Korean J Physiol Pharmacol
Vol 5: 165--175, April, 2001

Activation of ATP-sensitive Potassium Channels by the Predominant Metabolite of Isoflurane in Rabbit Ventricular Myocytes

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Background: Recent *in vivo* experimental evidence suggests that isoflurane-induced cardioprotection may involve K_{ATP} channel activation. However, it was demonstrated that isoflurane inhibited K_{ATP} channel activities in the inside-out patch mode. To explain this discrepancy, the present investigation tested the hypothesis that a metabolite of isoflurane, trifluoroacetic acid (TFA), contributes to isoflurnae-induced cardioprotection via K_{ATP} channel activation during myocardial ischemia and reperfusion. **Methods:** Single ventricular myocytes were isolated from rabbit hearts by an enzymatic dissociation procedure. Patch-clamp techniques were used to record single-channel currents. K_{ATP} channel activities were assessed before and after the application of TFA with the inside-out patch mode. **Results:** TFA enhanced channel activity in a concentration-dependent fashion. The concentration of TFA for half-maximal activation and the Hill coefficient were 0.03 mM and 1.2, respectively. TFA did not affect the single channel conductance of K_{ATP} channels. Analysis of open and closed time distributions showed that TFA increased burst duration and decreased the interburst interval without changes in open and closed time distributions shorter than 5 ms. TFA diminished ATP sensitivity of K_{ATP} channels in a concentration-response relationship for ATP. **Conclusions:** TFA, a metabolite of isoflurane, enhanced K_{ATP} channel activity in a concentration-dependent fashion. These results imply that TFA could mediate isoflurane-induced cardioprotection via K_{ATP} channel activation during myocardial ischemia and reperfusion.

Key Words: K_{ATP} channels, Isoflurane, Patch clamp techniques, Rabbit ventricular myocytes

INTRODUCTION

A brief period of ischemic preconditioning has been proposed to mediate a protective effect against subsequent, more severe episodes of ischemia (Murry et al, 1986; Lawson & Downey, 1993; Yellon et al, 1993; Lawson, 1994). In general, ischemic preconditioning appears to provide electrical stability and tissue resistance to ischemia via some underlying mechanisms whereby metabolic conservation occurs (Walker & Yellon, 1992). Activation of the adenosine triphosphate (ATP)-sensitive potassium (K_{ATP}) channels may be an endogenous protective mechanism against the consequences of ischemia and reperfusion of the myocardium (Shigematsu et al, 1995). In addition, K_{ATP} channels are thought to play a role in the phenomenon of ischemic preconditioning in the heart (Gross & Auchampach, 1992; Grover et al, 1992; Parratt, 1994; Parratt & Kane, 1994). It has been reported that K_{ATP} channel openers mediate a cardioprotective effects (Downey, 1993; Mizumura et al, 1995; Kersten et al, 2000), whereas K_{ATP} channel antagonists preclude cardioprotection conferred by ischemic preconditioning in the heart (Auchampach et al, 1992; Speechly-Dick et al, 1995; Qian et al, 1996).

Volatile anesthetic isoflurane also exerts cardioprotective protective effects during ischemia and

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reperfusion (Belhomme et al, 1999; Ismaeli et al, 1999; Roscoe et al, 2000). Numerous mechanisms have been proposed to explain the cardioprotective action of isoflurane, including reductions in myocardial oxygen consumption, beneficial alterations in intracellular calcium homeostasis and the activation of ion channels (Piriou et al, 2000). Recently, both many *in vivo*, or isolated whole-heart experiments (Boutros et al, 1997; Crystal et al, 1997), and a clinical study (Belhomme et al, 1999), revealed that activation of K_{ATP} channels contributes to isoflurane-induced cardioprotection. However, controversy exists regarding the role that isoflurane plays in mediating cardioprotection via activation of K_{ATP} channels.

In these experiments on isolated rabbit ventricular myocytes using a patch-clamp technique, it was found that isoflurane inhibited K_{ATP} channel activities in a concentration-dependent manner. This finding suggests that isoflurane may attenuate the cardioprotective effects of K_{ATP} channels during ischemia and reperfusion in the myocardium. To date, possible explanations for the discrepancy between results in this (Han et al, 1996) and the previous (Boutros et al, 1997; Crystal et al, 1997) studies have not been suggested.

The objective of this study was to test the hypothesis that the major metabolite of isoflurane, trifluoroacetic acid (TFA), contributes to isoflurane-induced cardioprotection via the activation of K_{ATP} channels. For this purpose, The effects of TFA on the activity of single K_{ATP} channels in rabbit ventricular myocytes were evaluated. The results of this study support the hypothesis indicating that TFA enhances K_{ATP} channel activity and contributes to the cardioprotective effects of the volatile anesthetic isoflurane.

METHODS

Cell isolation

Single ventricular myocytes were isolated from rabbit hearts by enzymatic dissociation procedure, as discussed previously (Han et al, 1994; Han et al, 1998). 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 ml, Yakult, Japan). After 15~25 min of enzymatic treatment, Kraft's Bruhe (KB) solution was perfused. After 5 min of perfusion with 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. The pieces 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. The 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 (Hamill et al, 1981). 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 MΩ 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 0.4~20 kHz 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 (Colquhoun & Sigworth, 1983). The open time histogram was

formed from continuous recordings of more than 60 sec. The open probability (P_o) was calculated using the formula:

$$P_o = (\sum_{j=1}^{N} t_j * j)/(T_d * N)$$

where t_j is the time spent at current levels corresponding to j=0,1,2, ... 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. 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". To minimize the time-dependent decrease of the channel activity and to obtain an accurate TFA-K_{ATP} channel activity relation, we used the DAD-12 superfusion system (Adams & List Associates, NY, USA) for the rapid change of bath solution and drugs in most experiments. Data from patches exhibiting more than 50% rundown were discarded.

Solutions and drugs

Normal Tyrode solution contained (in mM): 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): 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): 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): 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 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 this concentration of DMSO 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 $25 \pm 2^{\circ}C$.

Statistics

Data are presented as mean $\pm$ SE of several experiments when appropriate. The statistical significance of the effects of TFA was evaluated by the Student's paired t-test to compare data before and after application of TFA. In experiments with different preparations, the statistical significance of the drug effect was evaluated using the Student's unpaired t-test. A probability of 0.05 or less was considered significant.

RESULTS

Effects of TFA on K_{ATP} channel activity of rabbit ventricular myocytes

To test the hypothesis that activation of K_{ATP} channels are involved in the actions of TFA, the effects of TFA on K_{ATP} channels were investigated with inside-out patch configuration using a symmetrical transmembrane K^+ concentration (140 mM). After giga-seal formation, the pipette potential was set to the test potentials and the bath solution was switched from the normal Tyrode solution to the high K^+ solution. On formation of the inside-out patches in ATP-free solution, the activation of K_{ATP} channels was observed. K_{ATP} channels were identified by their conductance over the voltage range of -80 to -20 mV (77.8 $\pm$ 3.5 pS), unitary current (2.8 $\pm$ 0.3 pA; $+40$ mV), kinetic properties, and the responses to potassium channel openers, ATP, and glibenclamide. These channel properties were consistent with K_{ATP} channel currents recorded previously (Han et al, 1993; Han et al, 1996A; Han et al, 1996B; Han et al, 1996C).

Fig. 1 shows a typical example of the effect of TFA on K_{ATP} channels. TFA (0.1 mM) reversibly enhanced K_{ATP} channel activity (Fig. 1A). Addition of 1 mM ATP (Fig. 1B) or 0.5 μM glibenclamide (Fig. 1C) suppressed the TFA-induced K_{ATP} channel activities, confirming that observed channel activities were due to K_{ATP} channels. We found these effects at both depolarizing (+40 mV; Fig. 1) and hyperpolarizing (−40 mV; data not shown) membrane potentials.

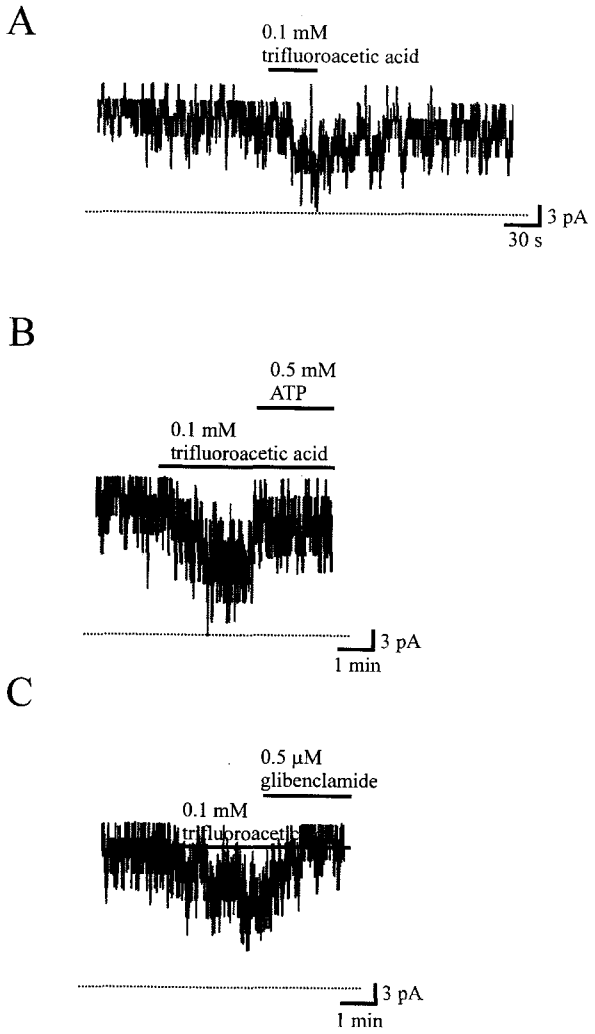

Fig. 1. Effects of TFA on ATP-sensitive K^+ (K_{ATP}) channel activity. Single-channel currents recorded from inside-out patches exposed to symmetrical 140 mM KCl. Shown are representative current records of TFA activation at a concentration of 0.1 mM (A). Note that the effect of TFA was reversible. 0.5 mM ATP (B) or 0.5 μM glibenclamide (C) inhibited the TFA-induced K_{ATP} channel activity, indicating that the channel was the K_{ATP} channel. A, B and C were separate experiments. The holding potential of the patch was +40 mV. The protocol for perfusing solutions is indicated by the bars above each current trace. Data were sampled at 10 kHz and filtered at 1 kHz. Dashed line indicates the zero current level.

The concentration-dependent effects of TFA on K_{ATP} channel activity

Fig. 2 shows the concentration-dependent effects of TFA on K_{ATP} channel activity. TFA increased K_{ATP} channel activity at a concentration as low as 0.001 mM and produced a concentration-dependent activation of K_{ATP} channel (Fig. 2A). The extent of activation was normalized by the maximum response to TFA (1 mM) and gave the concentration-response curve shown in Fig. 2B. The plot of relative channel activities as a function of the con-

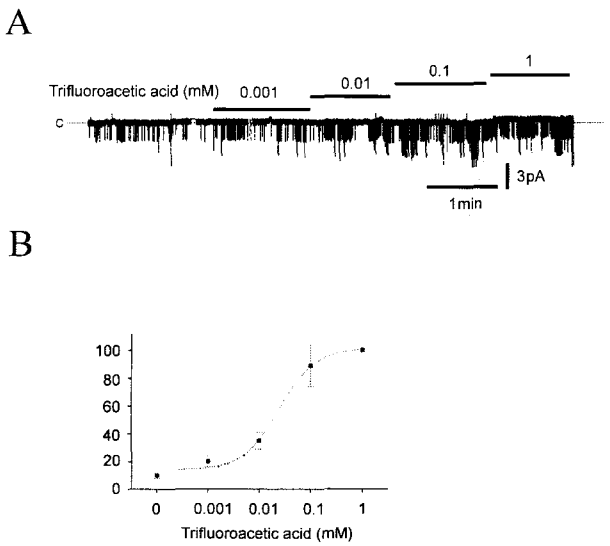

Fig. 2. Concentration-dependent effect of TFA on the K_{ATP} channel activity. (A) Representative current trace of concentration-dependent effects of TFA on K_{ATP} channel activity in an inside-out membrane patch containing one K_{ATP} channels. The membrane potential was held at −40 mV. Solution-exchange protocol for each concentration of TFA is shown above current trace. Data were sampled at 10 kHz and filtered at 1 kHz. Dashed line indicates the zero current level. (B) The relationship between the concentration of TFA and the relative channel activity of the K_{ATP} channel. The relative channel activity of the channel was obtained with reference to the value induced by 1 mM TFA in each patch. Values are means ± SE from 15 other experiments similar to that illustrated in A. The *solid line* in the graph was drawn from calculations that are described in the text.

centration of TFA ($[TFA]_i$) was fitted to the Hill equation using the least-squares method:

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

where K_d=$[TFA]_i$ at the half-maximal activation of the channel, and n=Hill coefficient. The K_d and n were 0.03 ± 0.01 mM and 1.2 ± 0.2, respectively (n=16 patches).

Effect of TFA on the conductance of K_{ATP} channels

The response of the unit amplitude to TFA was examined (Fig. 3). The traces (Fig. 3A) and all-point amplitude histograms (Fig. 3B) of the unit current are shown for the control (*left panel*) and 0.05 mM TFA (*right panel*) at -60 mV to demonstrate that single channel current amplitudes were not affected by TFA. Fig. 3C shows the current-voltage (*I-V*) relationships obtained from 15 patches before and after the application of 0.05 mM TFA. The *I-V* relationships before and after the application of TFA were linear in the negative membrane potential range, with slope conductance of 77.1 ± 4.1 picosiemens (pS) and 76.9 ± 3.4 pS before and after 0.05 mM TFA, respectively. There were no statistical differences between control and TFA-treated series. These results suggest that TFA does not affect the conductance of single-channel currents of K_{ATP} channels.

Effect of TFA on the kinetic properties of K_{ATP} channels

To examine the effect of TFA (0.05 mM) on the gating kinetics of K_{ATP} 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, which was analyzed from the current record filtered at a cut-off frequency of 10 kHz, revealed a single exponential distribution with a time constant (τ_o) of 1.5 ms under control condition (Fig. 4A, *left panel*). In the presence of TFA (Fig. 4A, *right panel*), the open time constant did not differ from that in the control (τ_o=1.6 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. 4C). Its time constant, designed as τ_b, was markedly prolonged by TFA (from 10 to 35 ms).

The histogram of closed time within bursts was best fitted to a single exponential function (Fig. 4B). This analysis was performed with closed times longer than 5 ms to be discarded, and filtered at a cutoff frequency of 10 kHz. The time constant of the closed time within bursts was designed as τ_c. The value of τ_c was not changed markedly by TFA (from 0.31 to 0.30 ms). The closed time between bursts was analyzed by using records filtered at a cut-off frequency of 0.1 kHz (Fig. 4D). The histogram was fitted using a biexponential function, with time constants of a fast (τ_{c1}) and a slow component (τ_{c2}). The τ_{c1} was equivalent to τ_c filtered at a cutoff frequency of 10 kHz, which was distorted by heavy filtering. The value of τ_{c1} was not influenced by TFA (from 18 to 14 ms). The value of τ_{c2} was 808 ms under control condition (Fig. 4D, *left panel*). This value was markedly decreased to 105 ms by TFA (Fig. 4D, *right panel*). The number of bursts was also analyzed. This value was markedly increased by TFA (Fig. 4E).

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

Interestingly, it was found that an application of ATP (0.5 mM) in the presence of TFA produced much less block (Fig. 1B). This suggests that the ATP sensitivity of the channel may have been changed by TFA. To determine the effects of TFA on ATP sensitivity of K_{ATP} channels, different concentrations of ATP were subsequently applied (Fig. 5). In the concentration range 1 μM to 10 mM, ATP exerted a greater inhibition of the channels in the absence (Fig. 5A) rather than in the presence of TFA (Fig. 5B). In Fig. 5C, the graph shows the does- response relationship for inhibition of K_{ATP} channel activity by ATP before (○) and after (●) the application TFA. From 25 patches, the inhibitory effects of ATP were quantified by measuring the open probability during ATP application, expressed relative to its value in ATP-free solution. The continuous lines in the graph were curves fitted to the Hill equation using the leastsquares method:

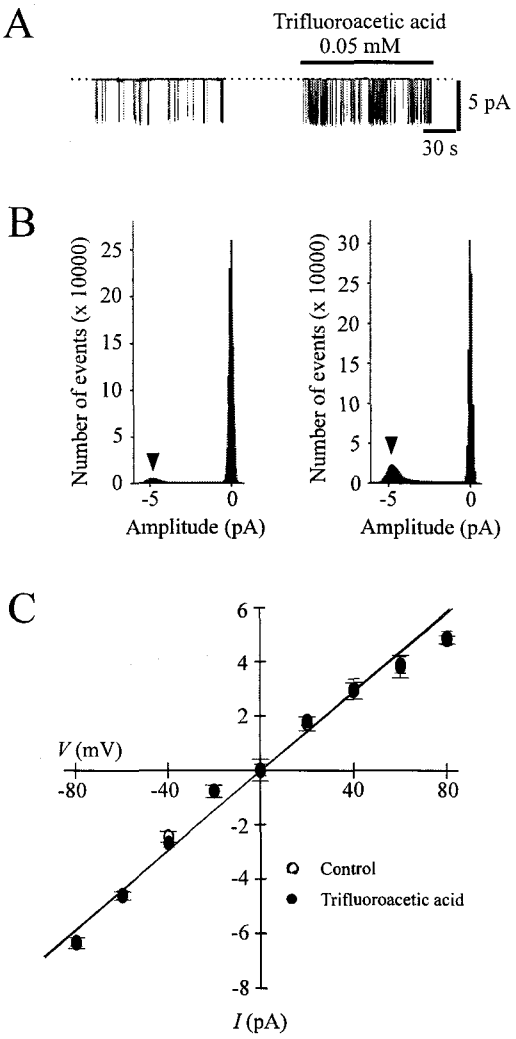

Fig. 3. Effect of TFA on the unit amplitude of K_{ATP} channel. (A) The traces of the unit current before (*left panel*) and after application of 0.05 mM TFA (*right panel*). Membrane potential was held at −60 mV. Both pipette and the internal solutions had 140 mM K^+. Data were sampled at 10 kHz and filtered at 1 kHz. Dashed line indicates the zero current level. (B) Amplitude histograms corresponding to the current traces in A. The number of events was plotted as a function of the current amplitude with fitted Gaussian distributions superimposed. *Arrowheads* indicate peaks corresponding to the unit amplitude, which was 4.9 and 4.8 before (*left panel*) and after application of TFA (*right panel*), respectively. (C) Current-voltage relationships of the unit current of K_{ATP} channel before (○) and after application of TFA (●). The unit current amplitude was measured in inside-out patches with symmetrical 140 mM K^+ conditions. Values are means ± SE. The *line* was drawn by linear least-squares fit to give a slope conductance of 77.1 pS and 76.9 pS before and after the application of TFA, respectively.

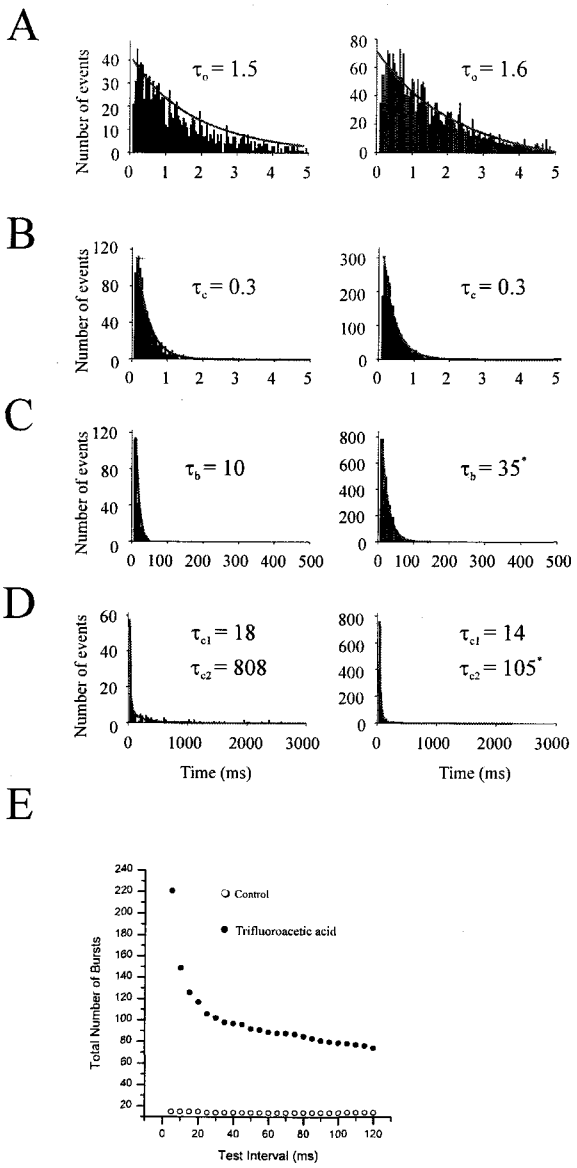

Fig. 4. Effects of TFA on the kinetic properties of K_{ATP} channel. Single-channel currents were recorded at −50 mV in inside-out patches. Histograms of open (A) and closed (B) times within bursts were analyzed from current records filtered at cutoff frequency of 10 kHz. Histograms of burst (C) and interburst (D) durations were analyzed from current records filtered at cutoff frequency of 0.1 kHz. Smooth curves were fitted by least-squares method. Each histogram was fitted either to a single exponential (A, B, C) or to the sum of two exponentials (D) to give the time constants shown in the panels. τ_o, Open-time constant; τ_c, closed-time constant; τ_b, burst time constant; τ_{c1}, τ_{c2}, fast and slow components of interburst time constant, respectively. (E) Graph showing the number of bursts in control and in the presence of TFA.

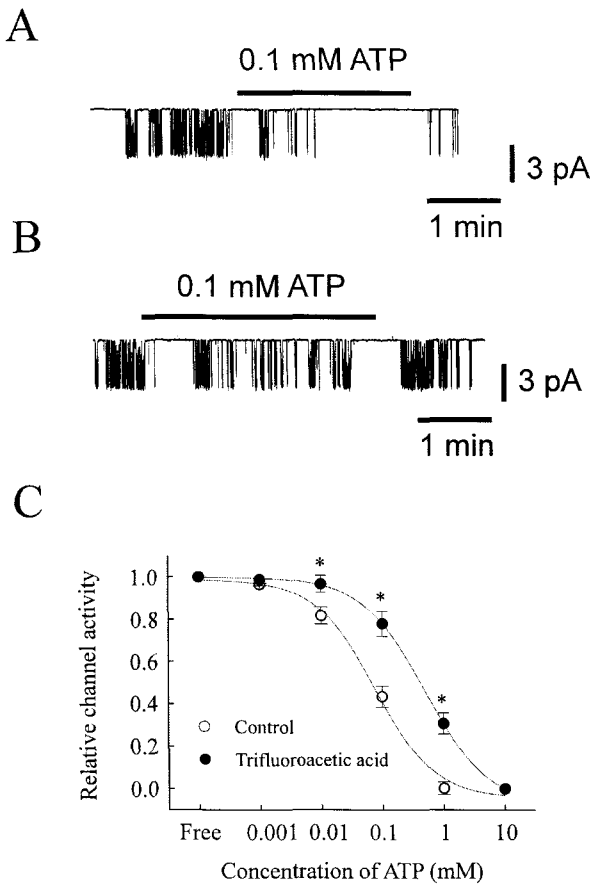

Fig. 5. Effect of TFA on the ATP sensitivity of K_{ATP} channel. Representative current traces of concentration-dependent effects of ATP on K_{ATP} channel activity in control (A) and in the presence of TFA (B). Single-channel currents recorded from inside-out patches exposed to symmetrical 140 mM KCl. The membrane potential was held at −40 mV. Solution-exchange protocol for each concentration of ATP is shown above current traces. Data were sampled at 10 kHz and filtered at 1 kHz. Dashed line indicates the zero current level. (B) The relationship between the concentration of ATP and the relative channel activity of the K_{ATP} channel in control (○) and in the presence of TFA (●). The relative channel activity of the channel was obtained with reference to the value in ATP-free solution in each patch. Values are means SE from 24 other experiments similar to that illustrated in A and B. The *solid line* in the graph was drawn from calculations that are described in the text. *Significantly different from control value ($P<0.05$).

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

where $[ATP]_i$ is ATP concentration in internal solution, K_i is the ATP concentration evoking the half-maximal inhibition, and n is the Hill coefficient. In control conditions, K_i and n were 71.5 ±7.6 and 1.9±0.6. In the presence of TFA, the dose-response relation for ATP had the same slope (1.7±0.7, $P>0.05$) as that in its absence but was shifted to higher ATP concentrations (K_i=233.2± 28.8 μM, $P<0.05$).

DISCUSSION

The objective of the current study was to determine if the metabolite of isoflurane TFA could modulate the K_{ATP} channels. The principal findings of this study are that TFA enhanced K_{ATP} channel activity by reducing the sensitivity of the K_{ATP} channels to ATP without changing the single-channel conductance, providing evidence toward a mechanism for isoflurane-induced cardioprotection during ischemia and reperfusion.

Myocardial ischemic preconditioning is an endogenous protection that renders the heart more resistant to prolonged ischemia (Murry et al, 1986; Reimer et al, 1986). Recent experimental and clinical evidence have indicated that volatile anesthetic isoflurane can precondition the ischemic heart (Piriou et al, 2000; Roscoe et al, 2000). The underlying mechanism of this isoflurane-induced preconditioning has not been completely elucidated. Several possible mechanisms for this phenomenon have been proposed, including: favorable alterations in myocardial oxygen supply-and-demand relations and in intracellular calcium homeostasis (Warltier et al, 1988); coronary vasodilation (Crystal et al, 1997); adenosine receptor activation (Kerstein et al, 1997); activation of ion channels such as stretch-activated channels (Piriou et al, 2000); and sarcolemmal (Boutros et al, 1997; Crystal et al, 1997) or mitochondrial K_{ATP} channels (Piriou et al, 2000).

Up to now, much interest has focused on the sarcolemmal K_{ATP} channels as a potential mechanism. This is mainly supported by the fact that, in most experimental and clinical studies, isoflurane-induced preconditioning is abolished by glibenclamide. Thus, it has been thought that cardiac K_{ATP} channels would be activated by isoflurane. To our knowledge, few studies to date specifically determine whether isoflurane exerts a direct effect on K_{ATP} channels. Moreover, controversy exists regarding the effect of isoflurane on K_{ATP} chan-

nels. Roscoe et al (2000) reported that, in human atrial myocytes, isoflurane fails to produce any significant changes in either inward or outward K_{ATP} currents in whole-cell voltage-clamp configurations when administered in concentrations as high as 3%. However, this study found that, in inside-out patch configurations, isoflurane inhibits K_{ATP} channel activities in a concentration-dependent manner. Differences in experimental conditions and animal species, between this and Roscoe's study, may be responsible for the discrepancy in results.

The major variations in experimental conditions are the mode of patch recordings and the compounds used. Roscoe et al (2000) carried out experiments in whole-cell mode of patch-clamp methods, whereas we performed inside-out patch mode. It is difficult to know how the mode of patch clamping can affect the action of isoflurane on the activity of K_{ATP} channels. Considering the previous results in inside-out patches (Han et al, 1996B) it may be plausible that several cellular components in the whole-cell mode can alter the effect of isoflurane. In addition, in their whole-cell mode, Roscoe et al (2000) used DNP to activate K_{ATP} currents. Because DNP activates K_{ATP} currents by inhibiting mitochondrial ATP synthesis as well as through a direct channel effect (Alekseev at al, 1997), it is possible that K_{ATP} channels were circumvented by the actions of DNP in their study.

In addition, the findings (Han et al, 1996B; Roscoe et al, 2000) that K_{ATP} channel activities are inhibited or not affected by isoflurane in experiments on isolated myocytes using patch-clamp techniques contradict previous studies that have demonstrated a cardioprotection by isoflurane-induced K_{ATP} channel activation in the majority of *in vivo* and *in vitro* studies. Nevertheless, there are several possible explanations for the discrepant data for isoflurane and its effect on K_{ATP} channel activity: (1) Isoflurane may activate K_{ATP} channels by decreasing intracellular ATP concentration and ATP/ADP ratio; (2) Isoflurane may diminish ATP sensitivity of K_{ATP} channels, indicating an increased likelihood of K_{ATP} channel activity for a given concentration of ATP after isoflurane anesthesia; (3) Isoflurane may activate K_{ATP} channels by its action on an upstream intermediate, such as an adenosine receptor.

The intracellular levels of ATP required to cause K_{ATP} channel activation are quite low when compared to normal intracellular levels, with half-maximal inhibition of channel activity at 20~100 μM (Han et al, 1993: Han et al, 1996A; Han et al, 1996C), whereas the normal intracellular ATP concentration is much higher (5~10 mM). However, isoflurane has been found to cause a small decrement (30% reduction at 2.8% isoflurane) (Kissch et al, 1990) or no reduction (Mattheussen et al, 1993) in intracellular ATP concentration at 2.3% isoflurane in ventricular myocytes. In addition, in whole-cell patch mode, isoflurane applied to the cell out of patch did not change the activity of K_{ATP} channel (Roscoe et al, 2000). These facts indicate that the isoflurane-induced activation of K_{ATP} channel is not mediated by decreasing intracellular ATP concentration, disproving the first possibility. Conversely, the finding that the dose-response curve for ATP-induced inhibition of K_{ATP} channel activity shifted to the right after isoflurane application (Han et al, 1996B) supports the second possibility. The third possibility also seems likely, because of the findings that the adenosine receptor has been found to be coupled to K_{ATP} channels via a G-protein mechanism (Kirsch et al, 1990) and the adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine inhibits the cardioprotective activity of isoflurane (Roscoe et al, 2000). In the previous study, we demonstrated that adenosine A_1 receptor activation activated of K_{ATP} channels by reducing the sensitivity of the K_{ATP} channel to ATP (Kim et al, 1997), supporting both the second and third possibilities.

Another possibility is that the metabolites of isoflurane may modulate K_{ATP} channels. Principal metabolites of isoflurane are TFA, fluoride ion and small quantities of other unidentified organic fluoride compounds (Hitt et al, 1974). All of these identified metabolites of isoflurane are non-toxic and administration of isoflurane has not been linked with anesthetic toxicity (Plummer et al, 1986). In human, the predominant metabolite has known to be TFA (Hitt et al, 1974).

In the present study, the effects of TFA on K_{ATP} channel activity in inside-out patches were tested. It was found that TFA activated K_{ATP} channels by reducing the sensitivity of the K_{ATP} channel to ATP. To our knowledge, these are first data obtained, which are relate to the effects of TFA on the K_{ATP} channels by using single channel patch clamp techniques. In the current study,

TFA was tested at a concentration ranging from 0.1 to 1000 μM. It cannot be ascertained that the concentrations of TFA tested would be found in humans after isoflurane anesthesia. However, the peak concentration of excreted TFA found in human urine was 1000~1300 μM (Hitt et al, 1974), when patients were exposed to isoflurane at a concentration of 0.9%. This could theoretically precondition the heart. This suggests that the concentrations of TFA used in this study may be sufficient to activate K_{ATP} channels and potentially contribute to the cardioprotective effects of isoflurane.

K_{ATP} channels have been identified both in cardiac myocytes (Noma, 1983) and in coronary vascular smooth muscle cells (Daut et al, 1990). Activation of these channels in vascular smooth muscle by ischemia, hypoxia, or K_{ATP} channel agonists causes vasodilation. Also isoflurane *in vivo* has been shown to produce coronary vasodilation through K_{ATP} channel activation (Daut et al, 1990; Crystal et al, 1997). Such findings, and the results of the current investigation, suggest that isoflurane and its metabolites should be further studied with regard to their cardioprotective effects, which may be specifically related to their modulation of the K_{ATP} channels in coronary vascular smooth muscle cells. Therefore, to further determine the nature of the interaction between isoflurane or its metabolites and the K_{ATP} channels in coronary arterial smooth muscle cells, single-channel recording using the patch clamp techniques in coronary arterial smooth muscles cells and isometric contraction experiments in arterial rings will be necessary. We are currently pursuing these studies.

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, non-ischemic stimuli in the heart such as ventricular pacing, stretch and heat stress and pharmacological agents that open K_{ATP} channels (Duncker & Verdouw, 2000). Although the exact mechanism by which K_{ATP} channel activation protects heart is still incompletely understood, recent evidence suggests that mitochondrial K_{ATP} channels mediate cardioprotection in ischemic preconditioning (Liu et al, 1998; Sato et al, 1998). This is mainly supported by the fact that, in most experimental models, preconditioning is blocked by 5-hydroxydecanoate, a selective blocker of mitochondrial K_{ATP} channels. 5-Hydroxydecanoate also blocked isoflurane-induced infarct size reduction, suggesting that this protection is, in part, mediated by mitochondrial K_{ATP} channels (Piriou et al, 2000). The current study does not provide direct experimental evidence supporting this interesting possibility. However, the finding that TFA can potentiate the K_{ATP} channel raises the intriguing possibility that TFA could modulate mitochondrial K_{ATP} channels in the heart and provides a possible mechanism of isoflurane-mediated cardioprotection. Indeed, every ligand that modulates sarcolemmal K_{ATP} channels has been known to regulate mitochondrial K_{ATP} channels (Sato et al, 1998; Grover et al, 2000). Interestingly, it has been demonstrated that the selective mitochondrial K_{ATP} channel blocker 5-hydroxydecanoate does not reduce protection from preconditioning in isolated rabbit hearts. This suggests that mitochondrial K_{ATP} channels may not mediate cardioprotection (Armstrong et al, 1997). It remains to be elucidated whether isoflurane-induced cardioprotection is due to mitochondrial K_{ATP} channels or sarcolemmal K_{ATP} channels or to a mixture of both.

In conclusion, these data show that TFA, the predominant metabolite of isoflurane, activates 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.

ACKNOWLEDGEMENT

This study was supported by the 2000 Inje University Research grant.

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