The Effect of Trifluoroacetic Acid, a Metabolite of Isoflurane on the ATP-sensitive Potassium Channel in Rabbit Ventricular Myocytes

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= Abstract =

Background: Activation of ATP-sensitive K^+ channels (K_{ATP} channels) in the cardiac muscle produces cardioprotective effects during myocardial ischemia. Previous experimental evidence indicates that volatile anesthetics exert beneficial actions in ischemic myocardium and enhance functional recovery of stunned myocardium. More recently, volatile anesthetics have been demonstrated to produce cardioprotective effects in stunned myocardium *in vivo*, and these effects are blocked by a K_{ATP} channel antagonist. This finding suggests that K_{ATP} channel activation by isoflurane may mediate antiischemic effects. However, it was demonstrated that isoflurane inhibited K_{ATP} channel activity in rabbit ventricular myocytes. To explain the discrepancy, the present investigation tested the hypothesis that isoflurane and its metabolite, trifluoroacetic acid, contributes to the activation of K_{ATP} channels in rabbit ventricular myocytes.

Methods: Single ventricular myocytes were isolated from rabbit hearts by an enzymatic dissociation procedure. Single-channel currents were measured in inside-out patch configurations of the patch-clamp technique. The perfusing liquid was equilibrated with isoflurane by passing 100% O_2 through a vaporizer.

Results: Isoflurane inhibited K_{ATP} channel activity without a change in the single-channel conductivity. Isoflurane decreased the burst duration and increased the interburst duration. In addition, isoflurane diminished the ATP sensitivity of K_{ATP} channels. Trifluoroacetic acid, a metabolite of isoflurane, enhanced the channel activity in a dose-dependent fashion. Trifluoroacetic acid increased the burst duration and decreased the interburst duration without a change in the single-channel conductivity. Isoflurane and trifluoroacetic acid diminished the ATP sensitivity of K_{ATP} channels.

Conclusions: These results imply that isoflurane and its metabolite could mediate cardioprotective effects via K_{ATP} channel activation during myocardial ischemia. (**Korean J Anesthesiol 2002; 43: S 1 ~ S 12**)

Key Words: ATP-sensitive potassium (K_{ATP}) channel; myocardial ischemia; rabbit ventricular myocytes; isoflurane; patch-clamp techniques; trifluoroacetic acid.

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INTRODUCTION

A brief period of ischemic preconditioning has been shown to protect the heart against subsequent prolonged ischemia in all species examined to date including human.¹⁾ In general, ischemic preconditioning appears to provide electrical stability and tissue resistance to ischemia via some underlying mechanisms whereby metabolic conservation occurs.²⁾ Studies of the mechanism of ischemic preconditioning suggest that protein kinase C (PKC) activity may be enhanced by ischemic preconditioning and, in turn, may activate the adenosine triphosphate-sensitive potassium (K_{ATP}) channels. K_{ATP} channels are thought to play a role in the phenomenon of ischemic preconditioning in the heart and the activation of these channels may improve recovery of regional contractile function of stunned myocardium by shortening action potential duration and attenuation of membrane depolarization, thus decreasing contractility and preserving energy during ischemia. These effects in turn reduce the duration of calcium influx through L-type calcium channels and increase the time when the sodium-calcium exchanger can extrude calcium from the cell, both of which will reduce intracellular calcium overload and myocardial tissue injury.³⁻⁵⁾

Previous experimental evidence indicates that volatile anesthetics exert beneficial actions in ischemic myocardium^{6,7)} and enhance functional recovery of stunned myocardium.^{6,8)} More recently, isoflurane, a volatile anesthetic, has been demonstrated to produce cardioprotective effects in stunned myocardium *in vivo*, and these effects are blocked by the glyburide, a K_{ATP} channel antagonist.⁹⁾ This finding suggests that K_{ATP} channel activation by isoflurane may mediate antiischemic effects.

However it was demonstrated that isoflurane inhibited K_{ATP} channel activity in rabbit ventricular myocytes.¹⁰⁾ To explain the discrepancy, the present investigation tested the hypothesis that the major metabolite of isoflurane, trifluoroacetic acid, contributes to activa-

tion of K_{ATP} channels using excised membrane patches from single ventricular myocytes of the rabbit.

METHODS

Cell isolation

Single ventricular myocytes were isolated from rabbit hearts by enzymatic dissociation procedure, as discussed previously.¹¹⁾ Briefly, rabbits weighing 800-1200 g were anesthetized by injection of sodium pentobarbital (50 mg/ml, 1 ml/kg body weight) and heparin (100 IU/ml) into the marginal ear vein. Hearts were rapidly removed via thoracotomy with artificial ventilation and the aorta was cannulated. A dissected heart was mounted on a Langendorff apparatus and perfused retrogradely with oxygenated normal Tyrode solution for 5-6 min until all signs of blood were removed with gentle squeezing of the heart. The hearts were then perfused with a normally Ca^{2+} -free Tyrode solution for 5 min, followed by perfusion with Ca^{2+} -free Tyrode solution containing 0.01% collagenase (5 mg/50 cc, Yakult, Japan). After 15-25 min of enzymatic treatment, Kraft's Bruhe (KB) solution was perfused. After 5 min of perfusion of KB solution, the hearts were removed from the cannula, the atria were discarded, and the ventricular walls and septum were cut vertically into four to six pieces. They were gently agitated in a small beaker with KB solution to obtain single cells. Isolated ventricular cells were stored in a KB solution at 4°C and used within 12 hours. Langendorff column was kept at 37°C during all previous steps.

Electrophysiological methods

Single-channel currents were measured in inside-out patch configurations of the patch-clamp technique.¹²⁾ Channel activity was measured using a patch-clamp amplifier (EPC-7, LIST, Darmstadt, Germany; Axopatch-1D, Axon Instruments, Foster City, CA). Pipettes of 5-10 M Ω resistance were pulled from borosilicate glass capillaries (Clark Electrochemical, Pangbourne, England) using a vertical puller (Narishige PP-83, Tokyo, Japan).

Their tips were coated with Sylgard and fire polished. Membrane currents were digitized at a sampling rate of 400 Hz and stored in digitized format on digital audiotapes using a Biologic DTR-1200 recorder (Grenoble, France). For the analysis of single channel activity, the data were transferred to a computer (IBM-PC, Pentium-III 450, Busan, Korea) with pCLAMP (version 6.3 software, Axon Instruments Inc., Burlingame, CA, USA) through an analogue-to-digital converter interface (Digidata-1200, Axon Instruments Inc., Burlingame, CA, USA).

Data analysis and quantification of channel activity

The threshold for judging the open state was set at half of the single-channel amplitude.¹³⁾ The open time histogram was formed from continuous recordings of more than 60 sec. The open probability (P_o) was calculated using the formula:

$${}_nP_o = \left(\sum_{j=1}^n t_j \times j \right) / (T_d \times N)$$

Where t_j is the time spent at current levels corresponding to $j = 0, 1, 2, \dots, N$ channels in the open state, T_d is the duration of the recording and N is the number of channels active in the patch. The number of channels in a patch was estimated by dividing the maximum current observed, during an extended period at zero ATP, by the mean unitary current amplitude. P_o was calculated over 30-sec records.

Rundown of K_{ATP} channels

The activity of K_{ATP} channels in rabbit ventricular myocytes decreases slowly with time after patches are excised into ATP-free solution. This phenomenon is known as "rundown". Upon excision, patches were continuously exposed to ATP ($100 \mu\text{M/L}$), except for a brief exposure to zero ATP at the beginning and end of experiments to estimate the number of channels in a patch and the degree of rundown. Data from patches exhibiting $> 50\%$ rundown were discarded. In experiments designed to test the effects of PKG activation on K_{ATP} channel activity, patches were con-

tinuously exposed to $100 \mu\text{M/L}$ ATP unless otherwise stated. This concentration of ATP was chosen to represent a half-maximal inhibition level of ATP, while giving a P_o to allow single-channel events to be observed.

Solution and drugs

Normal Tyrode solution contained (in mM/L): 143 NaCl, 5.4 KCl, 1.8 CaCl_2 , 0.5 MgCl_2 , 5.5 glucose, 5 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES); pH 7.4 with NaOH. The solutions facing the outside of the cell membrane in the excised patch recordings contained (in mM/L): 140 KCl, 2 CaCl_2 , 1 MgCl_2 , 10 glucose, 10 HEPES; pH 7.4 with KOH. The solutions facing the inside of the cell membrane in the excised patch recordings contained (in mM/L): 127 KCl, 13 KOH, 1 MgCl_2 , 5 ethylene glycol-bis-(aminoethyl ether)-N, N, N', N'-tetraacetic acid (EGTA), 10 glucose, 10 HEPES; pH 7.4 with KOH. The modified KB solution had the following composition (in mM/L): 25 KCl, 10 KH_2PO_4 , 16 KOH, 80 glutamic acid, 10 taurine 14 oxalic acid, 10 HEPES, and 11 glucose at pH 7.4 adjusted with KOH.

ATP and glibenclamide were added to either the extracellular or intracellular solutions according to the experimental protocols described in the text. Glibenclamide was dissolved as 0.2 mM/L stock solution in 2% dimethyl sulphoxide (DMSO) and diluted into the test solution appropriately before study. The final concentration of DMSO contained in the test solution was less than 0.01%. We confirmed that DMSO, at this concentration, had no effect on K_{ATP} channel activity. After addition of drugs to the test solution, the pH was re-adjusted to 7.4 with KOH. All agents used in the present study were obtained from Sigma (St. Louis, MO, USA). Experiments were done at a room temperature of $25 \pm 2^\circ\text{C}$.

Solution exchange system

In most experiments, the DAD-12 superfusion system (Adams & List Associates, New York, USA) was used for the change of bath solution and drugs. The system

was designed to simplify the application of various concentrations of drugs and solutions to cells. The system takes advantage of what many refer to as the "sewer pipe effect." When pointed at the cell to be studied, if the cell remains within the stream of the solution, the cell was essentially immersed completely in the solution that was applied. We could stop and start the flow quickly, and change it with up to 12 variables by using the system.

Statistics

Data are presented as mean $\pm$ SEM when appropriate. Student's unpaired *t* test was used to calculate statistical significance. A probability of 0.05 or less was considered significant.

RESULTS

K_{ATP} channels were identified by their conductance over the voltage range of -80 to -20 mV (77.8 ± 3.5 pS; *n* = 6 patches), unitary current (2.8 ± 0.2 pA; *n* = 6 patches; -40 mV), and the responses to potassium channel openers, ATP, and glibenclamide.

Effect of isoflurane on K_{ATP} channel activity of rabbit ventricular myocytes

In order to test the hypothesis that activation of K_{ATP} channels are involved in the actions of isoflurane,

the effects of isoflurane on K_{ATP} channels in inside-out patches were investigated (Fig. 1). After giga-seal formation, the pipette potential was set to -70 mV and the bath solution was switched from the normal Tyrode solution to the high K⁺ solution. After excision of the patch, ATP ($500 \mu\text{M/L}$ or $2 \mu\text{M/L}$) inhibited K_{ATP} channel activity rapidly and reversibly (Fig. 1A). In the concentration range (from 1 vol% to 5 vol%), the application of isoflurane to the bath induced a concentration-dependent inhibition of K_{ATP} channel activity (*n* = 23 patches). To obtain the concentration of isoflurane ([isoflurane]_i)-K_{ATP} channel activity relationship, we determined the effect of one concentration of isoflurane from each inside-out patch (Fig. 1B). A plot of relative channel activity as a function of [isoflurane]_i, using data obtained from a total of 5 patches, resulted in data points well fitted by straight line (the decrease in relative channel activity, 0.19 ± 0.01 per the increase in [isoflurane]_i, 1 vol%). Similar data were obtained with outside-out membrane patches where isoflurane was applied to the extracellular aspect of the membrane (*n* = 2 patches).

Effect of isoflurane on the conductance and kinetic properties of K_{ATP} channel activity

The current-voltage (I-V) relationship obtained from a total of 8 patches before and after the application of isoflurane is shown in Fig. 2A. The I-V curves before

Fig. 1. Effect of isoflurane on ATP-sensitive potassium channel. Concentration-response relationships. Single-channel recordings from inside-out patches. C, closed level. Patch membrane potential was held at -70 mV.

Fig. 2. Effect of isoflurane on current-voltage relationship and kinetic properties of ATP-sensitive potassium channel. The histograms of the open and closed time within bursts were analyzed. Isoflurane decreased the burst duration and increased the inter-burst duration.

and after the application of isoflurane (3 and 5 vol%) at negative membrane potentials displayed a linear relationship with a single channel conductance of 77.3 1.7 (before isoflurane, $\circ$), 76.8 2.2 (3 vol%, $\triangle$) and 76.1 2.5 (5 vol%, $\square$) pS, respectively. The open and closed time distributions were analyzed to determine the effect of isoflurane on the kinetic properties of the channel. Probability density histograms of the open and closed times measured at -50 mV are shown in Fig. 2B. Isoflurane had no effect on the fast open and closed kinetics within burst (Fig. 2B, upper panels). On the other hand, isoflurane decreased the burst duration and increased the interburst duration. The time constant of the burst duration (τ_b) was markedly decreased from 41.1 to 15.6 ms by isoflurane (Fig. 2B, left lower panel). τ_{c2} represents the time constant of the interburst duration (Fig. 2C, right lower panel). The value of τ_{c2} was increased from 72 to 213 ms by isoflurane.

Effect of isoflurane on the sensitivity of the K_{ATP} channels to ATP

Interestingly, we found that two successive appli-

cation of the same concentration of ATP (2 mM) after exposure to isoflurane produced much less block (Fig. 1A, middle and lower panels). This suggests that the ATP sensitivity of the channel may have been changed by isoflurane. To determine the effects of isoflurane on ATP sensitivity of K_{ATP} channels, different concentrations of ATP (2 μ M to 2 mM) were subsequently applied (Fig. 3). After the application of isoflurane (3 vol%), ATP produced much less block than before (Fig. 3A). In Fig. 3B, the graph shows the dose-response relationship for inhibition of K_{ATP} channel activity by ATP before ($\circ$) and after ($\bullet$) the application isoflurane. The continuous lines in the graph were the fitted curves to the Hill equation using the least-squares method as described previously.

$$\text{Relative channel activity} = 1 / \{1 + ([ATP]_i / K_i)^n\}$$

Where $[ATP]_i$ is ATP concentration in internal solution, K_i is the ATP concentration at half-maximal inhibition, and n is the Hill coefficient. Isoflurane changed the value of K_i from 67.6 ± 19.8 to $163.2 \pm 28.5 \mu$ M ($n = 7$ patches).

Fig. 3. Effect of isoflurane on the sensitivity of ATP-sensitive potassium channel to ATP. Concentration-response relationships between the relative channel activity and concentration of ATP before (open circle) and after application of trifluoroacetic acid (filled circle).

Effect of trifluoroacetic acid on K_{ATP} channel activity

In Fig. 4A, the concentration-dependent effects of trifluoroacetic acid on the K_{ATP} channel openings were examined. As the concentration of trifluoroacetic acid was increased in a concentration-dependent fashion. As the concentration of trifluoroacetic acid was increased from 0 to 0.001, 0.01, 0.1 and 1 mM, the channel openings increased in a concentration-dependent fashion. The curve was fitted by the Hill equation using least-squares method:

$$\text{Relative channel activity} = 100 / [1 + (K_d / [\text{trifluoroacetic acid}])^n]$$

Where K_d = the trifluoroacetic acid concentration at the half-maximal activation of the channel, and n = Hill coefficient. In the graph of Fig. 4B., K_d and n were

Fig. 4. Effect of trifluoroacetic acid on ATP-sensitive potassium channel. Concentration-response relationships. Single-channel recordings from inside-out patches. C, closed level. Patch membrane potential was held at -40 mV.

0.03 mM and 1.2, respectively.

Effect of trifluoroacetic acid on the conductance and kinetic properties of K_{ATP} channel activity

The I-V relationships obtained from patches under control and in the presence of trifluoroacetic acid are shown in Fig. 5A. Trifluoroacetic acid had no effect on I-V relationship and slope conductance value (from 76.9 ± 4.5 to 76.5 ± 3.9 pS, $n = 7$ patches).

To examine the effect of trifluoroacetic acid on the gating kinetics of the channels, the open-time and the closed-time histograms were calculated at a membrane potential of -50 mV negative to the reversal potential. The open time histogram (Fig. 5B, left upper panel), which was analyzed from the current record filtered at cut-off frequency of 1 kHz, revealed a single exponential distribution with a time constant of 2.7 ms under control condition. In the presence of trifluoroacetic acid, the open time constant (τ_o) did not differ from that in the absence of trifluoroacetic acid ($\tau_o =$

Fig. 5. Effect of trifluoroacetic acid on current-voltage relationship and kinetic properties of ATP-sensitive potassium channel. The histograms of the open and closed time within bursts were analyzed. Trifluoroacetic acid increased the burst duration and decreased the inter-burst duration.

2.8 ms). The lifetime of a burst was defined as an opening period observed in the records filtered at cut-off frequency of 0.1 kHz. The histogram of burst duration consisted of a single exponential distribution (Fig. 5B, left lower panel). Its time constant, designed as τ_b , was markedly prolonged by trifluoroacetic acid (from 41 to 60 ms). The histogram of closed time within bursts was best fitted to a single exponential function (Fig. 5B, right upper panel).

This analysis was performed with closed times longer than 4 ms to be discarded, and filtered at cut-off frequency of 1 kHz. The time constant of the

Fig. 6. Effect of trifluoroacetic acid on burst duration of ATP-sensitive potassium channel.

closed time within bursts was designed as τ_c . The value of τ_c was not changed markedly by trifluoroacetic acid (from 0.31 to 0.30 ms). The closed time between bursts was analyzed by using records filtered at cut-off frequency of 0.1 kHz (Fig. 5B, right lower panel). The histogram was fitted using a biexponential function, with time constants of a fast (τ_{c1}) and a slow component (τ_{c2}). The value of τ_{c1} was influenced by trifluoroacetic acid (from 7 to 5 ms). The value of τ_{c2} was 70 ms under control condition. This value was markedly decreased to 23 ms by trifluoroacetic acid. These findings suggest that trifluoroacetic acid increases the channel activity by an increase in burst durations and a decrease in interburst intervals. This was shown graphically in Fig. 6.

Effect of trifluoroacetic acid on the sensitivity of the K_{ATP} channels to ATP

We next investigated whether the sensitivity of the K_{ATP} channels to $[ATP]_i$ was regulated by trifluoroacetic acid. The protocols were the same as above. As shown in Fig. 7A, the K_{ATP} channel activity was shown in inside-out patches. Subsequently, addition of ATP to the bath solution significantly and reversibly inhibited the channel activities. However, the sensitivity of the K_{ATP} channels to $[ATP]_i$ in the presence of trifluoroacetic acid was lower than that in the absence of trifluoroacetic acid (control).

Fig. 7. Effects of trifluoroacetic acid on ATP-sensitive potassium channel. Concentration-response relationships between the relative channel activity and concentration of ATP before (open circle) and after application of trifluoroacetic acid (filled circle).

The continuous line in Fig. 7B. is a curve fitted to the Hill equation using the least squares method. Trifluoroacetic acid changed the value of K_i from 71.5 ± 7.6 to $233.2 \pm 28.8 \mu\text{M}$ ($n = 7$ patches).

In the present study, we demonstrated for the first time that activities of K_{ATP} channel in rabbit ventricular myocytes are enhanced by the major metabolite of isoflurane, trifluoroacetic acid. The results can be summarized as follows: (1) Isoflurane inhibited K_{ATP} channel activity without a change in the single-channel conductance. (2) Isoflurane decreased the channel activity by a decrease in burst duration and an increase in the interburst duration. (3) Isoflurane diminished the ATP sensitivity of K_{ATP} channels. (4) Trifluoroacetic acid, a metabolite of isoflurane, enhanced the channel activity

in a dose-dependent fashion. (5) Trifluoroacetic acid increased the burst duration and decreased the interburst duration without a change in the single-channel conductance. (6) Trifluoroacetic acid diminished the ATP sensitivity of K_{ATP} channels. These results imply that isoflurane and its metabolite could mediate cardioprotective effects via K_{ATP} channel activation.

DISCUSSION

Recently, experimental evidence has indicated that volatile anesthetics exert myocardial protective effects. Isoflurane and halothane enhanced the functional, metabolic, and ultrastructural recovery of myocardium after myocardial ischemia and reperfusion.^{6,7,14,15} Myocardial infarct size was reduced in dogs anesthetized with isoflurane and halothane after left anterior descending artery (LAD) ligation.^{16,17}

Halothane decreased S-T segment elevation produced by brief coronary artery occlusion.¹⁸ The mechanism responsible for these volatile anesthetic-induced cardioprotective effects has not been completely elucidated. A decrease in myocardial oxygen consumption produced by volatile anesthetics has been proposed as a potential mechanism of protection by these agents in ischemic myocardium. However, this is controversial. Halothane, not isoflurane, decreased myocardial oxygen consumption as estimated by the rate-pressure product, although both anesthetics were equally protective against myocardial stunning in a previous investigation.⁸ It was suggested that halothane may produce beneficial effects during complete functional cardiac arrest.⁷ Isoflurane has been shown to exert antiischemic actions during ischemia and reperfusion despite maintenance of heart rate and arterial pressure at control values.¹⁵ The findings suggest that favorable changes in myocardial oxygen supply-and-demand relations are not solely responsible for the beneficial actions of isoflurane and halothane, and they support the hypothesis that volatile anesthetics may also cause myocardial protection via other mechanisms, such as a reduction in excessive intracellular calcium accumulation. To date, the mecha-

nism of action of volatile anesthetics to reduce ischemia and reperfusion injury remains speculative. The present investigation tested the hypothesis that the protective effects of isoflurane are mediated by K_{ATP} channel activation.

Activation of K_{ATP} channels was shown to enhance the functional recovery of stunned myocardium, reduce myocardial infarct size, and modulate ischemic preconditioning.^{4,19,20} K_{ATP} channel activation may exert these cardioprotective effects via a reduction in abnormal intracellular calcium concentration associated with myocardial injury. Gross et al²⁰ reported that glibenclamide, a K_{ATP} channel blocker, abolished the effects of preconditioning. Our results demonstrate that isoflurane inhibits K_{ATP} channel activity in rabbit ventricular myocytes. These results suggest that inhibition of K_{ATP} channels by isoflurane may aggravate ischemia-reperfusion injury and attenuate ischemic preconditioning. In a different set of experiments, we also observed the both volatile anesthetic, halothane and intravenous anesthetic, pentobarbiton inhibited the channel activity in a manner similar to isoflurane (not shown). As the concentration of the anesthetics is important for determining the degree of K_{ATP} channel inhibition, it is possible that they act by a non-specific membrane interaction or by an action on specific hydrophobic protein region.

Extracellular potassium concentration increases during myocardial ischemia because of increased potassium efflux. Weiss et al²¹ reported that potassium efflux during metabolic inhibition was most likely to arise from the increase membrane potassium conductance possibly due to the activation of K_{ATP} channels. Increased extracellular potassium promotes depolarization of tissue in the vicinity of the ischemic region. The flow of this injury current has been implicated as a potential cause for the initiation of premature ventricular beats.²² Although K_{ATP} channel activation is thought to be protective in the ischemic myocardium, activation of these channels may prove to be arrhythmogenic. It has been shown that K_{ATP} channel antagonists can prevent ventricular arrhythmias in *in vitro*

and *in vivo*, whereas potassium channel openers reduce the time for the onset of fibrillation.^{23,24} Like the action of other K_{ATP} channel antagonists, isoflurane may protect against arrhythmia formation during myocardial ischemia. Alternatively, isoflurane has been demonstrated to exert antiischemic effects via activation of K_{ATP} channels both in the *in vivo* and *in vitro* experiments^{9,25} and more recently in a clinical study.²⁶ Contrary to these results, we did not find the activation of K_{ATP} channels elicited by isoflurane in rabbit ventricular myocytes. One possible explanation for the discrepant data for isoflurane and its effect on K_{ATP} channel activity may be a possible competition between ATP and isoflurane for the ATP-binding site. Our result supports this possibility as isoflurane diminishes ATP sensitivity, indicating an increased likelihood of K_{ATP} channel opening for a given concentration of ATP after isoflurane anesthesia.

The result supports, at least in part, the hypothesis that isoflurane mediates cardioprotective effects via K_{ATP} channel activation. However, our results cannot discriminate whether isoflurane act directly on the K_{ATP} channel protein or indirectly via an alteration of the physical characteristics of the membrane lipid. To discriminate between these possibilities, the molecular mechanism of the anesthetic-induced K_{ATP} channel inhibition remains to be further elucidated.

It is also possible that the metabolites of isoflurane activate the K_{ATP} channels although isoflurane itself inhibits K_{ATP} channel activity in cardiac myocytes. Principal metabolites of isoflurane are trifluoroacetic acid, fluoride ion and small quantities of other unidentified organic fluoride compounds, suggesting that cleavage occurs predominantly at the either linkage with the subsequent release of fluoride ions from the one-carbon fragment. However, there is a difference in the metabolism of isoflurane in rats and in human: the predominant metabolite in rats is ionic fluoride, whilst non-ionic fluoride (trifluoroacetic acid) predominates in human.²⁷ All of the identified metabolites of isoflurane are non-toxic and, to date, administration of isoflurane has not been linked with anesthetic toxicity.²⁸ To our

knowledge, these are first data obtained which relate to the effects of trifluoroacetic acid, the predominant metabolite of isoflurane, on the K_{ATP} channels by using single channel patch-clamp techniques. We found that trifluoroacetic acid activated K_{ATP} channels in rabbit ventricular myocytes. These results suggest that activation of the K_{ATP} channel by trifluoroacetic acid may protect myocardium against ischemia-reperfusion injury and serve to ischemic preconditioning.

Considering the results whereby myocardial protection was afforded by a volatile anesthetic, isoflurane during ischemia and reperfusion injury, it seems reasonable to hypothesize that isoflurane itself and its metabolite, trifluoroacetic acid, acts on ATP sensitivity of K_{ATP} channels to ATP, which serves to protect myocardium from a subsequent ischemic insult through activation of K_{ATP} channels. Additionally, it is possible that isoflurane specifically activates the K_{ATP} channels in coronary vascular smooth muscle cells^{29,30)} although isoflurane inhibits K_{ATP} channel activity in cardiac myocytes. Therefore, whether volatile anesthetics specifically activate the K_{ATP} channels in coronary vascular smooth muscle cells as demonstrated in *in vivo* and *in vitro* experiments remains to be assessed by using single channel activity.

K_{ATP} channel activation has been shown to be involved in cardioprotection by a variety of stimuli, including brief ischemia in the heart or remote organs and non-ischemic stimuli in the heart such as ventricular pacing, stretch and heat stress. Moreover, pharmacological agents that open K_{ATP} channels also produce cardioprotection.³¹⁾ Although the exact mechanism by which K_{ATP} channel activation protects is still incompletely understood, recent evidence suggests that mitochondrial K_{ATP} channels mediate cardioprotection in ischemic preconditioning.^{32,33)} Our results do not provide any experimental evidence to test this interesting possibility. However, our finding that trifluoroacetic acid can potentiate the K_{ATP} channel raises the intriguing possibility that trifluoroacetic acid could also modulate mitochondrial K_{ATP} channels in the heart and provides a possible mechanism of isoflurane-mediated

cardioprotection. Indeed, it has been known that mitochondrial K_{ATP} channel is regulated by every ligand that regulates sarcolemmal K_{ATP} channels.^{33,34)} However, it was interesting that the selective mitochondrial K_{ATP} channel blocker, 5-hydroxydecanoate, did not reduce protection from preconditioning in isolated rabbit hearts, which suggest that activation of mitochondrial K_{ATP} channels may not mediate cardioprotection.³⁵⁾ Therefore, it remains to be elucidated whether the cardioprotection of ischemic preconditioning is due to mitochondrial K_{ATP} channels or sarcolemmal K_{ATP} channels or to a mixture of both.

In conclusion, our data show that isoflurane and its major metabolite, trifluoroacetic acid modulate K_{ATP} channels by reducing the apparent affinity of the channel for ATP, suggesting that this mechanism may contribute, at least in part, to the isoflurane-induced cardioprotection during myocardial ischemia and reperfusion injury.

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