British Journal of Pharmacology (2006) 149, 573–580
 0007–1188/06 $30.00
www.brjpharmacol.org

RESEARCH PAPER

A key role for the subunit SUR2B in the preferential activation of vascular K_{ATP} channels by isoflurane

H Fujita[1,2], T Ogura[1], M Tamagawa[1], H Uemura[1], T Sato[1], A Ishida[2], M Imamaki[2], F Kimura[2], M Miyazaki[2] and H Nakaya[1]

[1]*Department of Pharmacology, Chiba University Graduate School of Medicine, Chuo-ku, Chiba, Japan and* [2]*Department of General Surgery, Chiba University Graduate School of Medicine, Chuo-ku, Chiba, Japan*

Background and purpose: It has been postulated that isoflurane, a volatile anaesthetic, produces vasodilatation through activation of ATP-sensitive K^+ (K_{ATP}) channels. However, there is no direct evidence for the activation of vascular K_{ATP} channels by isoflurane. This study was conducted to examine the effect of isoflurane on vascular K_{ATP} channels and compare it with that on cardiac K_{ATP} channels.
Experimental approach: Effects of isoflurane on K_{ATP} channels were examined in aortic smooth muscle cells and cardiomyocytes of the mouse using patch clamp techniques. Effects of the anaesthetic on the K_{ATP} channels with different combinations of the inward rectifier pore subunits (Kir6.1 and Kir6.2) and sulphonylurea receptor subunits (SUR2A and SUR2B) reconstituted in a heterologous expression system were also examined.
Key results: Isoflurane increased the coronary flow in Langendorff-perfused mouse hearts in a concentration-dependent manner, which was abolished by 10 μM glibenclamide. In enzymically-dissociated aortic smooth muscle cells, isoflurane evoked a glibenclamide-sensitive current (i.e. K_{ATP} current). In isolated mouse ventricular cells, however, isoflurane failed to evoke the K_{ATP} current unless the K_{ATP} current was preactivated by the K^+ channel opener pinacidil. Although isoflurane readily activated the Kir6.1/SUR2B channels (vascular type), the volatile anesthetic could not activate the Kir6.2/SUR2A channels (cardiac type) expressed in HEK293 cells. Isoflurane activated a glibenclamide-sensitive current in HEK293 cells expressing Kir6.2/SUR2B channels.
Conclusion and implications: Isoflurane activates K_{ATP} channels in vascular smooth muscle cells and produces coronary vasodilation in mouse hearts. SUR2B may be important for the activation of vascular-type K_{ATP} channels by isoflurane.
British Journal of Pharmacology (2006) **149**, 573–580. doi:10.1038/sj.bjp.0706891; published online 25 September 2006

Keywords: ATP-sensitive K^+ channel; isoflurane; coronary vasodilation

Abbreviations: HEK, human embryonic kidney; K_{ATP} channel, ATP-sensitive K^+ channel; NBD, nucleotide-binding domain; SUR, sulphonylurea receptor

Introduction

It has long been known that isoflurane, a volatile anaesthetic, produces vasodilatation (Eger, 1981). As the isoflurane-induced vasodilatation was inhibited by glibenclamide, it has been postulated that activation of ATP-sensitive K^+ (K_{ATP}) channels in vascular smooth muscle cells is involved in the vasodilatation (Cason *et al*., 1994; Crystal *et al*., 1997; Zhou *et al*., 1998). However, there is no direct evidence for the activation of vascular K_{ATP} channels by isoflurane. In terms of effects of isoflurane on cardiac K_{ATP} channels, apparently inconsistent results have been reported: Han *et al*. (1996) reported that isoflurane inhibited the openings of K_{ATP} channels in rabbit ventricular cells, whereas Kwok *et al*. (2002) indicated that isoflurane facilitated the K_{ATP} channel openings induced by metabolic blockade or the K^+ channel opener pinacidil. Therefore, the first aim of this study was to examine the effect of isoflurane on the K_{ATP} channel current in vascular smooth muscle cells and to compare it with the effect on cardiac K_{ATP} channel current.

Recently, the K_{ATP} channel has been described as a hetero-octamer comprising two subunits: the pore-forming Kir6.x (Kir6.1 or Kir6.2) and the regulatory sulphonylurea receptor SUR (SUR1, SUR2A or SUR2B) (Seino and Miki, 2003). Different combinations of Kir6.x and SUR constitute K_{ATP} channels with distinct electrophysiological properties (Inagaki *et al*., 1995, 1996; Gribble *et al*., 1997; Yamada *et al*., 1997). Functional studies using genetically engineered mice lacking various K_{ATP} channel subunits from this and other laboratories (Suzuki *et al*., 2001, 2002; Chutkow *et al*., 2002;

Correspondence: Professor H Nakaya, Department of Pharmacology, Chiba University Graduate School of Medicine, Inohana 1-8-1, Chuo-ku, Chiba 260-8670, Japan.
E-mail: nakaya@faculty.chiba-u.jp
Received 10 May 2006; revised 4 July 2006; accepted 9 August 2006; published online 25 September 2006

Miki *et al*., 2002) have indicated that Kir6.2 and SUR2A constitute the cardiac-type K_{ATP} channel, whereas Kir6.1 and SUR2B constitute the vascular smooth muscle-type K_{ATP} channel. If some difference could be detected between the effects of isoflurane on cardiac and vascular K_{ATP} channels, it would be of interest to identify which subunit(s) of the K_{ATP} channels were responsible for the different isoflurane effects. Accordingly, the second purpose of this study was to determine the effects of isoflurane on the K_{ATP} channels with different combinations of Kir6.x and SUR subunits reconstituted in a heterologous expression system. By doing so, we hoped to gain a greater insight into the molecule with which isoflurane interacts in K_{ATP} channels in cardiovascular tissues.

Methods

All experiments were performed according to the regulations of the Animal Research Committee of Chiba University Graduate School of Medicine and the Guide for the Care and Use of Laboratory Animals (NIH publication).

In vitro *functional study using Langendorff-perfused hearts*
C57BL/6 mice were purchased and used in this study. Mice were anaesthetized with urethane ($1.5\,mg\,g^{-1}$ body weight, intraperitoneally (i.p.)) and heparinized ($0.1\,U\,g^{-1}$ body weight, intravenously (i.v.)). Hearts were rapidly excised and connected to the perfusion cannula via the aorta, as described previously (Suzuki *et al*., 2001). Retrograde perfusion was maintained at a constant pressure of 80 cm H_2O with a modified Krebs–Henseleit solution containing (in mM): NaCl 119, KCl 4.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.8, glucose 10, $NaHCO_3$ 24.9. The perfusate was equilibrated with 95% O_2 and 5% CO_2 (pH 7.4, 37°C). Coronary flow was monitored continuously using an ultrasonic flow probe (Transonic Systems, Ithaca, NY, USA). Hearts were paced at a constant rate (480 beats/min) through a bipolar platinum electrode attached to the right atrium using an electronic stimulator (Nihon Kohden, Tokyo, Japan). After a 10-min stabilization period, the control perfusing solution was changed to one that was bubbled with various concentrations of isoflurane or others containing isoflurane and 10 μM glibenclamide.

Cell culture and transfection
Human embryonic kidney (HEK)293 cells (American Type Culture Collection, Rockville, MD, USA) were grown in Dulbecco's modified Eagle's Medium (Sigma-Aldrich Japan, Tokyo, Japan) supplemented with 10% foetal bovine serum (Invitrogen Corp., Carlsbad, CA, USA) and $100\,U\,ml^{-1}$ penicillin G and $100\,\mu g\,ml^{-1}$ streptomycin (Sigma-Aldrich Japan, Tokyo, Japan), and maintained at 37°C in a humidified atmosphere with 95% air and 5% CO_2. Transient transfection was performed using lipofectAMINE Plus reagent (Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer's instructions. HEK293 cells were seeded on glass coverslips and co-transfected with the expression vector pCMV containing rat SUR2A or rat SUR2B and the pCMV containing rat Kir6.1 or human Kir6.2 (these plasmids were kindly provided by Dr S Seino, Kobe University) at a molar plasmid ratio of 1:1. The amounts of vector per 35 mm dish were as follows: 0.9 μg plasmid containing SUR2A or SUR2B and 0.6 μg plasmid containing Kir6.1 or Kir6.2. In general, pEGFP-C1 vector (Clontech, Mountain View, CA, USA), encoding for green fluorescent protein, was added for easy identification of transfected cells; the procedure did not affect the electrophysiological properties. Cells were allowed to express transfected DNA for 48 h and were then used for electrophysiological experiments.

Electrophysiology
Vascular smooth muscle cells. Single smooth muscle cells were enzymically isolated from the adult mouse aorta. The thoracic aorta was isolated from anaesthetized mice and cleaned of fat and connective tissues. The aorta was incubated in Hanks' solution containing 0.4% of collagenase for 40 min at 37.0°C. The composition of the Hanks' solution was (in mM): NaCl 137, KCl 5.4, NaH_2PO_4 0.168, KH_2PO_4 0.44, glucose 5.6 and $NaHCO_3$ 4.17. The solution was aerated with a mixture of 95% O_2 and 5% CO_2, and pH of the solution was maintained at 7.2–7.4. The tissue was gently agitated with a glass pipette.

Membrane currents were recorded at room temperature using the whole-cell patch-clamp technique, as described previously (Suzuki *et al*., 2001). The composition of the extracellular high-K solution was (in mM): NaCl 2.9, KCl 140, $CaCl_2$ 2.2, $MgCl_2$ 1.2, glucose 14, HEPES-KOH buffer 10 (pH 7.4), and that of the pipette solution was (in mM): KCl 140, $MgCl_2$ 4, K_2-ATP 1, EGTA 10 and HEPES-KOH buffer 10 (pH 7.2).

Adult ventricular cells. Single ventricular cells of the adult mouse heart were isolated by conventional enzymic digestion (Sakamoto *et al*., 1998). Whole-cell membrane currents were recorded at 36.0°C by the patch-clamp method, as described previously (Suzuki *et al*., 2001). The composition of the pipette solution was (in mM): KCl 20, $MgCl_2$ 1, K-aspartate 110, K_2-ATP 1, phosphocreatine-K_2 1, $CaCl_2$ 1.41, EGTA 10 and HEPES 5 (pCa 8.0, pH 7.4). The external solution used was HEPES-Tyrode solution containing (in mM): NaCl 143, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, $NaHPO_4$ 0.33, glucose 5.5 and HEPES 5 (pH 7.4). A liquid junction potential between the internal solution and the bath solution of −8 mV was corrected. A ramp-pulse protocol was used to record the quasi-steady-state membrane current, as described previously (Sakamoto *et al*., 1998). The membrane potential was held at −40 mV and depolarized first to +50 mV at a rate of $1.2\,mV\,ms^{-1}$. It was then repolarized or hyperpolarized to −100 mV with a slope of $-1.2\,mV\,ms^{-1}$, during which time the changes in the membrane current was automatically plotted against the membrane potential. The current–voltage relation was measured during the repolarized or hyperpolarized phase and the current level at 0 mV was obtained. The ramp voltage pulses were applied at appropriate timing.

Transfected HEK cells. The ionic currents through the channels expressed in the HEK293 cells were recorded in the whole-cell configuration of the patch-clamp technique. The membrane potential was held at −40 mV and the same ramp-pulse protocol as that in the experiments using mouse ventricular cells was used to record the quasi-steady-state membrane current. The current level at 0 mV was measured and the current density was calculated by normalization with the membrane capacitance. The pipette solution and the extracellular solution were the same as those used for the recording of the membrane currents in vascular smooth muscle cells.

Statistics

All data are presented as mean ± s.e.m. Statistical analyses of the data were performed using two-way analysis of variance (ANOVA) combined with Fisher's *post hoc* test. Probability values less than 0.05 were considered significant.

Drugs

The following drugs were used: isoflurane (Merck, Osaka, Japan); glibenclamide (Sigma-Aldrich Japan, Tokyo, Japan); pinacidil (Sigma-Aldrich Japan, Tokyo, Japan); and theophylline (Wako, Osaka, Japan). Glibenclamide was dissolved in dimethyl sulphoxide (final concentration of the solvent was less than 0.1%) and pinacidil was dissolved in 0.1 N HCl as stock solutions. Isoflurane was introduced into the oxygen through Isoflurane Vapour (ACOMA I type MKIII, Tokyo, Japan) and dissolved in the external solution. From the data of a previous study in which isoflurane was vaporized into a physiological solution (Ozaki *et al*., 1990), the concentration of isoflurane in the solution dissolved at 4% would be expected to be around 1.36 mM.

Results

Effects of isoflurane on coronary flow in isolated mouse hearts

Representative changes of coronary flow after isoflurane alone and co-application of glibenclamide are shown in Figure 1. Isoflurane increased the coronary flow in a concentration-dependent manner. The coronary flow decreased below the control level after co-application of glibenclamide. Isoflurane dissolved at a concentration of 4% doubled the coronary flow and this effect was abolished by the addition of 10 μM glibenclamide. The K^+ channel opener, pinacidil (10 μM), also doubled coronary flow and this was also antagonized by the addition of glibenclamide.

Since it has been suggested that some of the activating effect of isoflurane on K_{ATP} channels is mediated by adenosine receptor stimulation (Gassmayr *et al*., 2003), effects of isoflurane on the coronary flow were examined in the presence of theophylline, a non-selective adenosine receptor antagonist. Even in the presence of 100 μM theophylline, isoflurane increased coronary flow from 1.15 ± 0.20 to 2.09 ± 0.09 ml min^{-1} ($P<0.01$, $n=5$).

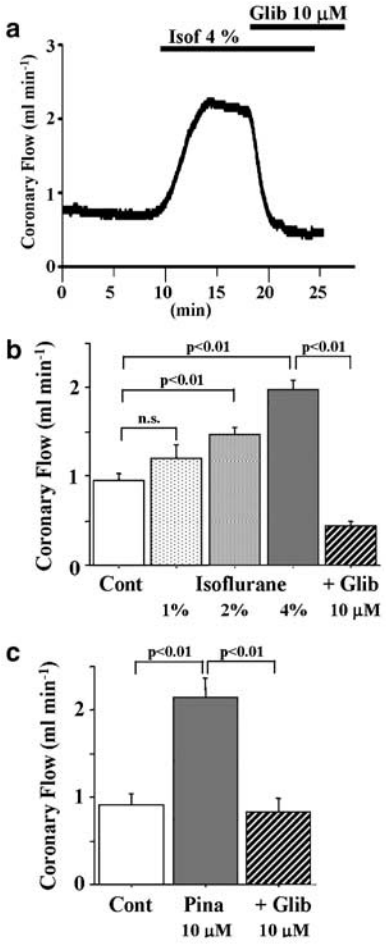

Figure 1 Changes of coronary flow after isoflurane and pinacidil in Langendorff-perfused mouse hearts. (**a**) A representative increase in coronary flow after isoflurane (Isof) bubbled at a concentration of 4%. The increase in coronary flow was antagonized by 10 μM glibenclamide (Glib). Summarized data of peak coronary flow after isoflurane and pinacidil (Pina) are indicated in (**b**) and (**c**), respectively. Values are expressed as mean ± s.e.m. of 6–8 preparations. Statistical analyses of the data were conducted using two-way ANOVA combined with Fisher's *post hoc* test.

Effects of isoflurane on K_{ATP} current in vascular smooth muscle cells

Effects of isoflurane and pinacidil on the whole-cell membrane current were examined in isolated aortic smooth muscle cells. Pinacidil (10 μM) activated a glibenclamide-sensitive inward current in smooth muscle cells held at −40 mV in the high K^+ solution, as described previously (Suzuki *et al*., 2001). As shown in Figure 2, isoflurane, dissolved at a concentration of 4%, also activated a glibenclamide-sensitive inward current in aortic smooth muscle cells increasing almost six-fold the density of the glibenclamide-sensitive inward current. The isoflurane-induced increase in the inward current at −40 mV was concentration-dependent and the current was readily blocked by 10 μM glibenclamide.

Effects of isoflurane on K_{ATP} current in ventricular cells

Effects of isoflurane and pinacidil on the whole-cell membrane current recorded using a ramp-pulse protocol

were examined in mouse ventricular cells. Isoflurane dissolved at a concentration of 4% hardly affected or slightly decreased the quasi-steady-state membrane current (Figure 3a and b). However, pinacidil (50 μM) increased the steady-state current at 0 mV from 3.4 ± 0.7 to 14.2 ± 3.1 pA pF^{-1} ($P<0.01$, $n=8$), which was readily blocked by 10 μM glibenclamide. Thus, pinacidil but not isoflurane activated the glibenclamide-sensitive K_{ATP} current in mouse ventricular myocytes.

Isoflurane was reported to sensitize the cardiac K_{ATP} channel to pinacidil in guinea-pig ventricular myocytes (Gassmayr *et al*., 2003). In their study, pretreatment with isoflurane produced a higher density of the pinacidil-induced K_{ATP} current than that induced by pinacidil alone in guinea-pig ventricular cells. In this study, we also examined effects of isoflurane on the K_{ATP} current pre-activated by pinacidil in mouse ventricular cells. Pinacidil at a concentration of 10 μM slightly increased the outward

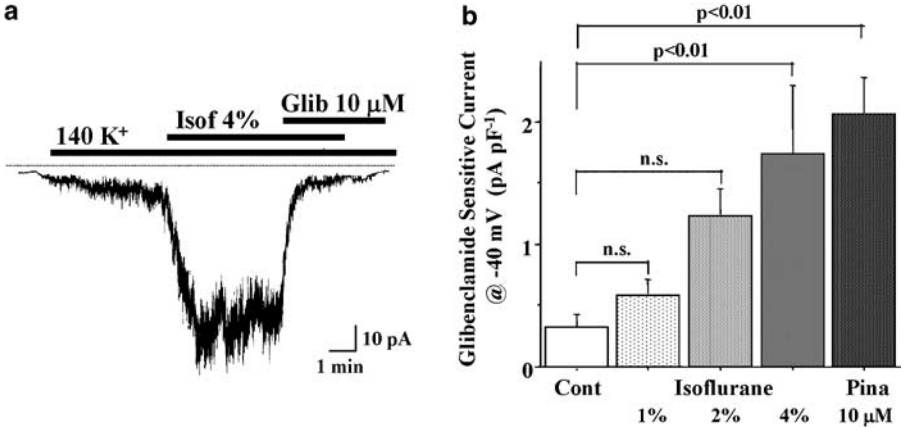

Figure 2 Isoflurane-induced current in mouse vascular smooth muscle cells. Representative change of the inward current after isoflurane (Isof) bubbled at a concentration of 4% is shown in (**a**). The inward current was sensitive to glibenclamide (Glib). The density of the glibenclamide-sensitive inward current after isoflurane and pinacidil (Pina) in aortic smooth muscle cells held at -40 mV are indicated in (**b**). Values are expressed as mean $\pm$ s.e.m. of 5–12 cells. Statistical analyses of the data were conducted using two-way ANOVA combined with Fisher's *post hoc* test.

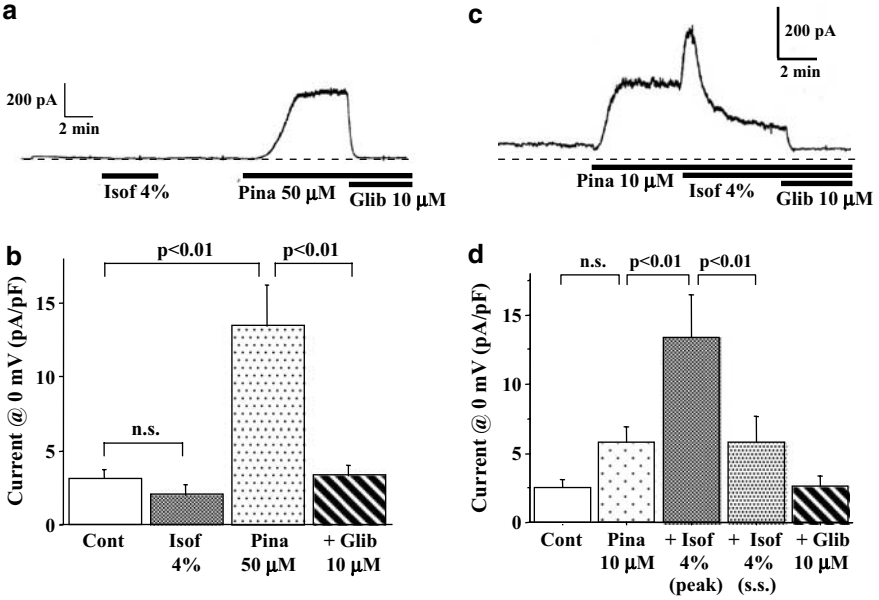

Figure 3 Effects of isoflurane on the membrane current in mouse ventricular myocytes. Actual traces of the holding current at -40 mV are depicted in (**a**) and (**c**). Current densities at 0 mV, measured by a ramp-pulse protocol (a voltage change from $+50$ to -100 mV at a rate of 1.2 mV ms^{-1}), are summarized in (**b**) and (**d**). Isoflurane (Isof) alone failed to activate a glibenclamide (Glib)-sensitive outward current (panels a and b). However, isoflurane transiently activated the K_{ATP} current when it was preactivated by a low concentration of pinacidil (Pina) (panels c and d). The densities of the outward current at 0 mV after pinacidil, pinacidil + isoflurane and pinacidil + isoflurane + glibenclamide are summarized in panel d. The peak and steady state (s.s.) current densities after the addition of isoflurane are shown. Values are expressed as mean $\pm$ s.e.m. of 8–10 cells. Statistical analyses of the data were conducted using two-way ANOVA combined with Fisher's *post hoc* test.

current, as shown in Figure 3c. Addition of isoflurane transiently increased and then suppressed the glibenclamide-sensitive outward current. When isoflurane (4%) and pinacidil (10 μM) were introduced simultaneously, the steady-state current at 0 mV was not different from control values.

Effects of isoflurane on recombinant K_{ATP} channels in HEK293 cells

In order to gain a greater insight into the molecular target for isoflurane, we examined effects of isoflurane on recombinant K_{ATP} channels expressed in HEK293 cells using patch-clamp techniques. HEK293 cells were co-transfected with a Kir6.1 or Kir6.2 subunit in combination with either of SUR2A or SUR2B. Effects of isoflurane on Kir6.1/SUR2B channels, representing smooth muscle-type K_{ATP} channels, are shown in Figure 4a. Isoflurane dissolved at a concentration of 4% increased an inward current, which was sensitive to 10 μM glibenclamide. Effects of isoflurane on Kir6.2/SUR2A channels, representing cardiac-type K_{ATP} channels, were also examined. Isoflurane did not increase the inward current in HEK293 cells transfected with Kir6.2 and SUR2A. After washout of isoflurane, however, pinacidil (50 μM) activated the inward current that was readily abolished by the addition of 10 μM glibenclamide (Figure 4b). Since isoflurane activated Kir6.1/SUR2B channels but not Kir6.2/SUR2A channels, we examined the effect of the volatile anaesthetic on

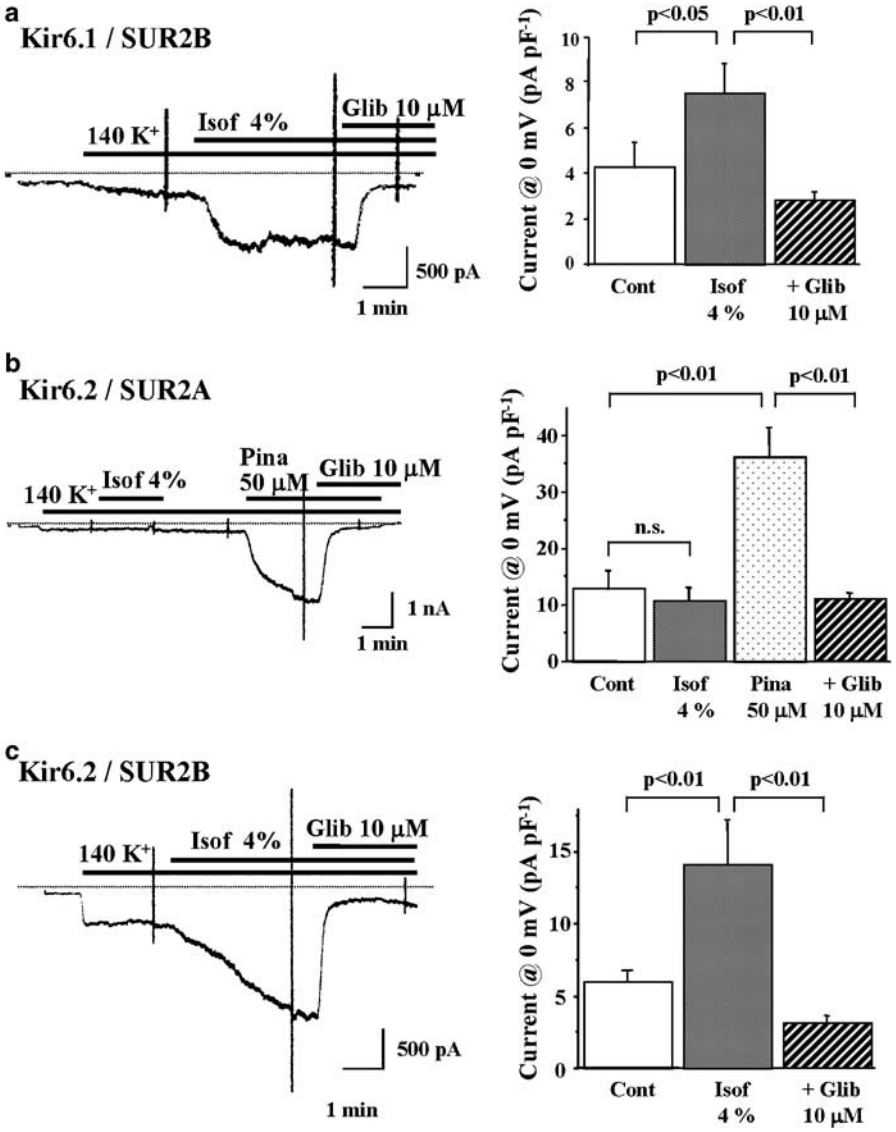

Figure 4 Effects of isoflurane on recombinant K_{ATP} channels expressed in HEK293 cells. Representative changes of Kir6.1/SUR2B, Kir6.2/SUR2A and Kir6.2/SUR2B channel currents after isoflurane (Isof), pinacidil (Pina) and glibenclamide (Glib) and the summarized data are shown in (**a**–**c**), respectively. The membrane potential was held at −40 mV and the same ramp-pulse protocol as that in Figure 3 was used to record the quasi-steady-state membrane current. The current level at 0 mV was measured and then the current density was calculated. Values are expressed as mean ± s.e.m. of nine cells. Statistical analyses of the data were conducted using two-way ANOVA combined with Fisher's *post hoc* test.

Kir6.2/SUR2B channels (Figure 4c). Isoflurane significantly increased the inward current in HEK293 cells transfected with Kir6.2 and SUR2B, although the time course of the current activation was relatively slow.

Discussion and conclusions

In this study, isoflurane increased coronary flow in isolated mouse hearts, which is consistent with coronary vasodilation observed in hearts of other species including humans (Reiz *et al*., 1983). As the isoflurane-induced increase of coronary flow was abolished by co-administration of glibenclamide, the involvement of activated K_{ATP} channel in this coronary vasodilatation seemed likely. We have provided direct evidence that isoflurane can activate the K_{ATP} current in isolated vascular smooth muscle cells. As far as we know, this is the first report showing direct evidence for the activation of K_{ATP} current by volatile anaesthetics in vascular smooth muscle cells.

In terms of effects of isoflurane on cardiac K_{ATP} channels, inconsistent results have been reported. Han *et al*. (1996) reported that isoflurane inhibited the openings of K_{ATP} channels in inside-out patches of rabbit ventricular cells. However, it was reported that isoflurane potentiated the K_{ATP} channel current induced by the K^+ channel opener pinacidil or metabolic inhibition in guinea-pig ventricular cells (Fujimoto *et al*., 2002; Kwok *et al*., 2002). In addition, Gassmayr *et al*. (2003) demonstrated that pretreatment with isoflurane increased the density of the K_{ATP} current induced by pinacidil in guinea-pig ventricular cells. In this study, isoflurane *per se* failed to activate the K_{ATP} current in mouse ventricular cells. However, isoflurane transiently enhanced the K_{ATP} current and then inhibited the current when the K_{ATP} current was pre-activated by pinacidil. When both isoflurane and pinacidil were applied simultaneously in mouse ventricular cells, pinacidil failed to activate the K_{ATP} current. Thus, the effects of isoflurane on cardiac K_{ATP} channels are undoubtedly complex. Isoflurane has inhibiting as well as activating effects on cardiac K_{ATP} channels and the isoflurane effect appears to be dependent on the experimental conditions, which might explain the inconsistencies in previous reports (Han *et al*., 1996; Kwok *et al*., 2002).

The mechanism(s) by which isoflurane produced dual effects on cardiac K_{ATP} channels could not be clarified from this study. It was reported that isoflurane reduced the openings of K_{ATP} channels at normal pH, but facilitated them at reduced pH in inside-out patches of guinea-pig ventricular cells (Stadnicka and Bosnjak, 2003). In addition, the isoflurane-induced sensitization of the cardiac K_{ATP} channel to pinacidil was ascribed to generation of reactive oxygen species and/or activation of protein kinase C (An *et al*., 2004; Marinovic *et al*., 2005). As anionic phospholipids such as phosphatidylinositol 4,5-diphosphate are known to modulate K_{ATP} channel activity (Baukrowitz *et al*., 1998; Shyng and Nichols, 1998), isoflurane might affect cardiac K_{ATP} channels through the changes of membrane phospholipids. One possibility is that isoflurane might directly inhibit cardiac K_{ATP} channels and indirectly activate them through changes in membrane composition or second messengers. However, this is entirely speculative and further experimentation is needed to prove it. In this context, halothane, another volatile anaesthetic, inhibited the K_{ATP} current induced by metabolic inhibition, but did not affect the current that evoked by pinacidil in guinea-pig ventricular cells (Kwok *et al*., 2002), although the same anaesthetic evoked coronary vasodilation probably through the activation of K_{ATP} channels (Crystal *et al*., 1997). Thus, it appears that volatile anaesthetics including isoflurane do not easily activate cardiac K_{ATP} channels under basal conditions, although isoflurane can activate the channels under certain conditions.

In this study, isoflurane activated the vascular-type Kir6.1/SUR2B channels but not the cardiac-type Kir6.2/SUR2A channels expressed in HEK293 cells, which is consistent with the results of the experiments using mouse perfused heart and vascular smooth muscle cells. Isoflurane also activated Kir6.2/SUR2B channels, although the time course of K^+ channel activation was relatively slow. Therefore, SUR2B may be important for the activation of K_{ATP} channels by isoflurane.

At the present time, it is difficult to decide whether the isoflurane effect on K_{ATP} channels is due to direct interaction with the K_{ATP} channel subunits or to secondary changes of intracellular messengers. SUR2A and SUR2B differ by only 42 amino acids in the C-terminus, caused by alternative splicing (Isomoto *et al*., 1996). The molecular mechanism by which these 42 C-terminal amino acids determine the pharmacological characteristics of SUR2A and SUR2B is not fully understood. Recently, it has been demonstrated that SUR2B shows greater affinity to K^+ channel openers compared with SUR2A, which is related to a difference in Mg-nucleotide handling between these two SUR2 isoforms (Reimann *et al*., 2000). In addition, several studies have suggested that interaction of nucleotide-binding domains (NBD1 and NBD2) with ATP and ADP and subsequent dimerization may allosterically regulate pore openings (Yamada and Kurachi, 2004; Yamada *et al*., 2004). They have suggested that nucleotide-bound NBD1 and NBD2 more strongly promote conformational changes in SUR2B than SUR2A (Yamada and Kurachi, 2005). Isoflurane might facilitate the dimerization of NBDs more efficiently in SUR2B than SUR2A. Further experiments are required to clarify the precise mechanism by which isoflurane activates K_{ATP} channels via interaction with SUR2B.

Isoflurane was reported to produce a cardioprotective effect, a phenomena known as cardiac preconditioning, in experimental animals (Kersten *et al*., 1997; Ismaeil *et al*., 1999). It has been postulated that cardiomyocytes have two distinct types of K_{ATP} channels, that is, sarcolemmal K_{ATP} channels and mitochondrial K_{ATP} channels, and mitochondrial K_{ATP} channels play an important role in cardiac preconditioning, although the molecular identity of mitochondrial K_{ATP} channels remains unclarified (O'Rourke, 2004). It has been demonstrated that isoflurane activates mitochondrial K_{ATP} channels, as shown by flavoprotein oxidation (Kohro *et al*., 2001) and enhancement of diazoxide-induced flavoprotein oxidation by isoflurane (Zaugg *et al*., 2002). Such anaesthetic preconditioning through the activation

of mitochondrial K_{ATP} channels may lead to more favourable outcomes in patients undergoing coronary artery bypass graft surgery (Zaugg *et al*., 2003a, b). In addition to the protective effect of isoflurane on cardiomyocytes, the coronary vasodilatation induced by isoflurane, as observed in this study, may improve the perioperative cardiovascular outcome in patients at high risk of cardiovascular complications.

In summary, this study has demonstrated that isoflurane activates K_{ATP} channels in vascular smooth muscle cells, thereby producing coronary vasodilation and that the SUR2B subunit is important for the isoflurane-induced activation of vascular-type K_{ATP} channels.

Acknowledgements

We thank Dr S Seino (Kobe University) for providing us the cDNAs of rat SUR2A, rat SUR2B, human Kir6.2 and rat Kir6.1. We also thank Y Reien and I Sakashita for excellent technical and secretarial assistance. This work was supported by research grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan and a grant from K Watanabe Research Foundation.

Conflict of interest

The authors state no conflict of interest.

References

An J, Standnicka A, Kwok WM, Bosnjak ZJ (2004). Contribution of reactive oxygen species to isoflurane-induced sensitization of cardiac sarcolemmal adenosine triphosphate-sensitive potassium channel to pinacidil. *Anesthesiology* **100**: 575–580.

Baukrowitz T, Schulte U, Oliver D, Herlitze S, Krauter T, Tucker SJ *et al*. (1998). PIP_2 and PIP as determinants for ATP inhibition of K_{ATP} channels. *Science* **282**: 1141–1144.

Cason BA, Shubayev I, Hickey RF (1994). Blockade of adenosine triphosphate-sensitive potassium channels eliminates isoflurane-induced coronary artery vasodilation. *Anesthesiology* **81**: 1245–1255.

Chutkow WA, Pu J, Wheeler MT, Wada T, Makielski JC, Burant CF *et al*. (2002). Episodic coronary artery vasospasm and hypertension develop in the absence of Sur2 K_{ATP} channels. *J Clin Invest* **110**: 203–208.

Crystal GJ, Gurevicius J, Salem MR, Zhou X (1997). Role of adenosine triphosphate-sensitive potassium channels in coronary vasodilation by halothane, isoflurane, and enflurane. *Anesthesiology* **86**: 448–458.

Eger EI (1981). Isoflurane: a review. *Anesthesiology* **55**: 559–576.

Fujimoto K, Bosnjak ZJ, Kwok WN (2002). Isoflurane-induced facilitation of the cardiac sarcolemmal K_{ATP} channel. *Anesthesiology* **97**: 57–65.

Gassmayr S, Stadnicka A, Suzuki A, Kwok WN, Bosnjak ZJ (2003). Isoflurane sensitizes the cardiac sarcolemmal adenosine triphosphate-sensitive potassium channel to pinacidil. *Anesthesiology* **98**: 114–120.

Gribble FM, Ashfield R, Ammla C, Ashcroft FM (1997). Properties of cloned ATP-sensitive K^+ currents expressed in *Xenopus* oocytes. *J Physiol (London)* **498**: 87–98.

Han J, Kim E, Ho WK, Earm YE (1996). Effects of volatile anesthetic isoflurane on ATP-sensitive K^+ channels in rabbit ventricular myocytes. *Biochem Biophys Res Commun* **229**: 852–856.

Inagaki N, Gonoi T, Clement IV JP, Namba N, Inazawa J, Gonzalez G *et al*. (1995). Reconstitution of I_{KATP}: an inward rectifier subunit plus the sulfonylurea receptor. *Science* **270**: 1166–1170.

Inagaki N, Gonoi T, Clement IV JP, Wang CZ, Agular-Bryan L, Bryan J *et al*. (1996). A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels. *Neuron* **16**: 1011–1017.

Ismaeil MS, Trachenko I, Gamperl AK, Hickey RF, Cason BA (1999). Mechanisms of isoflurane-induced myocardial preconditioning in rabbits. *Anesthesiology* **90**: 812–821.

Isomoto S, Kondo C, Yamada M, Matsumoto S, Higashiguchi O, Horio Y *et al*. (1996). A novel sulfonylurea receptor forms with BIR (Kir6.2) a smooth muscle type ATP-sensitive K^+ channel. *J Biol Chem* **271**: 24321–24324.

Kersten JR, Schmeling TJ, Pagel PS, Gross GJ, Warltier DC (1997). Isoflurane mimics ischemic preconditioning via activation of K_{ATP} channels: reduction of myocardial infarct size with an acute memory phase. *Anesthesiology* **87**: 361–370.

Kohro S, Hogan QH, Nakane Y, Yamakage M, Bosnjak ZJ (2001). Anesthetic effects on mitochondrial ATP-sensitive K channel. *Anesthesiology* **95**: 1435–1440.

Kwok WN, Martinelli AT, Fujimoto K, Suzuki A, Stadnicka A, Bosnjak ZJ (2002). Differential modulation of the cardiac adenosine triphosphate-sensitive potassium channel by isoflurane and halothane. *Anesthesiology* **97**: 50–56.

Marinovic J, Bosnjak ZJ, Stadnicka A (2005). Preconditioning by isoflurane induces lasting sensitization of the cardiac sarcolemmal adenosine triphosphate-sensitive potassium channel by a protein kinase C-δ-mediated mechanism. *Anesthesiology* **103**: 540–547.

Miki T, Suzuki M, Shibasaki T, Uemura H, Sato T, Yamaguchi K *et al*. (2002). Mouse model of Prinzmetal angina by disruption of the inward rectifier Kir6.1. *Nat Med* **8**: 466–472.

O'Rourke B (2004). Evidence for mitochondrial K^+ channels and their role in cardioprotection. *Circ Res* **94**: 420–432.

Ozaki S, Nakaya H, Gotoh Y, Azuma M, Kemmotsu O, Kanno M (1990). Effects of isoflurane on conduction velocity and maximum rate of rise of action potential upstroke in guinea pig papillary muscles. *Anesth Analg* **70**: 618–623.

Reimann F, Gribble FM, Ashcroft FM (2000). Differential response to K_{ATP} channels containing SUR2A or SUR2B subunits to nucleotides and pinacidil. *Mol Pharmacol* **58**: 1318–1325.

Reiz S, Balfors E, Sorensen MB, Ariola Jr S, Friedman A, Truedsson H (1983). Isoflurane: a powerful coronary vasodilator in patients with coronary artery disease. *Anesthesiology* **59**: 91–97.

Sakamoto N, Uemura H, Hara Y, Saito T, Masuda Y, Nakaya H (1998). Bradykinin B2-receptor-mediated modulation of membrane currents in guinea-pig cardiomyocytes. *Br J Pharmacol* **125**: 283–292.

Seino S, Miki T (2003). Physiological and pathophysiological roles of ATP-sensitive K^+ channels. *Prog Biophys Mol Biol* **81**: 133–176.

Shyng S, Nichols CG (1998). Membrane phospholipid control of nucleotide sensitivity of K_{ATP} channels. *Science* **282**: 1138–1141.

Stadnicka A, Bosnjak ZJ (2003). Isoflurane decreases ATP sensitivity of guinea pig cardiac sarcolemmal K_{ATP} channel at reduced intracellular pH. *Anesthesiology* **98**: 396–403.

Suzuki M, Li RA, Miki T, Uemura H, Sakamoto N, Ohmoto-Sekine Y *et al*. (2001). Functional roles of cardiac and vascular ATP-sensitive potassium channels clarified by Kir6.2-knockout mice. *Circ Res* **88**: 570–577.

Suzuki M, Sasaki N, Miki T, Sakamoto N, Ohmoto-Sekine Y, Tamagawa M *et al*. (2002). Role of sarcolemmal K_{ATP} channels in cardioprotection against ischemia/reperfusion injury in mice. *J Clin Invest* **109**: 509–516.

Yamada M, Ishii M, Hibino H, Kurachi Y (2004). Mutation in nucleotide-binding domains of sulfonylurea receptor 2 evokes Na-ATP-dependent activation of ATP-sensitive K^+ channels: Implication for dimerization of nucleotide-binding domains to induce channel opening. *Mol Pharmacol* **66**: 807–816.

Yamada M, Isomoto S, Matsumoto S, Kondo C, Shindo T, Horio Y *et al*. (1997). Sulphonylurea receptor 2B and Kir6.1 form a sulphonylurea-sensitive but ATP-insensitive K^+ channel. *J Physiol (London)* **499**: 715–720.

Yamada M, Kurachi Y (2004). The nucleotide-binding domains of sulfonylurea receptor 2A and 2B play different functional roles in nicorandil-induced activation of ATP-sensitive K^+ channels. *Mol Pharmacol* **65**: 1198–1207.

Yamada M, Kurachi Y (2005). A functional role of the C-terminal 42 amino acids of SUR2A and SUR2B in the physiology and pharmacology of cardiovascular ATP-sensitive K^+ channels. *J Mol Cell Cardiol* **39**: 1–6.

Zaugg M, Lucchinetti E, Garcia C, Pasch T, Scaub MC (2003a). Anaesthetics and cardiac preconditioning. Part II. Clinical implications. *Br J Anaesth* **91**: 566–576.

Zaugg M, Lucchinetti E, Spahn DR, Pasch T, Schaub MC (2002). Volatile anesthetics mimic cardiac preconditioning by priming the activation of mitochondrial K_{ATP} channels via multiple signaling pathways. *Anesthesiology* **97**: 4–14.

Zaugg M, Lucchinetti E, Uecker M, Pasch T, Schaub MC (2003b). Anaesthetics and cardiac preconditioning. Part I. Signalling and cytoprotective mechanisms. *Br J Anaesth* **91**: 551–565.

Zhou X, Abboud W, Manabat NC, Salem MR, Crystal GJ (1998). Isoflurane-induced dilation of porcine coronary arterioles is mediated by ATP-sensitive potassium channels. *Anesthesiology* **89**: 182–189.