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Prevention of Isoflurane-induced Preconditioning by 5-Hydroxydecanoate and Gadolinium

Possible Involvement of Mitochondrial Adenosine Triphosphate–sensitive Potassium and Stretch-activated Channels

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Background: **Both mitochondrial adenosine triphosphate–sensitive potassium (MK_{ATP}) channels (selectively blocked by 5-hydroxydecanoate) and stretch-activated channels (blocked by gadolinium) have been involved in the mechanism of ischemic preconditioning. Isoflurane can reproduce the protection afforded by ischemic preconditioning. We sought to determine whether isoflurane-induced preconditioning may involve MK_{ATP} and stretch-activated channels.**

Methods: **Anesthetized open-chest rabbits underwent 30 min of coronary occlusion followed by 3 h of reperfusion. Before this, rabbits were randomized into one of six groups and underwent a treatment period consisting of either no intervention for 40 min (control group; n = 9) or 15 min of isoflurane inhalation (1.1% end tidal) followed by a 15-min washout period (isoflurane group; n = 9). The two groups received an intravenous bolus dose of either 5-hydroxydecanoate (5 mg/kg) or gadolinium (40 μmol/kg) before coronary occlusion and reperfusion (5-hydroxydecanoate, n = 9; gadolinium, n = 7). Two additional groups received 5-hydroxydecanoate or gadolinium before isoflurane exposure (isoflurane–5-hydroxydecanoate, n = 10; isoflurane–gadolinium, n = 8). Area at risk and infarct size were assessed by blue dye injection and tetrazolium chloride staining.**

Results: **Area at risk was comparable among the six groups (29 ± 7, 30 ± 5, 27 ± 6, 35 ± 7, 31 ± 7, and 27 ± 4% of the left ventricle in the control, isoflurane, isoflurane–5-hydroxydecanoate, 5-hydroxydecanoate, isoflurane–gadolinium, and gadolinium groups, respectively). Infarct size averaged 60 ± 20% (SD) in untreated controls versus 54 ± 27 and 65 ± 15% of the risk zone in 5-hydroxydecanoate– and gadolinium-treated controls (P = nonsignificant). In contrast, infarct size in the isoflurane group was significantly reduced to 26 ± 11% of the risk zone ($P < 0.05$ *vs.* control). Both 5-hydroxydecanoate and gadolinium prevented this attenuation: infarct size averaged 68 ± 23 and 56 ± 21% of risk zone in the isoflurane–5-hydroxydecanoate and isoflurane–gadolinium groups, respectively (P = nonsignificant *vs.* control).**

Conclusion: **5-Hydroxydecanoate and gadolinium inhibited pharmacologic preconditioning by isoflurane. This result suggests that MK_{ATP} channels and mechanogated channels are probably involved in this protective mechanism. (Key words: Halogenated; ischemia; myocardial protection; rabbits.)**

MYOCARDIAL ischemic preconditioning is an endogenous protection that renders the heart more resistant to prolonged ischemia. It is usually elicited by one or several episodes of brief ischemia followed by brief reperfusion periods that delay the occurrence of infarction during a consecutive sustained ischemic insult. Several nonischemic stimuli can precondition the heart, including mechanical stretch by transient volume overload[1] or

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pharmacologic challenge by adenosine,[2] opioids,[3] and isoflurane.[4] Although the precise mechanism of this protective phenomenon is poorly understood, activation of adenosine triphosphate–sensitive potassium (K_{ATP}) channels and stretch-activated channels is important. It is unknown whether sarcolemmal K_{ATP} or mitochondrial membrane K_{ATP} (MK_{ATP}) channels are involved in isoflurane-induced protection. Although recent evidence suggests that isoflurane can activate mechanogated channels *in vitro*,[5] it remains elusive whether these stretch sensors are important for this isoflurane-induced antiischemic protection *in vivo*.

Therefore, the objective of this study was to determine whether MK_{ATP} and stretch-activated channels are involved in isoflurane-induced cardioprotection. Specifically, we investigated whether 5-hydroxydecanoate, a selective blocker of MK_{ATP},[6,7] and gadolinium, a potent blocker of stretch-activated channels,[8] can prevent isoflurane-induced preconditioning.

Material and Methods

All experiments performed in this study conformed to the Guiding Principles in the Care and Use of Animals approved by the American Physiologic Society. The Laboratoire de Physiologie Lyon Nord and the investigators received authorization from the French government to perform such animal studies.

A total of 68 New Zealand white rabbits of either sex (2.0–2.5 kg) were premedicated with xylazine (5 mg/kg intramuscularly) and anesthetized with ketamine HCl (50 mg/kg intramuscularly). Before surgical procedures were performed, adequate depth of anesthesia was ensured after determination of the absence of pedal and palpebral reflexes. After tracheostomy, animals were underwent mechanical ventilation (Servo ventilator 900B; Siemens-Elema, Solna, Sweden) with use of 100% oxygen. The tidal volume was set at 15 ml/kg and the respiratory rate at 35 strokes/min. Ventilation was adjusted to keep the end-tidal carbon dioxide in the physiologic range. End-tidal gas concentrations were measured continuously (gas analyzer; Capnomac Ultima, Datex, Helsinki, Finland). Body temperature, recorded by use of a thermistor inserted into the esophagus, was maintained between 39.0 and 40.5°C by means of a servocontrolled heating element incorporated into the operating table. Limb lead II of the electrocardiograph was continually monitored by means of subcutaneous needle electrodes. Anesthesia was maintained by infusion of a mixture of ketamine (3 $mg \cdot kg^{-1} \cdot h^{-1}$) and xylazine (1.5 $mg \cdot kg^{-1} \cdot h^{-1}$). Fentanyl 50 μg was injected systematically and intravenously in the animals before thoracotomy to provide adequate analgesia. Systemic blood pressure was monitored by use of a Gould pressure transducer connected to a 1-mm fluid-filled catheter inserted in the right carotid artery. The right internal jugular vein was catheterized with a 1-mm catheter to infuse fluids and drugs. Hetastarch (5 $ml \cdot kg^{-1} h^{-1}$) was continually infused *via* this intravenous cannula. The heart was exposed *via* a left thoracotomy and suspended in a pericardial cradle. The first large marginal branch of the circumflex artery was identified, and a 5/0 Dexon suture was passed around this artery approximately halfway between the apex and the base. The suture ends were threaded through a small vinyl tube to make a snare to perform further coronary occlusion and reperfusion. After the surgical procedure, a 20-min stabilization period was allowed.

In all groups, the coronary artery was occluded for 30 min. Myocardial ischemia was confirmed by the appearance of a regional cyanosis on the epicardium distal to the snare, akinesia or bulging in this area, and a progressive marked ST segment elevation in the electrocardiogram. After 30 min, the snare was released and reperfusion was allowed for a period of 3 h. Reperfusion was visually confirmed by the appearance of hyperaemia. The thread passed around the marginal artery was left in place.

At the end of the reperfusion period, the coronary artery was briefly reoccluded and diluted. Uniperse blue (Ciba-Geigy, Hawthorne, NY) was injected in the jugular vein to delineate the *in vivo* area at risk. With this technique, the previously non–area at risk appears blue, whereas the area at risk remains unstained. Anesthetized rabbits were then killed by an intravenous injection of KCl (4 mEq). The heart was excised and cut into five or six 2-mm transverse slices. After removing right ventricular tissue, each slice was weighted and identified. The basal surface of each slice was photographed for further measurement of area at risk. Each slice was then incubated for 15 min in tetrazolium chloride to differentiate infarcted (pale) from viable (red) myocardial area.[9] Each slice was then rephotographed. Enlarged projections of these slices were traced for determination of the boundaries of the different areas. Extent of left ventricle area, area at risk, and infarct size were quantified by computerized planimetry and corrected for the weight of tissue slices. Total weights of area at risk and area of necrosis were then calculated and expressed as weight (grams) or

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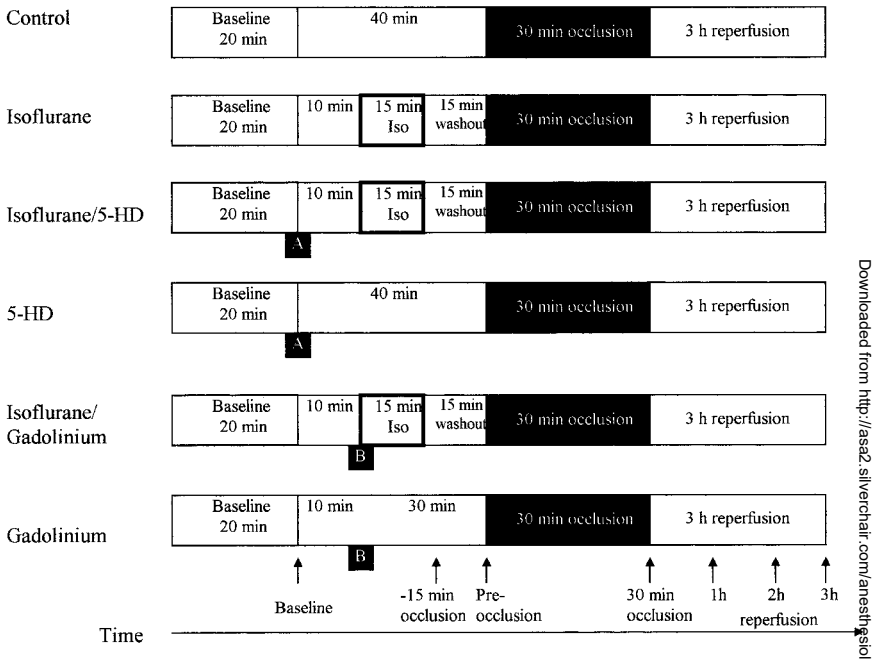

Fig. 1. Experimental protocol. Filled bars = ischemia; unfilled bars = perfusion. Isoflurane administration consisted of 1.0% end-tidal inhalational administration. (*A*) Intravenous injection of 5 mg/kg 5-hydroxydecanoate 40 min before coronary occlusion. (*B*) Intravenous injection of 40 μmol/kg gadolinium (Gd^{3+}) 30 min before ischemia.

as percentages of total left ventricle (LV) weight.[1] We decided prospectively that hearts with a risk region less than 10% of the LV weight would be excluded from the study. 5-Hydroxydecanoate (ICN Biomedicals, Aurora, OH) and gadolinium 3+ chloride hexahydrate (molecular weight = 371.7; Sigma Aldrich, Milwaukee, WI) were dissolved in NaCl 0.9% (wt/vol). Isoflurane was purchased from Abbott laboratories (Queensborough, United Kingdom).

Experimental Groups

The rabbits were randomly divided into six groups (fig. 1). All groups underwent a 30-min coronary artery occlusion and 3 h of reperfusion. Before this prolonged ischemia, they underwent a 40-min treatment period.

After 30 min of stabilization, the isoflurane group received 15 min of inhalational isoflurane (1% end tidal) followed by a 15-min washout period before the 30-min coronary artery occlusion.

To determine whether isoflurane-induced preconditioning is attenuated by the blocking MK_{ATP} channel, 5 mg/kg 5-hydroxydecanoate was intravenously injected 10 min before isoflurane administration in the isoflurane–5-hydroxydecanoate group. Similarly, 5 mg/kg 5-hydroxydecanoate was injected intravenously 40 min before coronary occlusion in the 5-hydroxydecanoate group to check the lack of 5-hydroxydecanoate effect on myocardial protection.

To determine whether stretch-activated ion channels are involved in myocardial preconditioning afforded by isoflurane, gadolinium, a blocker of stretch-activated ion channels, was injected. Animals in the isoflurane–gadolinium group received 40 μmol/kg gadolinium intravenously injected just before isoflurane administration. The gadolinium group received 40 μmol/kg gadolinium 30 min before ischemia to determine the lack of effect of gadolinium on infarct size.

For each animal receiving isoflurane inhalation, end-tidal isoflurane concentration was less than 0.1% at the end of the washout period.

Statistics

Statistical analyses of hemodynamics and end-tidal carbon dioxide were performed using two-way analysis of variance with repeated measures on one factor. LV weight and area at risk were analyzed by analysis of variance. Effect of pretreatment on percent of risk zone infarcted was analyzed by analysis of variance followed by *post hoc* Tukey's test. Differences of infarct zone among groups were evaluated by analysis of covariance and *post hoc* Tukey's test, with infarct zone as the dependent variable and area at risk as the covariant. Statistical calculations were performed using Statistica 5.0 computer software (Stasoft Inc., Tulsa, OK). All values are expressed as mean ± SD. A P value < 0.05 was significant.

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Table 1. Hemodynamic Measurements in Different Experimental Groups

	Control	Isoflurane	Isoflurane + 5-HD	5-HD	Isoflurane + Gadolinium	Gadolinium
Systolic arterial pressure (mmHg)						
Baseline	73 ± 6	64 ± 10	71 ± 9	78 ± 15	76 ± 9	62 ± 6
−15 min occlusion	68 ± 4	52 ± 8	59 ± 13	66 ± 11	51 ± 19	63 ± 3
Preocclusion	67 ± 8	63 ± 7	68 ± 13	63 ± 12	62 ± 15	66 ± 5
30 min occlusion	77 ± 7	65 ± 11	72 ± 7	59 ± 10	71 ± 12	72 ± 19
1 h reperfusion	74 ± 6	61 ± 6	63 ± 13	62 ± 7	68 ± 15	65 ± 20
2 h reperfusion	70 ± 3	59 ± 13	64 ± 8	54 ± 11	57 ± 19	68 ± 15
3 h reperfusion	68 ± 8	59 ± 11	57 ± 18	47 ± 17	52 ± 26	57 ± 10
Heart rate (beats/min)						
Baseline	191 ± 17	187 ± 17	208 ± 30	193 ± 35	216 ± 23	209 ± 18
−15 min occlusion	201 ± 10	189 ± 22	213 ± 19	198 ± 36	215 ± 10	215 ± 36
Preocclusion	195 ± 18	194 ± 11	209 ± 25	205 ± 35	205 ± 19	213 ± 25
30 min occlusion	210 ± 12	187 ± 28	214 ± 29	203 ± 25	212 ± 25	181 ± 32
1 h reperfusion	211 ± 214	194 ± 16	201 ± 25	192 ± 23	211 ± 21	191 ± 14
2 h reperfusion	207 ± 19	203 ± 20	202 ± 22	202 ± 25	197 ± 32	210 ± 23
3 h reperfusion	215 ± 21	207 ± 23	189 ± 23	199 ± 25	212 ± 45	190 ± 20

Values are expressed as mean ± SD.

5-HD = 5-hydroxydecanoate.

Results

Mortality and Exclusion

Among the 68 rabbits that have been included into this study, 16 were excluded for the following reasons: fatal ventricular fibrillation during coronary occlusion in one rabbit (isoflurane–5-hydroxydecanoate group), severe heart failure (obvious cardiac dilatation and systolic blood pressure < 40 mmHg) during coronary occlusion or reperfusion in nine rabbits (one control, one isoflurane, one isoflurane–5-hydroxydecanoate, two 5-hydroxydecanoate, three isoflurane–gadolinium, and one gadolinium). One rabbit was excluded because the risk region was smaller than 10% of the LV weight (gadolinium group). Five rabbits were excluded for technical problems during surgical preparation. Data are therefore presented for 52 rabbits: 9 in the control group, 9 in the isoflurane group, 10 in the isoflurane–5-hydroxydecanoate group, 9 in the 5-hydroxydecanoate group, 8 in the isoflurane–gadolinium group, and 7 in the gadolinium group.

Hemodynamic Data

Hemodynamic data, including heart rate and systolic blood pressure, are summarized in table 1. There were no significant differences among the six groups, although isoflurane tended to decrease systolic blood pressure during the treatment period (but this reduction did not reach statistical significance). End-tidal carbon dioxide and body temperature did not differ among groups.

Infarct Size

The area at risk and infarct size data are presented in table 2 and figure 2. LV weight and area at risk were comparable among the different groups, with mean value of area at risk ranging from 27 ± 6 to 35 ± 7% of

Table 2. Area at Risk, Infarct Size, and Risk Zone Infarcted

	Control	Isoflurane	Isoflurane + 5-HD	5-HD	Isoflurane + Gadolinium	Gadolinium
LV weight (g)	3.48 ± 0.64	3.47 ± 0.45	3.46 ± 0.39	3.62 ± 0.49	3.47 ± 0.56	3.72 ± 0.38
Area at risk (%LV)	29 ± 7	30 ± 5	27 ± 6	35 ± 7	31 ± 7	27 ± 4
Area at risk (g)	1.01 ± 0.35	1.05 ± 0.16	0.92 ± 0.21	1.28 ± 0.33	1.08 ± 0.25	1.00 ± 0.16
Infarct size (%LV)	18 ± 8	8 ± 3*	17 ± 6	20 ± 12	17 ± 8	17 ± 4
Infarct size (g)	0.62 ± 0.35	0.26 ± 0.11*	0.6 ± 0.18	0.74 ± 0.51	0.63 ± 0.32	0.65 ± 0.21
Risk zone infarcted (%)	60 ± 20	26 ± 11*	68 ± 23	54 ± 27	56 ± 21	65 ± 15

Values are expressed as mean ± SD.

* Significantly different from control group ($P < 0.05$).

5-HD = 5-hydroxydecanoate; LV = left ventricle.

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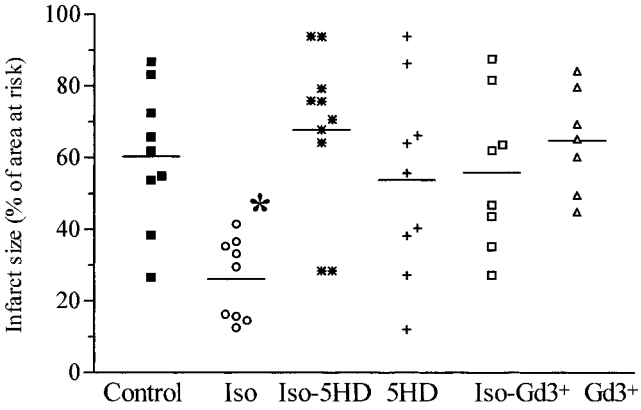

Fig. 2. Infarct size expressed as percentage of area at risk for each individual animals. Results are also expressed as mean values. Control = control group; Iso = isoflurane group; Iso-5-hydroxydecanoate = isoflurane–5-hydroxydecanoate group; 5-hydroxydecanoate = 5-hydroxydecanoate group; Iso-Gd^{3+} = isoflurane–gadolinium group; Gd^{3+} = gadolinium group. Lines indicate the mean values of the group. *Significantly different from control group ($P < 0.05$). Isoflurane preadministration significantly reduced infarct size expressed as percentage of risk area; 5-hydroxydecanoate and gadolinium preadministration significantly abolished this pharmacologic preconditioning. Gadolinium and 5-hydroxydecanoate alone had no effect compared with controls.

LV weight. Infarct size in the control group averaged 60 ± 20% of the area at risk. Neither 5-hydroxydecanoate nor gadolinium affected infarct size, which averaged 54 ± 27 and 65 ± 15% of the area at risk in the 5-hydroxydecanoate and gadolinium groups, respectively.

As expected, isoflurane-treated animals developed significantly smaller infarcts than controls, with mean infarct size averaging 26 ± 11% of the area at risk in this group ($P < 0.05$ *vs.* control). In addition, MK_{ATP} blockade by 5-hydroxydecanoate and stretch-activated channels blockade by gadolinium prevented this infarct size attenuation, because infarct size in these two groups was no longer different from that in the control group. Mean infarct size averaging 68 ± 23 and 56 ± 21% of the area at risk in the isoflurane–5-hydroxydecanoate and isoflurane–gadolinium groups, respectively (P = nonsignificant *vs.* control; $P < 0.05$ *vs.* isoflurane).

These data were confirmed when the weight of the infarct size was plotted *versus* the weight of the area at risk, its major determinant in the rabbit model of myocardial infarction. As depicted in figure 3, data points for the isoflurane group lie below the control line, indicating that for any size of the risk region, isoflurane-preconditioned hearts developed significantly smaller infarcts. Analysis of covariance confirmed this and showed that for any area at risk, the isoflurane group displayed smaller infarcts. In contrast, data points for isoflurane–5-hydroxydecanoate (fig. 3A), and data points for isoflurane–gadolinium (fig. 3B), lie close to the control line, indicating that 5-hydroxydecanoate or gadolinium preadministration prevented the protecting effect afforded by isoflurane. The regression line of the 5-hydroxydecanoate (fig. 3C) and gadolinium (fig. 3D) groups did not differ from the control group, indicating a lack of effect of these agents *per se*.

Discussion

In the current study, we demonstrated that pharmacologic preconditioning by isoflurane is prevented by 5-hydroxydecanoate and gadolinium. Recent studies have demonstrated that isoflurane can protect the ischemic heart. Kersten *et al.*[10] reported that isoflurane can attenuate postischemic contractile dysfunction (*i.e.*, myocardial stunning) in the canine heart. Cope *et al.*[11] showed in an *in vitro* rabbit model that a 5-min preadministration of volatile anesthetic agents (enfurane, halothane, and isoflurane) decreased infarct size. In another *in vivo* study, Kersten *et al.*[4] showed that the canine heart can be preconditioned by isoflurane preadministration, a protection that was blocked by the K_{ATP} antagonist glyburide. Cason *et al.*[12] showed that a 15-min isoflurane administration performed 15 min before a prolonged coronary occlusion significantly reduced infarct size in a rabbit model. Consistent findings have been reported by Ismaeil *et al.*[13] in propofol-anesthetized rabbits. These investigators showed that the beneficial effect of isoflurane-induced preconditioning on infarct size was abolished by glyburide, a K_{ATP} channel blocker, and by 8-(p-sulfophenyl)-theophylline, a nonspecific adenosine receptor antagonist. Our results are in close agreement with this: isoflurane was administrated 15 min before the sustained ischemic insult, and despite the fact that the end-tidal isoflurane concentration was almost null at the time of coronary occlusion, isoflurane-treated hearts developed significantly smaller infarcts. In other words, isoflurane can precondition the ischemic heart.

Role of Mitochondrial Adenosine Triphosphate–sensitive Potassium Channels

The underlying mechanism of this isoflurane-induced preconditioning remains unclear. Ischemic preconditioning has been initially described by Murry *et al.*[14] and Reimer *et al.*[15] on a canine model. These investigators showed that repetitive brief ischemic episodes before a

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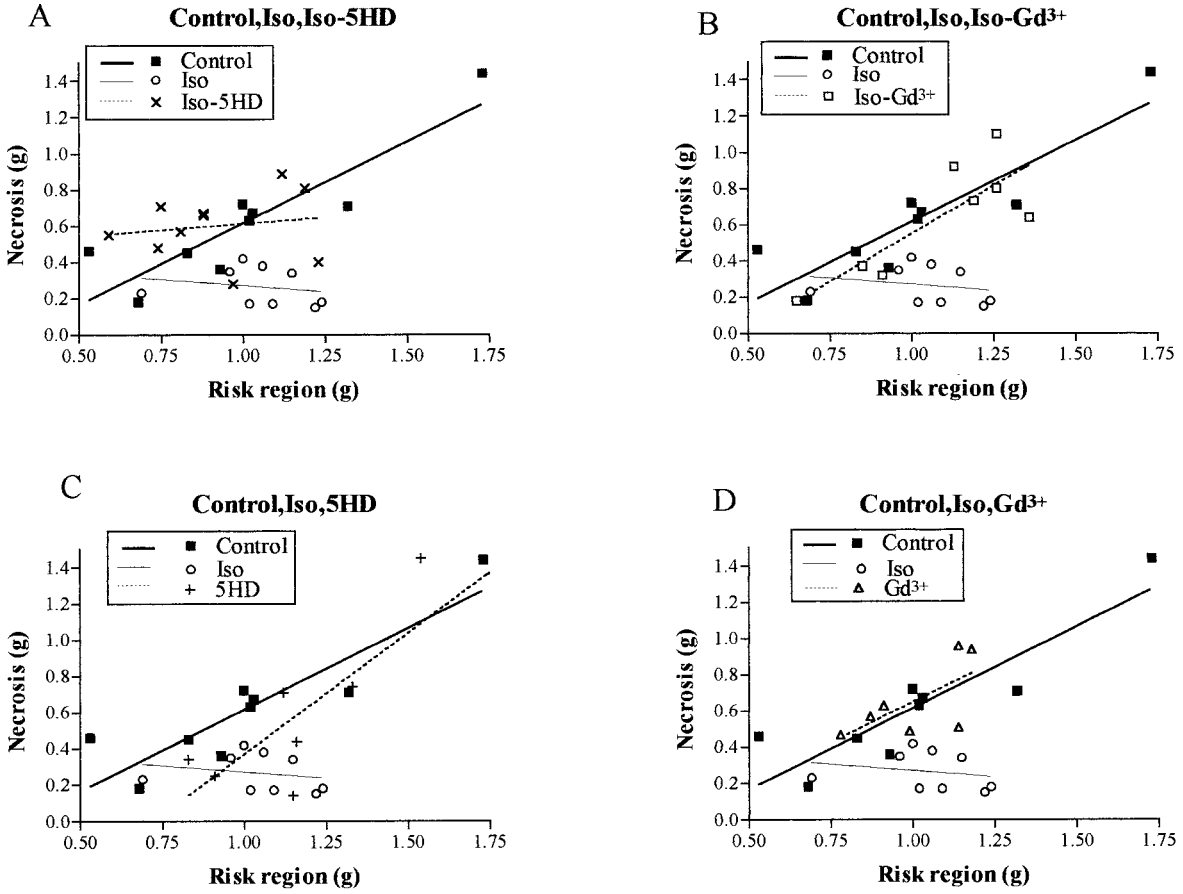

Fig. 3. Infarct size (necrosis in grams) plotted as a function of area at risk (grams). Each point represent one individual heart. Regression lines for each group are calculated by least square method. (*A* and *B*) Data points for isoflurane group are shifted downward *versus* control regression line, indicating that they develop a smaller infarct for any size of area at risk ($P < 0.05$). This effect is antagonized by 5-hydroxydecanoate (*A*) and gadolinium (*B*) preadministration. Data points for the 5-hydroxydecanoate (*C*) and gadolinium (*D*) groups lie close to those of the control group ($P > 0.05$), indicating that both agents had no effect on infarct size.

longer ischemia delayed myocardial infarction and ATP depletion. Ischemic preconditioning involves several sarcolemmal receptors, including adenosine A1, opioids, and bradykinin receptors, intracellular messengers or enzymes such as calcium G protein and protein kinase C, ion channels such as stretch-activated channels, and K_{ATP} channels.[16] So far, much interest has focused on the sarcolemmal K_{ATP} channel as a possible end-effector of preconditioning. Their activation would shorten action potential duration, limit calcium entry into the cell, and then reduce the detrimental energy-consuming process during ischemia–reperfusion. This is mainly supported by the fact that, in most experimental models, preconditioning is blocked by the sulfonylurea glibenclamide, a blocker of sarcolemmal K_{ATP} channels.[4,10,17,18]

However, several investigators reported that preconditioning can be observed in the absence of action potential duration shortening.[19,20] In addition, the K_{ATP} opener diazoxide can protect the ischemic heart without altering action potential duration.[21] In the whole cell patch-clamp preparation, diazoxide was 50-fold less potent than cromakalim, a K_{ATP} channel opener, to activate sarcolemmal K_{ATP} currents, yet both agents displayed a similar cardioprotective effect.[22] Both glibenclamide and 5-hydroxydecanoate antagonized the diazoxide effect. Taken together, these findings suggested that activation of the sole sarcolemmal K_{ATP} channel is not sufficient to explain the whole cardioprotective effect. In fact, MK_{ATP} channels are thought to be involved in the cardioprotection afforded by ischemic preconditioning.[23,24] MK_{ATP} channels have been discovered by Inoue *et al.*[25] on the inner face of rat liver mitochondrial membrane. Activa-

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tion of these channels depolarizes the inner membrane and leads to an influx of potassium into the mitochondria, accelerates the mitochondrial respiratory chain, slows the ATP production, releases accumulated calcium, and facilitates mitochondrial swelling.[26] Protein kinase C, which is known to be involved in the ischemic preconditioning pathway, can modulate MK_{ATP} activation.[7] The concomitant measure of sarcolemmal K_{ATP} current by patch clamp and flavoprotein fluorescence, an indicator of mitochondrial redox state that correlates with mitochondrial depolarization, confirmed this findings: Liu *et al.*[6] demonstrated that diazoxide can produce a reversible activation of flavoprotein without generating any surface K_{ATP} current. In contrast, pinacidil, another K_{ATP} channel opener, induced a mitochondrial oxidation associated with a sarcolemmal K_{ATP} current. 5-Hydroxydecanoate specifically blocked flavoprotein oxidation triggered by pinacidil without effect on surface current, indicating a specific effect on MK_{ATP} channels.[7] In contrast, glibenclamide acts as a nonspecific blocker, with inhibitory activity on both types of channels. 5-Hydroxydecanoate, which has shown to be a selective blocker of MK_{ATP} in *in vitro* experiments, blocks ischemic preconditioning in different species, such as the rabbit,[27] dog,[28] and rat.[29] All of these studies suggest that MK_{ATP} channels might be the end-effector of ischemic preconditioning. Other pharmacologic triggers of preconditioning, such as opioids, are sensitive to 5-hydroxydecanoate, and likely act through MK_{ATP} activation.[30] In the current study, 5-hydroxydecanoate clearly blocked isoflurane-induced infarct size reduction, suggesting that this protection is, in part, mediated by MK_{ATP}. Anesthetic-induced preconditioning seems to share common mechanisms with ischemic preconditioning, both depending on adenosine receptors[18] and protein kinase C,[11,12] and both being inhibited by 5-hydroxydecanoate, suggesting a specific role for MK_{ATP}.

Role of Stretch-activated Channels

It has been demonstrated that myocardial stretch, induced by acute volume overload, can precondition dog[1] and rabbit hearts.[31] These channels may also play a role in ischemic preconditioning (*i.e.*, in the absence of volume overload) because of their probable activation during possible distension of dysfunctional myocardium during preconditioning ischemia.[1] This protective effect is triggered by sarcolemmal stretch-activated ion channels, and is also mediated by K_{ATP} channels and protein kinase C.[31] Two pore-domain-potassium channels have been recently described[32] and individualized as TWIK-1, TREK-1, and TASK channels. Using a patch-clamp preparation, Patel *et al.* demonstrated that the two-pore-domain potassium channels TREK-1 and TASK can be activated both by mechanical stimulation (or stretch)[33] and by pharmacologic challenge by isoflurane and halothane.[5] These channels have been described in different tissues, including the heart.[34,35] In the current study, we found that the Lanthanide gadolinium, a potent blocker of mechanogated channels,[36] can attenuate isoflurane-induced preconditioning. This strongly suggests that gadolinium-sensitive channels, such as mechanogated channels, are involved in isoflurane-induced protection. However, the link between MK_{ATP} and stretch-activated channels remains unclear and was not specifically studied in the current work.

Limitation of the Study

Caution must be exercised before relating this experimental work to a clinical situation. All groups of animals received the same amount of ketamine and xylazine; however, we cannot exclude an interaction between intravenous anesthetics and isoflurane. The model we used is a classical one that has been validated in many studies. The rabbit model has two major advantages: (1) rabbits have a minimal collateral coronary flow degree,[37] diminishing confounding factors affecting the extension of infarction; and (2) the rabbit model is widely used in experiments on preconditioning, validating the pathophysiology knowledge of this phenomenon.[1,11,12,13,27] The end point of the current study is the percentage of infarct size expressed as percentage of risk area and not the ventricular functional improvement. It is justified because preconditioning delays the occurrence of infarction and likely has no effect on myocardial function in stunning models.[38] Most of the previous studies used glibenclamide to assess the role of K_{ATP} channels.[4,10,18,39] However, glibenclamide is not only specific of sarcolemmal K_{ATP} and MK_{ATP} channels, because it has been shown to inhibit other channels, such as Cl^- channels.[40] In the current study, we used 5 mg/kg 5-hydroxydecanoate and 40 μmol/kg gadolinium to block MK_{ATP} and stretch-activated channels, respectively. Theses doses are commonly used in similar myocardial protection models using 5-hydroxydecanoate[3,27,29] or gadolinium.[1,31] We cannot ascertain that the observed effects are fully related to a specific blockade of these channels. However, the doses we injected and the timing of injection are those usually used in similar *in vivo* rabbit models to antagonize these channels.[27,31] The action of 5-hydroxydecanoate on MK_{ATP} has been shown *in vitro*

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for a 100-μmol/l concentration; in our study we did not measure 5-hydroxydecanoate concentration. Gadolinium blocks *in vitro* mechanogated channels in a concentration ranging from 1 to 100 μmol/l. However, it can block other channels (for review see reference 36), such as calcium L- and T-type voltagegated channels[41] and sodium channels, and inhibit the delayed rectifier K^+ current IKs.[42] Therefore, caution must be exercised before concluding there is a direct effect of gadolinium on stretch-activated channels.

In addition, we cannot rule out the possibility that gadolinium also blocked MK_{ATP} channels. Sarcolemmal K_{ATP} can be activated by membrane deformation.[43] Although there is no report that MK_{ATP} channels are mechanosensitive, or even blocked by gadolinium, there is some evidence that they are involved in the regulation of mitochondrial volume.[26]

Although there was no statistical difference in hemodynamic parameters, we cannot strictly rule out the effect of isoflurane-induced hypotension on the decrease of infarct size in the groups receiving isoflurane. Similar experiments[44] using a different halogenated agent, such as sevoflurane, showed that preadministration of this agent significantly decreased blood pressure (mean blood pressure was 97 ± 3 mmHg in controls *vs.* 65 ± 4 mmHg in the sevoflurane-preconditioned group) without altering infarct size (23 ± 1% of the area at risk in controls *vs.* 21 ± 4% in the sevoflurane-preconditioned group). Kersten *et al.*[4] suggested that isoflurane preconditioned the heart by a direct effect on K_{ATP} channels. Using a similar *in vivo* rabbit model, Cope *et al.*[11] examined the effect of hypotension on halogenated cardioprotection. They showed that there was no correlation between infarction area and decrease in blood pressure. In an *in vitro* Langendorff model, the investigators showed that halogenated agents exerted cardioprotection without alteration in perfusion pressure.

Conclusion

In conclusion, the current study demonstrated that preadministration of isoflurane preconditions the rabbit heart. 5-Hydroxydecanoate, a specific blocker of MK_{ATP} channels, and gadolinium, a potent inhibitor of mechanogated channels, abolished this pharmacologic anesthetic preconditioning. These data indicate that MK_{ATP} channels are probably involved in isoflurane ischemic-like preconditioning, and that mechanogated channels play possibly a role in this phenomenon. Further studies are needed to determine the link between MK_{ATP} and stretch-activated channels in this protective phenomenon.

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