Effect of sevoflurane on the vascular reactivity of rabbit mesenteric artery

A. YAMAGUCHI AND E. OKABE

Summary
Sevoflurane is well known to cause depression of cardiovascular function, but detailed information on its actions on the contractility and reactivity of blood vessels is lacking. We have assessed therefore the direct effect of this anaesthetic on the functional reactivity of isolated rabbit mesenteric artery ring preparations. We found that contractions of endothelium intact rings induced by noradrenaline and phenylephrine were significantly attenuated by 4% sevoflurane; the observation that the maximal tension generation decreased without a significant reduction in $pD_2$ is consistent with the view that receptor dysfunction was not involved. The effect of sevoflurane was not affected by $N^\omega$-monomethyl-L-arginine. Sevoflurane 4% also produced attenuation of noradrenaline-induced contractions of endothelium denuded ring preparations. The contractions of endothelium denuded ring preparations produced by noradrenaline in Ca$^{2+}$-free media in the presence of K$^+$ were not affected by 4% sevoflurane, but sevoflurane depressed external Ca$^{2+}$-dependent contractions. When vasodilators (acetylcholine and nitroglycerin) were added to the bathing media in the presence of 2% sevoflurane, the endothelium-dependent relaxation produced by acetylcholine, but not the endothelium-independent relaxation produced by nitroglycerin, was attenuated; superoxide dismutase inhibited the effect of sevoflurane. These results are consistent with the view that sevoflurane inhibits $\alpha$ adrenoceptor-mediated contractions of isolated rabbit mesenteric artery ring preparations; this effect may be caused by reduced Ca$^{2+}$ influx, as estimated from the effect on external Ca$^{2+}$-dependent contractions, but is unlikely to be caused by reduced Ca$^{2+}$ release from the sarcoplasmic reticulum of vascular smooth muscle, as estimated from noradrenaline-induced contractions in Ca$^{2+}$-free bathing media. Sevoflurane may selectively attenuate endothelium-dependent relaxation by an oxygen free radical mechanism as opposed to endothelium-independent relaxation. (Br. J. Anaesth. 1995; 74: 576-582)

Key words

Several studies have demonstrated that volatile anaesthetics can alter endothelium-dependent relaxation in vitro. Thus, Muldoon and colleagues [1] suggested an effect of halothane on the synthesis, release or transport of endothelium-derived relaxing factor (EDRF) in rabbit aorta and canine femoral and carotid arteries and proposed that halothane interferes with EDRF-mediated relaxation of vascular smooth muscle. Stone and Johns [2] have also suggested that halothane, enflurane and isoflurane cause vasoconstriction via inhibition of basal EDRF production, stimulation of the release of an endothelium-derived constricting factor, or both. We have found previously that sevoflurane produced oxygen-derived free radicals which were measured using highly sensitive electron spin resonance spectroscopy and the spin-trap 5,5-dimethyl-pyrroline-N-oxide. We suggested that sevoflurane selectively impairs endothelium-dependent relaxation compared with endothelium-independent relaxation (produced by nitroglycerin) in canine mesenteric arteries by an oxygen-derived free radical mechanism, mainly because of interaction with EDRF [3]. Enhanced oxygen-derived free radical production has been associated with several vascular abnormalities ranging from loss of endothelial injury to total vascular collapse.

Although administration of some volatile anaesthetics to animals and humans may not always result in peripheral vasodilatation, it is agreed that deep anaesthesia, when induced by these agents, results in dilatation and hypotension [4, 5]. Until recently, it was thought that such anaesthetic-induced vasodilatation was caused exclusively by the effects of these anaesthetics on the central [6] and autonomic nervous systems [7] and on the myocardium [8, 9], and that concentrations of anaesthetics used to induce surgical anaesthesia could exert direct depressant and vasodilator effects on vascular smooth muscle [10-12]. Sevoflurane has been reported to decrease coronary flow in pigs [13] and rats [14]; however, Bernard and colleagues [15] and Crawford and colleagues [16] observed an increase in coronary flow induced by sevoflurane in dogs and rats. This
difference may be caused partly by differences in experimental situations. Here, we report that although sevoflurane attenuated endothelium-dependent relaxation by an oxygen-derived free radical mechanism, the modification elicited by sevoflurane on noradrenaline-induced contraction of endothelium-denuded rabbit mesenteric arteries was not attributed to the oxygen-derived free radicals generated (from sevoflurane itself).

Materials and methods

With approval from our institutional Animal Care Committee, we obtained mesenteric arteries from male albino (New Zealand) rabbits (2.0-2.5 kg) after exsanguination during anaesthesia with sodium pentobarbitone 40 mg kg⁻¹ i.v. Fat and other non-vascular tissues were gently removed from the blood vessels, which were cut into rings (2 mm width; mean weight of the tissue 2.79 (SEM 0.07) mg, n = 57), without disturbing the intimal layer, after immersion in ice-cold modified Krebs-Henseleit solution (mmol litre⁻¹): NaCl 118.0, KCl 4.7, MgSO₄ 1.18, CaCl₂ 2.5, KH₂PO₄ 1.18, NaHCO₃ 25.0 and 5.5 glucose, aerated with 95% oxygen and 5% carbon dioxide; pH 7.2-7.3. The endothelium was removed from some rings by gently rubbing the luminal surface with a wooden implement. The rings were allowed to equilibrate for 90 min in modified Krebs-Henseleit solution which was changed at 15-min intervals. During this time, the rings were stretched to a passive tension of 1.5 g. Endothelial integrity was checked in all experiments by the presence of the characteristic relaxation response to acetylcholine 10⁻⁶ mol litre⁻¹.

Sevoflurane (MAC value in rabbit 3.70 (SD 0.16) %) [17] was delivered from a vaporizer (Ohmeda Sevotec 3, Streton, England) in the oxygen-carbon dioxide mixture aerating the bathing media; the gas was humidified before entering the four-serial tissue baths. The concentration in the resulting gas mixture was monitored continuously by a recalibrated multi-gas anaesthetic agent analyser (Datex Capnomac, Helsinki, Finland). The tissue bath was covered with plastic to prevent the aerating gas from immediately escaping into the atmosphere. To determine the time of equilibration of sevoflurane, the concentration of sevoflurane in the bathing media was measured by gas chromatography (Shimazu GC-9A, Kyoto). It was found that equilibration of the bathing media with sevoflurane (1-4%) was complete within 10 min and that stable bath concentrations were achieved at a sevoflurane-oxygen-carbon dioxide mixture flow rate of 300 ml min⁻¹ of gas flow through the frittered glass discs at the bottom of the four-serial bath chambers. The mean bath anaesthetic concentrations after 30 min equilibration for 1%, 2% and 4% sevoflurane were 2.04 (SEM 0.15), 4.14 (0.34) and 8.42 (0.68) x 10⁻⁴ mol litre⁻¹ (n = 3), respectively. On the basis of this, we chose the preincubation conditions used to assess the effect of sevoflurane on vessel preparations; we used 30 min preincubation. All experiments in the presence of sevoflurane were carried out 30 min after beginning anaesthetic delivery, thereby giving 20 min of exposure of the vessel preparations to the stable concentrations of sevoflurane.

Means (SEM) are given throughout. Two sets of statistical comparisons were made. Student’s t test for paired samples was used when comparing two populations. Comparisons of subsequent interventions with controls were made using one-way analysis of variance, followed by Duncan’s multiple range test. Differences were considered significant at P < 0.05.

The following drugs and chemicals were used: sevoflurane (Maruishi Pharmaceuticals, Osaka, Japan), (-)-noradrenaline hydrochloride (Sigma Chemical Co., St Louis, MO), L-phenylephrine hydrochloride (Wako Chemicals, Osaka, Japan), acetylcholine chloride (Sigma), nitroglycerin (Nihon Kayaku, Tokyo, Japan), superoxide dismutase (SOD; from bovine liver, 3000 u./mg protein, Sigma), N⁶-monomethyl-L-arginine acetate salt (L-NMMA; Sigma), ascorbic acid (Wako Chemicals), indomethacin (Sigma), propranolol hydrochloride (Wako Chemicals), ethylenediaminetetra-acetic acid (EDTA; Wako Chemicals) and glycolediaminetetra-acetic acid (EGTA; Wako Chemicals). All drugs except sevoflurane and indomethacin were dissolved in pure water and diluted in the Krebs-Henseleit solution gassed with a mixture of 95%...
oxygen–5% carbon dioxide before being added to the tissue bath. Indomethacin stock solution was prepared by dissolving three parts indomethacin and one part sodium bicarbonate in distilled water. All other reagents were of analytical grade.

Results

The potentiating effect of several compounds on noradrenaline-induced vascular responses is known to result from protection of the catecholamine from oxidation, and Furchgott [18] recommended testing for this possibility by including in the bathing media sufficient EDTA, a heavy metal chelating agent, to completely inhibit the oxidation of catecholamines. Several other compounds, including ascorbic acid, potentiate noradrenaline by preventing its oxidation [18]. Therefore, EDTA and ascorbic acid were added simultaneously to the bathing media; propranolol and indomethacin were also added to block β adrenoceptors and to prevent volatile anaesthetic-induced release of a vasodilating prostanoid from endothelium [2], respectively, in most experiments.

Figure 1 shows the experimental protocol for assessing the effect of sevoflurane on noradrenaline-induced responses of the ring preparations. In all ring preparations tested, the pre- (fig. 1, lane a) and postanaesthetic (fig. 1, lane c) values did not differ significantly from each other, and the noradrenaline-induced response was stable (time-matched control, fig. 1n) during the experimental period. Therefore, to simplify the presentation of data, preanaesthetic values were used as controls, and data for postanaesthetic and time-matched control values are not presented. Figure 2 shows the effect of sevoflurane 30 min after beginning delivery on the concentration–response curves to noradrenaline and phenylephrine in endothelium intact artery rings; the experimental conditions for the phenylephrine studies were the same as those for noradrenaline depicted in figure 1. In control experiments, noradrenaline and phenylephrine contracted the ring preparations in a concentration-dependent fashion. Sevoflurane 4% significantly attenuated these responses with rightward shifts of the concentration–contraction curves and a decrease in the maximum contraction (fig. 2a, 2b). Sevoflurane 1% and 2% were ineffective (data not shown). Noradrenaline $2.58 \times 10^{-6}$ mol litre$^{-1}$ elicited approximately 50% of the maximum tension that developed in response to noradrenaline in the absence of sevoflurane (control in fig. 2a).

We next determined whether or not the endothelium-dependent relaxation induced by acetyl-
Sevoflurane in mesenteric artery

100

T

579

7 6 5

-log [NA] (mol litre^-1)

7 6 5

-log [NAXmol litre^-1]

Figure 3 Effect of L-NMMA on 4% sevoflurane (#)-induced attenuation of noradrenaline contraction of endothelium intact (A, B and C) and endothelium denuded (p) ring preparations of rabbit mesenteric artery. The experimental conditions were similar to those described in figure 1 except that L-NMMA 3 x 10^-4 mol litre^-1 was added before the start of experiments and endothelium denuded rings were used in some experiments. In this series of studies, parallel control experiments (O) (A and C) for the effect of L-NMMA (B) or removal of endothelium (D) were performed. Preanaesthetic responses to noradrenaline (NA) 10^-4 mol litre^-1 in the absence (6.51 (0.71)g, A; 6.90 (0.96)g, C; 7.45 (0.39)g, D) or presence of L-NMMA (7.50 (0.71)g, B) are taken as 100% and other data are plotted in relation to them. Each point represents mean (SEM) (n = 5); n = number of rabbits from which the mesenteric artery was taken. *P < 0.05, and **P < 0.01 vs corresponding control value.

Choline (Ach) was altered by sevoflurane. Relaxation responses to the stepwise cumulative addition of Ach and nitroglycerin were determined in rings contracted to a stable plateau tension by the addition of noradrenaline 2.58 x 10^-6 mol litre^-1 in the presence or absence of sevoflurane. Sevoflurane 1% and 2% significantly attenuated the response elicited by Ach with a decrease in the maximum contraction (fig. 2c, 2d); the endothelium-independent relaxation induced by nitroglycerin 10^-12-10^-5 mol litre^-1 was not affected significantly by the anaesthetic (data not shown).

We next determined the effect of SOD on the attenuation of endothelium-dependent relaxations of the ring preparations to Ach 10^-7 mol litre^-1, a concentration that produces approximately 50% of the maximum relaxation (derived from the data of control in fig. 2c) produced by 2% sevoflurane. In this series of experiments, the conditions were similar to those described in figure 2 (c and d) except that a single dose of Ach 10^-7 mol litre^-1, SOD 60 u. ml^-1, or both, was added. SOD was added simultaneously on commencing sevoflurane delivery or 30 min before precontraction induced by noradrenaline 2.58 x 10^-6 mol litre^-1 in the absence of sevoflurane. Sevoflurane reduced Ach-induced relaxations (average percentage of preanaesthetic responses to Ach: 72.4 (14.50), n = 5; P < 0.05 vs preanaesthetic value). This reduction was restored to near normal when Ach was added after sevoflurane was removed from the tissue bath (103.2 (5.92), n = 5). Further, SOD, when added to the bathing media simultaneously with initiation of sevoflurane delivery, almost prevented the sevoflurane-induced attenuation of the relaxation (100.1 (4.10), n = 5; P < 0.05 vs sevoflurane); SOD alone (added in a time-matched fashion) had no effect on Ach-induced relaxations (102.3 (2.05), n = 5). This suggests that the effect of sevoflurane on endothelium-dependent relaxation of ring preparations could result from its ability to inactivate released EDRF from endothelial cells in response to Ach, perhaps via generation of oxygen free radicals, possibly superoxide anion radicals (O_2^-). This finding would appear to fit with our previous data obtained in canine mesenteric artery ring preparations [3].

Recent evidence has suggested that EDRF may be nitric oxide (NO-) or a similar nitrogen oxide-containing compound [19]. Under pathological conditions, EDRF/NO- can combine with O_2^- in aqueous solution to produce the potentially cytotoxic substance, peroxynitrite (ONOO^-) [20] which then decays homolytically after protonation to the hydroxyl radical (HO^-) and nitrogen dioxide. Therefore, we proposed that O_2^- produced from sevoflurane itself [3], could combine with basally released NO^- [21] from endothelium, and the resultant ONOO^- could damage vascular reactivity. A test of this hypothesis is presented in figures 3 and 4.
the NO synthase inhibitor L-NMMA (fig. 3B). Sevoflurane 4% also attenuated noradrenaline-induced contraction of endothelium denuded ring preparations (fig. 3D). The effect elicited by sevoflurane on noradrenaline-induced contraction in endothelium intact or denuded rings was unaffected by SOD (fig. 4). The reduced response to noradrenaline of the ring preparations caused by sevoflurane was therefore not caused by the NO⁻ and O₂⁻ interaction.
Attempts to evaluate the effect of vasoactive agents in the presence of the oxygen free radical generator sevoflurane [3] are risky; the stability of the vasoactive agents (noradrenaline 10⁻³ mol litre⁻¹, phenylephrine 10⁻⁵ mol litre⁻¹, ACh 10⁻⁵ mol litre⁻¹ and nitroglycerin 10⁻⁶ mol litre⁻¹) used in the present study during the experiments was therefore investigated. Vascular reactivity to the agents, which were each preincubated with 4% sevoflurane for 30 min before being added to the tissue bath, was nearly the same as that to the untreated agents (average percentages of the responses to the agents which were preincubated in "aerated-no sevoflurane" bathing media: noradrenaline 95.9 (2.71); phenylephrine 99.9 (0.60); ACh 99.3 (2.37); nitroglycerin 98.2 (1.36); n = 5).

Noradrenaline has been shown to release Ca²⁺ from intracellular storage sites, the sarcoplasmic reticulum, in rabbit mesenteric artery [22]. To examine further the effect of sevoflurane on vascular smooth muscle contractility, we studied noradrenaline-induced contraction of the ring preparations in the external Ca²⁺-free conditions in the presence of KCl 40 mmol litre⁻¹. The contraction produced by noradrenaline of endothelium denuded ring preparations, which was effectively impaired by 4% sevoflurane under normal experimental conditions, was not affected by 4% sevoflurane in a Ca²⁺-free media; in contrast, sevoflurane depressed externally added Ca²⁺ (CaCl₂ 100 mmol litre⁻¹)-induced contraction (fig. 5), suggesting that sevoflurane may be able to reduce Ca²⁺ influx, thereby attenuating external Ca²⁺-dependent contractions.

Discussion

The present results demonstrate that sevoflurane may inactivate released EDRF from endothelial cells, in response to ACh, via generation of oxygen free radicals, possibly O₂⁻. When vessel rings were rinsed thoroughly after treatment with sevoflurane, the response to ACh was completely restored. Thus our data provide strong evidence for the direct inactivation of, but not for the synthesis, release or transport of, EDRF. The fate of O₂⁻ is unclear. It may react with biological molecules to cause cellular dysfunction or with water to form hydrogen peroxide, a spontaneous reaction the rate of which is enhanced by SOD. The present data support a role for O₂⁻; sevoflurane-induced attenuation of the endothelium-dependent relaxation caused by ACh was prevented by SOD. If hydrogen peroxide had been involved directly in the effect of sevoflurane, SOD should not have been protective, as the main product of SOD is hydrogen peroxide.

Interaction of O₂⁻ with NO⁻ can lead to the spontaneous formation of the ONOO⁻ radical [20]. Under the appropriate conditions, hydrogen peroxide and ONOO⁻ can generate the highly reactive HO⁻ (see Results). O₂⁻, in submicromolar concentrations, depresses contractile tension in vessels tonically contracted by noradrenaline [23]. Although this suggests a role for oxidant-dependent attenuation of vascular contraction, the experimental preparation did not allow the investigators to determine if the loss of contractile tension was caused by oxidation and inactivation of noradrenaline or by direct damage of the vascular preparation. If sevoflurane reduced noradrenaline-induced contractile tension by generating O₂⁻, ONOO⁻, or both, then when endothelial NO synthase had been inhibited by L-NMMA and when O₂⁻ generated from sevoflurane itself had been scavenged by SOD, this reduction would be removed. However, in the event, SOD and L-NMMA had no effect, while removal of endothelial cells was also without effect (figs 3, 4). Therefore, it appears that the effect of sevoflurane on noradrenaline-induced contractile tone is independent of oxygen free radicals, and that the arterial smooth muscle, rather than the noradrenaline molecule, is the site of action of the anaesthetic. The latter proposal is inferred further from the following observations: (1) the magnitude of the contractile responses of the ring preparations to noradrenaline was unaffected, although noradrenaline was preincubated with sevoflurane for a relatively long time, and (2) sevoflurane attenuated the response of the ring preparations to both noradrenaline and phenylephrine (fig. 2A and 2B). As both agents cause contraction via stimulation of α adrenoceptors, the effect of sevoflurane on noradrenaline- and phenylephrine-induced contractile tone must reflect on action at or after agonist-receptor interaction.

Endothelium-dependent relaxation was more sensitive to sevoflurane than were noradrenaline- and phenylephrine-induced contractions (because endothelium-dependent relaxation was attenuated by 1% and 2% sevoflurane, while attenuation of the contraction could only be accomplished with 4% sevoflurane) and clearly established a role for oxygen free radicals in the anaesthetic-induced loss of endothelium-dependent relaxation. However, the cellular mechanism involved in sevoflurane-induced loss of noradrenaline contraction remains unclear. Potential sites of action of sevoflurane include α adrenoceptors, alterations in second messenger function, defects in Ca²⁺ mobilization and disruption of contractile protein function. The observation that the maximal tension generation decreased without a significant reduction in ρD₂ (range: noradrenaline 5.27–5.50; phenylephrine 4.93–5.37; see fig. 2A, 2B) is consistent with the view that receptor dysfunction may not be involved in the effects of sevoflurane.

It has been postulated that the phasic response of vascular smooth muscle is evoked by release of Ca²⁺ from the sarcoplasmic reticulum after activation of the influx of Ca²⁺, and that the tonic response is generated by the amount of free Ca²⁺, comprising the Ca²⁺ influx, release and uptake at the sarcoplasmic reticulum and Ca²⁺ efflux. Noradrenaline can produce both phasic and tonic contractions [24]. It was shown that theophylline anaesthetics suppress membrane Ca²⁺ and K⁺ channel currents in cardiac myocytes [25, 26]. In in vitro preparations, halothane directly relaxed isolated coronary artery rings contracted previously with K⁺ or prostanoid [27]. This would suggest that volatile anaesthetics are suppressors of voltage-dependent Ca²⁺ entry, as voltage-gated Ca²⁺ influx is the mechanism underlying K⁺-induced
contractions [28]. On the basis of our results (fig. 5), one could propose that the attenuated noradrenaline-induced contraction of the ring preparations caused by sevoflurane might be produced by a reduced influx of Ca\(^{2+}\), possibly through voltage-dependent Ca\(^{2+}\) channels (because the experiments were carried out in the presence of KCl), rather than by reduced release of Ca\(^{2+}\) from the sarcoplasmic reticulum. As oxygen free radicals reduce Ca\(^{2+}\) release from the sarcoplasmic reticulum and activate or sensitize voltage-dependent Ca\(^{2+}\) channels in vascular smooth muscle [24], the effect of sevoflurane is thus oxygen free radical-independent.

In conclusion, the results presented here suggest that sevoflurane can inhibit \(\alpha\) adrenoceptor-mediated contraction of rabbit mesenteric artery ring preparations; this effect was caused by reduced Ca\(^{2+}\) influx, as estimated from the effect on external Ca\(^{2+}\)-dependent contraction, but was unlikely to be caused by reduced Ca\(^{2+}\) release from the sarcoplasmic reticulum of vascular smooth muscle, as estimated from noradrenaline-induced contraction in Ca\(^{2+}\)-free bathing media. Sevoflurane may also inactivate released EDRF from endothelial cells in response to ACh.

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