Neuroanesthesia and Intensive Care

Isoflurane tolerance against focal cerebral ischemia is attenuated by adenosine A_1 receptor antagonists

[La tolérance à l'isoflurane contre l'ischémie cérébrale focale est diminuée par les antagonistes des récepteurs de l'adénosine A_1]

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Purpose: To investigate the role of the adenosine A_1 receptor in the rapid tolerance to cerebral ischemia induced by isoflurane preconditioning.

Methods: Seventy-five rats were randomly assigned into five groups (n = 15 each): Control, 8-cyclopentyl-1,3-dipropulxanthine (DPCPX), Isoflurane, DPCPX+Isoflurane and Vehicle+Isoflurane groups. All animals underwent right middle cerebral artery occlusion (MCAO) for two hours. Isoflurane preconditioning was conducted one hour before MCAO in Isoflurane, DPCPX+Isoflurane and Vehicle+Isoflurane groups by exposing the animals to 1.5% isoflurane in 98% oxygen for one hour. In the Control and DPCPX groups, animals were exposed to 98% oxygen one hour before MCAO for one hour. A selective adenosine A_1 receptor antagonist, DPCPX, was administered (0.1 mg·kg^{-1}) 15 min before isoflurane/oxygen exposure in the DPCPX and DPCPX+Isoflurane groups to evaluate the effect of adenosine A_1 receptor antagonist on isoflurane preconditioning. Dimethyl sulfoxide, the solvent of DPCPX, was administered (1 mL·kg^{-1}) 15 min before isoflurane exposure in the Vehicle+Isoflurane group. Neurological deficit scores and brain infarct volumes were evaluated 24 hr after reperfusion.

Results: Animals in the Isoflurane and Vehicle+Isoflurane groups developed lower neurological deficit scores and smaller brain infarct volumes than the Control group ($P < 0.01$). Animals in the DPCPX+Isoflurane group developed higher neurological deficit scores and larger brain infarct volumes than the Isoflurane and Vehicle+Isoflurane groups ($P < 0.01$).

Conclusion: The present study demonstrates that preconditioning with isoflurane reduces focal cerebral ischemic injury in rats, and the adenosine A_1 receptor antagonist (DPCPX) attenuates the neuroprotection induced by isoflurane preconditioning.

***Objectif** : Explorer le rôle des récepteurs de l'adénosine A_1 dans la tolérance rapide à l'ischémie cérébrale induite par le préconditionnement à l'isoflurane.*

***Méthode**: Nous avons réparti 75 rats en cinq groupes de 15 : Témoin, 8-cyclopentyl-1,3-dipropulxanthine (DPCPX), Isoflurane, DPCPX+Isoflurane et Véhicule+Isoflurane. Tous les rats ont subi une occlusion de deux heures de l'artère cérébrale moyenne droite (OACM). Le préconditionnement avec l'isoflurane a été fait une heure avant l'OACM dans les groupes Isoflurane, DPCPX+Isoflurane et Véhicule+Isoflurane par une exposition à un mélange de 1,5 % d'isoflurane et de 98 % d'oxygène pendant une heure. Dans les groupes Témoin et DPCPX, les rats ont été exposés à 98 % d'oxygène une heure avant l'OACM qui a duré une heure. Un antagoniste sélectif des récepteurs de l'adénosine A_1, DPCPX, a été administré (0,1 mg·kg^{-1}) 15 min avant l'exposition à l'isoflurane/oxygène dans les groupes DPCPX et DPCPX+Isoflurane pour évaluer l'effet de l'antagoniste des récepteurs de l'adénosine A_1 sur le préconditionnement à l'isoflurane. Le sulfoxyde de diméthyle, le solvant de DPCPX, a été administré (1 mL·kg^{-1}) 15 min avant l'exposition à l'isoflurane dans le groupe Véhicule+Isoflurane. Les scores de déficit neurologique et les volumes d'infarctus cérébral ont été évalués 24 h après la reperfusion.*

***Résultats** : Dans les groupes Isoflurane et Véhicule+Isoflurane, les scores de déficit neurologique étaient plus bas et les infarctus cérébraux de moindre volume que dans le groupe Témoin ($P < 0,01$). Dans le groupe DPCPX+Isoflurane, les scores de déficit neurologique étaient plus élevés et les infarctus cérébraux de plus grand volume que dans les groupes Isoflurane et Véhicule+Isoflurane ($P < 0,01$).*

***Conclusion** : Le préconditionnement avec de l'isoflurane réduit la lésion ischémique cérébrale focale chez les rats et l'antagoniste (DPCPX) des récepteurs de l'adénosine A_1 atténue la neuroprotection induite par le préconditionnement à l'isoflurane.*

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THE phenomenon of ischemic tolerance in the heart was first reported by Murry et al.[1] in 1986. Four years later, the phenomenon in the brain was reported by Kitagawa et al.[2] Since then, studies on the mechanism and potential clinical applications of the phenomenon have been intense. A pharmacological agent to induce cerebral ischemic tolerance before surgery would be valuable. Unfortunately most pharmacological or chemical agents that induce cerebral tolerance, such as lipopolysaccharide,[3] tumour necrosis factor-α,[4] and interleukin-1,[5] are limited in their application to patients due to their toxicity.

Isoflurane is a volatile anesthetic that is often used as a primary anesthetic agent during neurosurgical procedures. In a recent study of severe forebrain ischemia, isoflurane-anesthetized rats had a better histological outcome than those administered fentanyl-nitrous oxide.[6] The neuroprotective effect persists beyond the time of isoflurane administration and has therefore been termed anesthetic- or isoflurane-induced tolerance. This emphasizes the similarity between the isoflurane-induced neuroprotection and ischemic tolerance, the neuroprotection conferred by transient, sublethal cerebral ischemia. Indeed, the mechanisms underlying isoflurane-induced tolerance are not completely known, but studies of myocardial ischemia have shown that isoflurane-induced tolerance shared several cellular mechanisms with ischemic tolerance including opening of adenosine triphosphate-sensitive potassium channels (K_{ATP} channels), an adenosine receptor-mediated pathway, and a protein kinase C (PKC)-mediated pathway.[7,8]

Involvement of adenosine A_1 receptors and K_{ATP} channels in the development of cerebral ischemic tolerance induced by sublethal ischemia has been well studied. In our previous study, we found that glibenclamide, an adenosine triphosphate-regulated potassium channel blocker, abolished the tolerance to focal cerebral ischemia induced by repeated isoflurane anesthesia.[9] Other findings suggest that adenosine receptor activity is modified by isoflurane and that adenosine receptor activation is a trigger by which K_{ATP} channels are activated.[7,10] Based on these findings, we hypothesized that the adenosine A_1 receptor activation is involved in the cerebral tolerance induced by isoflurane. Therefore, the present study was conducted to determine whether antagonism of the adenosine A_1 receptor with 8-cyclopentyl-1,3-dipropulxanthine (DPCPX) attenuates the rapid cerebral tolerance induced by isoflurane preconditioning, in a rat model of focal cerebral ischemia and reperfusion.

Methods

The experimental protocol was approved by the Ethics Committee for Animal Experimentation and was performed according to the Guidelines for Animal Experimentation of the Fourth Military Medical University. The animals were provided by the Experimental Animal Center of the Fourth Military Medical University.

Experiment protocol

Seventy-five male Sprague-Dawley rats weighing 280 to 320 g were randomly assigned into five groups (n = 15 each) using the following randomization procedure. First, the rats were numbered from 1 to 75. Second, 75 random numbers were generated by a computer and each random number was assigned to a rat. The numbers were then arranged in numerical sequence. Rats in Control, DPCPX, Isoflurane, DPCPX+Isoflurane and Vehicle+Isoflurane (Figure 1) were 1 to 15, 16 to 30, 31 to 45, 46 to 60 and 61 to 75 respectively. All animals were subjected to transient focal cerebral ischemia by occlusion of the right middle cerebral artery for two hours. Isoflurane preconditioning was conducted one hour before cerebral ischemia in Isoflurane, DPCPX+Isoflurane and Vehicle+Isoflurane groups by exposing the animals to 1.5% Isoflurane in 98% oxygen for one hour. In the Control and DPCPX groups, animals were exposed to 98% oxygen one hour before cerebral ischemia for one hour. A selective adenosine A_1 receptor antagonist, DPCPX (Sigma Chemical Co., St. Louis, MO, USA), was intraperitoneally administered (0.1 $mg \cdot kg^{-1}$) 15 min before isoflurane/oxygen exposure in the DPCPX and DPCPX+Isoflurane groups to evaluate the role of adenosine A_1 receptor antagonist on isoflurane preconditioning. Dimethyl sulfoxide, the solvent of DPCPX, was intraperitoneally administered (1 $mL \cdot kg^{-1}$) 15 min before isoflurane exposure in the Vehicle+Isoflurane group. Neurological deficit scores and brain infarct volumes were evaluated 24 hr after reperfusion.

Isoflurane or oxygen treatment was processed in an airtight container (50 cm × 30 cm × 20 cm) with an inlet and an outlet. The container was filled with 98% oxygen or 1.5% isoflurane in 98% oxygen according to the groups. The fresh gas flow was infused into the container at the rate of 4 $L \cdot min^{-1}$ via the inlet during the pretreatment. An anesthetic gas analyzer (Brüel & Kjae, Naerum, Denmark) was used to monitor the gas concentration inside. Soda lime was placed in the bottom of the container. The CO_2 pressure inside was maintained lower than 1 mmHg during the treatment. The animals were kept spontaneously breathing during the pretreatment.

In a separate experiment, we measured the physiological variables in nine additional rats weighing 280 to 320 g during the isoflurane treatment. The animals were randomly assigned into one of three groups (n = 3 each): Isoflurane, DPCPX+Isoflurane and Vehicle+Isoflurane groups. Anesthesia was induced with 4% isoflurane and was maintained with 2% isoflurane delivered by a mask. The right femoral artery was cannulated for continuous monitoring of blood pressure and for arterial blood sampling. A rectal probe was inserted to monitor core temperature. After five minutes stabilization, animals were put into the container for isoflurane treatment (1.5% isoflurane in oxygen for one hour). Arterial blood gases and plasma glucose were measured five minutes before isoflurane treatment and at the end of the treatments.

Focal cerebral ischemia

The rats were fasted for 12 hr but were allowed free access to water before surgery. Anesthesia was induced with 4% isoflurane and was maintained with 2% isoflurane delivered by a mask. Focal cerebral ischemia was induced as described by Longa *et al.*[11] Briefly, the right common carotid artery and the right external carotid artery were exposed through a ventral midline neck incision and were ligated proximally. A 3-0 nylon monofilament suture (Ethicon nylon suture; Ethicon Inc., Tokyo, Japan) with its tip rounded by heating near a flame was inserted through an arteriectomy in the common carotid artery just below the carotid bifurcation, and then advanced into the internal carotid artery approximately 17 to 18 mm distal to the carotid bifurcation until a mild resistance was felt. Occlusion of the origins of the anterior cerebral artery, the middle cerebral artery, and the posterior communicating artery was thereby achieved. Reperfusion was accomplished by withdrawing the suture after 120 min of ischemia. After withdrawing the suture, the rats were returned to their cages with free access to food and water. The incision sites were infiltrated with 0.25% bupivacaine hydrochloride for postoperative analgesia. Rectal temperature was monitored (Spacelabs Medical Inc., Redmond, WA, USA) and maintained at 37.0 to 37.5°C by surface heating and cooling.

Neurological deficit score

The animals were neurologically assessed 24 hr after reperfusion by an investigator who was unaware of animal grouping. A six-point scale modified from that previously described by Longa *et al.*[11] was used for neurological assessment. 0 = no deficit; 1 = failure to extend left forepaw fully; 2 = circling to the left; 3 = falling to the left; 4 = no spontaneous walking with a depressed level of consciousness; 5 = dead.

Infarct volume assessment

After the neurological assessment, the rats were reanesthetized with 4% isoflurane in oxygen and decapitated. The brains were rapidly removed and cooled in iced saline for ten minutes. Six 2-mm-thick coronal sections were cut with the aid of a brain matrix. Sections were incubated for 30 min in a 2% solution of 2, 3, 5-triphenyltetrazolium chloride at 37°C and fixed by immersion in a 10% buffered formalin solution. Unstained areas were defined as infarcted tissue. Brain slices on a piece of plotting paper were photographed with a digital camera (Kodak DC240, Eastman Kodak Co., Rochester, NY, USA) connected to a computer with an image analysis software (Adobe Photoshop 6.0.1, Windows). An investigator blinded to the experimental groups then outlined the zones of infarction, the zones

TABLE I Physiological variables

				Arterial blood gases		
	MAP (mmHg)	*T (°C)*	*pH*	*PaO_2 (mmHg)*	*$PaCO_2$ (mmHg)*	*Glucose ($mmol \cdot L^{-1}$)*
Five minutes before isoflurane exposure						
Iso	102.7 ± 6.2	37.4 ± 0.06	7.40 ± 0.03	319.4 ± 11.7	41.1 ± 5.0	6.07 ± 0.32
Vehicle+Iso	103.7 ± 13.0	37.4 ± 0.10	7.38 ± 0.03	314.5 ± 61.8	36.2 ± 2.1	6.10 ± 1.57
DPCPX+Iso	100.7 ± 11.5	37.6 ± 0.47	7.38 ± 0.02	312.2 ± 15.1	40.5 ± 4.1	6.73 ± 1.45
At the end of one hour isoflurane exposure						
Iso	94.4 ± 2.6	37.3 ± 0.38	7.37 ± 0.03	364.4 ± 56.2	42.0 ± 3.8	6.73 ± 0.31
Vehicle+Iso	98.1 ± 10.9	37.1 ± 0.26	7.39 ± 0.04	361.1 ± 10.6	34.0 ± 5.2	6.50 ± 1.13
DPCPX+Iso	96.5 ± 12.9	37.2 ± 0.32	7.43 ± 0.04	317.6 ± 20.5	38.4 ± 2.0	6.23 ± 0.64

MAP = mean arterial blood pressure; T = temperature; Iso = isoflurane; DPCPX = 8-cyclopentyl-1,3-dipropulxanthine. Iso = pretreatment with one hour of 1.5% Iso in oxygen at one hour before middle cerebral artery occlusion (MCAO); Vehicle+Iso = administration of dimethyl sulfoxide before pretreatment with one hour of 1.5% Iso in oxygen at one hour before MCAO; DPCPX+Iso = administration of DPCPX before pretreatment with one hour of 1.5% Iso in oxygen at one hour before MCAO. All variables are presented as mean ± SD, and were analyzed using multivariate analysis of variance.

of 1 mm^2 from a piece of photographed plotting paper, as well as the outlines of both hemispheres in each section on the computer screen. The unstained areas and the areas of both hemispheres were calculated for each brain slice. The uncorrected infarct volume was calculated by measuring the unstained area in each slice, multiplying it by slice thickness, and then summating all six slices. The corrected infarct volume was calculated to compensate for the effect of cerebral edema. The difference between the areas of the right and the left hemisphere in a slice was considered to be edema and subtracted from the infarct area of that slice (corrected infarct volume = uncorrected infarct volume – [right hemisphere's volume - left hemisphere's volume]).[12] The result was multiplied by slice thickness and all six slices were summated to find the total corrected infarct volume.

Statistical analysis

Physiological data and infarct volumes are expressed as mean ± SD. Physiological variables were analyzed using multivariate analysis of variance. The infarct volumes were analyzed by one-factor analysis of variance (ANOVA) followed by post hoc least significant difference test. Neurological deficit scores (NDS) were analyzed using Kruskal-Wallis test followed by the Mann-Whitney U test with Bonferroni correction. $P < 0.05$ was considered statistically significant.

Results

No differences were found in the rectal temperature, mean arterial blood pressure, arterial pH, $PaCO_2$, PaO_2 and blood glucose values during treatment with 1.5% isoflurane among groups (Table I). Isoflurane tended to decrease mean arterial blood pressure and core temperature during the treatment period, but the reduction did not reach statistical significance. Arterial blood gases showed that there was no respiratory depression during isoflurane treatment. After recovery from isoflurane pretreatment, no animal showed abnormal behaviour.

Focal cerebral ischemia was performed on 75 rats; all rats survived until 24 hr after reperfusion. Animals in Control and DPCPX groups developed severe neurological damage, with NDS of 2 (1–3) and 2 (1–4) respectively (Table II). Isoflurane pretreatment reduced the neurological damage with the NDS of 1 (0–3) in Isoflurane and Vehicle+Isoflurane groups ($P < 0.01$ *vs* Control group). Administration of DPCPX before isoflurane treatment attenuated the neuroprotective effect of isoflurane with the NDS of 2 (1–3) in the DPCPX+Isoflurane group ($P < 0.01$ *vs* Isoflurane group).

TABLE II Neurological deficit scores 24 hr after reperfusion from 120 min of MCAO in rats

Groups	*Neurological deficit score*						*Median (range)*
	0	1	2	3	4	5	
Control	0	2	9	4	0	0	2 (1–3)
DPCPX	0	5	6	3	1	0	2 (1–4)
Iso	2	10	2	1	0	0	1 (0–3)*
Vehicle+Iso	2	11	1	1	0	0	1 (0–3)*
DPCPX+Iso	0	4	7	4	0	0	2 (1–3)

MCAO = middle cerebral artery occlusion; DPCPX = 8-cyclopentyl-1,3-dipropulxanthine; Iso = isoflurane. Iso = pretreatment with one hour of 1.5% Iso in oxygen at one hour before MCAO; DPCPX+Iso = administration of DPCPX before pretreatment with one hour of 1.5% Iso in oxygen at one hour before MCAO; DPCPX = administration of DPCPX without Iso pretreatment. The neurological deficit scores among groups were analyzed using Kruskal-Wallis test followed by the Mann-Whitney U test with Bonferroni correction.* $P < 0.01$ compared with the Control group.

The uncorrected and corrected brain infarct volumes are presented in Figure 2. Both the uncorrected and corrected infarct volumes in three isoflurane-treated groups were smaller than those of the Control group (uncorrected volume 287.2 ± 90.3 mm^3 and corrected volume 217.3 ± 79.5 mm^3). A significant reduction was observed in the Isoflurane group (uncorrected volume 137.4 ± 84.8 mm^3 and corrected volume 106.5 ± 60.0 mm^3) and the Vehicle+Isoflurane group (uncorrected volume 138.0 ± 84.2 mm^3 and corrected volume 104.7 ± 60.9 mm^3), ($P < 0.01$). The infarct volumes of DPCPX+Isoflurane group (uncorrected volume 250.1 ± 102.8 mm^3 and corrected volume 191.0 ± 80.0 mm^3) were significantly larger than those of the Isoflurane and Vehicle+Isoflurane groups ($P < 0.01$). Eight-cyclopentyl-1,3-dipropulxanthine treatment without isoflurane preconditioning in the DPCPX group did not change the infarct volume compared with the Control group.

Discussion

The purpose of the present study was to evaluate the beneficial effects of isoflurane preconditioning on focal cerebral ischemia in rats and whether the presence of DPCPX, a selective adenosine A_1 receptor antagonist, would alter the effects of isoflurane preconditioning. The results show that brief preconditioning with isoflurane diminishes the focal cerebral ischemic injury with improved neurological outcome and reduced brain infarct volume in rats, and DPCPX attenuated the neuroprotective effect of isoflurane preconditioning.

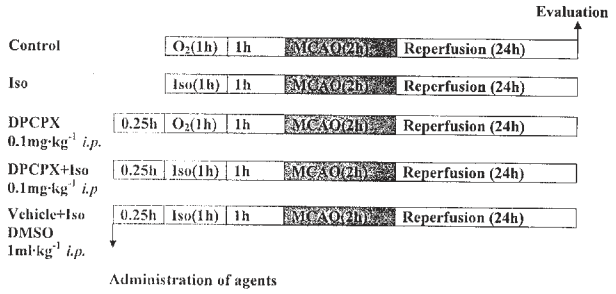

FIGURE 1 Diagram of different experimental protocols. Iso = 1.5% Isoflurane (Iso) pretreatment for one hour; 8-cyclopentyl-1,3-dipropulxanthine (DPCPX) = *ip* 0.1 mg·kg^{-1} DPCPX was administered 15 min (0.25 hr) before 98% oxygen exposure for one hour; DPCPX+Iso = *ip* 0.1 mg·kg^{-1} DPCPX was administered 15 min (0.25 hr) before Iso pretreatment for one hour; Vehicle+Iso = *ip* 1 mL·kg^{-1} dimethyl sulfoxide (DMSO) was administered 15 min (0.25 hr) before Iso pretreatment for one hour.

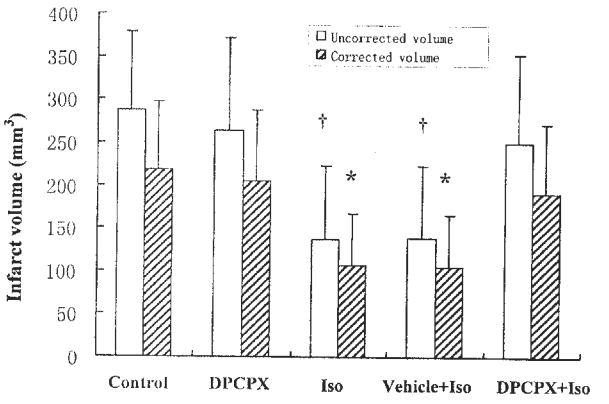

FIGURE 2 Uncorrected and corrected brain infarct volumes 24 hr after reperfusion from 120 min middle cerebral artery occlusion (MCAO). Data are presented as mean ± SD, and were analyzed by one-factor analysis of variance followed by post hoc least significant difference test. *$P <$ 0.01 compared with the control, 8-cyclopentyl-1,3-dipropulxanthine (DPCPX) and DPCPX+Iso groups. Iso = pretreatment with one hour of 1.5% isoflurane (Iso) in oxygen one hour before MCAO; Vehicle +Iso = administration of DMSO before pretreatment with one hour of 1.5% Iso in oxygen one hour before MCAO; DPCPX+Iso = administration of DPCPX before pretreatment with one hour of 1.5% Iso in oxygen one hour before MCAO; DPCPX = administration of DPCPX without Iso pretreatment.

It has been reported previously that preconditioning with volatile anesthetics such as isoflurane induces tolerance to myocardial ischemia.[7,13] In our previous study, we found that repeated isoflurane anesthesia induced cerebral ischemic tolerance in rats in a dose-dependent manner.[9] Kapinya *et al.* demonstrated that pretreatment with 1.4% isoflurane for three hours at zero, 12, and 24 hr before middle cerebral artery occlusion (MCAO) could induce neuroprotection in a rat focal cerebral ischemia model.[14] The results of this study confirm our previous findings and those from Kapinya *et al.*[14] Furthermore, we demonstrated that isoflurane preconditioning for one hour could induce acute tolerance to subsequent transient MCAO.

The concentration and duration of isoflurane inhalation chosen in this study were based on our previous report.[9] The concentration of 1.5% isoflurane equals 1.0 minimum alveolar anesthetic concentration.[15,16] To exclude the possible influence of hypotension, hypothermia, or respiratory depression on the beneficial effects of isoflurane, we measured the physiological variables during isoflurane pretreatment. The results showed no obvious changes in physiological variables during isoflurane preconditioning. In the present study, we did not measure the cerebral blood flow after the occlusion of the middle cerebral artery. In previous reports, it has been demonstrated that cerebral blood flow does not explain the beneficial effects of isoflurane on outcome from near-complete forebrain ischemia in rats.[17]

In the brain, preconditioning required several hours to develop. However, recent studies suggest that rapid preconditioning with a time course similar to that in the heart, can protect synaptic activity after anoxia in the brain slices and after ischemia in the intact brain.[18–20] The similarities in time course for preconditioning in the heart and brain suggest that preconditioning mechanisms may be similar in both organs. This assumption was supported by findings that activation of K_{ATP} channel plays important roles both in the cardiac and cerebral preconditioning.[21–24] The possible involvement of adenosine A_1 receptors as mediators of cardiac preconditioning has been proven by studies in several animal models.[7,25,26] It has been suggested that adenosine receptors may be activated by preconditioning and, in turn, activate K_{ATP} channels, possibly through an intermediate such as PKC.[27,28] Whether adenosine receptors play important roles in isoflurane preconditioning in cerebral ischemia is unclear. Nevertheless, as a ubiquitous neuromodulator, adenosine and its analogue have been proposed frequently as candidates for clinical development in

treatment of cerebral ischemia and stroke. Substantial studies have shown that pre- and postischemic administration of these drugs result in a significant reduction of postischemic brain damage while A_1 receptor antagonists blocked such neuroprotection effect induced by adenosine and its analogue.[29–31] We hypothesized that adenosine and the adenosine A1 receptor were essential for the induction of ischemic tolerance induced by isoflurane preconditioning in the brain. The results of the present study prove that the neuroprotection induced by isoflurane pretreatment was significantly attenuated by DPCPX.

Besides activating PKC and K_{ATP} channels, the activation of the adenosine receptor could also inhibit neurotransmitter release, stabilize the membrane potential and limit postsynaptic depolarization.[25,32,33] It has been suggested that excitatory amino acids are involved in the induction of ischemic/hypoxic damage in the brain. Excitatory amino acids are released immediately after ischemic injury, and they induce a sequence of changes ranging from excessive membrane depolarization to a rise in intracellular Ca^{2+} level which leads to cell death. Selective agonists of A_1 receptors, such as 2-chloroadenosine, R-N^6-phenylisopropyloadenosine, cyclohexyladenosine or N^6-cyclopentyladenosine, given either systemically or locally into the striatum or hippocampus attenuate the neuronal loss induced by non-depolarizing blocking agents, kainate, quisqualate or ibotenate. On the other hand, adenosine receptor antagonists such as DPCPX exacerbate the neurotoxic effect of kainic acid in the hippocampus.[34] Therefore, it seems that, as in animal models of ischemia/hypoxia, A_1 receptor agonists exert neuroprotective effects on the excitatory amino acid-induced excitotoxicity, a process which has been implicated in a variety of neuropathological conditions.[35]

The ischemic model used in our experiments is widely accepted.[3,9,12,36] In the present study, the infarct areas were measured by using Adobe Photoshop, the method was also used for measuring the infarct areas in the heart.[37] Many other image analysis softwares are used for measuring the infarct areas in different experiments.[3,12,14] In our experiment, two hours of MCAO in control animals resulted in the infarct volume of 217.3 ± 79.5 mm^3 (corrected infarct volume), which is similar with the infarct volume of 210.39 ± 31.25 mm^3 (corrected infarct volume) after two hours of MCAO in another report.[36]

The isoflurane-induced rapid tolerance to cerebral ischemia might be used to manage patients undergoing surgical procedures in which cerebral ischemia may occur. The results of the present study also provide a clue for further preclinical studies on the possibility of developing selective agonists of the adenosine A_1 receptors as neuroprotective agents. However, substantially more information on isoflurane-induced tolerance and the agonists of adenosine A1 receptors must be obtained before these indications can be advocated in humans.

In conclusion, isoflurane preconditioning induces neuroprotection to subsequent transient MCAO in rats, and administration of the adenosine A_1 receptor antagonist (DPCPX), shortly before isoflurane pretreatment, significantly attenuates the beneficial effects of isoflurane. Our work suggests that isoflurane-induced ischemic tolerance in the brain may depend on the activation of adenosine A_1 receptors.

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