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Activation of the Adenosine Triphosphate Sensitive Mitochondrial Potassium Channel is Involved in the Cardioprotective Effect of Isoflurane

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Abstract

The adenosine triphosphate-dependent potassium (K_{ATP}) channel has been proposed to play an important role in the cardioprotective effect of isoflurane (ISO). However, the question of whether the K_{ATP} channel, sarcolemmal or mitochondrial is the main contributor to the effect has not been clarified. The major aim of the present study was to determine whether or not the mitochondrial potassium channel was a site of action for ISO. Whether there was an acute “memory phase”, in which drugs were not detected in the tissues, but the protective effect still remained in the ischemic preconditioning (IP)-like effect of ISO was also investigated. Dangling participle isolated rat hearts, a 20-min normothermic nonperfused phase was maintained to produce a global ischemia. Under these ischemic conditions, the effects of ISO, sodium 5-hydroxydecanoate (5 HD: a selective mitochondrial K_{ATP} channel antagonist), and ISO combined with 5 HD on cardiac performance were examined. To all these four groups, (non-treated group, ISO group, 5 HD group and ISO plus 5 HD group, n=6 each) drugs were given for 30 min. After 10 min of drug-free perfusion (pre-ischemia restabilization period), 20 min of ischemia followed. Then the cardiac performance and the creatine kinase (CK) release during the reperfusion period were tested. In the non treated group and 5 HD group, cardiac performance was stable during the treated period and pre-ischemia the restabilization period. In the ISO group and ISO plus 5 HD group, heart rate (HR), left ventricular (LV) systolic pressure, and LV maximum rate of development of tension (dP/dtMax) during the drug-treated period became gradually and linearly worse.

However, these values were the same as in the non-treated group and 5 HD group at the end of the pre-ischemia restabilization period. So 5 HD itself had no hemodynamic effect; nor did it have any influence on the actions of ISO. At the end of the pre-ischemia restabilization period, the significant hemodynamic differences among the groups diminished and ISO was not detected in the solution. In the post-reperfusion period, except for the ISO group, (non treated group, 5 HD group and ISO plus 5 HD group) cardiac performances were drastically decreased. ISO significantly ameliorated the dysfunction of cardiac output, LV systolic pressure and LV+dP/dtMax. The CK level in the coronary effluent during reperfusion was also significantly reduced by ISO. 5 HD completely inhibited these cardiac effects of ISO. Activation of the adenosine triphosphate sensitive mitochondrial potassium channel is involved in the cardioprotective effect of ISO, and the action of this agent has an acute “memory phase” like ischemic preconditioning. (J Nippon Med Sch 2001; 68: 238—245)

Key words: isoflurane, ischemia-reperfusion injury, sodium 5-hydroxydecanoate, mitochondrial K_{ATP} channel, acute memory phase

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Introduction

The adenosine triphosphate-dependent potassium (K_{ATP}) channel plays an important role in the cardioprotective effect of ischemic preconditioning (IP)¹. Volatile anesthetics such as isoflurane (ISO) also induce an IP-like effect²⁻⁴, which is blocked by the non-specific K_{ATP} channel antagonist glyburide. Therefore, the K_{ATP} channel is thought to be involved in IP. K_{ATP} channels have recently been identified in the sarcolemma as well as in the mitochondrial membrane⁵. Sarcolemmal K_{ATP} (sar K_{ATP}) channels were initially linked to cardioprotection during ischemia, but subsequent evidence has suggested that these channels are not solely responsible⁶. More recently, the role of mitochondrial K_{ATP} (mit K_{ATP}) channels in ischemic preconditioning has also been suggested^{7,8}. The subcellular location of the K_{ATP} channels that are activated by ISO is unknown. We tested the hypothesis that ISO directly preconditions the myocardium against cardio-depression via activation of mit K_{ATP} channels in an ischemic model of the working rat heart ischemic model, and we also investigated whether the protection afforded by ISO was associated with an acute memory phase similar to that for ischemic preconditioning.

Materials and Methods

This study was approved by the animal ethics committee of Nippon Medical School. Male Sprague-Dawley rats (240 to 260 g) were anesthetized using 100 mg/kg of intraperitoneal sodium pentobarbital. After tracheal intubation, heparin (1,000 U/kg) was infused intravenously.

(1) General preparation

The hearts were removed from fully anesthetized rats and were arrested in cold Krebs-Henseleit bicarbonate (KHB) solution (4°C) containing the following (mg/ml): NaCl 6.90, NaHCO₃ 2.10, KCl 0.35, MgSO₄ 0.14, KH₂PO₄ 0.16, CaCl₂ 0.33, and glucose 1.80. The aorta was transected 4~5 mm above the aortic valve and was mounted on an aortic steel cannula, after which retrograde perfusion was started. While the

heart was preperfused with a circulating KHB solution, the perfusate was oxygenated with 95% O₂/5% CO₂ and the temperature was kept constant at 37°C. An angled steel cannula was advanced into the left atrium via a pulmonary vein. Then the preperfusion tube was clamped and the left atrial tube was unclamped for antegrade perfusion of the heart (working heart mode). Perfusate was ejected from the heart into an aortic bubble trap, which was placed above the heart, and the afterload could be maintained at a constant level (70 mmHg) by setting the height of this bubble trap.

(2) Preliminary study (Fig. 1 A)

To evaluate the extent of ischemia-perfusion injury, we tested the appropriate duration of ischemia. The preparations were randomised into three groups that were subjected to 18 min, 19 min and 20 min of ischemia (n=3 each). After 50 min, global ischemia was induced by shutting off the perfusate and setting the afterload to zero. The temperature of the heart during ischemia was also kept at 37°C by pouring the non-oxygenated warm perfusate on it. Reperfusion of the heart after this ischemic period was performed by opening the reperfusion circuit, so that oxygenated perfusate was pumped from the bottom of the chamber to the top of the circuit, the height of which determined the driving pressure (70 mmHg) for reperfusion, and preload clamping was continued. After 30 min, reperfusion was ceased and working heart mode was started again. At 15 min after the end of reperfusion, we measured cardiac function.

(3) Main study (Fig. 1 B)

The hearts were randomly allocated to the following four groups (n=6 each): a control group (no drugs), a group treated with isoflurane (ISO; purchased from Abbott Ireland (Sligo, Ireland)), a group treated with sodium 5-hydroxydecanoate (5 HD; purchased from Sigma Chemical Co. [St. Louis, Mo]), a selective mitochondrial K_{ATP} channel antagonist, and a group treated with ISO plus 5 HD (ISO+5 HD). After an initial stabilization period (10 min), the heart was exposed for 30 min to the perfusate equilibrated with or without a 2 minimum alveolar concentration (MAC) of ISO in the oxygenating chamber. The MAC

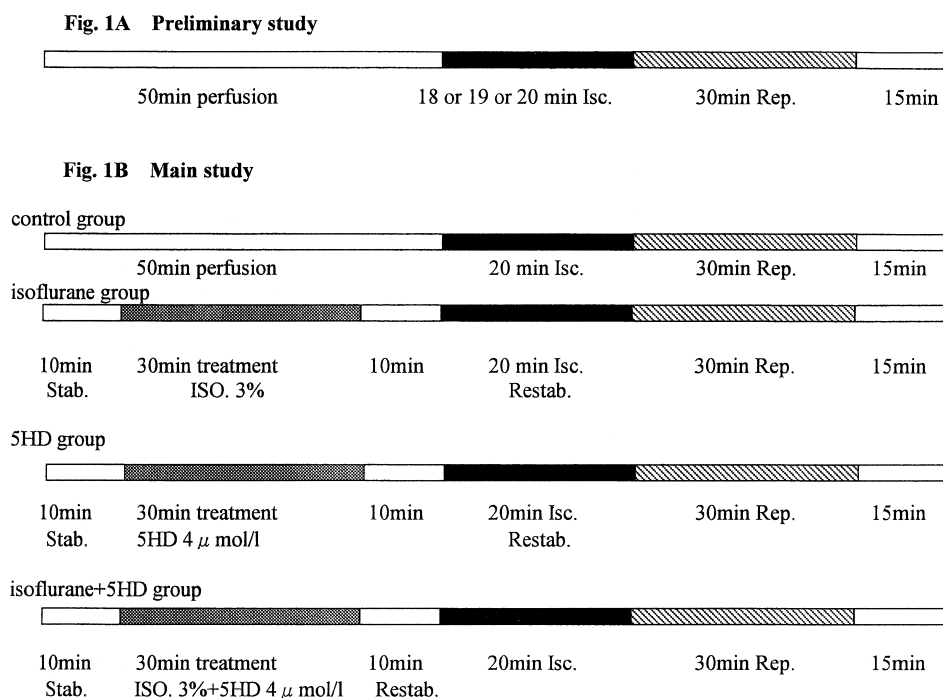


Fig. 1 Experimental protocol

Isc.=ischemia; Rep.=reperfusion; Stab.=Stabilization; Restab.=restabilization

5 HD=sodium 5-hydroxydecanoate; ISO=isoflurane

Control=non treated group; 5 HD=sodium 5-hydroxydecanoate (5 HD) group; ISO=isoflurane group, ISO+5 HD=isoflurane+5 HD group

of isoflurane used was 1.5%, which is the MAC value reported in male rats⁹. The concentration of the anesthetic was continuously measured in the gas phase of the oxygenating chamber using a Datex anesthetic agent monitor (Datex-Ohmeda, Finland). After 30 min, the perfusate was completely changed to a new perfusate and a restabilizing period of 10 min was provided. In this period, ISO was totally cleared from the heart. Subsequently, global ischemia was induced for 20 min, followed by reperfusion of the heart (as above) for 30 min. During the reperfusion period, coronary effluent was sampled to determine the cumulative creatine kinase (CK) release. After 30 min, the working heart mode was started again.

Left ventricular pressure was measured with a transducer (Abbott Ireland, Sligo, Ireland) connected to an 18-gauge catheter (Argyle Intramedicut Catheter, Sherwood, Tokyo, Japan) inserted into the left ventricle through the mitral valve from an angled steel cannula in the left atrium. The rate of development of tension (dP/dt) was measured from the derivatives of left ventricular pressure recorded electronically. Aortic flow was measured by collecting the

effluent from the air trap. Coronary flow was measured by timed collection of the pulmonary artery outflow and the surface run-off of the heart arising from the coronary sinus and the thebesian vessels. Cardiac output was considered to be the sum of the aortic and coronary outflows. The coronary effluent was not recirculated. Coronary resistance was calculated using the total coronary flow and the perfusion pressure (70 mmHg) during 30 min of reperfusion.

For determination of creatine kinase (CK) release, the total amount of coronary effluent during the reperfusion period was collected. CK was measured using a kit from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). After each experiment, the dry weight of the heart was measured and CK release was expressed per gram of dry tissue.

(4) Statistical analysis

Data are expressed as the mean \pm SD. The significance of differences among the groups was tested by one-way ANOVA, followed by Duncan's multiple range test and the significance of differences within the group was tested by two-way ANOVA, followed

Table 1 Baseline status

	Preliminary study			Conclusive study			
	18min	19min	20min	control	5HD	ISO	ISO+5HD
body weight (g)	242±10.4	243±21	242±16	241±12	254±9.2	242±15	243±8.2
dry heart weight (g)	0.2±0.03	0.2±0.01	0.2±0.02	0.2±0.02	0.2±0.03	0.2±0.01	0.2±0.01

values are mean ± SD. 18min, 19min and 20min means duration of ischemic period in each groups.
 control = non treated group ; 5HD = sodium 5-hydroxydecanoate group ; ISO = isoflurane group
 ISO + 5HD = isoflurane plus 5HD group
 no statistical difference was found among four groups.

Table 2 Summary of hemodynamic variables in the preliminary study

		HR	LVPSP	LV + dP /dtMax	CO
18 min	pre	325 ± 10	105 ± 9	3,300 ± 495	58 ± 4
	post	295 ± 14	83 ± 6	2,347 ± 150	24 ± 3
19 min	pre	312 ± 10	99 ± 2	2,867 ± 416	60 ± 6
	post	227 ± 21	71 ± 5	1,470 ± 296	18 ± 1
20 min	pre	297 ± 25	98 ± 1	2,700 ± 436	57 ± 2
	post	122 ± 107	11 ± 10*	109 ± 101*	3 ± 3*

Values are the mean ± SD, HR = heart rate (beats/min);
 LVPSP = left ventricular peak systolic pressure (mmHg);
 LV + dP/dtMax = maximum positive left ventricular rate of developed tension (mmHg/s)
 CO = cardiac output (ml/min)
 Pre = before ischemia (at 40 min after starting the experiment); post = at 15 min after reperfusion.
 p < 0.05, 20 min group vs. the 18 min and 19 min groups.

by Dunnett's test. P < 0.05 was regarded as statistically significant.

Result

There were no differences observed between any of the groups (three groups in the preliminary study and four groups in the main study) with respect to body weight and dry heart weight (Table 1).

1. Preliminary study

Data from the preliminary study are summarized in Table 2. Preischemic conditions were not different among the groups. In the 20-min group, the postischemic heart rate (HR) showed no statistical difference, but cardiac output, left ventricular (LV) systolic pressure, and LV + dP/dtMax were significantly lower than in the other groups. Neither 18 min nor 19 min of ischemia had such a marked effect as 20 min.

2. Hemodynamic changes in the preischemic period

No differences in baseline hemodynamics were observed among the experimental groups (Table 3). In all experimental stages, 4 µmol/l 5 HD had no hemodynamic effects. At the end of drug administration, ISO caused a significant decrease in HR, LV systolic pressure, and LV + dP/dtMax. The same trend was seen in the ISO + 5 HD group, with respect to HR, LV systolic pressure, and LV + dP/dtMax. The cardiovascular actions of ISO were not affected by 5 HD. Throughout the experimental period, there were no differences between the groups in coronary flow or cardiac output.

All significant hemodynamic discrepancies between the groups diminished during the preischemia reestablishment period.

3. After ischemia-reperfusion

After 15 min of reperfusion, hemodynamic parameters were measured; the data are summarized in Table 4. HR showed no statistical difference, but cardiac output, LV systolic pressure, and LV + dP/dtMax were significantly higher in the ISO group than in the other groups. The results indicated that concomitant application of 5 HD blocked the protective effect of ISO.

The CK level of coronary effluent during reperfusion was significantly lower in the ISO group than in the other groups (Fig. 2 A) (12.1 ± 1.6 IU/g in the ISO group versus 22.4 ± 4.5 IU/g in the control group, 21.9 ± 3.7 IU/g in the 5 HD group, and 29.6 ± 6.2 IU/g in the ISO+5 HD group, p < 0.05, n = 6 each). On the other hand, total coronary flow during the same period (30 min) and at the same perfusion pressure (70 mmHg) was not significantly different among the groups

Table 3 Summary of preischemic hemodynamic variables

		Ao (ml/min)	CF (ml/min)	HR (beats/min)	LVPSP (mmHg)	LV + dP/dtMax (mmHg/s)
control	Predrug	45.3 ± 4.0	20.3 ± 4.2	307 ± 21	112 ± 8	3,800 ± 267
	postdrug	41.3 ± 7.6	17.9 ± 2.9	306 ± 27	106 ± 4	3,178 ± 122
	preischemia	39.3 ± 3.3	18.1 ± 2.6	307 ± 24	98 ± 2	2,816 ± 319
5HD	predrug	49.3 ± 2.8	19.0 ± 3.4	339 ± 21	116 ± 2	3,850 ± 259
	postdrug	37.5 ± 2.3	13.9 ± 3.1	302 ± 8	101 ± 3	3,150 ± 217
	preischemia	37.5 ± 3.8	15.2 ± 2.7	304 ± 20	98 ± 2	2,708 ± 169
isoflurane	predrug	42.5 ± 5.2	19.5 ± 2.1	323 ± 34	111 ± 6	3,817 ± 362
	postdrug	29.5 ± 6.9	16.1 ± 2.2	265 ± 20*	90 ± 8*	2,617 ± 298*
	preischemia	37.5 ± 6.2	15.4 ± 2.3	279 ± 25	97 ± 8	2,950 ± 326
isoflurane + 5HD	predrug	50.5 ± 7.5	20.7 ± 2.0	346 ± 27	117 ± 7	4,108 ± 456
	postdrug	35.8 ± 3.5	18.1 ± 5.3	265 ± 20*	90 ± 5*	2,408 ± 169*
	preischemia	41.9 ± 4.3	19.3 ± 5.4	312 ± 6	96 ± 3	2,683 ± 397

Values are the mean ± SD.

Ao = aortic flow rate ; CF = coronary flow rate ; HR = heart rate ;

LVPSP = left ventricular peak systolic pressure ;

LV + dP/dtMax = maximum positive left ventricular rate of developed tension

Predrug = before exposure to drug (10 min after starting the experiment)

post drug = at the end of drug exposure (40 min after starting the experiment)

preischemia = at the end of restabilization period (50 min after starting the experiment)

There were no significant differences among the four groups in the predrug and preischemia periods.

p < 0.05, predrug vs. postdrug values.

Table 4 Hemodynamics after ischemia-reperfusion

	HR	LVPSP	LV + dP/dtMax	CO
control	91 ± 79	10 ± 8	91 ± 79	3 ± 3
5HD	110 ± 131	7 ± 8	175 ± 214	2 ± 2
ISO	206 ± 90	67 ± 15*	1,608 ± 526*	19 ± 5*
ISO + 5HD	131 ± 152	6 ± 8	140 ± 187	1 ± 2

Values are the mean ± SD.

HR = heart rate (beats/min)

LVPSP = left ventricular peak systolic pressure (mmHg)

LV + dP/dtMax = maximum positive left ventricular rate of developed tension (mmHg/s)

CO = cardiac output (ml/min)

*p < 0.05, ISO group vs. the control, 5HD, and ISO + 5HD groups.

(Fig. 2 B). However, coronary resistance was lower in the ISO group than in the other groups (208 ± 65 ml in the ISO group versus 176 ± 59 ml in the control group, 133 ± 8 ml in the 5 HD group, and 136 ± 19 ml in the ISO+5 HD group, n=6 each).

Discussion

In the present study, ISO had a cardioprotective ef-

fect against ischemia-reperfusion injury. Before ischemia, HR, cardiac contractility, and cardiac work were not different between the ISO group and the control group, but all of these parameters were higher in the ISO group after reperfusion. In addition to hemodynamic parameters, the smaller CK release during reperfusion in the ISO group suggests that it could protect cardiac myocytes from injury.

5 HD alone did not have any effect on cardiac ischemia-reperfusion. Before and after ischemia, none of the indicators were different between the 5 HD group and the control group. In the ISO+5 HD group, preischemic parameters also did not differ from those in the ISO group, but after reperfusion there were differences between the ISO and ISO+5 HD groups. In the ISO+5 HD group, cardiac function was lower after reperfusion and CK release during reperfusion was higher than in the ISO group. These results suggested that 5 HD was able to completely inhibit the cardioprotective effect of ISO.

A "memory phase" is one of the features of IP. During this phase, the myocardium remains resistant to infarction for up to 2 hr after preconditioning in dogs¹⁰ and 1 hr in rats¹¹. K_{ATP} activation has been

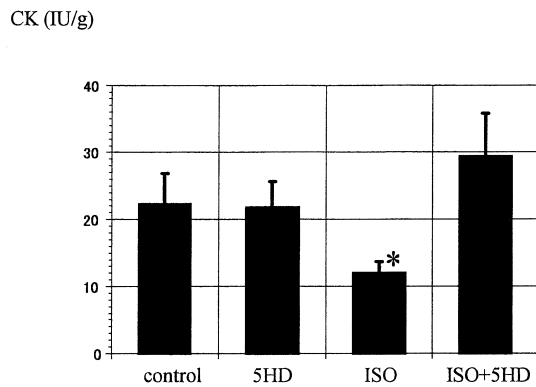


Fig. 2 A Creatine kinase (CK) release of coronary effluent (per gram of dry heart weight). control=non treated group; 5 HD=sodium 5-hydroxydecanoate (5 HD) group; ISO = isoflurane group, ISO+5 HD=isoflurane+5 HD group
* $P < 0.05$, ISO group vs. the control, 5 HD and ISO+5 HD groups.

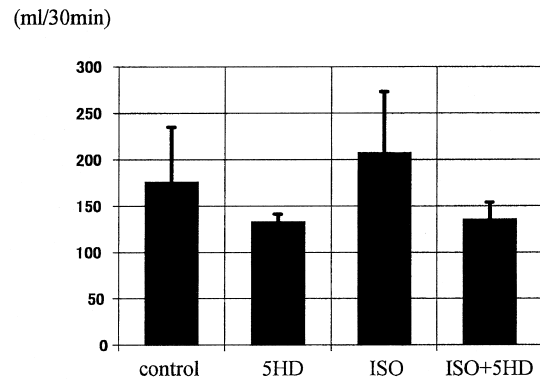


Fig. 2 B Coronary flow during the reperfusion period (30 min).

This value represents the coronary resistance because it was measured at a constant perfusion pressure and duration. No statistical difference was found among the four groups. However, coronary resistance tended to decrease more in the ISO group than in the others.

shown to play an important role in the “memory phase”¹². In our model, the cardioprotective effect of ISO persisted after the preischemia restabilization period, thus resembling the “memory phase” of IP. A “memory phase” of cardioprotection by volatile anesthetics was indicated in dogs⁴, but previous studies did not suggest any “memory phase” in rats. Some studies have used volatile anesthetics just before ischemia¹³ and others have used the anesthetics during ischemia¹⁴⁻¹⁶. In such cases, volatile anesthetics may still be present at the onset of reperfusion, causing suppression of intracellular Ca^{2+} and free radical generation¹⁷. Volatile anesthetics are thought to have a direct cardiovascular effect through inhibition of Ca^{2+} channels¹⁸. These channels induce a decrease in the intra-cellular Ca^{2+} concentration and cardiac contractility, perhaps thus resulting in cardioprotection. Halothane¹⁹ and ISO¹⁷ also reduce free radical formation and protect the reperfused heart from injury. In this study, ISO was not detected before ischemia and no intergroup variation of its hemodynamic effects was found. Therefore, we concluded that the protection afforded by ISO has a “memory phase” and that the cardioprotective effect suggested by our study is not related to ISO’s suppressive effect of intracellular Ca^{2+} and free radical formation during the ischemia-reperfusion period.

After 10 to 15 min of exposure to ISO, no memory

phase was found in our model; nor was one seen at a lower concentration of ISO (1 MAC). After 30 min, no memory phase was found with a 1 MAC concentration of ISO, and a higher concentration of 2 MAC was essential to produce a “memory phase”.

Liu and Downey found that three cycles of three minutes of ischemia and reperfusion could precondition the rat heart²⁰. With our working heart model, three cycles of five minutes of global ischemia plus reperfusion produced a preconditioned heart, and the heart could beat even after 25 min of ischemia (data not shown). In contrast, the heart did not beat after the same ischemic period when treated with ISO, so the protective effect of ISO seems to be weaker than that of IP. This conclusion is the same as that of a previous study²¹. The $mitK_{ATP}$ pathway is probably not the sole mechanism of the IP-like effect of ISO.

Previous studies have used 100 $\mu\text{mol/l}$ of 5 HD as an antagonist of the mitochondrial potassium channels^{7, 22-24}. However, because at 100 $\mu\text{mol/l}$ cardiac contractility, coronary flow and cardiac output were significantly depressed after 30 min of exposure and this did not occur at 4 $\mu\text{mol/l}$ (data not shown), we applied 4 $\mu\text{mol/l}$ of 5 HD in this study. In previous studies, 10 to 15 min was the usual exposure time of 5 HD and during that period cardiac performances did not change. But in our study, exposure time was 30 min. This is probably why hemodynamic depression

occurred only in our case. At 4 $\mu\text{mol/l}$, 5 HD did not have any effect on the recovery from ischemia-reperfusion or on CK release (an indicator of cell injury), but it was able to antagonize the effect of ISO. Therefore, we concluded that this concentration was sufficient to assess the role of mitK_{ATP} in the IP-like effect of ISO.

Several recent studies have used CK as an indicator of myocardial injury in isolated rat heart ischemia-reperfusion models^{15,25-27}. In these studies, CK values obtained from non treated groups were 10 to 30 times higher than those obtained from our control group. And the same trend was seen between the CK values of these drug-treated groups and that of our ISO group. These discrepancies were due to differences in the protocol, since the other studies employed a Langendorff apparatus with a constantly higher coronary reperfusion rate or pressure than we used, as well as a longer period of ischemia and a longer duration of reperfusion over which CK was measured.

The present study tested the hypothesis that ISO directly preconditions the myocardium against ischemia-reperfusion induced cardiac depression via activation of mitK_{ATP} channels. The selective mitK_{ATP} antagonist 5 HD completely inhibited ISO-induced cardioprotection, indicating that the mitK_{ATP} channel is relevant to this IP-like cardioprotective effect.

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