J Anesth (2005) 19:343–346
DOI 10.1007/s00540-005-0343-z

Journal of
Anesthesia

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Short communication

Volatile anesthetics constrict pulmonary artery in rabbit lung perfusion model

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Abstract
Volatile anesthetics are generally considered to possess a vasodilator action. Some of their actions on pulmonary vessels, however, are not clearly understood. We examined the effects of various volatile anesthetics on pulmonary vessels using an in situ rabbit isolated-lung perfusion model. We prepared a rabbit constant-flow lung-perfusion model by sending blood to the pulmonary artery and removing blood from the left atrium, and observed the changes in pulmonary arterial perfusion pressure caused by inhalation of 0.5, 1, 2, and 3 minimum alveolar concentration (MAC) volatile anesthetics: halothane, enflurane, isoflurane, and sevoflurane, in random order. These volatile anesthetics increased pulmonary arterial perfusion pressure in a dose-dependent manner and caused the pulmonary arteries to constrict. In particular, halothane at all concentrations induced significantly greater pulmonary vasoconstriction than the other volatile anesthetics. Therefore, it is suggested that volatile inhalation anesthetics induce the pulmonary arteries to constrict, and halothane exhibits the most potent pulmonary vasoconstrictor effect among the volatile anesthetics tested.

Key words Volatile inhalation anesthetic · Pulmonary vasoconstriction · Pulmonary perfusion model · In situ rabbit model

Volatile anesthetics are generally considered to possess a potent vasodilator action. An in vitro study has suggested that halothane induces constriction of the pulmonary arteries [1]. There are a number of reports regarding the effects of volatile anesthetics on tension in various smooth-muscle tissues. According to these reports, volatile anesthetics have inhibitory effects on smooth-muscle constriction [2–6]. Regarding pulmonary vessels, many articles have reported the effects of volatile anesthetics on the hypoxic pulmonary vasoconstriction (HPV) [7,8] response. However, there are only a few articles reporting how volatile anesthetics may affect pulmonary vessels in normoxia [1,9]. In this study, we observed the effects of various volatile anesthetics: halothane, enflurane, isoflurane, and sevoflurane, on pulmonary vessels, using an in situ rabbit constant-flow isolated-lung perfusion model. We conducted this experiment according to a protocol approved by the Institutional Animal Care and Use Committee of Kinki University School of Medicine.

White male Japanese rabbits were anesthetized with pentobarbiturate (20 mg·kg^{-1}, IV) and ketamine (35 mg·kg^{-1}, IM), followed by anticoagulation with heparin sodium. The trachea was dissected, a tube was inserted into the trachea, and controlled ventilation was performed using a ventilator (model 681; Harvard Apparatus, Cambridge, MA, USA) for small animals. The composition of inhaled gas was 21% O_2, 5% CO_2 and N_2 for balance. The right ventricle was incised at the origin of the pulmonary artery, and a cannula for sending blood was inserted through the incision site into the main pulmonary artery. The left ventricle was dissected in the apical region, and a cannula for removing blood was inserted backward into the left atrium and secured in the left ventricle by ligation. With the heart and lungs remaining in the thoracic cavity, pulmonary perfusion was conducted by a constant-flow reperfusion method with a peristaltic pump (model 1215; Harvard Apparatus) at a flow rate of 30 ml·kg^{-1}·min^{-1}, monitored with an electromagnetic blood flowmeter (MF-1200; Nihon Kohden, Tokyo, Japan). Controlled ventilation was performed at the following settings: volume per ventilation, 10 ml·kg^{-1}; respiratory rate, 40 min^{-1}; and positive end-expiratory pressure (PEEP), 2 cmH_2O. Partial pressure of End-tidal carbon dioxide ($P_{ET_{CO_2}}$) was monitored with a gas analyzer (Capnomac Ultima; Datex, Tewksburg, MA, USA) and confirmed at 35–40 mmHg.

Address correspondence to: M. Takemura
We have presented a summary of this study at the Japanese Society of Anesthesiologists' 49th Annual Meeting (Fukuoka)
Received: July 23, 2003 / Accepted: June 18, 2005

As the perfusion solution, collected autologous blood was diluted with physiological salt solution (PSS) and the hematocrit was adjusted to about 10%. Then, 3% albumin-PSS was prepared by adding 100 mg dextrose, 20 mU insulin, and 3 g bovine plasma albumin to 100 ml PSS.

Following completion of the model preparation, perfusion was initiated and continued for 30 min. After confirming that pulmonary arterial perfusion pressure had become stable, the study was started. Twenty-four (24) male rabbits were divided into four groups, consisting of a halothane group ($n = 6$), enflurane group ($n = 6$), isoflurane group ($n = 6$), and sevoflurane group ($n = 6$), and the volatile inhalation anesthetics at concentrations corresponding to 0.5, 1, 2, and 3 minimum alveolar concentration (MAC) were given at random, by 5 min of inhalation, and 10 min of washout, to observe changes in pulmonary arterial perfusion pressure. One MAC (%) for halothane, enflurane, isoflurane, and sevoflurane in rabbits was 1.4, 2.8, 2.0 [10], and 3.7 [11], respectively.

The vaporizers used were Fluotec 3 (Ohmeda, Madison, WI, USA) for halothane, Enfluwick (Muraco, Tokyo, Japan) for enflurane, Forawick (Muraco) for isoflurane, and Sevotec 3 (Ohmeda) for sevoflurane. In particular, Sevotec 3 was used by connecting vaporizers in series in order to obtain a high sevoflurane concentration.

Concentrations of oxygen and volatile anesthetics were monitored with a gas analyzer. The PO_2 of the perfusion solution under volatile inhalation anesthetics was measured and confirmed to be over 140 mmHg. The mean pulmonary artery (PA; mmHg) was used as an index of pulmonary arterial perfusion pressure. The difference between pulmonary arterial perfusion pressure before inhalation of volatile anesthetics and that after inhalation was defined as Δmean PA (mmHg), to compare differences among the groups (Fig. 1).

Data values were expressed as means ± SD. For statistical analysis, one-way analysis of variance (ANOVA) and the Tukey post-hoc test were used. We defined statistical significance as $P < 0.05$.

After completion of the models preparation, each model showed stable pulmonary arterial perfusion pressure of 6–7 mmHg. The various volatile anesthetics rapidly increased the pulmonary arterial perfusion pressure. After suspension of inhalation, pulmonary arterial perfusion pressure returned to near its original value and became stable. When pulmonary arterial perfusion pressure after inhalation was compared to that before inhalation, significant increases were observed with the inhalation of halothane at 0.5 MAC or more (all MAC), enflurane and isoflurane at 1 MAC or more, and sevoflurane at 2 MAC and 3 MAC (Table 1). Regarding the pulmonary vasoconstrictor effect at each MAC, the halothane group showed a significantly

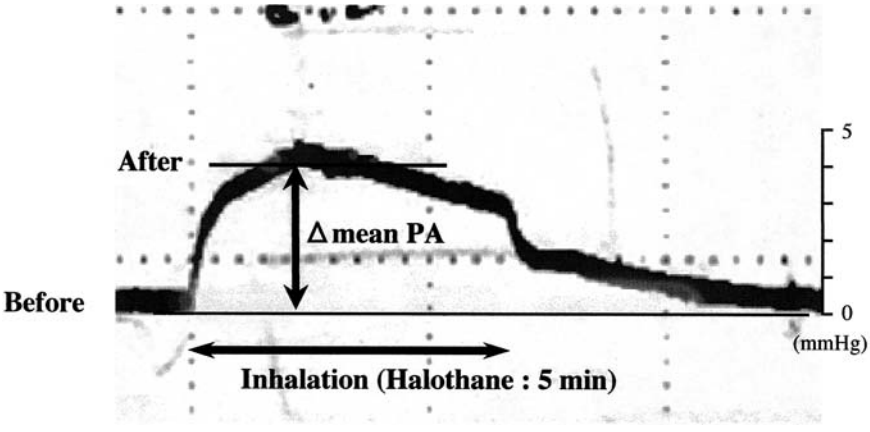

Fig. 1. Representative waveform of pulmonary arterial perfusion pressure measured with a pressure transducer (mean pressure). The difference between pulmonary pressure before inhalation of volatile inhalation anesthetics (*before*; mmHg) and that after inhalation (*after*; mmHg) was defined as Δmean pulmonary artery (*PA*; mmHg)

Table 1. Changes in pulmonary arterial perfusion pressure

	Halothane		Enflurane		Isoflurane		Sevoflurane	
	Before	After	Before	After	Before	After	Before	After
0.5 MAC	7.3 ± 1.5	8.3 ± 1.7*	6.6 ± 1.7	7.1 ± 2.5	6.8 ± 1.4	7.0 ± 1.6	6.0 ± 1.1	6.1 ± 1.1
1 MAC	7.7 ± 1.6	9.6 ± 2.2*	7.1 ± 2.5	8.7 ± 3.7*	7.0 ± 1.5	7.5 ± 1.8*	6.3 ± 1.4	6.5 ± 1.5
2 MAC	7.9 ± 2.1	11.1 ± 2.8*	7.6 ± 2.9	9.6 ± 3.7*	7.3 ± 1.7	8.1 ± 1.9*	6.6 ± 1.3	7.2 ± 1.5*
3 MAC	8.2 ± 2.1	13.5 ± 3.2*	7.6 ± 2.6	10.4 ± 3.6*	7.3 ± 1.7	8.6 ± 1.9*	7.4 ± 1.7	8.3 ± 1.8*

* $P < 0.05$ vs Before
Values are means ± SD

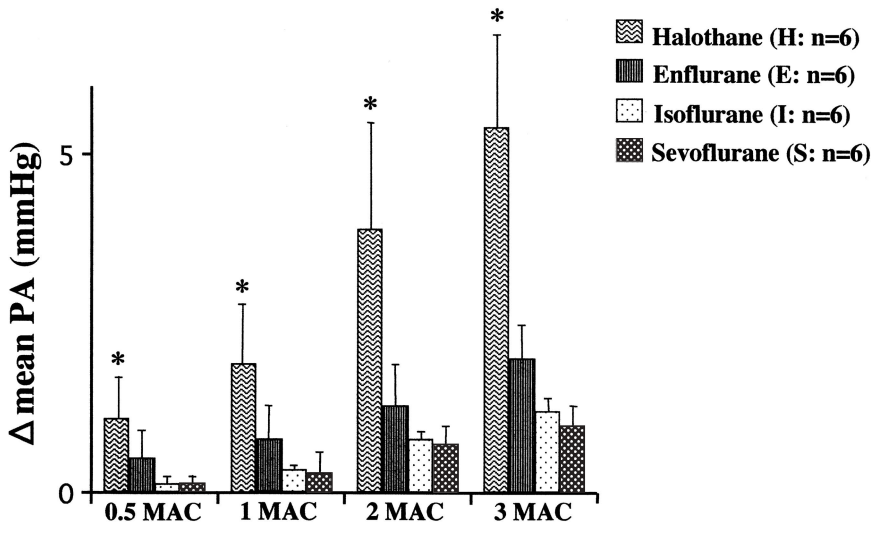

Fig. 2. Comparison of Δmean PAs. A significantly greater increase in pulmonary arterial perfusion pressure was observed in the halothane group, at all concentrations, compared to the other three groups (*$P < 0.05$ vs E, I, and S groups, respectively). Values are means ± SD. *MAC*, mean alveolar concentration

more potent vasoconstrictor action at each MAC compared to the findings in the other three groups (Fig. 2).

According to the results of our experiment, the volatile anesthetics caused pulmonary vessels to constrict. Especially, significantly more potent pulmonary vasoconstrictor responses to halothane were observed compared to the responses to the other three volatile anesthetics.

In the present study, we used an in situ experimental model. This model is more effective than an in vivo model, because effects caused by the dynamics of the systemic circulation, such as cardiac output and neural factors, can be eliminated and relatively pure evaluation of the tonus of the pulmonary vessels is possible [12]. It is well known that volatile anesthetics have a wide range of action sites and exert a variety of effects on vascular responses through various mechanisms. The results of this experiment using an in situ isolated perfusion model are considered to represent the pure effects of volatile anesthetics on pulmonary vessels.

Many reports [2–6] have shown an inhibitory effect on tension in smooth muscle as a direct action of volatile anesthetics. It has also been reported, however, that, depending on the type of stimulant or smooth muscle, the actions of volatile anesthetics vary; some show no vasodilator action, some show a vasoconstrictor action, and others show a vasodilator action following a vasoconstrictor action [13]. It is considered that the vasodilator actions of volatile anesthetics are mainly attributable to neural activity, especially the inhibition of norepinephrine release from sympathetic nerve endings [2–6,14].

As a direct inhibitory mechanism of the action of volatile anesthetics on smooth-muscle tissue itself, inhibition of the voltage-dependent calcium channel and an increase in cyclic adenosine 3′, 5′-monophosphate (c-AMP) have been suggested [13]. On the other hand, as a mechanism of the vasoconstrictor action, the promotion of calcium release from the sarcoplasmic reticulum and inhibition of the calcium channel have been suggested [13]. An experiment conducted in an isolated rat endothelium-denuded mesenteric artery showed that halothane and enflurane increased the intracellular calcium level and generated tension in calcium-free solution; however, isoflurane and sevoflurane did not produce the same results [15]. Similar results were reported in an experiment using a dog mesenteric artery model [16]. These vasoconstrictor responses were inhibited by caffeine [15] and ryanodine [16]. Accordingly, it could be concluded that the vasoconstrictor responses induced by volatile anesthetics are related to calcium release from intracellular calcium stores. All of these studies were conducted using systemic vessels.

An experiment using a bovine pulmonary vessel ring elucidated that the pulmonary vasoconstrictor response to halothane was inhibited by cyclopyazonic acid (a Ca^{2+}-adenosine triphosphatase [ATPase] inhibitor) and ryanodine; therefore, this vasoconstrictor action was considered to have been caused by calcium release from the sarcoplasmic reticulum [1]. In an experiment using saponin-treated, skinned-fiber-prepared rabbit pulmonary artery smooth muscle, halothane promoted calcium release from the sarcoplasmic reticulum, and this effect was attenuated by caffeine and inositol 1,4,5-triphosphate (IP3) [9]. Based on the results obtained from the above experiments, we consider that calcium release from the sarcoplasmic reticulum may be the main mechanism of the results in our experiment.

On the other hand, another mechanism of the vasoconstrictor action is suggested to be inhibition of the potassium channel. In canine coronary arterial

cells, halothane and isoflurane were observed to inhibit the potassium channel [17]. The mechanism of the vasoconstrictor response was reported to be that the volatile anesthetics regulated pulmonary vascular tension through the potassium channel [18]. Halothane, enflurane, and isoflurane showed constrictive effects on pulmonary vessels in the presence of a voltage-sensitive potassium channel inhibitor and a high conductance calcium-activated potassium channel inhibitor. The volatile anesthetics had the effect of inhibiting the potassium channel, showing a vasoconstrictor action. However, we consider that the effect on the potassium channel may not be the main mechanism of the vasoconstrictor action of the volatile anesthetics.

Acknowledgments. This study was funded, in part, by the Osaka Medical Research Foundation for Incurable Diseases.

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