

Sevoflurane postconditioning against cerebral ischemic neuronal injury is abolished in diet-induced obesity: Role of brain mitochondrial K_{ATP} channels

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Abstract. Obesity is associated with increased infarct volumes and adverse outcomes following ischemic stroke. However, its effect on anesthetic postconditioning-induced neuroprotection has not been investigated. The present study examined the effect of sevoflurane postconditioning on focal ischemic brain injury in diet-induced obesity. Sprague-Dawley rats were fed a high-fat diet (HF; 45% kcal as fat) for 12 weeks to develop obesity syndrome. Rats fed a low-fat diet (LF; 10% kcal as fat) served as controls. The HF or LF-fed rats were subjected to focal cerebral ischemia for 60 min, followed by 24 h of reperfusion. Postconditioning was performed by exposure to sevoflurane for 15 min immediately at the onset of reperfusion. The involvement of the mitochondrial K_{ATP} (mitoK_{ATP}) channel was analyzed by the administration of a selective inhibitor of 5-hydroxydecanoate (5-HD) prior to sevoflurane postconditioning or by administration of diazoxide (DZX), a mitoK_{ATP} channel opener, instead of sevoflurane. The cerebral infarct volume, neurological score and motor coordination were evaluated 24 h after reperfusion. The HF-fed rats had larger infarct volumes, and lower neurological scores than the LF-fed rats and also failed to respond to neuroprotection by sevoflurane or DZX. By contrast, sevoflurane and DZX reduced the infarct volumes and improved the neurological scores and motor coordination in the LF-fed rats. Pretreatment with 5-HD inhibited sevoflurane-induced neuroprotection in the LF-fed rats, whereas it had no effect in the HF-fed rats. Molecular studies demonstrated that the expression of Kir6.2, a significant mitoK_{ATP} channel component, was reduced in the brains of the HF-fed rats compared with the LF-fed rats. The results of this study indicate that diet-induced obesity eliminates the ability of anesthetic sevoflurane postconditioning to protect the brain against cerebral ischemic neuronal injury, most likely due to an impaired brain mitoK_{ATP} channel.

Introduction

Obesity is a growing worldwide epidemic and is considered a primary risk factor for stroke, which has become the third leading cause of mortality in the United States and a major source of debilitation among adults (1,2). Ischemic brain injury is the underlying pathophysiological cause of stroke. Following a cerebrovascular ischemic event, obesity is associated with increased infarct volumes and adverse outcomes in terms of morbidity and mortality (2,3). Therefore, the effect of any developed neuroprotective strategy would be particularly beneficial in this population.

Volatile anesthetics have been demonstrated to exert direct cardioprotective and neuroprotective effects when they are applied prior to ischemia-reperfusion (anesthetic preconditioning) and also when they are administered immediately following ischemia or during early reperfusion (anesthetic postconditioning) in *in vitro* and *in vivo* studies (4-6). The clinical use of anesthetic preconditioning is limited, as the majority of ischemic episodes are unpredictable. By contrast, the onset of reperfusion is more frequently predictable. Therefore, anesthetic postconditioning by modulation of reperfusion rather than ischemia may be more clinically useful for cardioprotection, as well as for neuroprotection. It has been demonstrated that pathological situations, including obesity, may affect the effectiveness of well-established cardioprotective strategies (7,8). However, the majority of studies regarding anesthetic postconditioning-induced neuroprotection have been conducted in healthy animals (4,9,10). To the best of our knowledge, there are no studies that have evaluated the neuroprotective effects of anesthetic postconditioning in obesity.

Although many signaling pathways have been proposed to be involved in anesthetic postconditioning induced neuroprotective action, the precise mechanism remains unknown. Previous studies have demonstrated that volatile anesthetic postconditioning with sevoflurane provides neuroprotection via the activation of mitochondrial KATP (mitoKATP) channels in a rat model of ischemic stroke (4,9). It remains unclear whether this strategy-induced neuroprotection is also mediated by the similar signaling pathway in obesity, where the expression and function of mitoKATP channels have been reported to be impaired in the brain and peripheral tissues (11-13).

The aim of the present study was to determine whether obesity affects volatile anesthetic sevoflurane postconditioning-induced

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neuroprotection against cerebral ischemic neuronal injury *in vivo*, and to evaluate whether this effect is mediated via the mitoK_{ATP} channels. For this purpose, the high-fat diet (HF)-induced obese rat model was selected, which has been shown to represent human obesity syndrome (14).

Materials and methods

Animals. Adult male Sprague-Dawley rats weighing 83.8±0.9 g (Beijing Laboratory Animal Research Center, Beijing, China) were randomly assigned to receive a HF (45% kcal as fat; Research Diets, New Brunswick, NJ, USA) or a low-fat diet (LF; 10% kcal as fat) for 12 weeks. All rats were housed in a room maintained between 23 and 25°C with a 12-h light/dark cycle, and were provided with food and water *ad libitum*. The animal protocol was approved by the Institutional Animal Care and Use Committee of Jilin University (Jilin, China). All experiments were performed in accordance with the 'Guiding principles for research involving animals and human beings' (15).

Induction of focal cerebral ischemia. Middle cerebral artery occlusion was performed to induce focal cerebral ischemia as described previously (4). Briefly, 300 mg/kg chloral hydrate was administered to the rats intraperitoneally as anesthesia. A temperature controlled heating pad was used to maintain the core body temperature within a normothermic range of 37–38°C. A midline cervical incision was made to isolate the right common, external and internal carotid arteries. A 4/0 surgical nylon monofilament was inserted into the internal carotid artery and advanced until its tip occluded the ipsilateral middle cerebral artery. After a 60 min occlusion, the reperfusion was elicited by removing the monofilament.

Blood pressure (BP), heart rate (HR) and arterial blood gas were monitored during the experiment by cannulating the right femoral artery. Aortic pressure signals were connected to an analog digital converter and analyzed using the PowerLab software (PowerLab/8SP, Chart 5.0; AD Instruments Pty, Ltd., Castle Hill, Australia). BP and HR were measured at the following time points: prior to ischemia, 15 and 35 min after the onset of ischemia and 5 and 15 min after the onset of reperfusion. Arterial blood gas analysis was conducted with a blood gas analyzer (Compact 3, AVL Medizintechnik, Graz, Austria) at 15 min after the onset of ischemia or reperfusion. After recovery from the anesthesia, the rats were returned to their cages with free access to food and water.

Experimental protocol. The experimental design is illustrated in Fig. 1. Rats were randomly assigned to the following eight groups: i) The LF-fed ischemia-reperfusion alone group (LF-Control, n=10); ii) the LF-fed sevoflurane postconditioning group (LF-sevo, n=10); iii) the LF-fed sevoflurane postconditioning plus 5-hydroxydecanoate (5HD) group (LF-5HD+sevo, n=10). This group was identical to ii), but was treated with 5HD, a selective mitoK_{ATP} channel blocker (40 mg/kg, i.p.), 30 min prior to sevoflurane postconditioning (4); iv) the LF-fed diazoxide (DZX) alone group (LF-DZX, n=8). These rats were treated with DZX, a mitoK_{ATP} channel opener (10 mg/kg, i.p.), 30 min prior to reperfusion (4); v) the HF-fed ischemia-reperfusion alone

group (HF-control, n=10); vi) the HF-fed sevoflurane postconditioning group (HF-sevo, n=10); vii) the HF-fed sevoflurane postconditioning plus 5HD group (HF-5HD+sevo, n=10); and viii) the HF-fed DZX alone group (HF-DZX, n=8).

Following a 30-min stabilization period, all the rats were subjected to 60 min of focal cerebral ischemia followed by 24 h of reperfusion. The rats that had been assigned to sevoflurane postconditioning were exposed to sevoflurane for 15 min at a concentration of 2.6% in a gas-tight anesthetic chamber immediately at the onset of reperfusion. A gas analyzer was connected to the chamber to constantly monitor and maintain the concentrations of inspired oxygen and sevoflurane. The dose of sevoflurane was selected according to the results of a previous study in which this dose was determined to result in optimal neuroprotective action *in vivo* in rats (4). At the end of the protocol (24 h after the 60 min ischemia period), the neurological deficit scores and motor coordination were evaluated, and the rats were then sacrificed by an overdose of pentobarbital. The blood samples were collected for biochemical measurements, the brain and epididymal fat were removed and weighed and the brains were then processed for infarct volume assessment.

Evaluation of motor coordination, neurological deficit scores and infarct volumes. The neurological deficit scores were evaluated 24 h after ischemia based on an eight-point scale (4,16). The scores were as follows: 0, no apparent deficits; 1, failure to fully extend left forepaw; 2, decreased grip of the left forelimb; 3, spontaneous movement in all directions, contralateral circling only if pulled by the tail; 4, circling or walking to the left; 5, walking only if stimulated; 6, unresponsiveness to stimulation and with depressed level of consciousness; and 7, fatality.

Motor coordination was evaluated 24 h prior to and 24 h after ischemia (4,17). The rats were placed on an accelerating rotarod (Columbus Inst., Columbus, OH, USA). The speed of the rotarod was increased from 4 to 40 rpm over 5 min. Each rat was tested three times and the mean time spent on the accelerating rotarod was used to evaluate the coordination function. All rats were trained for three continuous days prior to the formal tests.

Following the termination of the observation period, the rats were sacrificed by an overdose of pentobarbital. The brains were removed and snap-frozen in chilled 2-methylbutane (–50 to –60°C) for cryostat sectioning. Sections (50 µm) at 800-µm intervals were stained with cresyl violet (Sigma-Aldrich, St. Louis, MO, USA), and the area of the infarct was determined in each section using an image analysis system (National Institutes of Health, Bethesda, MD, USA). The infarct volume was calculated by multiplying the sum of the areas by the distance between the sections. The difference between the volumes of the ipsilateral and contralateral hemispheres was used to calculate the edema volume. The correction of the infarct size for edema was calculated according to the method from a previous study (17).

Biochemical measurements. Blood glucose levels were measured immediately following sampling using a glucose analyzer (Prestige Smart System, Fort Lauderdale FL, USA). The total cholesterol and triglyceride levels were determined

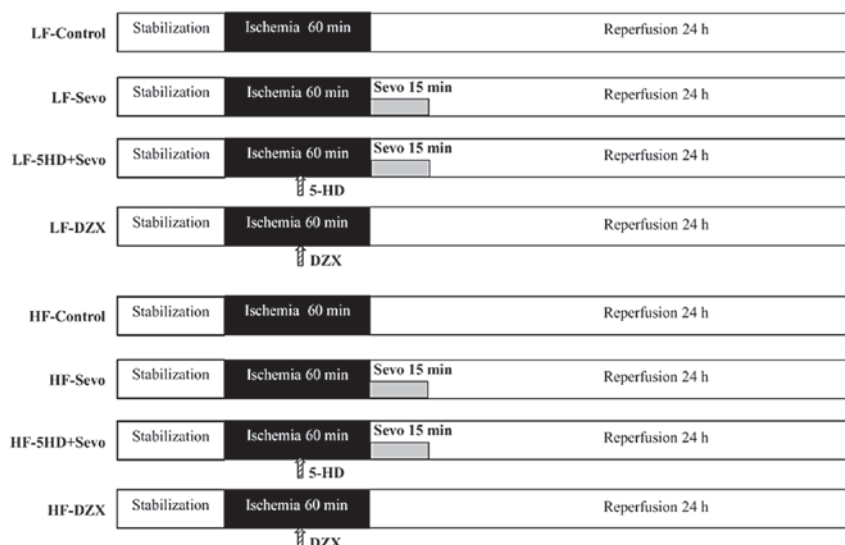


Figure 1. Diagram of the experimental protocol. Animals were subjected to focal cerebral ischemia for 60 min followed by 24 h of reperfusion. Postconditioning was performed by exposure to sevoflurane for 15 min immediately at the onset of reperfusion. A selective mitoK_{ATP} channel blocker, 5-hydroxydecanoate (5-HD), was administered 30 min prior to sevoflurane postconditioning, and a mitoK_{ATP} channel opener, diazoxide (DZX), was administered 30 min prior to reperfusion. LF, low-fat diet; HF, high-fat diet; sevo, sevoflurane; mitoK_{ATP}, mitochondrial K_{ATP}.

by an automatic analyzer (Hitachi 7170A, Tokyo, Japan). The plasma levels of leptin and insulin were measured by commercial ELISA kits (leptin kit, Morinaga Institute of Biological Science, Inc., Yokohama, Kanagawa, Japan; insulin kit, Shibayagi, Gunma, Japan; Assaypro, St. Charles, MO, USA).

Analysis of K_{ATP} channel gene expression. Additional HF-fed (n=15) or LF-fed rats (n=15) that were not subjected to ischemia-reperfusion were used for the analysis of K_{ATP} channel gene and protein expression or for immunofluorescence studies.

The reverse transcription-polymerase chain reaction (RT-PCR) was used to measure mRNA expression of the K_{ATP} channel subunits. The total RNA in the brain tissue was extracted using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) and reverse transcribed into cDNA with 10X buffer (MgCl₂ free; PerkinElmer, Waltham, MA, USA), 10 mM dNTPs, 25 mM MgCl₂, random hexamer primers, RNasin (33 U/μl, Promega Corporation, Madison, WI, USA), and Moloney murine leukemia virus reverse transcriptase (10 U/μl, Promega). The reaction mixture for RT was incubated at 21°C for 10 min and maintained at 42°C for 75 min, then 5 min at 95°C. The 50-μl PCR reaction mixture including 5 μl of each RT product was used for PCR to detect the K_{ATP} channel subunits Kir6.2 and SUR1. The primers used were as follows: Kir6.2 forward, 5'-CGCATGGTGACAGAGGAATG-3' and reverse, 5'-GTGGAGAGGCACAACCTTCGC-3'; SUR1 forward, 5'-TGCCAGCTCTTTGAGCATTG-3' and reverse, 5'-AGGATGATACGGTTGAGCAGG-3'; GAPDH forward, 5'-CTCAAGATTGTCAAGCAATGC-3' and reverse, 5'-CAGGATGCCCTTTAGTGGGC-3'. The PCR reaction (total of 35 cycles) was as follows: initial denaturation at 94°C for 4 min, then 94°C for 10 sec, 56°C for 30 sec and 72°C for 2 min, followed by a final extension at 72°C for 10 min. RT was omitted for the negative control experiments. The PCR products were separated on a 1.5% agarose gel by and

visualized using ethidium bromide. Photographs of the ethidium bromide-stained gels were analyzed with a Molecular Imager (Bio-Rad, Hercules, CA, USA).

Analysis of K_{ATP} channel protein expression. Mitochondria were isolated from brain tissue as previously described (18). Briefly, the brain tissue was homogenized (Glen Mills Inc., Clifton, NJ, USA) in ice cold isolation buffer containing 225 mmol/l mannitol, 75 mmol/l sucrose, 5 mmol/l 3-(N-morpholino)propanesulfonic acid (MOPS), 0.5 mmol/l EGTA and 2 mmol/l taurine, with 0.2% bovine serum albumin (BSA) (pH 7.25). After centrifugation of homogenate twice at 1,000 x g for min (4°C), the supernatant was transferred into a new tube and centrifuged at 10,000 x g for 10 min (4°C). The pellet was gently washed with washing buffer and then resuspended in buffer containing 225 mmol/l mannitol, 25 mmol/l sucrose, 5 mmol/l MOPS, 1 mmol/l EGTA, 5 mmol/l KH₂PO₄ and 2 mmol/l taurine supplemented with 0.2% BSA (pH 7.4). The Bradford method was used to measure the concentration of mitochondrial protein. Electrophoresis with 4-20% SDS PAGE was performed with equal quantities of protein from the mitochondrial lysate samples which were then transferred onto PVDF membranes. The membranes were blocked with blocking buffer including 5% skimmed milk, Tris-buffered saline and 0.1% Tween-20, then incubated with rabbit polyclonal anti-Kir6.2, rabbit polyclonal anti-SUR1 (EMD Millipore, Billerica, MA, USA) and rabbit polyclonal anti-β Actin (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) antibodies at 4°C overnight. After being washed three times, the membranes were incubated with goat anti rabbit horse-radish peroxidase IgG (Santa Cruz Biotechnology, Inc.) and developed by enhanced chemiluminescence, and the densities of the bands were then analyzed.

Immunofluorescence studies. Rats (n=3 for each group) were transcardially perfused with ice-cold heparinized saline and

Table I. Body weight and metabolic parameters in each group.

Group	Body weight, g	Epididymal fat, g	Plasma glucose, mg/dl	Plasma insulin, ng/ml	Plasma leptin, ng/ml	Plasma triglycerides, mg/dl	Plasma cholesterol, mg/dl
LF-control	341±11	5.34±1.09	93±7	3.54±0.22	5.03±0.76	83±11	91±11
LF-sevo	345±16	5.25±1.17	89±5	3.61±0.26	5.13±0.45	90±13	88±13
LF-5HD+sevo	350±14	5.30±1.20	90±5	3.47±0.54	4.98±0.63	88±10	85±17
LF-DZX	347±11	5.22±1.24	88±4	3.50±0.61	5.19±0.50	85±7	90±15
HF-control	471±16 ^a	12.27±1.42 ^a	100±9	9.25±1.20 ^a	8.79±1.33 ^a	97±19	174±19 ^a
HF-sevo	469±15 ^a	12.38±1.59 ^a	96±7	9.83±1.18 ^a	8.65±1.07 ^a	115±21	168±17 ^a
HF-5HD+sevo	475±12 ^a	12.70±1.45 ^a	97±6	9.74±1.16 ^a	8.87±1.18 ^a	121±18	179±19 ^a
HF-DZX	470±13 ^a	12.99±1.63 ^a	92±7	9.96±1.53 ^a	9.00±1.79 ^a	118±23	166±14 ^a

Values are presented as the mean ± standard error of the mean; n=8-10 for each group. ^aP<0.05 vs. respective LF. LF, low-fat diet; HF, high-fat diet; 5HD, 5-hydroxydecanoate; sevo, sevoflurane; DZX, diazoxide.

4% paraformaldehyde. Next, the brains were removed and post-fixed in 4% paraformaldehyde at 4°C overnight and then dehydrated in 30% sucrose for 3 days. Frozen 16-μm coronal brain sections were cut on a cryostat and incubated with rabbit polyclonal anti-Kir6.2 (EMD Millipore) antibodies at 4°C overnight followed by Alex Fluor 488 goat anti-rabbit IgG at room temperature for 2 h. The intensity of fluorescence was analyzed using National Institutes of Health image analysis software. Quantification with a confocal laser scanning microscope (Zeiss LSM 510, Carl Zeiss, Cambridge, MA, USA) was performed on ≥5 representative tissue samples from the brain cortex for each rat and an average value was used for data analysis.

Statistical analysis. All data are expressed as the mean ± standard error of the mean. Data for the neurological deficit scores, motor coordination, infarct volumes and biochemical parameters were analyzed by Student's t-test with Bonferroni's correction for multiple comparisons. Changes in BP and HR between groups or between time-points in a group were observed using two-way analysis of variance followed by Tukey's post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Body weight and metabolic characterization. The body weight and metabolic characteristics of the LF- and HF-fed rats are summarized in Table I. Subsequent to feeding the rats with a HF for 12 weeks, the body weight was significantly higher in all the HF-fed rats compared with the LF-fed control rats. The HF-fed rats also exhibited a significant increase in the epididymal fat mass. The levels of blood glucose and plasma triglycerides were similar among all groups, but the levels of plasma insulin, leptin and cholesterol were elevated and the level of plasma adiponectin was reduced in the HF-fed rats.

Hemodynamic and physiological variables. To eliminate confounding factors on neurological outcomes, physiological

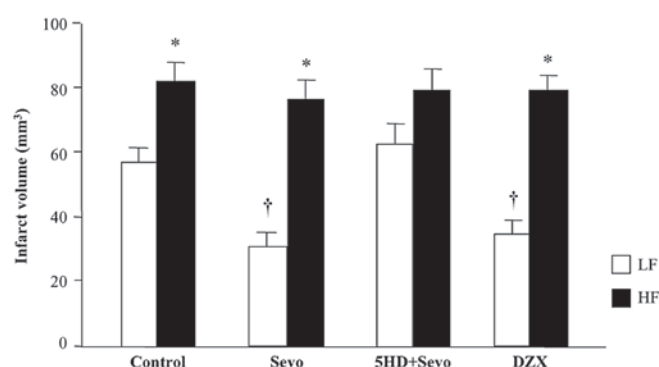


Figure 2. Effect of sevoflurane (sevo) postconditioning or mitoK_{ATP} channel opener, diazoxide (DZX), on infarct volume in each group. Values are expressed as the mean ± standard error of the mean (n=8-10 for each group). *P<0.05 vs. the respective LF; †P<0.05 vs. the respective control. LF, low-fat diet; HF, high-fat diet; 5HD, 5-hydroxydecanoate; mitoK_{ATP}, mitochondrial K_{ATP}.

parameters, including BP, HR and arterial blood gases, were monitored and controlled prior to, during and following focal cerebral ischemia. The hemodynamic and physiological variables in the experimental groups are presented in Tables II and III, respectively. No significant differences were identified in the mean BP, HR and arterial pH, carbon dioxide tension and oxygen tension at each time-point prior to and during focal cerebral ischemia and during reperfusion among the groups.

Effect of sevoflurane postconditioning or mitoK_{ATP} channel opener, DZX, on infarct size. The infarct volume at 24 h after reperfusion was significantly greater in the HF-control rats compared with that in the LF-control rats (81±10 versus 57±8 mm³; P<0.05; Fig. 2). Anesthetic postconditioning with sevoflurane reduced the total infarct volume by 47% (30±9 mm³ versus the LF-control; P<0.05) in the LF-fed rats, whereas it failed to protect the HF-fed rats (78±17 mm³ versus HF-control; P>0.05) against ischemic cerebral injury. Administration of the mitoK_{ATP} channel blocker, 5-HD, prior to sevoflurane postconditioning completely inhibited the protective effect of

Table II. Systemic hemodynamic variables in experimental groups.

A, Mean blood pressure (mmHg)					
Experimental groups	Pre-ischemia	Ischemia for 15 min	Ischemia for 35 min	Reperfusion for 5 min	Reperfusion for 35 min
LF-control	96±11	103±15	90±13	93±9	96±10
LF-sevo	98±6	101±9	89±8	87±11	90±8
LF-5HD+sevo	95±10	99±12	94±9	90±8	92±14
LF-DZX	97±12	98±8	92±6	95±9	90±9
HF-control	100±9	99±11	98±10	91±5	95±7
HF-sevo	95±12	100±13	91±14	89±12	88±10
HF-5HD+sevo	94±13	96±11	89±12	87±13	97±11
HF-DZX	99±10	102±9	95±10	90±9	89±6

B, Heart rate (beats/min)					
Experimental groups	Pre-ischemia	Ischemia for 15 min	Ischemia for 35 min	Reperfusion for 5 min	Reperfusion for 35 min
LF-control	336±12	340±15	352±13	344±10	343±9
LF-sevo	349±17	351±17	342±17	335±16	346±13
LF-5HD+sevo	350±15	346±11	339±10	351±9	339±11
LF-DZX	337±19	339±16	340±16	342±12	345±10
HF-control	341±10	344±9	339±12	341±15	338±15
HF-sevo	349±12	352±11	340±11	338±17	345±10
HF-5HD+sevo	346±9	342±14	337±15	346±9	350±16
HF-DZX	342±15	343±12	340±10	350±13	347±14

LF, low-fat diet; HF, high-fat diet; 5HD, 5-hydroxydecanoate; sevo, sevoflurane; DZX, diazoxide.

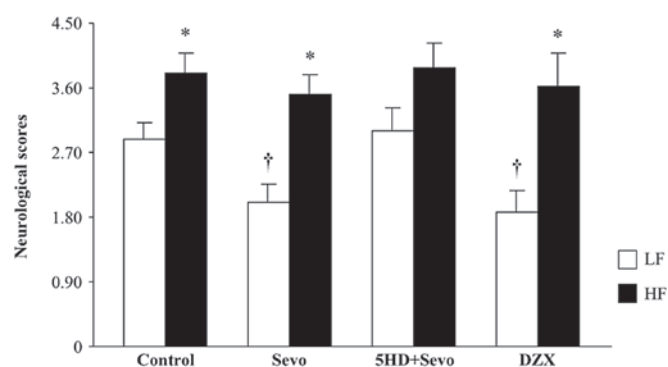


Figure 3. Effect of sevoflurane (sevo) postconditioning or mitoK_{ATP} channel opener, diazoxide (DZX), on neurological deficit scores in each group. Values are expressed as the mean ± standard error of the mean (n=8-10 for each group). *P<0.05 vs. the respective LF; †P<0.05 vs. the respective control. LF, low-fat diet; HF, high-fat diet; 5HD, 5-hydroxydecanoate; mitoK_{ATP}, mitochondrial K_{ATP}.

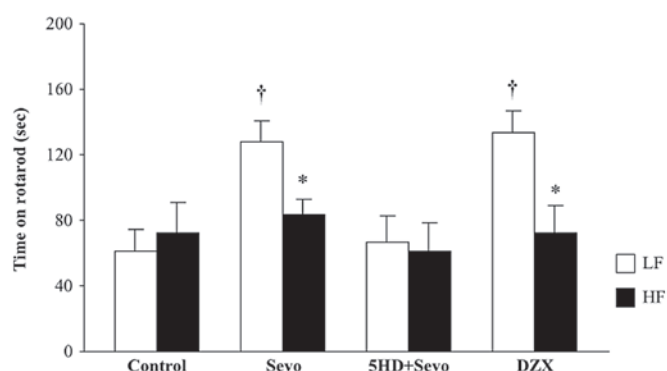


Figure 4. Effect of sevoflurane (sevo) postconditioning or mitoK_{ATP} channel opener, diazoxide (DZX), on motor coordination in each group. Values are expressed as the mean ± standard error of the mean (n=8-10 for each group). *P<0.05 vs. the respective LF; †P<0.05 vs. the respective control. LF, low-fat diet; HF, high-fat diet; 5HD, 5-hydroxydecanoate; mitoK_{ATP}, mitochondrial K_{ATP}.

sevoflurane postconditioning in the LF-fed rats (63±11 mm³ versus LF-sevo; P<0.05) whereas it had no effect on sevoflurane postconditioning in the HF-fed rats (79±12 mm³ versus HF-sevo; P<0.05). Administration of the mitoK_{ATP} channel opener, DZX, alone prior to reperfusion reduced the infarct volume similar to sevoflurane postconditioning in the LF-fed

rats (35±8 mm³ versus the LF-control; P<0.05). By contrast, DZX administration had no effect on the infarct volume in the HF-fed rats (79±9 mm³ versus HF-control; P>0.05).

Effect of sevoflurane postconditioning or mitoK_{ATP} channel opener, DZX, on neurological deficit scores. The results of

Table III. Physiological variables in experimental groups.

Experimental groups	pH	Ischemia PaO ₂ , mm Hg	PaCO ₂ , mm Hg	pH	Reperfusion PaO ₂ , mm Hg	PaCO ₂ , mm Hg
LF-control	7.35±0.04	130±18	45±6	7.39±0.04	143±19	42±7
LF-sevo	7.37±0.05	132±19	43±7	7.36±0.03	138±15	41±5
LF-5HD+sevo	7.41±0.03	136±16	39±5	7.40±0.02	139±12	38±6
LF-DZX	7.38±0.03	138±10	36±7	7.37±0.04	141±15	39±3
HF-control	7.39±0.02	141±12	42±5	7.37±0.03	140±17	40±4
HF-sevo	7.40±0.04	138±14	38±4	7.39±0.06	142±10	39±5
HF-5HD+sevo	7.36±0.06	140±17	41±5	7.38±0.05	138±11	43±7
HF-DZX	7.39±0.03	133±20	39±3	7.40±0.05	136±18	38±4

PaCO₂, arterial carbon dioxide tension; PaO₂, arterial oxygen tension; LF, low-fat diet; HF, high-fat diet; 5HD, 5-hydroxydecanoate; sevo, sevoflurane; DZX, diazoxide.

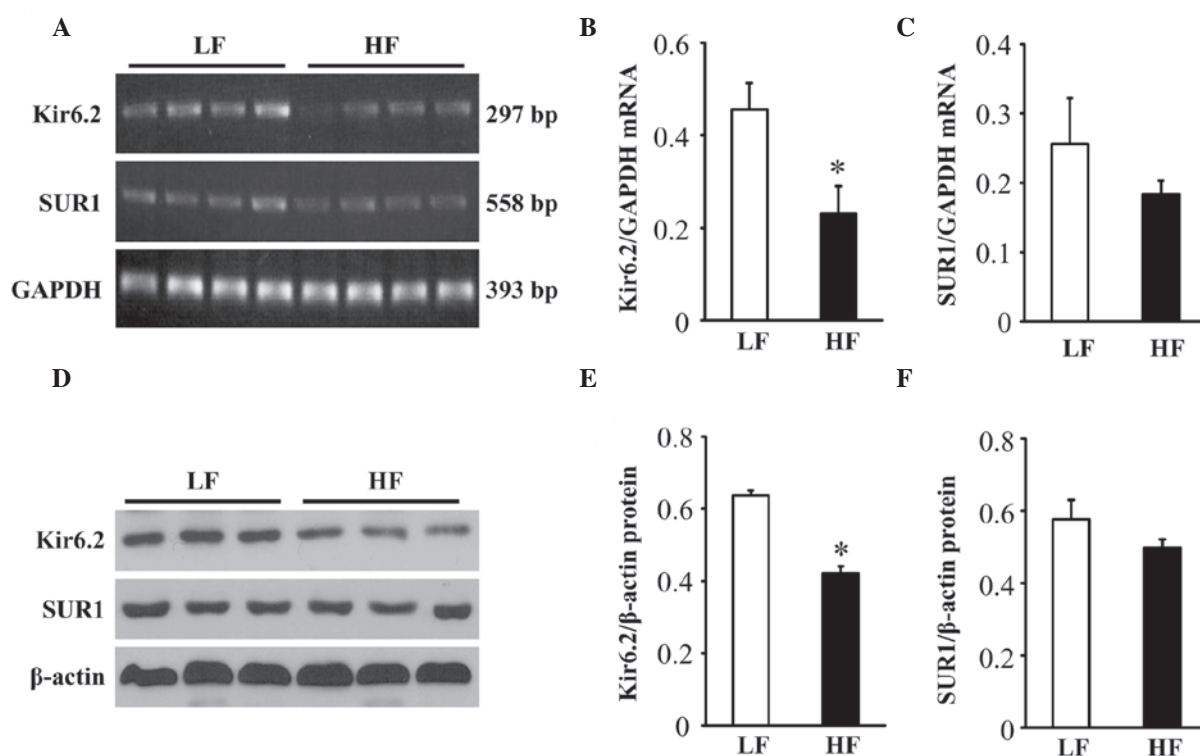


Figure 5. Effects of diet-induced obesity on the expression of mitoK_{ATP} channel subunits in the brain. (A, B and C) Representative RT-PCR product bands and quantitative comparison of mRNA expression for the mitoK_{ATP} channel subunits, Kir6.2 and SUR1, from HF- or LF-fed rats; (D, E and F) Representative western blotting and quantitative comparison of protein levels for Kir6.2 and SUR1 from HF- or LF-fed rats. Values are expressed as the mean ± standard error of the mean (n=5-7 for each group). *P<0.05 vs. LF. RT-PCR, reverse transcription-polymerase chain reaction; LF, low-fat diet; HF, high-fat diet; 5HD, 5-hydroxydecanoate; mitoK_{ATP}, mitochondrial K_{ATP}.

the neurological severity score tests are presented in Fig. 3. Compared with the LF-control rats, the HF-control rats had significantly higher neurological scores 24 h after reperfusion. Sevoflurane postconditioning reduced the neurological scores in the LF-fed rats (P<0.05 versus LF-control), whereas it had no effect on the HF-fed rats. Pretreatment with 5HD reversed the sevoflurane-induced decrease in neurological scores in the LF-fed rats, but did not change the score in the HF-fed rats. Similar to the response to sevoflurane postconditioning, administration of DZX alone also resulted in decreased

neurological scores in the LF-fed rats, but not in the HF-fed rats. These data are consistent with previous studies (5,16), indicating that changes in neurological deficit scores are associated with infarct volumes.

Effect of sevoflurane postconditioning or mitoK_{ATP} channel opener, DZX, on motor coordination. The rotarod performance results were similar between the two control groups (Fig. 4). Sevoflurane postconditioning significantly improved the rotarod performance in the LF-fed rats (P<0.05 versus

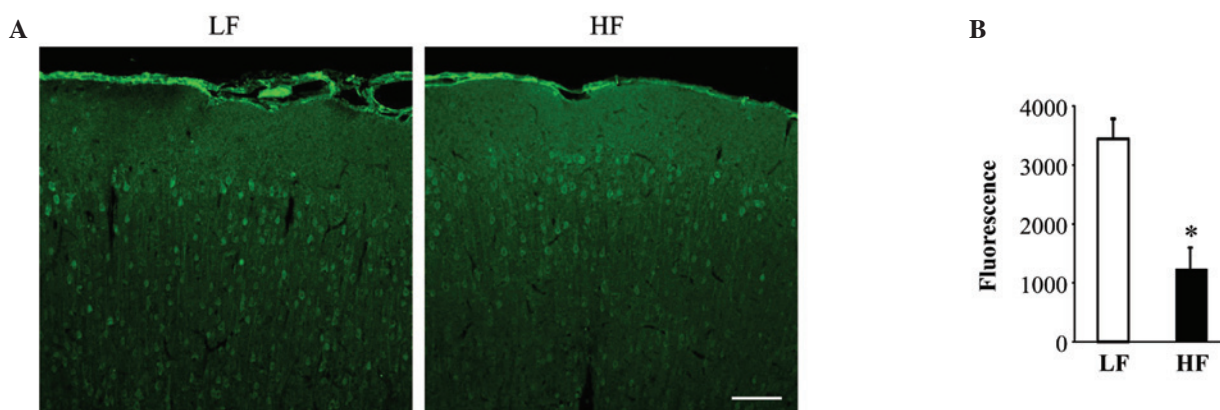


Figure 6. (A) Representative laser confocal images stained with Alex Fluor 488 goat anti-rabbit IgG and (B) quantitative comparison of Kir6.2 fluorescence in the brain cortex of LF- and HF-fed rats. Magnification $\times 20$; scale bar, 200 μm . Values are expressed as the mean \pm standard error of the mean ($n=3$ for each group). * $P<0.05$ vs. LF. LF, low fat diet; HF, high fat diet.

LF-control) and this improvement was eliminated by 5-HD. Neither sevoflurane postconditioning nor 5-HD changed the rotarod performance in the HF-fed rats. DZX administration improved the rotarod performance in the LF-fed rats, but not in the HF-fed rats.

Expression of brain K_{ATP} channels. To determine whether the K_{ATP} channels were altered in the brain in HF-induced obesity, the expression of the brain K_{ATP} channel subunits, Kir6.2 and SUR1, were measured using RT-PCR and western blotting. As shown in Fig. 5, the mRNA and protein levels of Kir6.2 in the brain were significantly reduced in the HF-fed rats compared with the LF-fed rats. However, the mRNA or protein levels of SUR1 in the brain were similar between the two groups.

Immunostaining of the mito K_{ATP} channel subunit, Kir6.2, in the brain. Laser confocal microscopy revealed that the HF-fed rats had less Kir6.2 immunoreactivity in the brain cortex compared with the LF-fed rats Fig. 6. This observation is consistent with the data from the RT-PCR and western blot analysis.

Discussion

The major findings of the present study are as follows: i) Anesthetic sevoflurane postconditioning failed to confer neuroprotection in the HF-fed rats compared with the LF-fed rats; ii) inhibition of mitochondrial K_{ATP} channels eliminated the neuroprotective effect of sevoflurane postconditioning in the HF-fed rats, whereas it had no effect in the LF-fed rats; iii) the mito K_{ATP} channel opener, DZX, induced a neuroprotective effect in the LF-fed rats, but not in the HF-fed rats, similar to sevoflurane postconditioning; and iv) expression of the K_{ATP} channel subunit, Kir6.2, in the brain was significantly reduced in the HF-fed rats compared with the LF-fed rats. The results of this study demonstrated for the first time that HF diet-induced obesity eliminates sevoflurane postconditioning-induced neuroprotective actions, possibly due to the alteration of the K_{ATP} channels in the brain.

A diet consisting of high levels of fat, also termed the 'Western diet', has been linked to a marked rise in obesity, type II diabetes and metabolic syndrome (3,17). The incidence

of stroke is correlated with the occurrence of obesity with metabolic syndrome, which has been shown to affect the outcome following stroke (3). In the present study, it was identified that focal cerebral ischemia-reperfusion without postconditioning induced a larger infarct volume and increased functional deficits in the HF-fed rats compared with the LF-fed rats. This result confirmed findings of an earlier study, which revealed that long-term exposure to a HF exacerbated focal ischemic brain injury (3), indicating that HF-induced obesity may increase susceptibility to ischemic brain injury. Significantly, sevoflurane postconditioning was identified to significantly reduce the ischemia-reperfusion-induced infarct volume and improve the neurological outcome in the LF-fed rats, but not in the HF-fed rats. The present study extended these previous results and demonstrated that HF-induced obesity was inhibited by sevoflurane postconditioning-induced neuroprotective actions.

Multiple intracellular mechanisms, including the activation of protein kinase C, mitogen-activated protein kinase, adenosine receptors and mito K_{ATP} channels, have been indicated to be involved in anesthetic preconditioning-induced cardioprotection and neuroprotection (8,10,20). It is widely accepted that the activation/opening of mito K_{ATP} channels is a significant mechanism for ischemic preconditioning-induced protection in various organs and for ischemic postconditioning-induced cardioprotection (10). Previous studies have indicated that anesthetic postconditioning-induced neuroprotection is also mediated by mito K_{ATP} channels (4,9,10). The present results revealed that the inhibition of the mito K_{ATP} channels in the LF-fed rats with 5-HD eliminated the protection conferred by sevoflurane postconditioning, indicating that under normal conditions, the opening of the mito K_{ATP} channels is critical for sevoflurane postconditioning, which is consistent with results from previous studies (4,10). By contrast, 5-HD did not change the infarct volume and functional deficits in the HF-fed rats following sevoflurane postconditioning. In addition, DZX, which has been used extensively to study pharmacological preconditioning to confer cardioprotection or neuroprotection by activating mito K_{ATP} channels (18,21), induced a similar significant reduction in the infarct volume and an improvement in functional deficits in the LF-fed rats, but not in the HF-fed rats, indicating that brain mito K_{ATP} channels were impaired

in the HF-fed rats. Therefore, the expression of the mitoK_{ATP} channels in the brains of the HF-fed rats was further evaluated and compared with that in the LF-fed rats.

It has been established that functional mitoK_{ATP} channels consist of two subunits in a hetero-octameric compound; the pore-forming Kir6.x subunits, Kir6.1 or Kir6.2, and the sulfonylurea receptor regulatory subunits, SUR1 or SUR2 (22,23). The various combinations of Kir6.x and SURx are expressed in various tissues, comprising mitoK_{ATP} channels with distinct electrophysiological and pharmacological characteristics (24). The mRNA transcripts of Kir6.2 and SUR1, which have been predominantly identified in the brain, are significant in protecting neurons against ischemic damage (23,24). A previous study revealed that the expression of the mitoK_{ATP} channel subunit, Kir6.2, in the brain hypothalamus was reduced in obese Zucker diabetic fatty rats (11). Other studies reported that the sensitivity or expression of the mitoK_{ATP} channel subunits, Kir6.1 and SUR2, in the peripheral tissues were impaired or reduced in obese Zucker or diet-induced obese rats (12,13). In the present study, the mRNA and protein expression levels of Kir6.2 in the brains of the HF-fed rats were observed to be significantly decreased compared with the LF-fed rats, but the expression of SUR1 was similar between the two groups. These results indicated that HF-induced obesity selectively caused dysfunction of the mitoK_{ATP} channel subunit, Kir6.2, in the brain by downregulating its expression. Reduced Kir6.2 expression may diminish mitoK_{ATP} channel density and lead to impaired mitoK_{ATP} channel activity, contributing to the inability to postcondition the brain against ischemia-reperfusion injury. Chronic hyperglycemia has been reported to reduce the expression of Kir6.2 in the brain and peripheral tissues in the presence and absence of hyperinsulinemia (11). However, the level of blood glucose was not significantly different between the HF and LF-fed rats in the present study. Further studies are required to examine the mechanism responsible for the decreased expression of the mitoK_{ATP} channels in the brain in HF-induced obesity.

In conclusion, the present study demonstrated that diet-induced obesity eliminated the ability of anesthetic sevoflurane postconditioning to protect the brain against ischemic neuronal injury. The impaired brain mitoK_{ATP} channel possibly contributes to the loss of neuroprotection in this experimental model of obese rats. Restoration of the brain mitoK_{ATP} channels may be critical to elicit neuroprotection by anesthetic postconditioning in human obesity.

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