Laboratory Report

Halothane inhibition of acetylcholine-induced relaxation in rat mesenteric artery and aorta

Hiroshi Iranami MD,
Yoshio Hatano MD,
Yoshi Tsukiyama MD,
Manabu Yamamoto MD,
Hiroshi Maeda MD,
Kazuhiro Mizumoto MD

Purpose: The effect of halothane was compared on acetylcholine (ACh)-induced relaxation of the mesenteric artery and the aorta in rats.

Methods: The responses of isolated rat aortic and mesenteric arterial ring segments precontracted with phenylephrine to ACh (10^{-8}–10^{-5} M), in the presence of halothane 0–3%, were compared using isometric force tension recordings. Effects of N^G-nitro-l-arginine (L-NOARG, 3 × 10^{-5}), methylene blue (MB, 5× 10^{-6} M), oxyhaemoglobin (OxyHB, 10^{-7} M), and various potassium channel inhibitors; tetraethylammonium (TEA, 10^{-5} M, 10^{-3} M), apamin (AP, 10^{-7} M), charybdotoxin (ChTx, 10^{-7} M) and glibenclamide (GC, 10^{-5} M) on ACh-induced relaxation in mesenteric artery were tested. Using radioimmunoassay, ACh (10^{-6} M)-induced guanosine 3':5'-cyclic monophosphate (cGMP) accumulation of mesenteric arterial rings pretreated with L-NAORG were also measured.

Results: L-NOARG partially inhibited ACh-induced relaxation in mesenteric arterial rings ($P < 0.05$, maximum relaxation reduced by approximately 50%), whereas it abolished them in aortic rings. The remaining relaxation resistant to L-NOARG in mesenteric arterial rings was insensitive to additional MB or OxyHB, and was not accompanied by increases in cGMP contents of rings. Halothane inhibited endothelium-dependent relaxation in aorta and mesenteric arterial rings. This inhibitory effect was larger in aorta. Halothane also inhibited NO independent EDHF-dependent relaxation in the mesenteric arterial rings.

Conclusion: Despite a similar inhibitory effect on the EDHF relaxing pathway, halothane has a larger effect on endothelium-dependent relaxation in the aorta (NO dependent mainly) than in the mesenteric rings (NO and EDHF dependent).

Objectif : Comparer sur l'artère mésentérique et sur l'aorte du rat l'influence de l'halothane sur la vasodilatation induite par l'acétylcholine (ACh).

Méthodes : On a comparé les réactions d'anneaux artériels isolés de segments d'aorte et d'artére mésentérique préalablement contractés avec de la phénylephrine à l'ACh (10^{-8}–10^{-5} M), en présence d'halothane à 0 à 3% sur des enregistrements de la force de la tension isométrique. On a vérifié les effets de la N^G-nitro-l-arginine (L-NOARG, 3 × 10^{-5} M), du bleu de méthylène (MB, 5 × 10^{-6} M), de l'oxyhémoglobine (OxyHB, 10^{-7} M) et de plusieurs inhibiteurs des canaux potassiques : le tétraéthylammonium (TEA, 10^{-5} M, 10^{-3} M), l'apamine (AP, 10^{-7} M), la charybdotoxine (ChTx, 10^{-7} M) et le glibenclamide (GC, 10^{-5} M) sur la vasodilatation de l'artère mésentérique induite par l'ACh. Le radioimmunodosage a servi en outre à mesurer l'accumulation de guanosine 3':'5 - monophosphatase cyclique (cGMP) dans les anneaux artériels mésentériques prétraités à la L-NOARG.

Résultats : La L-NOARG n'abolissait que partiellement la vasodilatation des anneaux artériels mésentériques induite par l'ACh ($P < 0.05$, réduction de la dilatation maximale d'environ 50%) alors qu'elle abolissait celle des anneaux aortiques. La vasodilatation résiduelle L-NOARG-résistante des anneaux artériels mésentériques ne réagissait pas à l'ajout de MB ou d'OxyHB et ne s'accompagnait pas d'une augmentation du contenu en cGMP des anneaux. L'inhibition par l'halothane de la vasodilatation endothélium-dépendante des anneaux aortiques et artériels mésentériques était plus importante dans l'aorte. L'halothane inhibait aussi la vasodilatation NO-indépendante EDHP-dépendante des anneaux mésentériques.

Conclusion : Malgré des effets inhibiteurs similaires sur les voies de la vasodilation EDHF, l'halothane a un effet plus prononcé sur la vasodilatation endothélium-dépendante au niveau de l'aorte (principalement NO-dépendante) qu'au niveau des anneaux mésentériques (NO-et EDHF-dépendante).

ACETYLCHOLINE (ACh) stimulates vascular endothelium to release endothelium-derived nitric oxide (NO), resulting in vascular smooth muscles (VSMs) relaxation via a guanosine 3':5'-cyclic monophosphate (cGMP)-mediated mechanism.[1,2] Numerous investigations have revealed that volatile anaesthetics can inhibit this relaxation mechanism in isolated blood vessels.[3–8] Almost of all these findings were based on the isometric force tension experiments using isolated rat and rabbit aorta, which were categorized into large conduit vessel.

Isolated smaller arteries were reported to have different responses to vasoconstrictive substances or to transmural electrical stimulation from those of the aorta.[9] Su *et al.* elucidated the responses of rabbit aortic and femoral arterial segments to vasoactive substance, norepinephrine, in the presence of enflurane and indicated that it produced different modulation of norepinephrine-induced tone in the femoral artery from that in the aorta.[10] These findings suggest that volatile anaesthetics have different modulations on the vasorelaxation mechanism in smaller arteries.

The present study was designed to examine the inhibitory potency of halothane, the strongest inhibitor among the available volatile anaesthetics,[4] on ACh-induced relaxation in a smaller conduit artery (mesenteric artery) in comparison with that in a larger one (aorta). The findings of the present study might provide further insight into the actions of volatile anaesthetics on the endothelium-dependent vasorelaxation mechanism in more peripheral vessels.

Methods

Male Wistar rats weighing 200 to 300 g were anaesthetized with intraperitoneally injected 50 mg·kg^{-1} pentobarbital. Midline incisions followed by cross-clamping of the aortic above and below the mesenteric artery were performed, after which the main mesenteric arteries were removed rapidly. The rats were killed by releasing the two aortic clamps and the thoracic aortas were isolated. The samples were rapidly dissected free from surrounding connective tissues and divided into 3 mm-long ring segments. The aortic and mesenteric arterial rings were mounted on two parallel tungsten wire hooks, with diameters of 100 and 40 µm, respectively and placed in water-jacketed 10-ml organ baths containing modified Krebs' solution (composition in mM: NaCl 119, KCl 4.7, MgSO$_4$ 1.17, NaHCO$_3$ 25, KH$_2$PO$_4$ 1.18, CaCl$_2$ 2.5 and glucose 11), which was gassed with CO$_2$ 5% v/v in O$_2$ and maintained at 37°C. The lower hook was attached to a support leg and the upper to a force transducer (Nihondenki-Sanei Co. Tokyo, Japan). Changes in

isometric force were amplified (Nihondenki-Sanei Co. Tokyo, Japan) and displayed on an ink-writing recorder (Nihondenki-Sanei Co. Tokyo, Japan).

The aortic and mesenteric arterial rings were placed under resting tensions of 3.0 and 1.5 g, respectively and equilibrated for 60 min, during which the bath fluids were exchanged every 15 min. Optimal resting tensions for the aortic and the mesenteric arterial rings had been preliminary determined by the resting tension-contraction relationship. Then, the rings were contracted submaximally with phenylephrine 3 × 10^{-7} M (aorta) and 10^{-6} M (mesenteric arteries) in the absence (control) or presence of halothane (1, 2 and 3%). The amount of phenylephrine had been determined as ED$_{80}$ for aortic and mesenteric arterial rings, respectively. Halothane was introduced into the aerating gas through the agent-specific vaporizer, Fluotec 3 (BOC Co, USA), and its concentration was regulated by the anaesthetic gas monitor (Atom Co., Tokyo, Japan). Preliminary experiments had revealed that the concentrations of dissolved anaesthetics into the nutritional solution measured by the Gas chromatographic method had a linear relationship with the concentrations which were introduced into the insufflating gas mixture through this monitor. When a steady plateau contraction to phenylephrine was obtained, the responses to cumulatively applied ACh were then tested on the control rings and those treated with halothane.

In order to compare the inhibitory effects of N^G-L-nitro arginine (L-NOARG), an inhibitor of NO synthase, on ACh-induced relaxations of aortic and mesenteric arterial rings, each ring was first pretreated with L-NOARG (3 × 10^{-5} M) for 20 min, and then phenylephrine was added to induce a sustained contraction. When the contraction had reached a plateau, each ring had been treated with L-NOARG for at least 30 min, and the responses to ACh were tested, as described above.

In an attempt to characterize the remaining ACh-induced relaxation resistant to L-NOARG in arterial rings, each ring was pretreated with L-NOARG (3 × 10^{-5} M) for 20 min, contracted with phenylephrine as described above and then the relaxation evoked by ACh (10^{-6} M) was tested (the control response). Then, the rings were treated with oxyhemoglobin (OxyHB, 10^{-7} M) or methylene blue (MB, 10^{-6} M) for 30 min, and otherwise treated with tetraethylammonium (TEA, 10^{-5} M, 10^{-3} M), glibenclamide (GC, 10^{-5} M), apamin (AP, 10^{-7} M) or charybdotoxin (ChTx, 10^{-7} M) for 30 min, after which ACh (10^{-6} M)-induced relaxation was tested again.

The contents of guanosine 3':5'-cyclic monophosphate (cGMP) of mesenteric arterial rings precontracted with phenylephrine (10^{-6} M) followed by exposure to ACh (10^{-6} M) for 10 min in the presence of

L-NOARG (3×10^{-5} M) were measured. These rings were the same size as those used in the isometric force tension experiment and connective tissues around the rings had been cleaned free. Following exposure to ACh, they were frozen quickly in liquid nitrogen, and the contents of cGMP were measured by radioimmunoassay (Yamasa cGMP assay kit, Yamasa Shoyu Co. Chiba, Japan). All the rings were treated with indomethacin (10^{-5} M) throughout the experiments.

Data analysis

The relaxations induced by ACh were expressed as percentages relative to the maximum relaxation induced by papaverine 10^{-4} M (% Relaxation). Papaverine caused relaxation below the resting passive tension in all rings, therefore, maximum relaxation was determined by the difference between the initial tension induced by phenylephrine and the tension after the introduction of papaverine. The concentration of ACh that produced 50% of the maximal relaxation induced by papaverine (EC_{50}) was determined in aortic and mesenteric arterial rings, respectively. The negative logarithm to base 10 of EC_{50} (pD_2) and maximum % relaxation (induced by ACh 10^{-5} M) were used for the statistical comparison of relaxation responses of both rings to ACh. The data are expressed as mean $\pm$ SEM. The inhibitory potencies of halothane were expressed as % inhibitions, which were calculated by the following formula:

$$\left(1 - \frac{\% \text{ relaxation in the presence of halothane}}{\% \text{ relaxation in the absence of halothane}}\right) \times 100 \ (\%)$$

Data from studies using various inhibitors and radioimmunoassay were analysed by Student's t test for paired samples. The comparisons of % inhibitions, pD_2 values and maximum % relaxations between aortic and mesenteric arterial rings were performed by Student's t test for unpaired samples. The other data were evaluated by analysis of variance (ANOVA) and Scheffe's F test for repeated measurements. Responses that differed from control values at $P < 0.05$ were considered to be significant. All statistical analyses were performed using Statview II on an Apple MacIntosh computer.

The chemicals used for the experiments were phenylephrine hydrochloride, indomethacin, methylene blue, N^G-1-nitro-arginine, haemoglobin, tetraethylammonium (Sigma chemical Co, St. Louis, USA), halothane (Takeda Pharmaceutical Co, Osaka, Japan), ACh (Dai-ichi Pharmaceutical Co, Tokyo, Japan), apamin, charybdotoxin, glibenclamide (Research Biochemical Inc., Natick, USA) and papaverine hydrochloride (Dai-Nippon Pharmaceutical Co, Tokyo, Japan). Indomethacin was

dissolved in ethanol 99% and all the other drugs were dissolved in distilled water. Oxyhaemoglobin was prepared using sodium hydrosulphite (Sigma chemical Co, St. Louis, USA) as follows: 10 ml freshly prepared sodium hydrosulphite (10^{-3} M) were added to 10 ml commercial haemoglobin (10^{-3} M), and the sodium hydrosulphite was removed by dialysis against a sufficient volume of distilled water for two hours at 4°C. The conversion of methaemoglobin to oxyhaemoglobin was checked spectrophotometrically. If satisfactory conversion had occurred, 1 ml aliquots were frozen at –20°C and stored for up to one week before use.

Results

Active plateau force induced by phenylephrine plus maximum negative force induced by papaverine yielded 1.84 ± 0.03 (n = 22) and 0.74 ± 0.02 g (n = 22) in aortic and mesenteric arterial rings, respectively. Acetylcholine (from 10^{-8} M to 10^{-5} M) relaxed the phenylephrine-precontracted thoracic aortic and mesenteric arterial rings in a dose-relation manner and in endothelium-dependent manner (data were not shown). The pD_2 values of aortic and mesenteric arterial rings in response to ACh were 6.75 ± 0.05 and 7.34 ± 0.03, respectively ($P < 0.05$, n = 16, each). The maximal %relaxations of both rings were $73 \pm 0.45\%$ and $84 \pm 0.32\%$, respectively ($P < 0.05$, n = 16, each). An endothelium-independent vasodilator, papaverine (10^{-4} M) fully relaxed the phenylephrine-induced tone of aortic and mesenteric arterial rings.

In the presence of halothane (1, 2 and 3%), active plateau force induced by phenylephrine plus negative force induced by papaverine in aortic rings yielded 1.77 ± 0.02, 1.65 ± 0.04 and 1.54 ± 0.01 g (n = 6, each), respectively, and those in mesenteric arterial rings yielded 0.65 ± 0.05, 0.54 ± 0.07 and 0.39 ± 0.04 g (n = 6, each), respectively. Comparison between those in the absence and presence of halothane revealed that halothane 2 and 3% reduced relaxation in both rings ($P < 0.05$, n = 6, each). Therefore, additional application of phenylephrine (up to 10^{-6} M or 2×10^{-6} M in aortic or mesenteric arterial rings, respectively) was performed to maintain the pre-anaesthetic forces. The ACh-induced relaxations in the presence of halothane (1, 2 and 3%) are shown in Figure 1. Halothane (1, 2 and 3%) showed concentration-dependent inhibitory effects on the ACh-induced relaxations of aortic ($P < 0.05$ or 0,001) and mesenteric arterial rings ($P < 0.05$ or 0,001). Percent inhibitions (% inhibition) of halothane (2 and 3%) in aortic and mesenteric arterial rings are summarized in the table. The % inhibitions in aortic rings were greater than in mesenteric arterial
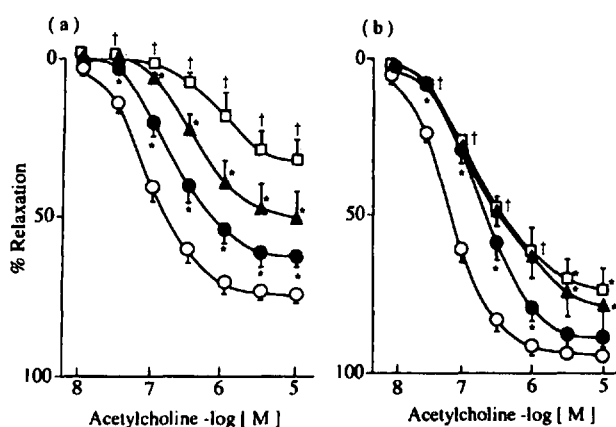
FIGURE 1   Effects of halothane on the relaxation responses to ACh of aortic (a) and mesenteric arterial rings (b) precontracted with phenylephrine. (O) denotes the rings without halothane (control). (●), (▲) and (□) denote the rings treated with halothane 1%, 2% and 3%, respectively. All rings represent mean ± SEM of six separate experiments.
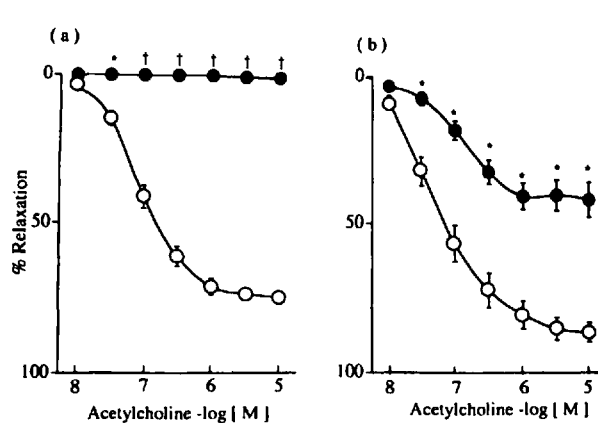
$*P < 0.05$ vs control
$†P < 0.001$ vs control

FIGURE 2   Effects of L-NOARG (3 × 10^{-5} M) on relaxation responses to ACh of aortic (a) and mesenteric arterial rings (b). (O) denotes the rings without L-NOARG (control). (●) denote the rings treated with L-NOARG. All rings represent mean ± SEM of six separate experiments.

$*P < 0.05$ vs control
$†P < 0.001$ vs control

rings at each concentration of ACh and at both concentrations of halothane ($P < 0.05$, n = 6, each).

The effect of L-NOARG on ACh-induced relaxation in both rings is shown in Figure 2. In the presence of L-NOARG, active plateau force induced by phenylephrine plus negative force induced by papaverine in aortic and mesenteric arterial rings yielded 1.89 ± 0.05 and 0.79 ± 0.06 g, respectively (*P*: NS. Pretreatment with L-NOARG (3 × 10^{-5} M) almost abolished the ACh-induced relaxations in aortic rings ($P < 0.001$, n = 6, each). It also produced an inhibitory action on those in mesenteric arterial rings ($P < 0.05$, n = 6), but this inhibition was incomplete: the maximum % relaxation induced by ACh (10^{-5} M) was only reduced to 42 ± 2.6% from 84 ± 0.32%.

In a further attempt to determine whether the NO-cGMP relaxation mechanism involved in the L-NOARG resistant component of the ACh-induced relaxations in mesenteric arterial rings, the effects of other inhibitors of NO-cGMP pathway, MB (5 × 10^{-6} M) and OxyHB (10^{-7} M), on the ACh (10^{-6} M)-induced relaxations of mesenteric arterial rings pretreated with L-NOARG (3 × 10^{-5} M) were tested (Figure 3). In the presence of MB or OxyHB, active tone induced by phenylephrine plus negative force induced by papaverine yielded 0.79 ± 0.02 or 0.74 ± 0.06 g, respectively. The phenylephrine-induced tones were not changed by these inhibitors (n = 6, each). The ACh (10^{-6} M)-induced relaxation of mesenteric arterial rings pretreated with L-NOARG (3 × 10^{-5} M) showed 41.7 ±

TABLE   Comparisons of the inhibitory effects of halothane on ACh (10^{-8}–10^{-5} M)-induced relaxation in aortic and mesenteric arterial rings. All data are expressed as "% inhibition" (halothane 2% and 3%). All values represent mean ± SEM of six separate experiments.

| ACh | Halothane 2% | | Halothane 3% | |
	Aortic rings	Mesenteric arterial rings	Aortic rings	Mesenteric arterial rings
10^{-8}M	96.2 ± 0.12*	81.1 ± 0.06	97.1 ± 0.11*	79.8 ± 0.03
3 × 10^{-8}M	95.5 ± 0.21*	61.1 ± 0.02	95.5 ± 0.22*	66.7 ± 0.11
10^{-7}M	84.4 ± 0.19*	52.2 ± 0.31	95.3 ± 0.07*	56.5 ± 0.34
3 × 10^{-7}M	63.8 ± 0.16*	43.2 ± 0.42	87.2 ± 0.12*	43.8 ± 0.56
10^{-6}M	43.7 ± 0.20*	30.2 ± 0.23	74.5 ± 0.31*	34.3 ± 0.48
3 × 19^{-6}M	36.8 ± 0.31*	19.7 ± 0.23	59.6 ± 0.35*	25.3 ± 0.39
10^{-5}M	32.8 ± 0.33*	16.4 ± 0.18	56.9 ± 0.28*	23.3 ± 0.33

$*P < 0.05$ between both rings
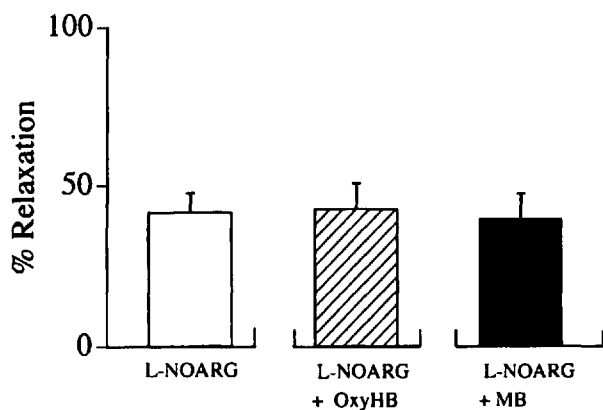
FIGURE 3   Effects of oxyhaemoglobin (OxyHB, 10^{-7} M) or methylene blue (MB, 5×10^{-6}) on the relaxation responses to ACh (10^{-6} M) of the mesenteric arterial rings pretreated with L-NOARG (3×10^{-5} M). All rings represent mean ± SEM of five separate experiments.

FIGURE 4   Effect of ACh (10^{-6} M) on the cGMP contents of mesenteric arterial rings precontracted with phenylephrine (10^{-6} M) in the absence (a) or presence of L-NOARG (3×10^{-5} M, b). All rings represent mean ± SEM of five separate experiments.

$*P < 0.05$ vs pre-ACh (white bar)

5.6% (n = 6). These relaxations were not affected in the presence of additionally applied MB (44.4 ± 6.9%, n = 5) or OxyHB (40.3 ± 7.6%, n = 5).

Furthermore, the changes in the cGMP content of mesenteric arterial rings were examined (Figure 4). Mesenteric arterial rings precontracted with phenylephrine (10^{-6} M) had mean cGMP content of 137 ± 9.6 fmol·mg^{-1}, wet tissue (n = 5). The application of ACh (10^{-6} M) to these rings increased the cGMP content (207 ± 14.3 fmol·mg^{-1}, wet tissue, $P < 0.05$, n = 5). Treatment with L-NOARG (3×10^{-5} M) did not change the cGMP content (116 ± 18.1 fmol·mg^{-1}, wet tissue, n = 5). The application of ACh (10^{-6} M) to L-NOARG-treated rings failed to increase the cGMP content (89 ± 10.6 fmol/mg, wet tissue, n = 5).

To examine the association of potassium channels with the L-NOARG resistant component of ACh-induced relaxation, four distinct inhibitors; TEA (10^{-5} M, 10^{-3} M), AP (10^{-7} M), ChTx (10^{-7} M) or GC (10^{-5} M), were tested (Figure 5). In the presence of TEA (10^{-5} M), TEA (10^{-3} M), AP, ChTx or GC, active tone induced by phenylephrine plus negative force induced by papaverine yielded 0.82 ± 0.01, 0.74 ± 0.04, 0.80 ± 0.02, 0.73 ± 0.01 or 0.71 ± 0.08 g, respectively. Phenylephrine-induced tone was not changed by these inhibitors (n = 6, each). The ACh (10^{-6} M)-induced relaxations of the rings were inhibited by pretreatment with TEA 10^{-5} M ($P < 0.05$, n = 6), TEA 10^{-3} M ($P < 0.001$, n = 6), AP ($P < 0.05$, n = 6) and ChTx ($P < 0.05$, n = 6), but not by GC (n = 6).

The effect of halothane on the L-NOARG resistant components of ACh-induced relaxation of mesenteric arterial rings was examined (Figure 6). In the presence
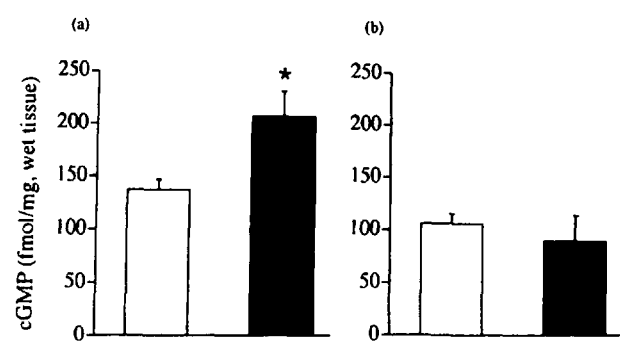
of halothane (1, 2 or 3%), active tone induced by phenylephrine plus negative force induced by papaverine yielded 0.77 ± 0.02, 0.72 ± 0.03 or 0.69 ± 0.04 g, respectively. The phenylephrine-induced tones were not changed by halothane (n = 6, each). Halothane (1, 2 and 3%) inhibited the L-NOARG-resistant components of ACh-induced relaxations of mesenteric arterial rings ($P < 0.05$, n = 6, each).

Discussion

The most important finding in the present study was that halothane produced an inhibitory action on ACh-induced relaxation in both aortic and mesenteric arterial rings, but its action was less potent in the mesenteric artery than in the aorta.

This study confirmed that the mechanism underlying ACh-induced relaxation in rat mesenteric artery is not solely derived from the NO-cGMP relaxation pathway, which has been proved to be a major one in rat aorta. Pretreatment with L-NOARG inhibited the ACh-induced endothelium-dependent relaxations of the mesenteric artery only partially, whereas it abolished those of the aorta. These findings with rat mesenteric arteries are in good agreement with those reported by Garland et al.[11] Moreover, our observations that OxyHB and MB did not affect the L-NOARG-resistant component of ACh-induced mesenteric arterial relaxation and that this component was not accompanied with cGMP accumulation, strongly support the view that endothelium-dependent and non NO-cGMP mechanisms are involved in the ACh-induced relaxation in rat mesenteric artery. Prostacyclin, a cyclo-oxygenase product generated from arachidonic acid, is known to be one of the media-
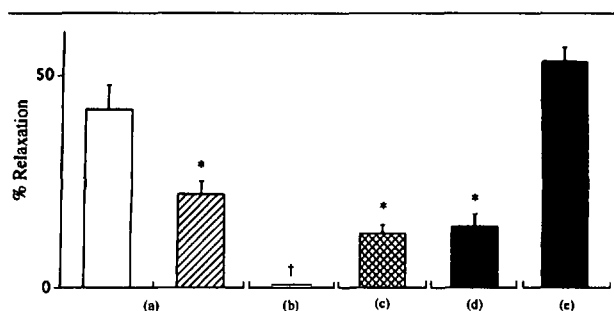
FIGURE 5   Relaxation responses to ACh (10^{-6} M) of the mesenteric arterial rings pretreated with L-NOARG (3×10^{-5} M) in the absence (a, white bar) or presence of potassium channel inhibitors; TEA(10^{-5} M, a, shaded bar), TEA (10^{-3} M, b), ChTx (10^{-7} M, c), AP (10^{-7} M, d) and GC (10^{-5} M, e). All rings represent mean ± SEM of five separate experiments.

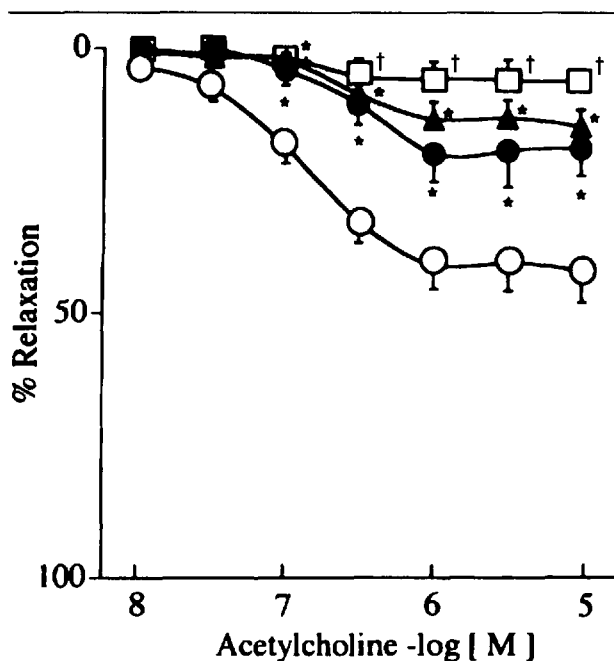$*P < 0.05$
$\dagger P < 0.001$ *vs* pre-ACh (white bar)

FIGURE 6   Effect of halothane on ACh-induced relaxation of mesenteric arterial rings pretreated with L-NOARG (3×10^{-5} M). (O) denotes the rings without halothane (control). (●), (▲) and (□) denotes the rings treated with halothane 1%, 2% and 3%, respectively. All rings represent mean ± SEM of six separate experiments.

$*P < 0.05$ *vs* control
$\dagger P < 0.001$ *vs* control

tors released from vascular endothelium. However, this mediator was not likely to be attributed to the relaxation mechanism, since, in the present study, the arterial rings were treated with sufficient amount of indomethacin for preventing its synthesis during the experiments. Recently,

another factor which mediates endothelium-dependent ACh-induced relaxation of isolated arteries has been reported.[12-15] This factor characteristically hyperpolarizes VSM membrane to evoke vasorelaxation, independently of the cGMP- and/or cAMP-mediated mechanisms. This mediator was termed endothelium-derived hyperpolarization factor. Garland *et al.* recorded the membrane potentials of VSMs and isometric tension of rat isolated mesenteric artery simultaneously, and demonstrated that ACh caused vasorelaxation accompanying with membrane hyperpolarization of VSM even in the presence of L-NOARG (10^{-4} M).[11] Their findings confirm our view that, unlike in the aorta, both NO and EDHF were involved in the ACh-induced relaxation of the rat isolated mesenteric artery.

Although the nature of EDHF has not been fully clarified, recent investigations suggested that an increase in the membrane conductance for potassium is involved in EDHF-mediated relaxations of VSMs.[16-18] In most species, including the rat, Ca^{2+}-dependent potassium channel is involved in EDHF-mediated relaxation mechanism, but ATP-dependent potassium channel in others. In any channels activated by EDHF, it seems likely to cause strong relaxation co-operated with NO. A more recent report suggested that EDHF is the P450 metabolite of arachidonic acid.[19] In the present study, several potassium channel inhibitors, including selective inhibitors of Ca^{2+}- activated (AP and ChTx), ATP-activated (GC) and non-specific (TEA), were tested. Our findings that TEA, apamin and charybdotoxin but not glibenclamide inhibited the L-NOARG-resistant component of ACh-induced relaxation in the mesenteric artery indicate that Ca^{2+}-activated potassium channel opening is associated with this relaxation mechanism and this is in good agreement with the findings of Kajioka *et al.* who demonstrated that Ca^{2+}-activated potassium channel opening greatly contributed to the EDHF-mediated relaxation mechanism in rat mesenteric arterial vasculature.[20] Therefore, it seems reasonable to suggest that the L-NOARG-resistant component of ACh-induced relaxation is derived from EDHF.

The findings with rat aorta reported by other investigators[4,5,7] are in good agreement with our findings. Although the mechanism underlying the inhibitory action on the NO-mediated mechanism has not been fully clarified, several sites of action are postulated: within endothelium; calmodulin sites, Ca^{2+} mobilization, release of NO, within VSMs; activity of soluble guanylyl cyclase.[21] Also, it has been demonstrated that volatile anaesthetics can inhibit the EDHF-mediated relaxation in rabbit mesenteric arteries,[22] or that halothane can inhibit the release of EDHF from endothelium of rabbit carotid artery.[23] Our finding

that halothane produced an inhibitory action on the relaxation of mesenteric arteries pretreated with L-NOARG also supports them.

Su *et al.* reported that enflurane enhanced norepinephrine-induced tone in the aorta, whereas it decreased that in the femoral artery, although it had similar mechanisms of action in aorta and femoral artery: blocking Ca^{2+} influx and causing Ca^{2+} release from sarcoplasmic reticulum through the ryanodine-receptor channel. They concluded the differences in intracellular Ca^{2+} modulations evoked by agonist between aorta and femoral artery was the reason for the differences in cellular reactions exposed to enflurane between these arteries.[10] Their findings suggest that the responses of smaller arteries exposed to volatile anaesthetics are not always the same as those of larger arteries. Muldoon *et al.* reported that halothane inhibited ACh-induced relaxation in canine carotid and femoral arteries. However, comparisons of inhibitory potencies of halothane in these vessels and in larger ones were not be stated.[3] The present study focussed on the differences in inhibitory potency of halothane on the ACh-induced relaxations of larger conduit (aorta) and smaller vessels (mesenteric artery) and showed that the inhibitory potency of halothane was greater in the former than in the latter. As described above, the ACh-induced relaxation in the aorta is predominantly derived from NO, whereas, that in the mesenteric artery is derived not only from NO but also from EDHF. These findings may raise the question as to why halothane inhibits ACh-induced relaxation of rat mesenteric artery to a lesser extent than that of the aorta despite the fact that halothane can inhibit relaxation mediated not only via the NO-cGMP but also by the EDHF pathways. We hypothesize that vasorelaxation would be amplified when the two distinct mechanisms, which are initiated by muscarinic receptor stimulation, act cooperatively. Although further study is needed to clarify this hypothesis, the finding that ACh relaxed mesenteric arterial rings more potently than aortic rings, whereas, the endothelium-independent vasodilator, papaverine, could relax both rings fully, is supportive of the concept.

In conclusion, we report, for the first time, that inhibitory effect of halothane on ACh-induced relaxation is less potent in smaller conduit arteries (mesenteric artery) than in larger vessels (aorta), even though halothane can produce strong inhibition not only of the EDHF-mediated mechanism but also of NO-mediated relaxation. The mesenteric artery is only one of the small conduit vessels. The contribution of EDHF to relaxation responses of similar small calibers of upper or lower limb to endothelium-dependent dilator, ACh, might be different from that of mesenteric artery. However, our findings provide further insight into the

haemodynamic effects of halothane on endothelium-dependent relaxation mechanism of small calibre vessels whose tone contribute more to arterial pressure than does the tone of the large calibre aorta.

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