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Effects of halothane and isoflurane on acetylcholine-induced, endothelium-dependent vasodilation in perfused rat mesenteric arterial beds

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Abstract

Purpose. The present study was designed to examine the effects of halothane and isoflurane on acetylcholine-induced, endothelium-dependent vasodilation in rat mesenteric arterial beds perfused at a constant flow both in vitro and in situ.

Methods. In the in-vitro preparation, the mesenteric artery was cannulated and perfused (5 ml·min^{-1}). The perfusion pressure was continuously monitored. Under active tone induced by methoxamine, the effects of halothane and isoflurane on the vasodilator response to acethylcholine in either the presence or absence of N^G-nitro-L-arginine (L-NA), tetraethylammonium (TEA), or KCl (30 mM)-depolarization were examined. All experiments in these preparations were performed in the presence of indomethacin (10 mM). In the in-situ experimental model, rats were anesthetized with pentobarbital and the lungs were mechanically ventilated via a tracheostomy with a ventilator. The superior mesenteric artery was cannulated and used for the monitoring of the perfusion pressure. Blood shunting with constant flow (2 ml·min^{-1}) from the carotid artery to the superior mesenteric artery was introduced with clamping at the immediately distal portion of the mesenteric artery branching. Following 20-min ventilation with halothane or isoflurane at 1 minimum alveolar concentration (MAC) in oxygen, acetylcholine was given from the mesenteric artery, under active tone induced by norepinephrine (100 mg·kg^{-1}·hr^{-1}).

Results. In the in-vitro preparation, the nitric oxide synthase inhibitor, L-NA (100 μM) did not affect vasodilations to acetylcholine (1, 10 nM), while the K^+ channel inhibitor TEA (10 mM), as well as KCl (30 mM), significantly reduced these vasodilations. However, only in the presence of L-NA, TEA and KCl completely abolished the vasodilations produced by acetylcholine. The higher concentrations of halothane (2.0%, 3.0%), but neither isoflurane (3.0%) nor the lower concentration of halothane (1.0%), significantly impaired vasodilator responses to acetylcholine in the presence of L-NA, whereas the volatile anesthetics did not affect these vasodilations in the absence of L-NA. Halothane (2.0%) did not alter the vasodilation produced by acetylcholine in the presence of TEA or KCl. In the in-vivo preparation, the vasodilator effects of acetylcholine (1 and 10 nmol) were not affected by the inhalation of halothane (1.0%) or isoflurane (1.3%).

Conclusion. These results suggest that, in resistance arteries in conditions of constant flow, halothane and isoflurane do not affect vasodilations in response to an endothelium-dependent agonist. However, in these preparations, once the enzymatic activity of nitric oxide synthase is inhibited, higher concentrations of halothane, but neither isoflurane nor the lower concentration of halothane, appear to impair endothelium-dependent relaxations, probably mediated by TEA-sensitive K^+ channels.

Key words Volatile anesthetics · Nitric oxide · Endothelium-dependent hyperpolarizing factor (EDHF) · Mesenteric artery

Introduction

Vascular endothelium plays a pivotal role in the modulation of vascular tone [1]. Cumulative findings have demonstrated that endothelial cells are capable of producing vasoactive substances, including nitric oxide, endothelium-derived hyperpolarizing factor (EDHF), and prostacyclin [2]. Previous studies documented the inhibitory effects of volatile anesthetics on the endothelium-dependent relaxations produced by pharmacological and physiological stimuli acting on endothelial cells via receptor-dependent and receptor-independent mechanisms [3–5]. Most of these studies focused on the modulatory effects of the anesthetics on relaxations in response to endothelium-derived nitric oxide [3–5]. Whether volatile anesthetics are capable of modifying EDHF-mediated relaxation is not yet fully understood. Endothelial cells in blood vessels increase nitric oxide production dynamically in response to increased shear stress [6,7]. A recent study has demonstrated that increased shear stress can augment the

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production of prostacyclin, as well as that of nitric oxide, in endothelial cells [8,9]. These results suggest that shear stress may be a determinant of the balance between nitric oxide-dependent and independent (EDHF) relaxations in arteries with intact endothelial function. Although recent studies using isolated arteries suggested the inhibitory effect of volatile anesthetics on relaxations to nitric oxide-independent, endothelium-dependent factors [10–12], these studies were conducted in the ring segment of conduit artery without flow-dependent shear stress.

Therefore, the present study was designed to examine whether the presence of intravascular pressure and flow stress modify the inhibitory effects of volatile anesthetics on acetylcholine-induced vasodilation in rat mesenteric arterial beds, in the presence of a nitric oxide synthase inhibitor, in perfused rat mesenteric arterial beds.

Materials and methods

The study was approved by the Animal Care and Use Committee of Wakayama Medical University.

Preparation of in-vitro mesenteric arterial beds

Male Wistar rats (280–350 g) were anesthetized with intraperitoneal pentobarbital (50 mg·kg^{-1}), and mesenteric arterial beds, including small intestine, were rapidly isolated and set up for a perfused preparation by a modification of the technique originally described in the study by McGregor [13]. A schematic diagram of the experiment is shown in Fig. 1. The mesenteric artery was cannulated with a polyethylene catheter (internal diameter, 3 Fr) through an incision in the abdominal aorta. The mesenteric arterial beds were flushed with modified Krebs-Ringer bicarbonate solution containing heparin (10 U/ml; control solution) of the following composition (mM): NaCl, 120; $NaHCO_3$, 25; KCl, 5.9; $MgSO_4$, 1.2; $CaCl_2$, 2.4: ethylenediaminetetraacetic acid (EDTA)-2Na, 0.027, and glucose 11, and they were then separated from the intestine. A long duration of perfusion with Krebs solution is likely to cause intestinal edema, leading to a change in perfusion pressure. The distal portions of the arteries corresponded to the sixth to eighth branches of the main mesenteric artery. The preparation was mounted on a stainless steel grid in a chamber (37°C) with a plastic cover. The arterial bed was constantly perfused at a flow rate of 5 ml·min^{-1} by a peristaltic pump (MP-3; EYELA, Tokyo Rikakikai, Tokyo, Japan), with Krebs Ringer solution (37°C, pH 7.4) bubbled with 95% O_2 −5% CO_2 gas mixture. The perfusion pressure was continuously monitored by a pressure transducer, and this, in turn, was connected to a recorder (RMC-1100; Nihon Kohden, Tokyo, Japan). The perfusion pressure was calibrated as 0 mmHg at zero flow. Because the flow rate was constant throughout the experiments, the changes in the perfusion pressure were directly proportional to changes in vascular resistance. After an equilibration period of 30 min, active tone was induced by a constant infusion of methoxamine (30 to 80 μM), which was added to the perfusate to maintain the perfusion pressure at approximately 90 mmHg. When the tone became stable, acetylcholine (1 or 10 nmol), as a bolus, was added to the perfusate. In the preliminary experiments, vasodilator responses to acetylcholine (1 and 10 nmol) were repeatedly obtained three times for each preparation, and this revealed that the responses to acetylcholine were not altered by the repeated exposure to the compound. Indeed, the vasodilator responses to acetylcholine, expressed by the percentage of maximal increase in the perfusion pressure induced by methoxamine, were 75.2 ± 6.3%, 77.6 ± 6.3%, and 79.2 ± 5.8% for the first, second and third exposures to acetylcholine 1 nmol, re-

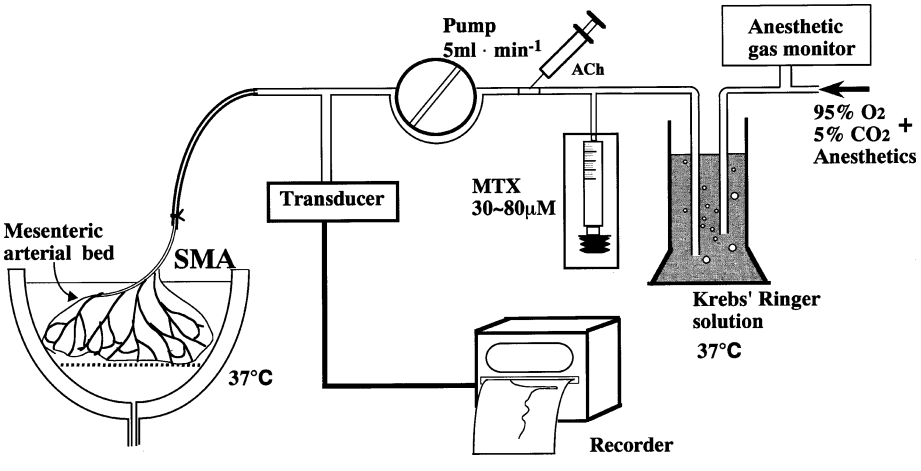

Fig. 1. Schematic diagram of the in-vitro experiment. *SMA*, Superior mesenteric artery; *MTX*, methoxamine; *ACh*, acetylcholine

spectively ($n = 15$, each), and 85.2 ± 6.9%, 85.6 ± 6.0%, and 90.2 ± 8.7% for the first, second, and third exposures to acetylcholine 10 nmol, respectively ($n = 15$ each; differences not significant among the first, second, and third exposures in each concentration of acetylcholine). Therefore, in all experiments, responses induced by the first exposure to acetylcholine served as the control, and the responses to the second exposure were used to determine the effect of several compounds on the acetylcholine-induced vasodilations. The concentrations of halothane at 1%, 2%, and 3% in Krebs bicarbonate solution were $2.9 \pm 0.2 \times 10^{-4}$ M, $5.7 \pm 0.3 \times 10^{-4}$ M, and $8.4 \pm 0.2 \times 10^{-4}$ M, respectively, and that of isoflurane at 3% was $6.3 \pm 0.2 \times 10^{-4}$ M. In the preliminary experiment, the increased perfusion pressure caused by methoxamine infusion fell gradually during 40-min exposure to halothane at a concentration of 3.0% and isoflurane at concentrations of 2.0% to 3.0%. Thus, the responses to acetylcholine at 1 nmol and 10 nmol were observed after 20-min exposure to the volatile anesthetics.

All experiments were performed in the presence of indomethacin (10 μM) to inhibit the possible production of prostanoids by the cyclooxygenase pathway. In the presence of active tone induced by methoxamine (30 to 80 μM) infusion, vasodilator responses to acetylcholine (1 or 10 nmol) were obtained in the absence or in the presence of halothane (1.0%, 2.0%, or 3.0%), isoflurane (1.0%, 2.0%, or 3.0%), N^G-nitro-L-arginine (L-NA; 100 μM), tetraethylammonium (TEA; 10 mM), potassium chloride (KCl; 30 mM), charybdotoxin (100 nM), and apamin (100 nM). We confirmed, in a preliminary study, that higher concentrations of L-NA, up to 300 μM, did not further inhibit the response to acetylcholine. We determined the concentration of TEA that completely abolished the response to acetylcholine in the presence of L-NA. Mesenteric arterial beds were treated with halothane and isoflurane, L-NA, TEA, and KCl 20 or 30 min before and during the addition of acetylcholine. The relaxations were expressed as percentages of the active tone induced by methoxamine (30 to 80 μM).

Preparation of in-situ mesenteric arterial beds

The in-situ experimental model of rat mesenteric arterial beds was prepared by the method of Jackson and Campbell [14], with some modifications. Male Wistar rats (280–350 g) were anesthetized with intraperitoneal pentobarbital (50 mg·kg^{-1}). Rats were performed tracheostomy and their lungs were mechanically ventilated with a ventilator (SN-480-7; Shinano, Tokyo, Japan), with 100% oxygen (flow, 0.5 l·min^{-1}; respiratory rate, 45·min^{-1}). By introducing these procedures, normocapnia ($PaCO_2$, 36–44 mmHg), normal acid-base balance (pH, 7.35–7.45), and adequate arterial oxygenation (PaO_2, 250–280 mmHg) were obtained throughout the experiments. The right carotid artery was cannulated with a 24-gauge polyethylene catheter, and it was flushed with 2 ml saline containing heparin (1500 U). Then, a midline laparotomy was performed and the abdominal aorta was clamped at the immediately distal portion of the superior mesenteric artery branching. The superior mesenteric artery was cannulated with a polyethylene catheter (internal diameter, 3 Fr) through the incision of the abdominal aorta, and the cannula was used for monitoring the perfusion pressure and the route of drug administration. The perfusion pressure was measured by a pressure transducer connected to a recorder (RMC 1100; Nihon Koden, Tokyo, Japan). After these procedures, constant flow blood shunting (2 ml·min^{-1}) from the left carotid artery to the mesenteric artery, using a roller pump (MP-3, EYELA; Tokyo Rikakikai) was started. A schematic diagram of the in-situ experiment is shown in Fig. 2. Rectal temperature was maintained at 37 ± 0.5°C, using a radiant warmer. After an equilibration period of 20 min, active tone was induced by norepinephrine (100 μg·kg^{-1}·h^{-1}), which was directly administered into the mesenteric artery. When the tone became stable, intraarterial acetylcholine (1 nmol) was given. After 30 min more of equilibration, the rat was ventilated with halothane (1.0%) or isoflurane (1.3%) (end-tidal concentration, 1 minimum alveolar concentrations [MAC] for rat) in 100% oxygen [15]. After 20-min exposure to volatile anesthetics, norepinephrine and acetylcholine were administered. The vasodilator effects of acetylcholine in these preparations were demonstrated by the changes in perfusion pressure.

Drugs

The following pharmacological agents were used: indomethacin, methoxamine, N^G-nitro-L-arginine (L-NA), tetraethylammonium (TEA; Sigma, St. Louis, MO, USA), acetylcholine (Daiichi Pharmaceutical, Tokyo, Japan), halothane (Takeda Pharmaceutical, Tokyo, Japan), and isoflurane (Dinabot Pharmaceutical, Tokyo, Japan). Stock solutions of indomethacin (10^{-5} M) were prepared in equal molar concentrations of Na_2CO_3. The concentrations of drugs are expressed as final molar (M) concentrations.

Statistical analysis

The data values were expressed as means ± SE; n refers to the number of rats from which the mesenteric arterial preparation was made. Statistical analysis was performed using repeated-measures analysis of variance (ANOVA), followed by Scheffe's F test. Differences were considered to be statistically significant when P was less than 0.05.

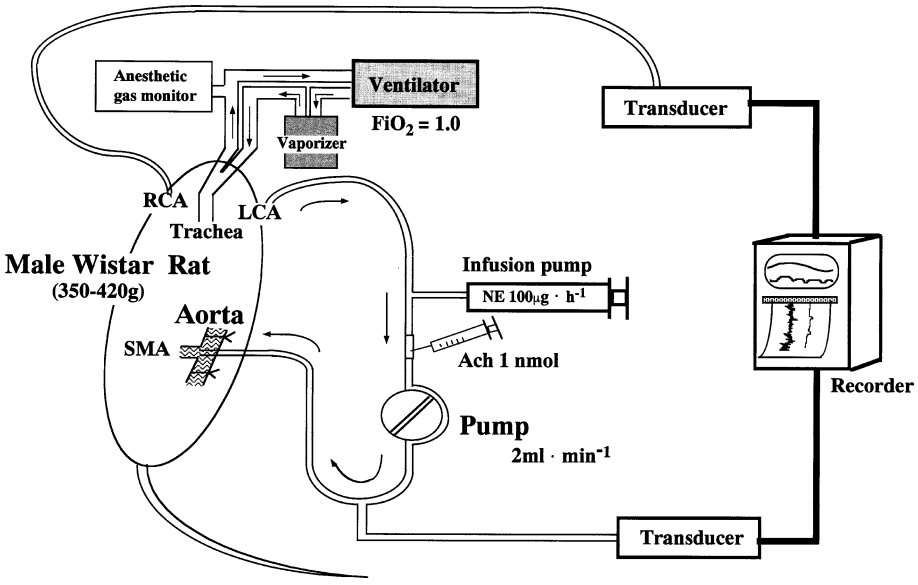

Fig. 2. Schematic diagram of the in-situ experiment. *RCA*, Right carotid artery; *LCA*, left carotid artery; *FiO_2*, fraction of inspiratory oxygen; *NE*, norepinephrine

Results

In-vitro mesenteric arterial beds

The basal perfusion pressure in mesenteric arterial beds was 46.8 ± 2.1 mmHg (n = 15). Methoxamine (30 to 80 μM) produced a sutained increased in perfusion pressure to 74.0 ± 2.8 mmHg (n = 15).

Perfusion with the solution containing the nitric oxide synthase inhibitor, L-NA (100 μM), caused a transient increase in the perfusion pressure, to 100.2 ± 8.6 mmHg (n = 15), followed by a decrease in the pressure to the levels observed prior to addition of this compound. Decreases in perfusion pressure caused by acetylcholine 1 nmol and 10 nmol in the absence of L-NA were 77.2 ± 4.1% and 85.3 ± 4.5%, respectively, and those in the presence of L-NA (100 μM) were 71.9 ± 4.2% and 77.3 ± 5.0% (n = 10), respectively, relative to the contraction induced by methoxamine. Although slight inhibition by L-NA was observed, the difference was not significant. The K^+ channel inhibitors, TEA (10 mM) and KCl (30 mM), significantly increased the perfusion pressure, from 73.2 ± 5.6 to 93.1 ± 6.2 mmHg (n = 6; $P < 0.05$), and from 74.8 ± 4.3 to 95.7 ± 9.7 mmHg (n = 6; $P < 0.05$), respectively. TEA (10 mM) and KCl (30 mM) significantly reduced the vasodilations produced by acetylcholine (Fig. 3), whereas, in the presence of L-NA (100 μM), these K^+ channel inhibitors completely abolished these vasodilations (n = 5). In perfused rat mesenteric arterial beds, charybdotoxin at 100 nM and apamin at 100 nM inhibited the vasodilator response to acetylcholine (1 nmol) by 93.7 ± 3.7% (n = 4) and 69.1 ± 1.1% (n = 4), respectively.

Exposure for 20 min to halothane (1.0% to 3.0%), as well as to isoflurane (1.0% to 3.0%), did not affect the

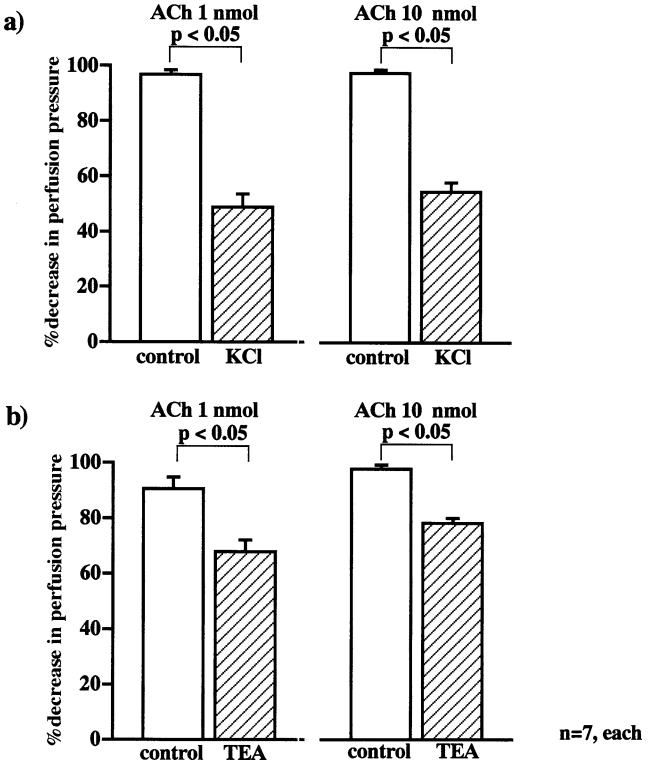

Fig. 3a,b. Effects of KCl (30 mM; **a**) and tetraethylammonium (*TEA*; 10 mM; **b**) on acetylcholine (1 nmol and 10 nmol)-induced vasodilation in perfused mesenteric arterial beds. As described in "Methods", the first response to acetylcholine served as the control, and the second response was obtained in the presence of testing agents in the same preparation. KCl (30 mM) or TEA (10 mM) completely abolished acetylcholine-induced vasodilation in the presence of N^G-nitro-L-arginine (L-NA; 100 μM; not shown). The number of preparations was seven in each experiment. *ACh*, Acetylcholine

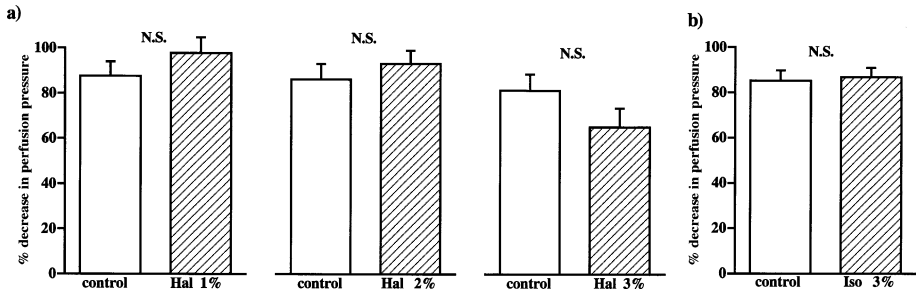

Fig. 4a,b. Effects of halothane (*Hal*; **a**) and isoflurane (*Iso*; **b**) on acetylcholine (1 nmol)-induced vasodilation. *n*-7 in each experiment. *N.S.*, Not significant (difference between without [*control*] and with anesthetics). Isoflurane at 1.0% and 2.0% did not affect acetylcholine-induced vasodilation (data not shown)

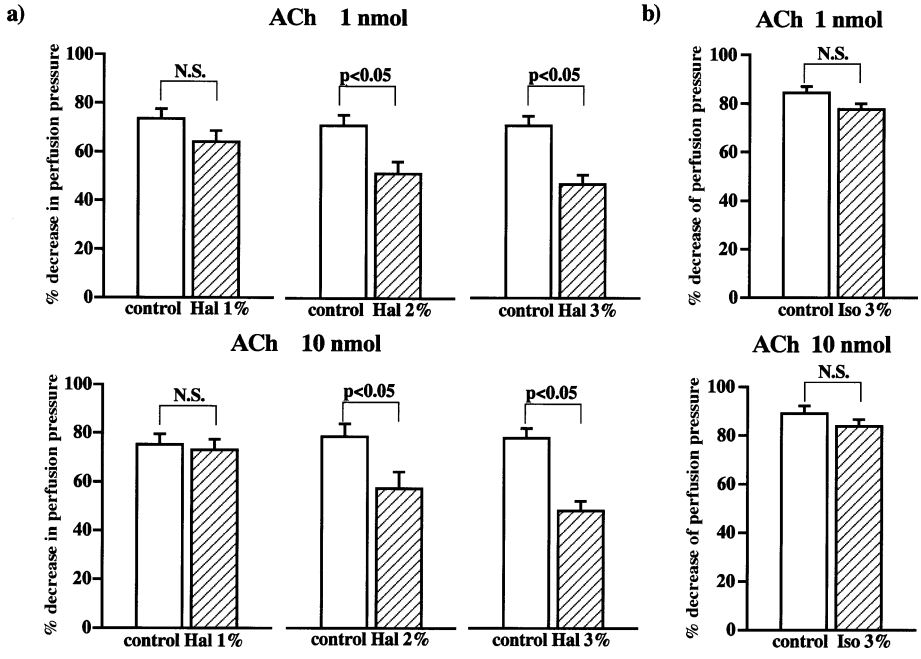

Fig. 5a,b. Effects of halothane (**a**) and isoflurane (**b**) on acetylcholine-induced vasodilation in the presence of L-NA. Isoflurane at concentrations of 1% and 2% did not affect acetylcholine-induced vasodilation (data not shown). *N.S.*, Not significant; *Hal*, halothane; *Iso*, isoflurane; *ACh*, acetylcholine

vasodilator responses produced by acetylcholine (1 nmol) (Fig. 4). In the presence of L-NA (100 μM), higher concentrations of halothane (2.0% and 3.0%), but neither isoflurane (1.0% to 3.0%) nor the lower concentration of halothane (1.0%), significantly impaired the vasodilation in response to acetylcholine (Fig. 5). The results with isoflurane 3% are presented in Fig. 5. Actual recordings are presented in Fig. 6. Neither halothane (2.0%) nor isoflurane (3%) altered vasodilator responses to acetylcholine, in the presence of TEA (10 mM) (Fig. 7) or KCl (30 mM) (Fig. 8).

In-situ mesenteric arterial beds

Before the inhalation of the volatile anesthetics, mean systemic arterial pressure measured in a carotid artery was 74.2 ± 5.5 mmHg. The perfusion pressures in the mesenteric artery after the inhalation of halothane or isoflurane were decreased from 107.0 ± 8.1 to 40.6 ± 3.1 mmHg (n = 6) and from 110.2 ± 9.3 to 48.3 ± 8.1 mmHg (n = 6), respectively. Vasodilations in response to acetylcholine (1 nmol) were not affected by the inhalation of halothane (1.0%) or isoflurane (1.3%) (1 MAC for rat) (Fig. 9).

Discussion

The present study has demonstrated the following findings. Firstly, in the in-vitro preparation, the nitric oxide synthase inhibitor, L-NA, did not affect vasodilations to acetylcholine, and the K^+ channel inhibitors, TEA and KCl, significantly reduced these vasodilations. However, only in the presence of L-NA, these K^+ channel inhibitors completely abolished the vasodilations produced by acetylcholine. Secondly, in these preparations, the higher concentrations of halothane, but neither isoflurane nor the lower concentration of halothane, significantly impaired vasodilator responses to acetylcholine in the presence of L-NA, whereas the volatile anesthetics did not affect these vasodilator responses in the absence of L-NA. Halothane did not alter the

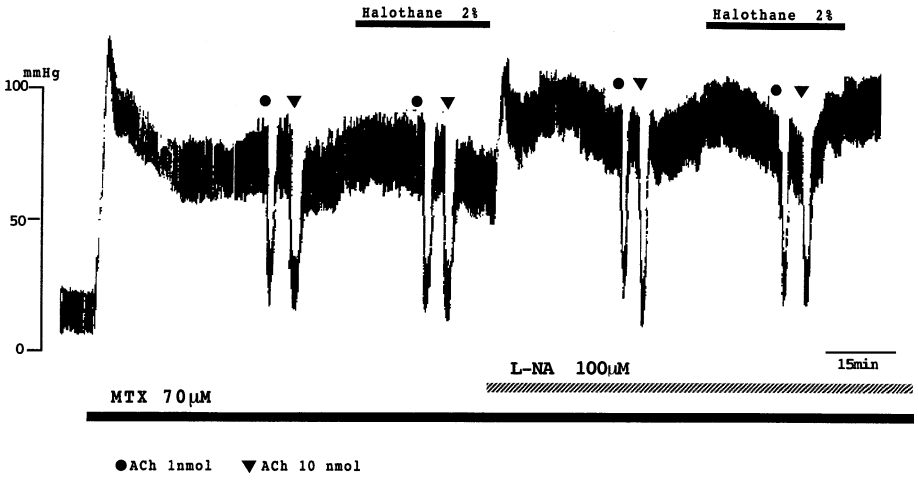

Fig. 6. The effects of halothane (2%) on acetylcholine-induced vasodilation in the absence or presence of L-NA. *MTX*, Methoxamine; *ACh*, acetylcholine

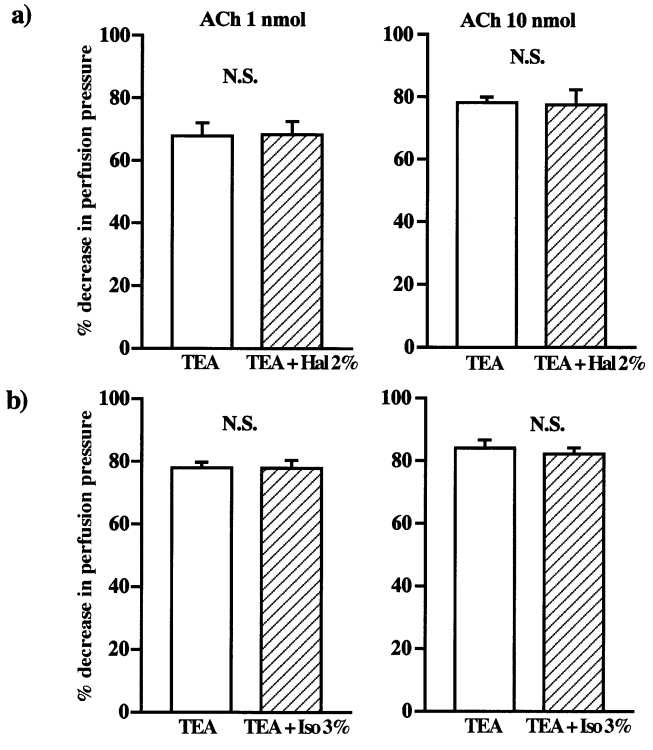

Fig. 7a,b. Effects of halothane (2%) and isoflurane (3%) on acetylcholine-induced vasodilation in the presence of TEA. *ACh*, Acetylcholine

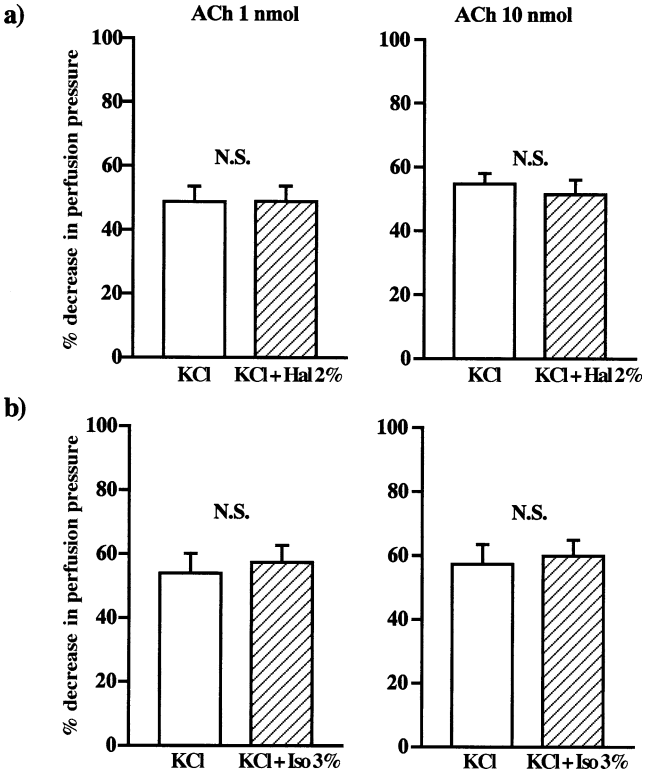

Fig. 8a,b. Effects of halothane (2%) and isoflurane (3%) on acetylcholine-induced vasodilation in the presence of KCl. *ACh*, Acetylcholine

vasodilation produced by acetylcholine, in the presence of TEA or KCl. Thirdly, in the in-situ preparation of the arterial beds, the vasodilator effects of acetylcholine were not affected by the inhalation of either halothane or isoflurane at 1 MAC. These results suggest that, in the resistance arteries in conditions of constant flow stress, halothane and isoflurane do not affect vasodilations in response to an endothelium-dependent agonist. However, in these in-vitro preparations, once the enzymatic activity of nitric oxide synthase is inhibited, higher concentrations of halothane, but neither isoflurane nor the lower concentration of halothane, appear to impair endothelium-dependent relaxations, probably mediated by TEA-sensitive K^+ channels.

In the perfused rat mesenteric artery beds, vasodilator responses to acetylcholine were not significantly altered by L-NA at a concentration of 10^{-4}M. These

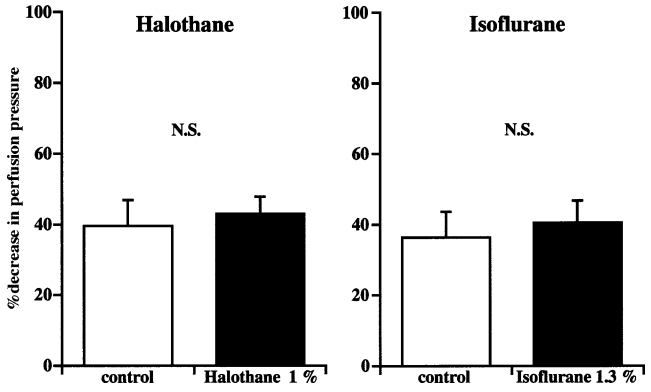

Fig. 9. Effects of inhalation of halothane and isoflurane at 1 minimum alveolar concentration (MAC) on acetylcholine-induced vasodilation in perfused rat mesenteric arterial beds

results are in contrast to results in our previous study, conducted on isolated rat aorta and mesenteric conduit arterial rings in nonperfused conditions, in which L-NA, at 2×10^{-5}M, an even lower concentration than that used in the present study, abolished relaxations to acetylcholine [12]. Moore et al. [16] have demonstrated that, in isolated blood vessels, the concentration of L-NA used in the present study can potently inhibit endothelial nitric oxide biosynthesis via impairment of the enzymatic activity of nitric oxide synthase. Therefore, it is unlikely that the failure of L-NA at 100 μM to inhibit vasodilatation to acetylcholine is due to an incomplete inhibitory effect of L-NA on endothelial nitric oxide synthase. In more peripheral arteries used in a nonperfused preparation, EDHF contributed to vasodilation in response to acetylcholine to a greater extent than nitric oxide [8]. Further, the mesenteric arterial beds used in the present study were constantly exposed to intravascular pressure and flow stress during the experiments. It has been shown that even a constant flow elicits vasodilations via a "shear stress" mechanism in rat mesenteric artery [7]. Thus, it appears that the existence of pressure and flow in peripheral arterial beds may contribute to these different results in perfused and nonperfused isolated mesenteric arteries. Further, in rat mesenteric arteries, L-NA in combination with indomethacin does not reduce relaxations to shear stress, suggesting that, in this arterial bed, endothelium-derived nitric oxide may not play a major role in endothelium-dependent relaxations in the presence of shear stress [7]. A study in isolated porcine coronary arteries indicated that shear stress may augment the contribution of EDHF-mediated relaxations to endothelium-dependent relaxation [17].

In the perfused mesenteric artery, TEA or KCl, in combination with L-NA, completely abolished vasodilations in response to acetylcholine. Higher concentrations of TEA, as well as KCl, are known to inhibit K^+ channels nonselectively, including voltage-dependent, Ca^{2+}-dependent, and ATP-sensitive K^+ channels [18]. Therefore, our results with TEA and KCl suggest that, in these preparations treated with inhibitors of nitric oxide synthase and cyclooxygenase, endothelium-dependent relaxations to acetylcholine are mediated by K^+ channels. Our previous study on isolated rat mesenteric conduit arteries treated with L-NA and indomethacin documented that vasorelaxations to acetylcholine were reduced by charybdotoxin and apamin, and completely abolished by a rather low concentration of TEA (1 mM) [12]. In fact, the data for the inhibitory effects of charybdotoxin and apamin on relaxation to acetylcholine in perfused rat mesenteric arterial beds are in agreement with our previous findings. These results indicate that, in rat mesenteric arterial beds, TEA-sensitive K^+ channels play a major role in vasodilations when the enzymatic activities of nitric oxide synthase and cyclooxygenase are inhibited, and that Ca^{2+}-dependent K^+ channels, at least in part, contribute to these relaxations. Previous studies in rat mesenteric arteries have demonstrated hyperpolarization concurrently with endothelium-dependent relaxation which is almost totally resistant to inhibitors of nitric oxide synthase and cyclooxygenase [19]. The vasodilator substance excluding nitric oxide and prostacyclin has been termed endothelium-derived hyperpolarizing factor (EDHF) [2]. Although we did not measure the membrane potentials during vasodilations to acetylcholine in the present study, the vasodilator component resistant to inhibitors of nitric oxide synthase and cyclooxygenase is probably mediated by EDHF. Recent studies have suggested several specific substances as candidates for EDHF, including cytochrome P450-derived arachidonic acid metabolites and anandamide [2]. However, because it has been proposed that these substances are not involved in the acetylcholine-induced hyperpolarization in rat mesenteric arteries [20,21], we did not conduct experiments by using inhibitors of cytochrome P450-derived arachidonic acid metabolites and anandamide.

In perfused rat mesenteric arteries, TEA and KCl reduced the vasodilations produced by acetylcholine, whereas the K^+ channel inhibitors completely abolished these vasodilations only in the presence of L-NA. In addition, although L-NA caused a transient increase in perfusion presssure, it did not affect the vasodilations induced by acetylcholine. Therefore, it is likely that the EDHF-mediated vasorelaxations may be unaffected by the inhibition of nitric oxide production, and, rather this may compensate for the loss of nitric oxide. Indeed, a recent finding in rat mesenteric arterial beds demonstrated that an analogue of cyclic GMP enhanced the inhibitory effect of a nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester, on carbachol-induced

relaxations, which are not altered by indomethacin [22], suggesting that, in these preparations, basal nitric oxide may modulate EDHF-mediated relaxations and, hence, on the loss of basal nitric oxide production, the EDHF component of these relaxations may become functionally greater [22]. A similar inhibitory effect of nitric oxide on relaxations in response to EDHF was reported in mesenteric arteries isolated from mice lacking the gene for constitutive endothelial nitric oxide synthase [23].

In the perfused mesenteric arterial beds, halothane (2.0% and 3.0%), but neither isoflurane (1.0%–3.0%) nor halothane (1.0%), impaired vasodilator responses to acetylcholine in the presence of L-NA and indomethacin. Therefore, in mesenteric arterial beds under conditions of shear stress, when the enzymatic activities of nitric oxide synthase and cyclooxygenase are inhibited, higher concentrations of halothane, but neither isoflurane nor the lower concentration of halothane, appear to impair endothelium-dependent relaxations. This conclusion is in agreement with previous studies on isolated arteries, suggesting that halothane reduces EDHF-mediated relaxations resistant to inhibitors of nitric oxide synthase and cyclooxygenase [10–12]. However, in contrast to our negative results with isoflurane, a previous study on isolated rabbit carotid arteries demonstrated that isoflurane reduced relaxations induced by EDHF [11]. Although we do not have a clear explanation for these differential effects of volatile anesthetics on the resistance of endothelium-dependent relaxations to inhibitors of nitric oxide synthase and cyclooxygenase, the most likely explanations are species and/ or regional differences in these arteries. In the present in-vitro and in-situ studies, halothane and isoflurane did not affect vasodilations in response to an endothelium-dependent agonist. In contrast to these results, previous studies on isolated arteries have demonstrated that, even in arteries in which EDHF contributes to endothelium-dependent relaxations, halothane and isoflurane are capable of inhibiting endothelium-dependent relaxations in the absence of inhibitors of nitric oxide synthase and cyclooxygenase [10–12]. In the present study, the perfusion of arteries itself appeared to change the balance between nitric oxide and EDHF-dependent components of endothelium-dependent relaxations, suggesting that these different results are probably due to the existence of shear stress in perfused arteries.

Halothane significantly inhibited the vasodilatation produced by acetylcholine at concentrations up to 2% in the presence of L-NA, but did not affect the vasodilatation at concentrations of up to 3% in the presence of TEA or KCl. These findings suggest that the effect of halothane on the resistance of endothelium-dependent relaxation to inhibitors of nitric oxide synthase and cyclooxygenase is mediated via a mechanism that is related to TEA-sensitive K^+ channels [18]. Previous studies on isolated arteries have also indicated that volatile anesthetics can reduce endothelium-dependent relaxations in response to EDHF mediated by TEA-sensitive K^+ channels [10–12]. Hecker and coworkers (Lischke et al. [11]) reported that volatile anesthetics may inhibit EDHF-mediated vasorelaxation in these arteries. However, because a direct inhibitory effect of volatile anesthetics on TEA-sensitive K^+ channels was also documented in the smooth muscle cells of guinea pig portal vein, we cannot rule out a direct effect of halothane on TEA-sensitive K^+ channels in our preparations [24].

Our results in the in-situ preparation of the mesenteric artery perfused with blood demonstrated that the 1 MAC of halothane and isoflurane did not affect vasodilations in response to acetylcholine. However, there are several limitations with these preparations. In the present study, more than 1 MAC of halothane or isoflurane induced severe hypotension (data not shown). In this situation, the drawing of blood from the internal carotid artery was not capable of supplying sufficient blood flow to the mesenteric artery to obtain a stable perfusion pressure. Therefore, we had to conduct these experiments only at the lower concentrations of anesthetics.

The results in the in-vitro and in-situ perfusion models of mesenteric resistance arterial beds suggest that halothane and isoflurane at clinically relevant concentrations may not affect endothelium-dependent vasodilator mechanisms in the mesenteric vasculature. The present results, obtained from experiments using perfused mesenteric arterial beds, in combination with the results of our previous studies using nonperfused aorta and mesenteric conduit arteries, support the idea that the effect of volatile anesthetics on endothelium-dependent vasodilatations may be altered by the experimental conditions, such as the absence or presence of intravascular pressure and flow [7].

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