexamethasone (5 mg/kg, intraperitoneally) and metyrapone (100 mg/kg, intraperitoneally) on ischemia-induced changes in brain concentrations of adenosine 5′-triphosphate (ATP), adenosine 5′-diphosphate (ADP), and adenosine 5′-monophosphate (AMP). Each value represents the mean ± SD of 6 animals. $*P<0.05$, $**P<0.01$ compared with the value in corresponding saline-injected group at each time point. $\dagger P<0.01$ compared with the value in corresponding dexamethasone-injected group at each time point. D indicates dexamethasone-injected group; M, metyrapone-injected group; S, saline-injected group.

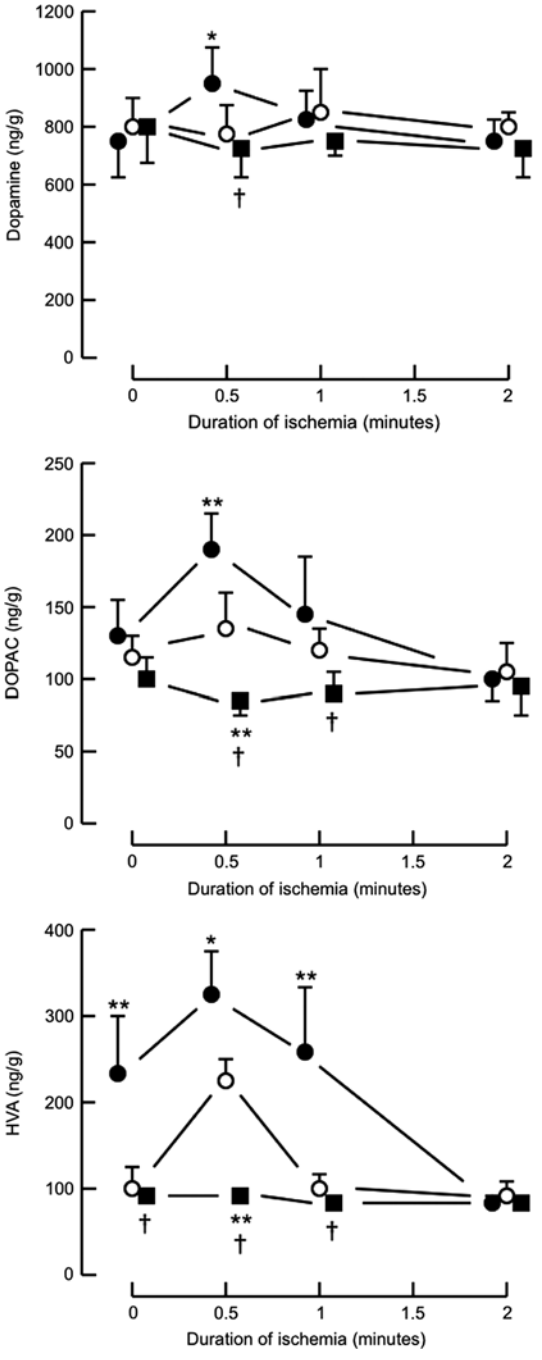

FIGURE 3. Effects of pretreatment with dexamethasone (5 mg/kg, intraperitoneally) and metyrapone (100 mg/kg, intraperitoneally) on ischemia-induced changes in brain concentrations of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA). Each value represents the mean ± SD of 6 animals. $*P<0.05$, $**P<0.01$ compared with the value in corresponding saline-injected group at each time point. $\dagger P<0.01$ compared with the value in corresponding dexamethasone-injected group at each time point. ○, saline-injected group; ●, dexamethasone-injected group; and ■, metyrapone-injected group.

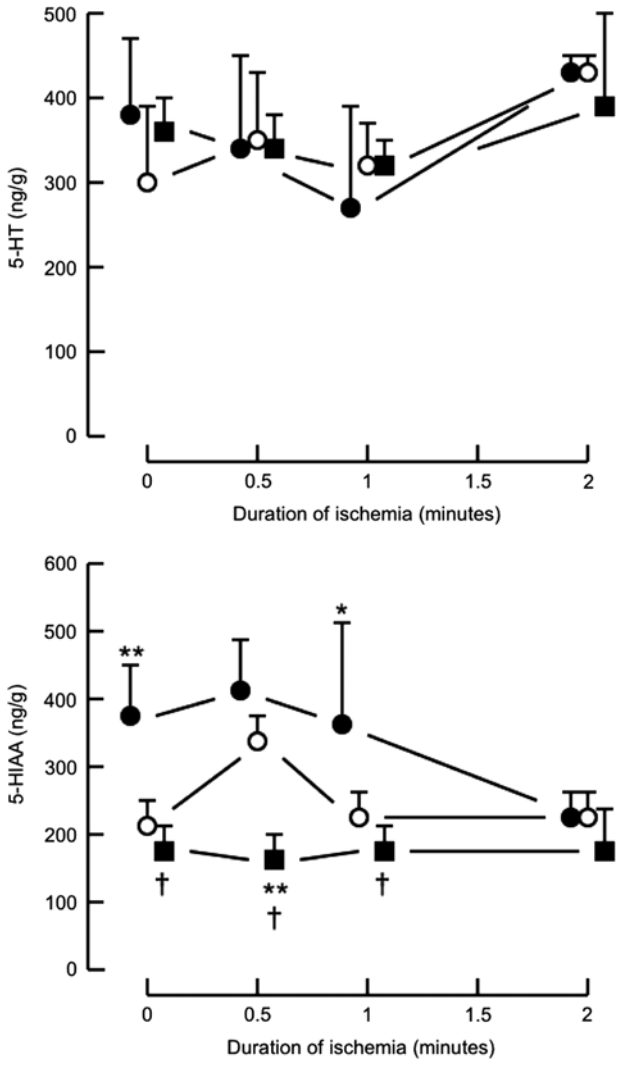

FIGURE 4. Effects of pretreatment with dexamethasone (5 mg/kg, intraperitoneally) and metyrapone (100 mg/kg, intraperitoneally) on ischemia-induced changes in brain concentrations of serotonin (5-hydroxytryptamine, 5-HT) and 5-hydroxyindoleacetic acid (5-HIAA). Each value represents the mean ± SD of 6 animals. $*P<0.05$, $**P<0.01$ compared with the value in corresponding saline-injected group at each time point. $\dagger P<0.01$ compared with the value in corresponding dexamethasone-injected group at each time point. ○, saline-injected group; ●, dexamethasone-injected group; and ■, metyrapone-injected group.

dexamethasone treatment. Furthermore, it increased the heart ADP content for the entire duration of ischemia. Metyrapone is reported to reduce oxygen consumption and decrease body temperature as observed in the present study.[33,34] Although the precise mechanism underlying hypothermia is obscure, hypothermia may have resulted in preservation of ATP during ischemia and accumulation of ADP by depressing hydrolysis of ATP and phosphorylation of ADP. For these reasons, the preservation of ATP in metyrapone-treated animals may be caused by another mechanism besides depletion of endogenous glucocorticoids.

Although there are no reports of metyrapone aggravating ischemia-induced myocardial injury, the agent has been shown to reduce brain damage induced by focal and global ischemia and seizure.[35] We also confirmed beneficial effects of metyrapone on ischemia-induced neuronal damage.[10,15] It reduced glutamate toxicity and ameliorated delayed neuronal death caused by transient ischemia. In the present study, however, the ATP concentration in the brain in the metyrapone group was markedly low, whereas the AMP concentration was high at the beginning of ischemia, showing suppression of the energy charge in the brain by metyrapone. Hence, changes in the energy level cannot explain beneficial effects of the agent in the brain.

There is a study in which protective effects of metyrapone independent of plasma corticosterone levels was demonstrated.[36] In the study, supplemental administration of corticosterone in metyrapone-treated rats did not result in a subsequent increase in brain damage and seizures when compared with metyrapone-treated animals.[36] In another study, the hippocampal levels of glutamate and γ-aminobutyric acid, neurotransmitter amino acids, have been shown to be suppressed by metyrapone.[37] These are consistent with our present results on the dopaminergic and serotonergic activity. The neuroprotective properties of metyrapone may be partly attributed to the metabolic modifications on the nervous system.

In conclusion, exogenous administration of dexamethasone and blockade of endogenous glucocorticoids by metyrapone did not act oppositely on energy levels in the present study. Further, we could not clearly elucidate the relationship between changes in energy levels by glucocorticoids and cellular viability, as morphologic and functional changes by the agents were not evaluated in the present animal model. Studies that can correlate functional outcomes to energy levels are required, at least, and the relevance to clinical practice of glucocorticoids remains to be clarified in future studies.

ACKNOWLEDGMENT

The authors thank Dr. Keizo Ikemune for his assistance on statistical data analysis.

REFERENCES

1. Varga E, Nagy N, Lazar J, et al. Inhibition of ischemia/reperfusion-induced damage by dexamethasone in isolated working rat hearts: the role of cytochrome C release. *Life Sci*. 2004;75:2411–2423.
2. Skyschally A, Haude M, Dorge H, et al. Glucocorticoid treatment prevents progressive myocardial dysfunction resulting from experimental coronary microembolization. *Circulation*. 2004;109:2337–2342.
3. Fan WJ, Genade S, Genis A, et al. Dexamethasone-induced cardioprotection: a role for the phosphatase MKP-1? *Life Sci*. 2009;84:838–846.
4. Engelman RM, Prasad MR, Rousou JA, et al. Steroid-induced myocardial preservation is associated with decreased cell membrane microviscosity. *Circulation*. 1989;80(suppl III):III-36–III-43.
5. Shatney CH, MacCarter DJ, Lillehei RC. Temporal factors in the reduction of myocardial infarct volume by methylprednisolone. *Surgery*. 1976;80:61–69.

6. Masters TN, Harbold NB, Hall DG, et al. Beneficial metabolic effects of methylprednisolone sodium succinate in acute myocardial ischemia. *Am J Cardiol*. 1976;37:557–563.
7. da Luz PL, Forrester JS, Wyatt HL, et al. Myocardial reperfusion in acute experimental ischemia. Beneficial effects of prior treatment with steroids. *Circulation*. 1976;53:847–852.
8. Giugliano GR, Giugliano RP, Gibson CM, et al. Meta-analysis of corticosteroid treatment in acute myocardial infarction. *Am J Cardiol*. 2003;91:1055–1059.
9. Sapolsky RM, Pulsinelli WA. Glucocorticoids potentiate ischemic injury to neurons: therapeutic implications. *Science*. 1985;229: 1397–1400.
10. Adachi N, Chen J, Liu K, et al. Dexamethasone aggravates ischemia-induced neuronal damage by facilitating the onset of anoxic depolarization and the increase in the intracellular Ca^{2+} concentration in gerbil hippocampus. *J Cereb Blood Flow Metab*. 1998;18:274–280.
11. Tsubota S, Adachi N, Chen J, et al. Dexamethasone changes brain monoamine metabolism and aggravates ischemic neuronal damage in rats. *Anesthesiology*. 1999;90:515–523.
12. Adachi N, Namba C, Nagaro T, et al. Dexamethasone reduces energy utilization in ischemic gerbil brain. *Eur J Pharmacol*. 2001;427:119–123.
13. Mitsuyo T, Adachi N, Yorozuya T, et al. Facilitation of ischemia-induced release of dopamine and neuronal damage by dexamethasone in the rat striatum. *Eur J Pharmacol*. 2003;465: 267–274.
14. Bramlett HM, Dietrich WD. Progressive damage after brain and spinal cord injury: pathomechanisms and treatment strategies. *Prog Brain Res*. 2007;161:125–141.
15. Adachi N, Chen J, Liu K, et al. Metyrapone alleviates ischemic neuronal damage in the gerbil hippocampus. *Eur J Pharmacol*. 1999;373:147–152.
16. Brambrink AM, Kopacz L, Astheimer A, et al. Control of brain temperature during experimental global ischemia in rats. *J Neurosci Methods*. 1999;92:111–122.
17. Lo EH, Steinberg GK. Effects of hypothermia on evoked potentials, magnetic resonance imaging, and blood flow in focal ischemia in rabbits. *Stroke*. 1992;23:889–893.
18. Magnusson O, Nilsson LB, Westerlund D. Simultaneous determination of dopamine, DOPAC and homovanillic acid. Direct injection of supernatants from brain tissue homogenates in a liquid chromatography-electrochemical detection system. *J Chromatogr*. 1980;221:237–247.
19. Murry CE, Richard VJ, Reimer KA, et al. Ischemic preconditioning slows energy metabolism and delays ultrastructural damage during a sustained ischemic episode. *Circ Res*. 1990;66: 913–931.
20. Jennings RB, Murry CE, Reimer KA. Preconditioning myocardium with ischemia. *Cardiovasc Drugs Ther*. 1991;5:933–938.
21. Sanada S, Komuro I, Kitakaze M. Pathophysiology of myocardial reperfusion injury: preconditioning, postconditioning, and translational aspects of protective measures. *Am J Physiol Heart Circ Physiol*. 2011;301:H1723–H1741.
22. Vogel WM, Lum D, Lucchesi BR. Methylprednisolone sodium succinate treatment in global ischemia of the cat isolated heart. *J Cardiovasc Pharmacol*. 1979;1:53–68.
23. Osher J, Lang TW, Meerbaum S, et al. Methylprednisolone treatment in acute myocardial infarction. Effect on regional and global myocardial function. *Am J Cardiol*. 1976;37:564–571.
24. Enc Y, Karaca P, Ayoglu U, et al. The acute cardioprotective effect of glucocorticoid in myocardial ischemia-reperfusion injury occurring during cardiopulmonary bypass. *Heart Vessels*. 2006;21:152–156.
25. Namba C, Adachi N, Liu K, et al. Suppression of sodium pump activity and an increase in the intracellular Ca^{2+} concentration by dexamethasone in acidotic mouse brain. *Brain Res*. 2002;957:271–277.
26. Maker HS, Weiss C, Silides DJ, et al. Coupling of dopamine oxidation (monoamine oxidase activity) to glutathione oxidation via the generation of hydrogen peroxide in rat brain homogenates. *J Neurochem*. 1981;36:589–593.
27. Hastings TG. Enzymatic oxidation of dopamine: the role of prostaglandin H synthase. *J Neurochem*. 1995;64:919–924.
28. Nakata N, Kato H, Kogure K. Protective effects of serotonin reuptake inhibitors, citalopram and clomipramine, against hippocampal CA1 neuronal damage following transient ischemia in the gerbil. *Brain Res*. 1992;590:48–52.
29. Prehn JH, Welsch M, Backhauss C, et al. Effects of serotonergic drugs in experimental brain ischemia: evidence for a protective role of serotonin in cerebral ischemia. *Brain Res*. 1993;630:10–20.
30. Munck A, Guyre PM, Holbrook NJ. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocr Rev*. 1984;5:25–44.
31. Hafezi-Moghadam A, Simoncini T, Yang Z, et al. Acute cardiovascular protective effects of corticosteroids are mediated by nontranscriptional activation of endothelial nitric oxide synthase. *Nat Med*. 2002;8:473–479.
32. Werner R. Effect of metopirone-ditartrate on thermogenesis in the guinea-pig. *Comp Biochem Physiol C*. 1988;90:445–450.
33. Werner R, Wünnenberg W. Effect of the adrenocorticostatic agent, metopirone, on thermoregulatory heat production in the European hedgehog. *Pflugers Arch*. 1980;385:25–28.
34. Massey TE, Walker RM, McElligott TF, et al. Acetaminophen-induced hypothermia in mice: evidence for a central action of the parent compound. *Toxicology*. 1982;25:187–200.
35. Smith-Swintosky VL, Pettigrew LC, Sapolsky RM, et al. Metyrapone, an inhibitor of glucocorticoid production, reduces brain injury induced by focal and global ischemia and seizures. *J Cereb Blood Flow Metab*. 1996;16:585–598.
36. Krugers HJ, Kemper RH, Korf J, et al. Metyrapone reduces rat brain damage and seizures after hypoxia-ischemia: an effect independent of modulation of plasma corticosterone levels? *J Cereb Blood Flow Metab*. 1998;18:386–390.
37. Drouet JB, Fauvelle F, Batandier C, et al. Metyrapone effects on systemic and cerebral energy metabolism. *Eur J Pharmacol*. 2012; 682:92–98.