Anesthesiology 2001; 95:939–46

Role of Potassium Channels in Isoflurane- and Sevoflurane-induced Attenuation of Hypoxic Pulmonary Vasoconstriction in Isolated Perfused Rabbit Lungs

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Background: **Although potassium channels are thought to be responsible for the initiation of hypoxic pulmonary vasoconstriction (HPV), their role in the HPV-inhibitory effect of volatile anesthetics is unclear. The current study tested if the HPV-inhibitory effect of isoflurane and sevoflurane can be affected by changing the potassium-channel opening status with specific potassium-channel inhibitors in isolated rabbit lungs.**

Methods: **Isolated rabbit lungs were divided into eight groups (n = 6 each in isoflurane groups and n = 8 in sevoflurane groups): those receiving no inhibitor treatment = control-isoflurane and control-sevoflurane groups; those treated with an adenosine triphosphate-sensitive potassium (K_{ATP})-channel inhibitor, glibenclamide = glibenclamide–isoflurane and glibenclamide-sevoflurane groups; those treated with a high-conductance calcium-activated potassium (K_{Ca})-channel inhibitor, iberiotoxin = iberiotoxin–isoflurane and iberiotoxin–sevoflurane groups; and those treated with a voltage-sensitive potassium (K_V)-channel inhibitor, 4-aminopyridine = 4-aminopyridine–isoflurane and 4-aminopyridine–sevoflurane groups. The effect of anesthetic on HPV was tested by exposure of the lungs to isoflurane at a concentration of 0, 0.5, 1, or 2 minimum alveolar concentration, or to sevoflurane at a concentration of 0, 0.5, 1, or 1.62 minimum alveolar concentration. The relation between anesthetic concentrations and the HPV response was analyzed by the Wagner equation.**

Results: **The inhibition of K_V channels by 4-aminopyridine and K_{Ca} channels by iberiotoxin augmented the HPV response. The isoflurane-induced attenuation of HPV was attenuated by voltage-sensitive potassium-channel inhibition with 4-aminopyridine, potentiated by K_{Ca}-channel inhibition with iberiotoxin, but not affected by K_{ATP}-channel inhibition with glibenclamide. The sevoflurane-induced attenuation of HPV was not affected by any of the potassium-channel inhibitors.**

Conclusions: **Isoflurane may modulate the HPV response partially through K_{Ca} and K_V channels, but sevoflurane may attenuate the HPV response through other pathways rather than through the currently investigated potassium channels in isolated rabbit lungs.**

HYPOXIC pulmonary vasoconstriction (HPV) is an important and unique response to protect against hypoxia by diverting blood flow away from poorly ventilated alveoli to well-ventilated regions. The exact mechanism of HPV is not fully understood, but it appears to involve direct effects on both the endothelium and vascular smooth muscle cells. There is growing evidence to suggest that hypoxia mediates vasoconstriction, at least in part, through various types of potassium channels.[1–4]

Although the inhibition of HPV is not a general characteristic of volatile anesthetics,[5] volatile anesthetics inhibit HPV during various circumstances.[6–9] The exact mechanisms of inhibition are not clear, although some possible mechanisms, such as cyclooxygenase pathways[8,10,11] and calcium channel pathways,[12] have been suggested. It is unclear if potassium channels also play an important role in the inhibitory effect of volatile anesthetics on HPV. Various subtypes of potassium channels are located on pulmonary vascular smooth muscle cells, including voltage-dependent potassium (K_V) channels, adenosine triphosphate–sensitive potassium (K_{ATP}) channels, and calcium-activated potassium (K_{Ca}) channels.[13] Volatile anesthetics can depress K_V channels in vascular smooth muscle cells of arteries[14,15] and veins.[16] It is also reported that isoflurane-mediated hyperpolarization (and associated relaxation) of mesenteric vascular smooth muscle can be attributed in part to an enhanced (or maintained) opening of K_{Ca} and K_{ATP} channels.[17] Based on these studies, it is possible that volatile anesthetics may modulate HPV through certain potassium channels. In the current study, we hypothesized that potassium channels may play an important role in modulating the effect of volatile anesthetics on HPV, and that the HPV-inhibitory effect of isoflurane and sevoflurane may be affected by changing the potassium-channel opening status with specific potassium-channel inhibitors. Isolated perfused rabbit lungs were used in this study.

Materials and Methods

Isolated Lung Preparation

The experimental protocol was approved by the Tottori University Faculty of Medicine Laboratory Animal Care Committee. Fifty-six female Japanese white rabbits (weighing 1.8–2.7 kg) were anesthetized with pentobarbital (20 mg/kg intravenously) and ketamine (30 mg/kg intramuscularly). Heparin was injected intravenously (500 U/kg) before surgery. After local anesthesia with 1.0% lidocaine and tracheostomy, the lungs were mechanically ventilated with a ventilator (model 681; Harvard Apparatus, Natick, MA) using room air at a rate of

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Received from the Department of Anesthesia and Reanimatology, Tottori University Faculty of Medicine, Tottori, Yonago, Japan, and the Department of Anesthesia, Toyooka Hospital, Toyooka, Hyogo, Japan. Submitted for publication December 10, 1999. Accepted for publication April 12, 2001. Support was provided solely from institutional and/or department sources. Presented in part at the annual meeting of the Japan Society of Pharmacoanesthesiology, Tokyo, Japan, June 11, 1999, and the 74th Clinical and Scientific Congress of the International Anesthesia Research Society, Honolulu, Hawaii, March 10–14, 2000.

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40 strokes per minute, a tidal volume of 10 ml/kg, and a positive end-expiratory pressure of 2 cm H_2O until the pulmonary artery and left atrium were cannulated, after which the lungs were ventilated at the ventilator settings with a warm humidified gas mixture of 21% O_2 and 5% CO_2, and balance N_2. The right common carotid artery was dissected, and a blood sample of approximately 70 ml was collected. After sternotomy, the pulmonary artery and left atrium were cannulated *via* the right and left ventricles and were secured in place with sutures. The heart, lungs, and mediastinal structures were removed *en bloc* and placed in a humidified chamber at a temperature of 37–38°C. The isolated lungs were perfused throughout the study at a flow rate of 40 ml · kg^{-1} · min^{-1} using a peristaltic pump (Harvard 1215; Harvard Apparatus) with a mixture of autologous whole blood with a physiological salt solution (PSS). The hematocrit of the perfusate was adjusted to approximately 15%. PSS contained 119 mM NaCl, 4.7 mM KCl, 1.17 mM $MgSO_4$, 22.61 mM $NaHCO_3$, 1.18 mM KH_2PO_4, and 3.2 mM $CaCl_2$. The following were added to each 100 ml of PSS stock solution: 100 mg dextrose, 20 mU insulin, and 3 g bovine serum albumin (Sigma Chemical Co., St. Louis, MO). The pH of the perfusate was adjusted to 7.35–7.45 by the addition of sodium bicarbonate. Indomethacin (Wako Pure Chemical Industries, Osaka, Japan) was added to the mixture at a dose of 20 μg/ml to reduce the production of vasoactive factors that can potentially produce lung injury. After removal from the chest cavity, the lungs were gently inflated several times until obvious atelectasis had cleared away.

Pulmonary arterial (P_{pa}) and venous (P_{pv}) pressures were continuously monitored by transducers connected to amplifiers (model 2238; San-ei, Tokyo, Japan). A flow probe (model FF-050T; Nihon Kohden, Tokyo, Japan) connected to an electromagnetic flow meter (MFV-3100; Nihon Kohden) was placed in the perfusion circuit for continuous monitoring of blood flow. P_{pv} was adjusted to 5 mmHg to ensure that P_{pv} exceeded the mean airway pressure. Zero level for pressures was assigned to the bottom of the lung in the chamber. All signals were digitized using an analog-to-digital converter (DigiData 1200; Axon Instruments, Foster City, CA) and were analyzed using commercially available software (Axograph version 3.0; Axon Instruments).

Experimental Protocol

After 30 min of stabilization, the 56 isolated rabbit lung preparations were divided into eight groups (n = 6 each in isoflurane groups and n = 8 each in sevoflurane groups) based on the anesthetic and potassium-channel inhibitor administered in the study. The concentrations of potassium-channel inhibitors in the perfusate were all between 0.5 and 2 orders of magnitude greater than those reported producing half block of the respective channels.[18,19] In the control-isoflurane (control-ISO) and control-sevoflurane (control-SEVO) groups, no potassium-channel inhibitors were added. In the glibenclamide–isoflurane (Glib-ISO) and glibenclamide–sevoflurane (Glib-SEVO) groups, the lungs were pretreated with 10^{-5} M glibenclamide (Wako Pure Chemical Industries). In the iberiotoxin–isoflurane (IbTX-ISO) and iberiotoxin–sevoflurane (IbTX-SEVO) groups, the lungs were pretreated with 10^{-7} M iberiotoxin (Wako Pure Chemical Industries), a highly selective K_{Ca}-channel inhibitor. In the 4-aminopyridine–isoflurane (4AP-ISO) and 4-aminopyridine–sevoflurane (4AP-SEVO) groups, the lungs were pretreated with 10^{-3} M 4-aminopyridine (Wako Pure Chemical Industries), a K_V-channel inhibitor. The inhibitors were freshly prepared in a small volume of *N*,*N*-dimethylformamide (Wako Pure Chemical Industries) and PSS mixture for glibenclamide and PSS mixture for 4-aminopyridine and iberiotoxin just before each of the experiments. The inhibitors were added into the reservoir at the beginning of stabilization, and their concentrations were calculated on the basis of the total volume of the perfusate. Isoflurane (Abbott Laboratories, North Chicago, IL) or sevoflurane (Maruishi Pharmaceutical, Osaka, Japan) was administered using isoflurane (Acoma Medical Industry, Tokyo, Japan) or sevoflurane (Penlon, Abing, Oxon, UK) vaporizers. The concentrations of isoflurane and sevoflurane in inspired gas were monitored with an anesthetic agent monitor (Anesthetic Gas Monitor Type 1304; Brüel & Kjær, Nærum, Denmark).

The HPV response was induced by switching the inspired gas to a hypoxic gas mixture (3% O_2, 5% CO_2, balance N_2) for 5 min. This period of 5 min was determined as a sufficient time to obtain the maximum HPV response based on our previous study.[8] Based on the previous report from another laboratory that the anesthetic concentration in the perfusate equilibrated with that in the lungs within 10 min,[6] the lungs were ventilated for 15 min with each concentration of volatile anesthetics in a normoxia condition, then for 5 min with the hypoxic gas mixture containing the same concentration of anesthetics. Three concentrations of isoflurane (0.5, 1, or 2 minimum alveolar concentration [MAC]) or sevoflurane (0.5, 1, 1.62 MAC) were administered randomly to each perfused lung. After the 5-min HPV response test, a fresh normoxic gas mixture was administered for 8 min to washout the anesthetics and allow P_{pa} to return to the baseline level.

Measurements

The pulmonary vascular response to HPV was expressed as the difference in P_{pa} (ΔP_{pa}) before and after 5 min of hypoxic stimuli. The inhibitory effect of anesthetics was expressed as a percentage of inhibition ($\%R_{max}$), which represented the value of HPV responses in the presence of anesthetic (R) over the value of HPV response in the absence of anesthetic (R_{max}). The inhibitory effects of anesthetics on HPV were compared by the concentrations in MAC at which 50% of depression of HPV

Table 1. Effect of Isoflurane on the Hypoxic Pulmonary Vasoconstriction Response in the Absence and Presence of Potassium Channel Inhibitors

	0 MAC		0.5 MAC		1 MAC		2 MAC	
	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia
Control isoflurane								
P_{pa}	11.3 ± 0.9	16.8 ± 2.5*	11.6 ± 1.2	15.9 ± 1.9*	11.6 ± 1.2	14.5 ± 2.4*	12.0 ± 1.6	12.9 ± 2.2*
P_{pv}	5.3 ± 0.4	5.2 ± 0.4	5.1 ± 0.5	5.0 ± 0.5	5.0 ± 0.6	5.0 ± 0.7	5.2 ± 0.5	5.2 ± 0.5
ΔP_{pa}	—	5.5 ± 2.3	—	4.3 ± 1.2	—	2.9 ± 1.3†	—	0.9 ± 0.7†
Flow	80.9 ± 2.3	81.4 ± 1.2	80.5 ± 2.4	81.3 ± 1.4	80.7 ± 2.6	80.7 ± 1.6	81.3 ± 2.6	80.9 ± 2.6
Glib isoflurane								
P_{pa}	11.0 ± 1.1	16.7 ± 2.3*	11.2 ± 1.1	15.5 ± 2.4*	11.4 ± 1.1	14.2 ± 1.8*	11.6 ± 1.1	12.5 ± 1.2*
P_{pv}	5.1 ± 0.6	5.0 ± 0.6	5.1 ± 0.7	5.0 ± 0.6	5.1 ± 0.6	5.0 ± 0.6	5.1 ± 0.7	5.0 ± 0.7
ΔP_{pa}	—	5.7 ± 2.1	—	4.3 ± 2.0	—	2.7 ± 1.4†	—	1.0 ± 0.2†
Flow	82.3 ± 2.7	82.0 ± 2.6	83.0 ± 1.4	82.8 ± 1.5	82.2 ± 2.4	82.0 ± 2.2	82.4 ± 2.4	82.2 ± 2.5
4-AP isoflurane								
P_{pa}	16.8 ± 4.1	24.8 ± 5.6*	17.4 ± 2.9	24.5 ± 5.0*	18.4 ± 3.0	22.8 ± 3.9*	19.1 ± 3.0	21.4 ± 3.5*
P_{pv}	5.4 ± 0.3	5.3 ± 0.4	5.2 ± 0.3	5.1 ± 0.2	5.3 ± 0.3	5.3 ± 0.3	5.4 ± 0.3	5.3 ± 0.3
ΔP_{pa}	—	8.1 ± 2.3	—	7.1 ± 2.8	—	4.4 ± 1.4†	—	2.3 ± 0.8†
Flow	79.4 ± 5.3	79.3 ± 4.5	79.2 ± 6.0	79.0 ± 5.8	79.0 ± 5.6	79.0 ± 5.5	78.4 ± 5.7	78.7 ± 5.1
IbTX isoflurane								
P_{pa}	11.8 ± 1.8	22.8 ± 1.8*	11.6 ± 1.6	17.9 ± 2.9*	11.3 ± 0.8	14.5 ± 1.5*	11.4 ± 0.8	12.0 ± 0.9*
P_{pv}	5.5 ± 0.3	5.3 ± 0.3	5.4 ± 0.3	5.3 ± 0.3	5.5 ± 0.3	5.3 ± 0.2	5.5 ± 0.2	5.2 ± 0.1
ΔP_{pa}	—	10.9 ± 1.9	—	6.3 ± 2.2†	—	3.1 ± 1.1†	—	0.7 ± 0.3†
Flow	83.2 ± 5.4	83.1 ± 5.7	83.3 ± 5.4	83.3 ± 5.7	82.8 ± 4.9	83.3 ± 5.7	83.4 ± 5.6	83.3 ± 5.6

Values are mean ± SD (n = 6 per group).

* $P < 0.01$ *versus* normoxia; † $P < 0.01$ *versus* 0 minimum alveolar concentration (MAC) in ΔP_{pa} within group.

P_{pa} = pulmonary arterial pressure (mmHg); P_{pv} = pulmonary venous pressure (mmHg); ΔP_{pa} = the difference in P_{pa} before and after hypoxic stimuli; flow = perfusion blood flow rate (ml/min); glib = glibenclamide, the adenosine triphosphate–sensitive potassium channel inhibitor; 4-AP = 4-aminopyridine, the voltage-sensitive potassium channel inhibitor; IbTX = iberiotoxin, the high-conductance calcium-activated potassium channel inhibitor.

(ED_{50}) occurred. The relation between the concentration in MAC units and response in $\%R_{max}$ was analyzed by the Wagner equation[20] for sigmoid curves:

$$\%Rmax = 100(MAC)^s/\{Q^{-1} + (MAC)^s\},$$

where Q is the value of $R/(R_{max} - R)$ at 1 MAC.

The lungs were used to estimate the tissue wet-to-dry weight ratio. After recording the wet weight of the lung tissue, the lungs were placed in a drying oven at 60°C for 2 weeks and reweighed. Wet-to-dry weight ratio was calculated using the formula:

$$(\text{wet weight} - \text{dry weight})/\text{dry weight}.$$

Statistical Analysis

All data are presented as mean ± SD. Differences within groups were analyzed by the one-way analysis of variance with repeated measures, followed by *post hoc* analysis with a Scheffé test (Statview 4.5, Abacus Concepts, Berkeley, CA). ED_{50} was compared by analysis of variance and *post hoc* analysis with a Scheffé test. ΔP_{pa} in the absence of volatile anesthetics among groups was compared by analysis of variance and *post hoc* analysis with Scheffé test. Significance was determined when P was less than 0.05.

Results

The hematocrit of the perfusate of each group ranged between 14.4 ± 0.5% to 15.2 ± 1.0%. Because there were no differences in pH or oxygen and carbon dioxide partial pressures among groups at different time intervals, we averaged the data of the eight groups. The mean values of pH, oxygen partial pressure, and carbon dioxide partial pressure were 7.37 ± 0.08, 150.1 ± 6.0 mmHg, and 37.4 ± 2.0 mmHg, respectively, during normoxia, and 7.38 ± 0.07, 30.6 ± 3.4 mmHg, and 37.3 ± 1.4 mmHg, respectively, during hypoxia. The lung wet-to-dry weight ratio of each group ranged between 5.0 ± 0.4 and 5.3 ± 0.3, and there was no difference among groups. The effects of isoflurane and sevoflurane on pulmonary vascular pressures and the HPV response in the absence and presence of potassium-channel inhibitors are shown in table 1 (isoflurane) and table 2 (sevoflurane).

Effects of Potassium-channel Inhibitors on Pulmonary Arterial Pressure and the Hypoxic Pulmonary Vasoconstriction Response in the Absence of Anesthetics in Normoxia and Hypoxia

The K_V-channel inhibition by 4-aminopyridine caused a significant increase in P_{pa}. The baseline P_{pa} in the 4AP-ISO group was significantly higher than that in the control-ISO group (16.8 ± 4.1 *vs.* 11.3 ± 0.9 mmHg, $P < 0.05$). P_{pa} was significantly higher in the 4AP-SEVO group than in the control-SEVO group (15.5 ± 1.2 *vs.* 12.1 ± 1.4 mmHg, $P < 0.05$). There were no significant differences in P_{pa} among other groups. ΔP_{pa} was significant augmented by K_V- and K_{Ca}-channel inhibition (4-

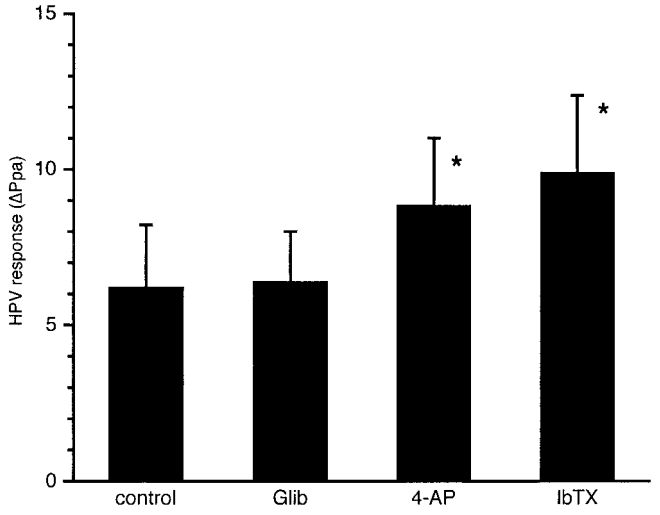

Fig. 1. Changes in hypoxic pulmonary vasoconstriction (HPV) response (ΔP_{pa}) by potassium channel inhibition in the absence of volatile anesthetics. Data are mean ± SD, n = 14 per group. *$P < 0.05$ *versus* control. Glib = glibenclamide, the adenosine triphosphate–sensitive potassium (K_{ATP})-channel inhibitor; 4-AP = 4-aminopyridine, the voltage-sensitive potassium (K_V)-channel inhibitor; IbTX = iberiotoxin, the high-conductance calcium-activated K (K_{Ca})-channel inhibitor.

aminopyridine and iberiotoxin), but was not affected by K_{ATP}-channel inhibition (glibenclamide) (fig. 1).

Effects of Isoflurane and Sevoflurane on Pulmonary Arterial Pressure in the Presence or Absence of Potassium-channel Inhibitors in Normoxia

Isoflurane did not affect P_{pa} in normoxia in the absence of potassium-channel inhibitors (table 1). Sevoflurane induced a slight but significant dilation of pulmonary vessels in a dose-dependent fashion: P_{pa} decreased from 12.1 ± 1.4 to 11.1 ± 0.6 mmHg ($P < 0.05$) with the increase of sevoflurane concentration from 0 to 1.62 MAC in the absence of potassium-channel inhibitors (table 2). The effect of isoflurane on P_{pa} during normoxia was changed by K_V-channel inhibition with 4-aminopyridine but was not affected by K_{Ca}- and K_{ATP}-channel inhibition. P_{pa} increased in a dose-dependent fashion from 16.8 ± 4.1 to 19.1 ± 3.0 mmHg ($P < 0.01$) with the increase of isoflurane concentration from 0 to 2 MAC when K_V channels were inhibited by 4-aminopyridine (table 1). The effect of sevoflurane on P_{pa} was not influenced by potassium-channel inhibitions (table 2).

Effect of Potassium-channel Inhibitors on Isoflurane-induced Hypoxic Pulmonary Vasoconstriction Inhibition

Change in P_{pa} was suppressed in a dose-dependent fashion in all isoflurane groups (table 1). The dose–response curve shifted to the right by K_V-channel inhibition in the 4AP-ISO group and to the left by K_{Ca}-channel inhibition in the IbTX-ISO group (fig. 2). The HPV response was inhibited even by 0.5 MAC isoflurane in the IbTX-ISO group, an effect not observed in the control-ISO group. ED_{50} obtained from each curve was 0.93 ± 0.08 MAC in the control-ISO group, 0.91 ± 0.22 MAC in the Glib-ISO group, 1.15 ± 0.10 MAC in the 4AP-ISO group, and 0.59 ± 0.18 MAC in the IbTX-ISO group. ED_{50} was significantly higher in the 4AP-ISO group ($P < 0.05$) and lower in the IbTX-ISO group ($P < 0.05$) than in the control-ISO group, but was not different in the Glib-ISO group from that in the control-ISO group.

Effect of Potassium-channel Inhibitors on Sevoflurane-induced Hypoxic Pulmonary Vasoconstriction Inhibition

Change in P_{pa} was suppressed in a dose-dependent fashion in all sevoflurane groups (table 2). There was no significant difference in the dose–response curve among sevoflurane groups (fig. 3). There was no significant difference in the dose–response curve between control-ISO and control-SEVO groups. ED_{50} calculated from each curve was 0.82 ± 0.21 MAC in the control-SEVO group, 0.87 ± 0.25 MAC in the Glib-SEVO group, 0.96 ±

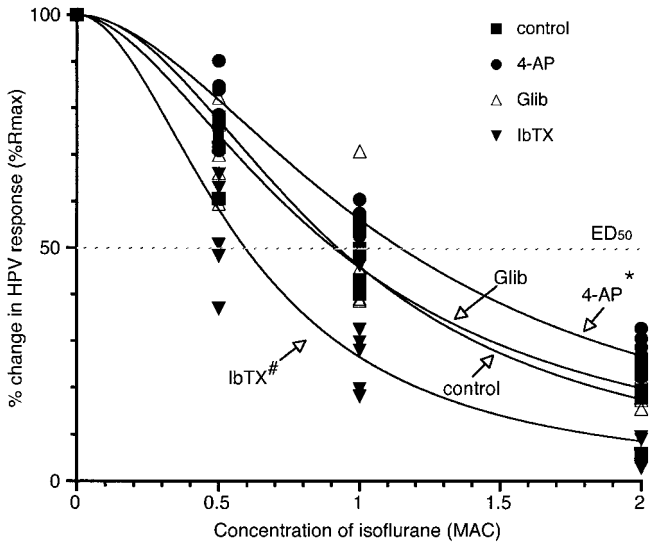

Fig. 2. Dose–response curve of the inhibitory effect of isoflurane on hypoxic pulmonary vasoconstriction (HPV) in the absence and presence of potassium channel inhibitors, n = 6 per group. *Right shift compared with the control-ISO group ($P < 0.05$); #left shift compared with the control-ISO group ($P < 0.05$). The concentration of isoflurane that induced 50% inhibition of HPV (ED_{50}) was 0.93 ± 0.08 MAC in the control-ISO group, 0.91 ± 0.22 MAC in the Glib-ISO group, 1.15 ± 0.10 MAC in the 4AP-ISO group ($P < 0.05$ *vs.* the control-ISO group), and 0.59 ± 0.18 MAC in the IbTX-ISO group ($P < 0.05$ *vs.* the control-ISO group). Glib = glibenclamide, the adenosine triphosphate–sensitive potassium (K_{ATP})-channel inhibitor; 4-AP = 4-aminopyridine, the voltage-sensitive potassium (K_V) channel inhibitor; IbTX = iberiotoxin, the high-conductance calcium-activated potassium (K_{Ca})-channel inhibitor; ISO = isoflurane.

Table 2. Effect of Sevoflurane on the Hypoxic Pulmonary Vasoconstriction Response in the Absence and Presence of Potassium Channel Inhibitors

	0 MAC		0.5 MAC		1 MAC		1.6 MAC	
	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia
Control sevoflurane								
P_{pa}	12.1 ± 1.4	18.8 ± 2.8*	11.7 ± 1.2	16.9 ± 2.2*	11.3 ± 1.0	13.8 ± 1.8*	11.1 ± 0.6	11.7 ± 0.7*
P_{pv}	4.9 ± 0.3	4.8 ± 0.3	4.9 ± 0.2	4.9 ± 0.3	4.9 ± 0.3	4.9 ± 0.4	4.9 ± 0.2	4.9 ± 0.2
ΔP_{pa}		6.7 ± 1.8		5.2 ± 1.4		2.5 ± 1.0†		0.6 ± 0.3†
Flow	80.0 ± 5.2	80.0 ± 5.0	80.0 ± 5.0	80.0 ± 5.3	80.0 ± 5.1	79.1 ± 4.6	80.0 ± 4.8	79.7 ± 5.0
Glib sevoflurane								
P_{pa}	11.8 ± 0.9	18.7 ± 1.3*	11.2 ± 0.9	17.3 ± 1.8*	11.1 ± 0.9	14.1 ± 1.7*	10.9 ± 0.9	11.7 ± 1.2*
P_{pv}	5.0 ± 0.2	4.9 ± 0.2	4.9 ± 0.3	4.9 ± 0.3	4.9 ± 0.2	4.8 ± 0.4	4.9 ± 0.3	4.8 ± 0.3
ΔP_{pa}	—	6.9 ± 1.0	—	6.0 ± 1.8	—	3.0 ± 1.1†	—	0.8 ± 0.6†
Flow	79.4 ± 4.7	79.4 ± 4.7	80.0 ± 4.2	79.6 ± 4.0	80.1 ± 3.9	80.1 ± 4.0	79.9 ± 4.0	79.7 ± 4.5
4-AP sevoflurane								
P_{pa}	15.5 ± 1.2	24.9 ± 2.4*	15.3 ± 1.3	22.9 ± 2.4*	15.0 ± 1.5	29.7 ± 2.0*	14.6 ± 1.0	16.8 ± 2.5*
P_{pv}	5.0 ± 0.5	5.0 ± 0.6	5.0 ± 0.5	5.0 ± 0.6	5.2 ± 0.7	5.1 ± 0.7	5.0 ± 0.6	5.0 ± 0.6
ΔP_{pa}	—	9.4 ± 2.1	—	7.6 ± 1.9	—	4.7 ± 1.5†	—	2.2 ± 0.8†
Flow	81.4 ± 3.4	81.4 ± 3.4	82.0 ± 3.5	81.6 ± 3.4	81.9 ± 3.5	81.7 ± 3.8	81.9 ± 3.8	81.6 ± 4.0
IbTX sevoflurane								
P_{pa}	11.9 ± 0.8	21.0 ± 3.0*	11.5 ± 0.5	18.5 ± 3.1*	10.9 ± 0.9	13.9 ± 1.0*	10.8 ± 1.0	11.4 ± 1.0*
P_{pv}	4.8 ± 0.3	4.8 ± 0.4	4.8 ± 0.4	4.7 ± 0.3	4.8 ± 0.4	4.8 ± 0.4	4.8 ± 0.4	4.8 ± 0.4
ΔP_{pa}	—	9.1 ± 2.7	—	7.0 ± 3.0	—	3.0 ± 0.8†	—	0.6 ± 0.3†
Flow	82.9 ± 4.6	82.7 ± 4.3	82.7 ± 4.7	83.4 ± 4.7	82.8 ± 4.4	83.2 ± 4.4	82.9 ± 4.8	82.8 ± 4.8

Values are mean ± SD (n = 8 per group).

* $P < 0.01$ *versus* normoxia; † $P < 0.01$ *versus* 0 minimum alveolar concentration (MAC) in ΔP_{pa} within group.

P_{pa} = pulmonary arterial pressure (mmHg); P_{pv} = pulmonary venous pressure (mmHg); ΔP_{pa} = the difference in P_{pa} before and after hypoxic stimuli; flow = perfusion blood flow rate (ml/min); glib = glibenclamide, the adenosine triphosphate–sensitive potassium channel inhibitor; 4-AP = 4-aminopyridine, the voltage-sensitive potassium channel inhibitor; IbTX = iberiotoxin, the high conductance calcium-activated potassium channel inhibitor.

0.24 MAC in the 4AP-SEVO group, and 0.76 ± 0.17 MAC in the IbTX-SEVO group.

Discussion

The new findings of this study were as follows: (1) the inhibition of K_V channels by 4-aminopyridine treatment and K_{Ca} channels by iberiotoxin treatment augmented the HPV response; (2) the isoflurane-induced attenuation of HPV was suppressed by K_V-channel inhibition with 4-aminopyridine, augmented by K_{Ca}-channel inhibition with iberiotoxin, and not affected by K_{ATP}-channel inhibition with glibenclamide; and (3) the sevoflurane-induced attenuation of HPV was not affected by any of the potassium-channel inhibitors investigated in the current study.

There is ample evidence suggesting that potassium channels play important roles in regulating pulmonary vascular tensioin.[1,2,21] In the current study, baseline P_{pa} increased significantly in 4-aminopyridine groups compared with that in control groups, suggesting that K_V channels regulate the basal pulmonary vascular tension in normoxia. The change in alveolar oxygen tension is the primary stimulus for triggering HPV. Although the mechanisms underlying this unique response are unclear, a number of studies suggest that some kinds of potassium channels in pulmonary vessels are sensitive to oxygen.[3,22–24] Hypoxia can inhibit K_V channels and result in depolarization and increase of Ca^{2+} entry through voltage-dependent Ca^{2+} channels, and vasoconstriction.[25,26] Activation of K_{Ca} channels may cause membrane hyperpolarization and inactivation of the voltage-gated Ca^{2+} channels and result in vasodilation.[13] It has been proposed that K_{Ca} channels appear to serve as a negative feedback pathway to regulate the degree of membrane depolarization and hence vasoconstriction caused by vasoconstrictors.[19,27] Increases in intracellular Ca^{2+} (by hypoxia) may activate K_{Ca} channels, which would increase potassium efflux, counteract the depolarization and constriction caused by vasoconstrictors.[27] This mechanism would also predict that blockage of K_{Ca} channels should cause depolarization and vessel constriction. K_{ATP} channels are reported to mediate, at least in part, the hypoxic vasodilatation in systemic vascular smooth cells.[28] However, K_{ATP} channels in pulmonary vessels are normally closed and not opened by hypoxia, and glibenclamide does not affect HPV.[29] The current results are consistent with these findings and indicate that K_V and K_{Ca} channels may play an important role in the HPV response because inhibition of both channels significantly augmented the HPV response, whereas the inhibition of K_{ATP} channel had no significant effect on the HPV response. However, enhanced HPV by inhibition of K_{ATP} channel by glibenclamide in the conscious state was reported in an *in vivo* study.[11] The different results may be a result of different models (*in vivo vs. in vitro*) used in these studies. Although K_{ATP} channels in pulmonary vessels are normally closed in isolated lung preparations,[29] they may be in an open status *in vivo*.

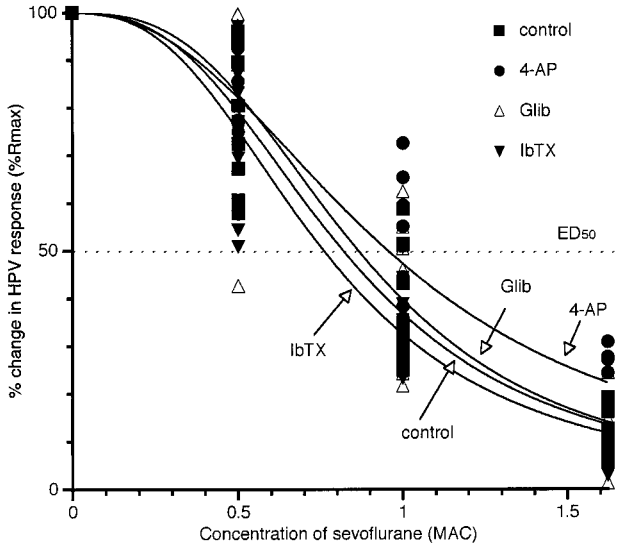

Fig. 3. Dose–response curve of the inhibitory effect of sevoflurane on hypoxic pulmonary vasoconstriction (HPV) in the absence and presence of potassium channel inhibitors, n = 8 per group. There are no significant differences among the groups. The concentration of isoflurane that induced 50% inhibition of HPV (ED_{50}) was 0.82 ± 0.21 MAC in the control-SEVO group, 0.87 ± 0.25 MAC in the Glib-SEVO group, 0.96 ± 0.24 MAC in the 4AP-SEVO group, and 0.76 ± 0.17 MAC in the IbTX-SEVO group. Glib = glibenclamide, the adenosine triphosphate–sensitive potassium (K_{ATP})-channel inhibitor; 4-AP = 4-aminopyridine, the voltage-sensitive potassium (K_V)-channel inhibitor; IbTX = iberiotoxin, the high-conductance calcium-activated potassium (K_{Ca}) channel inhibitor; SEVO = sevoflurane.

The effects of isoflurane on basal pulmonary vascular tension are consistent with our previous data[8] and those of other reports.[6,30] Although sevoflurane did not significantly change the pulmonary vascular tension in our previous study,[8] it dilated the pulmonary vessels slightly but significantly dose-dependently in the current study. The reason may be that the dilation effect of sevoflurane is subtle and can only be statistically detected with a high concentration of sevoflurane. The other possibility is that we used a higher perfusion flow (*vs.* 30 ml · kg^{-1} · min^{-1}), and the lungs were isolated and placed in the warm chamber in the current study (*vs.* left in the chest cavity in the previous study).

Although K_V channels in mesenteric vessels are not affected by isoflurane,[17] 4-aminopyridine–sensitive K_V channels are reversibly suppressed by clinically relevant concentrations of isoflurane in the canine coronary artery.[14] The results in the current study indicate that the pulmonary vascular effect of isoflurane was changed by K_V-channel inhibition both in normoxia and hypoxia, indicating that isoflurane regulates pulmonary vascular tension through K_V channels. The right-side shift of the dose–response curve by treatment with 4-aminopyridine indicates that isoflurane-induced HPV attenuation was partly abolished, suggesting that isoflurane inhibits HPV through the initiatory K_V-channel pathway, because K_V-channel inhibition is thought to be a primary step of the HPV initiation.[2,31] Although it is unknown if isoflurane can open or inhibit K_V channels in the lung, based on the current results, there is a possibility that isoflurane may strengthen K_V-channel blockage of the K_V-channel inhibitor; as a result, isoflurane caused vasoconstriction in normoxia and inhibited the HPV in a weaker extent in the presence of 4-aminopyridine.

Volatile anesthetics inhibit K_{Ca} channels in cerebral arteries[15] and aortic endothelial cells[32] but activate or have no effect on K_{Ca} channels in small mesenteric arteries.[17] The current results, with the dose–response curve shifted to the left side by iberiotoxin treatment, indicate that the HPV inhibitory effect of isoflurane was potentiated by K_{Ca}-channel inhibition, suggesting that isoflurane modulates HPV through K_{Ca} channels. It also suggests that isoflurane has a different effect on K_{Ca} channels compared with K_V channels. It is highly possible that isoflurane may activate K_{Ca} channels because the inhibition of these channels in the absence of volatile anesthetics can potentiate the HPV response.

Activation or inhibition of K_{ATP} channels changes the pulmonary vascular dilative or constrictive responses during anesthesia.[30,33] In the current study, however, the effect of both isoflurane and sevoflurane on HPV was not influenced by K_{ATP}-channel inhibition. These different results may be a result of the different models (*in vivo vs. in vitro*), as discussed previously. Both studies by Seki *et al.*[33] and Fujiwara and Murray[30] are *in vivo* studies. The current results are consistent with those of another *in vitro* study indicating that the K_{ATP} channel is not important in maintaining low pulmonary vascular tone in the isolated rat lungs.[34]

The inhibitory effect of sevoflurane is similar to that of isoflurane in the absence of potassium-channel inhibitors. This is consistent with our previous study.[8] However, the effect of sevoflurane on pulmonary vascular tension was not significantly affected by these potassium-channel inhibitors in both normoxia and hypoxia (we even increased the group size to 8 per group), suggesting that sevoflurane may regulate pulmonary vascular tension and inhibit HPV through other mechanisms rather than through the currently investigated potassium channels. This is not surprising, because sevoflurane has its own specific chemical structure and properties and therefore may affect the pulmonary vessels in a different fashion. The different effects of sevoflurane from other anesthetics on vessels have been demonstrated previously in the mesenteric,[35] coronary arteries,[36] and thoracic aorta.[37]

The perfusion flow rate used in the current study was 40 ml · min^{-1} · kg^{-1}, 25–35% of normal pulmonary blood in normal living rabbit. We used this flow rate because our previous study indicated that even a much lower flow (30 ml · min^{-1} · kg^{-1}) can produce a significant HPV response,[8] and we consider that higher flow

rates should be avoided in the isolated lung preparations, because high flow or high pressure may produce lung edema. Lung edema was not observed in the current study, as confirmed by the wet-to-dry lung weight ratios. Another concern about the current study is the use of the cyclooxygenase inhibitor, because it has been reported that cyclooxygenase inhibition potentiated the magnitude of HPV in both conscious and anesthetized states in a chronically instrumented canine model.[10,11] However, cyclooxygenase inhibition may not affect the HPV response in *in vitro* models.[1,8,38] Cyclooxygenase inhibition did not alter ED_{50} and the slope of the dose–response curve of sevoflurane in perfused rabbit lungs[8] and the HPV response in isolated pulmonary arteries.[1,38]

Although we demonstrated that potassium channels might play a role in HPV initiation and inhibition by isoflurane, we cannot conclude that the volatile anesthetics have a direct effect (activation or inhibition) on these potassium channels because of a lack of electrophysiological evidence. It is also possible that isoflurane may alter the downstream steps, including Ca^{2+} influx. Volatile anesthetics also inhibit HPV through Ca^{2+} channels.[12] The relation between potassium channels and the inward Ca^{2+} current in the effect of volatile anesthetics on HPV remains to be investigated.

In summary, K_V channel inhibition with 4-aminopyridine potentiated the HPV response and attenuated the isoflurane-induced HPV inhibition. K_{Ca} channel inhibition with iberiotoxin also potentiated the HPV response but enhanced the isoflurane-induced HPV inhibition. K_{ATP} channel inhibition with glibenclamide had no effect on HPV. The sevoflurane-induced attenuation of HPV was not affected by any of the potassium-channel inhibitors tested in the current study. Our results suggest that K_{Ca} and K_V channels may play an important role in the initiation of HPV, isoflurane may modulate the HPV response partially through K_{Ca} and K_V channels, and sevoflurane may attenuate HPV through other pathways rather than through the currently investigated potassium channels.

The authors thank Yasuaki Kawai, M.D., Ph.D. (Professor, Department of Physiology, Tottori University Faculty of Medicine, Japan), and Yoshimi Inagaki, M.D., Ph.D. (Associate Professor, Department of Anesthesia and Reanimatology, Tottori University Faculty of Medicine, Japan), for instructive suggestions during revision of the manuscript.

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