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Halothane inhibits endothelium-dependent relaxation elicited by acetylcholine in human isolated pulmonary arteries

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Abstract

This study examined whether a clinically relevant concentration of the volatile anaesthetic halothane modifies the endothelium-dependent relaxation produced by acetylcholine (3 nM–10 μM), histamine (1 pM–0.1 μM) and anti-human immunoglobulin E (1:1000) in human isolated pulmonary arteries submaximally precontracted with noradrenaline. An inhibitor of nitric oxide formation, N^G-nitro-L-arginine (100 μM), attenuated acetylcholine-induced relaxation but failed to inhibit histamine- and anti-human immunoglobulin E-induced relaxation. Indomethacin (2.8 μM, a cyclooxygenase inhibitor) preferentially reduced the relaxation to histamine and anti-human IgE. Halothane (2%) significantly attenuated the relaxation to acetylcholine but had no significant effect on the relaxation elicited by histamine and anti-human IgE. Halothane (2%) enhanced the basal release of prostaglandin I_2 by human pulmonary arteries (control 0.31 ± 0.04 ng mg^{-1}; treated tissues 0.50 ± 0.06 ng mg^{-1}; $n = 5$; $P < 0.05$). Halothane (2%) did not alter the responsiveness and sensitivity of preparations to relaxants acting through activation of adenylyl cyclase (forskolin) or guanylyl cyclase (sodium nitroprusside) or by the opening of K_{ATP} channels (cromakalim). In conclusion, halothane inhibits the endothelium-dependent relaxation of human pulmonary arteries to acetylcholine by interfering with the nitric oxide pathway at a site before activation of soluble guanylyl cyclase in vascular smooth muscle.

Keywords: Pulmonary artery, human; Halothane; Endothelium-dependent relaxation

1. Introduction

The endothelium plays an important role in the modulation of vascular tone through the production of vasoactive factors (Furchgott, 1983). In the pulmonary arterial bed, the endothelium produces vasodilators such as the endothelium-derived relaxing factors (EDRFs), prostaglandin I_2, and endothelium-derived hyperpolarizing factor (EDHF) that cooperate to maintain the normoxic low tone (Peach et al., 1989; Hasunuma et al., 1991). One of these EDRFs is nitric oxide (NO) which activates soluble guanylyl cyclase to form cyclic GMP in vascular smooth muscle cells thus producing relaxation (Palmer et al., 1987; Ignarro, 1990). Thus, EDRF/NO and prostaglandin I_2 induce relaxation via two different second messengers, cyclic GMP and cyclic AMP, respectively. EDHF relaxes by increasing membrane permeability of vascular smooth muscle cells to K^+ although the type of K^+ channel involved may differ in different vascular preparations (Standen et al., 1989; Hasunuma et al., 1991; Garland et al., 1995). Endothelium-dependent relaxation to acetylcholine, histamine, anti-human immunoglobulin E (IgE) as well as to other substances has been described in human isolated pulmonary arteries (Greenberg et al., 1987; Thom et al., 1987; Crawley et al., 1990; Dinh Xuan et al., 1990; Ortiz et al., 1992, 1993; Norel et al., 1996).

Volatile anaesthetics have been reported to affect EDRF/NO formation, release or action although some reports yielded conflicting results and the clinical relevance of this effect is yet uncertain (Johns, 1991). One of these volatile anaesthetics, halothane, was reported to inhibit endothelial-dependent relaxation to acetylcholine or bradykinin in canine femoral and carotid arteries, rabbit and rat aortae, and cultured bovine aortic endothelial cells

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(Muldoon et al., 1988; Toda et al., 1992; Blaise et al., 1994). Endothelium-dependent effects of halothane in rat isolated aorta have been interpreted as related to inhibition of EDRF production or action (Muldoon et al., 1988; Stone and Johns, 1989; Nakamura et al., 1991; Toda et al., 1992; Blaise et al., 1994) or to enhanced release of a vasodilating cyclooxygenase metabolite from vascular endothelium (Stone and Johns, 1989) as demonstrated to occur in cultured bovine pulmonary artery endothelial cells (Barnes et al., 1992).

The aim of the present study was to examine the effects of a clinically relevant concentration of halothane on the endothelium-dependent relaxation produced by acetylcholine and anti-human IgE in human isolated pulmonary arteries. The effects of halothane on endothelium-dependent relaxation were compared with those produced on relaxations elicited by forskolin (activator of adenylyl cyclase; Rabe et al., 1994), sodium nitroprusside (activator of guanylyl cyclase; Rabe et al., 1994), and cromakalim (a K_{ATP} channel opener; Hamilton and Weston, 1989). In addition, basal prostaglandin I_2 production by human pulmonary artery (Ortiz et al., 1993) was measured in halothane-treated tissues.

2. Materials and methods

2.1. Tissue preparation

Human pulmonary arteries (2–3 mm internal diameter) were obtained from macroscopically normal surgical specimens of patients with lung carcinoma. Tissues were always used within 12 h of surgery. The specimens were placed in physiological salt solution (PSS; composition in mM: NaCl 118, KCl 5.9, $MgSO_4 \cdot 7H_2O$ 1.2, $CaCl_2 \cdot 6H_2O$ 2.5, $NaH_2PO_4 \cdot H_2O$ 1.2, $NaHCO_3$ 25.5, glucose 5.6) at 4°C and oxygenated with 95% O_2 and 5% CO_2. Following removal of the surrounding connective tissue, the vessels were cut into rings (3–4 mm in length) and mounted over two parallel wires, one fixed and the other attached to a force-displacement transducer (FT.03 Grass, Quincy, MA, USA); then the wires were lowered into a 10 ml organ bath filled with PSS (37°C) and bubbled with 95% O_2 and 5% CO_2. Measurements of tension were recorded by using Proto5 software (Letica, Barcelona, Spain). Resting tensions of 1 g were applied to the preparations in order to obtain optimal responses. The vessels were then left to stabilize for 1 h before any drug addition and were washed with fresh PSS every 15 min.

2.2. Vascular muscle responses

Since human pulmonary arteries are known to have little or no inherent tone, drug-induced relaxations were studied in preparations contracted with noradrenaline. Each preparation was initially challenged with a maximally effective concentration of noradrenaline (10 μM) and when the contractile response had reached a plateau, histamine (1 μM) was added to assess endothelial integrity (Ortiz et al., 1992). After washout and recovery of resting tension, preparations were exposed to a concentration of noradrenaline (0.3–1 μM) that was titrated for each vascular ring to give a contraction amounting to about 70% of maximal contraction, i.e., the contraction obtained with 10 μM noradrenaline. When stable contractions were obtained, the relaxant responses were studied by making cumulative concentration–response curves to acetylcholine (3 nM to 10 μM), histamine (1 pM to 0.1 μM), forskolin (1 nM to 10 μM), sodium nitroprusside (1 nM to 10 μM) or cromakalim (10 nM to 10 μM). The relaxant response to anti-human IgE (1:1000 dilution) was also studied. The concentrations used for the relaxants examined in this study were derived from previous studies in human pulmonary arteries (Greenberg et al., 1987; Ellis and Müller-Schweinitzer, 1991; Ortiz et al., 1992, 1993; Rabe et al., 1994). The endothelium was mechanically removed in some pulmonary artery rings by lightly rubbing the vessel lumen with a moistened cotton swab and removal confirmed by the loss of the relaxation response to histamine in preconstricted tissues (Ortiz et al., 1992).

Test tissues were subjected to different pretreatments and then relaxant responses were obtained. Some tissues were pretreated with L-NOARG (100 μM) to inhibit NO synthesis (Furfine et al., 1993), with indomethacin (2.8 μM) to suppress prostaglandin production (Ortiz et al., 1993), or with a mixture of L-NOARG (100 μM) and indomethacin (2.8 μM). In other experiments, preparations were pretreated with D-NOARG (100 μM), the less active enantiomer of L-NOARG that serves as a negative control. In anaesthetic-pretreated tissues, halothane was introduced into the O_2-CO_2 mixture through a Fluotec Mark 3 vaporizer (Ohmeda, Keighly, UK). The concentration in the resulting gas mixture was monitored and adjusted to 2% using a Capnomac Ultima (Datex, Finland). Equilibration of halothane in PSS was reached within 10 min. The aqueous concentration in the bathing medium for halothane 2% was 0.45 ± 0.02 mM (mean ± S.E.M., $n = 5$), determined as in a previous study (Romero et al., 1987). In all experiments involving pretreatment, this was added 30 min before and during application of relaxants. Relaxant responses are expressed as a percentage of the noradrenaline-induced contraction (i.e. the difference between basal tension and that induced by noradrenaline is defined as 100%). Relaxant responses obtained in drug-pretreated tissues were compared to those of paired, time-matched, control tissues. Where appropriate, drug concentrations eliciting 50% (EC_{50}) of the maximum response (E_{max}) were calculated from concentration–response curves by nonlinear curve fitting (GraphPad software, San Diego, CA, USA).

2.3. Mediator release

Direct quantification of the stable metabolite of prostaglandin I_2, 6-keto-prostaglandin $F_{1\alpha}$, was carried out by

enzyme immunoassay (EIA) as previously outlined (Pradelles et al., 1985; Ortiz et al., 1993) and following the instructions of the manufacturer of the kit (Biotrak, RPN 221, Amersham, Madrid, Spain). Absorbances were measured at 450 nm with an automated microtitre plate photometer (Bio Kinetics Reader EL340; Bio-Tek Instruments, Winooski, VT, USA). The assay uses horseradish peroxidase-labelled 6-keto-prostaglandin $F_{1\alpha}$ and a rabbit specific antiserum. The sensitivity of the assay was 0.15 pg/well (equivalent to 3.0 pg/ml). Cross-reactivity for other related compounds was negligible. Unknown 6-keto-prostaglandin $F_{1\alpha}$ values were quantified by interpolation on the standard curve (0.5–64.0 pg/well). Results are expressed as ng mg^{-1} tissue for control (30 min incubation in PSS) or halothane (2%)-treated tissues.

2.4. Drugs and chemicals

Drugs were obtained from the following sources: acetylcholine, anti-human immunoglobulin E, forskolin, histamine dihydrochloride, indomethacin, N^G-nitro-L-arginine (L-NOARG), sodium nitroprusside (Sigma, Madrid, Spain). N^G-Nitro-D-arginine (D-NOARG) was from Calbiochem (La Jolla, CA, USA). Cromakalim was a gift from Smith Kline Beecham Pharmaceuticals (Surrey, UK). Halothane was obtained from Zeneca. The stock solutions of forskolin, indomethacin, and cromakalim were prepared in absolute ethanol, dimethyl sulphoxide and 70% (v/v) ethanol, respectively. The final bath concentrations of drug vehicles did not alter either baseline tension or drug-induced responses.

2.5. Statistical analysis

Data are mean ± S.E.M. The results were statistically analysed by analysis of variance followed by Bonferroni correction for multiple comparisons or by unpaired *t*-test for comparing two groups. $P < 0.05$ was considered significant.

3. Results

3.1. Vascular muscle responses

The submaximal contraction induced by noradrenaline in intact preparations was 0.98 ± 0.11 g (90 preparations from 15 lung samples). Intact pulmonary arterial rings precontracted with noradrenaline relaxed when stimulated with acetylcholine, histamine or anti-human IgE (Figs. 1 and 2). The contraction produced by noradrenaline in endothelium-denuded preparations was 1.06 ± 0.13 (9 preparations from 3 lung samples) which did not significantly differ from the contraction obtained in intact preparations. The relaxant response to acetylcholine, histamine or anti-human IgE was abolished in preparations where the endothelium had been removed (data not shown).

Addition of indomethacin (2.8 μM), L-NOARG (100 μM) or its combination induced a contraction that represented less than 10% of the contraction produced by noradrenaline (10 μM). The tension value at the end of the 30 min incubation period with enzyme inhibitors and the subsequent contractile response to noradrenaline did not significantly differ from the tension value and noradrenaline response obtained in control preparations incubated with PSS (data not shown). L-NOARG (100 μM) significantly inhibited the concentration-dependent relaxation to acetylcholine (Fig. 1) while indomethacin (2.8 μM) produced minor effects. The effects of each inhibitor on the E_{max} and the pD_2 values of the acetylcholine-induced relaxation are shown in Table 1. In human pulmonary arteries treated with the combination of both enzyme in-

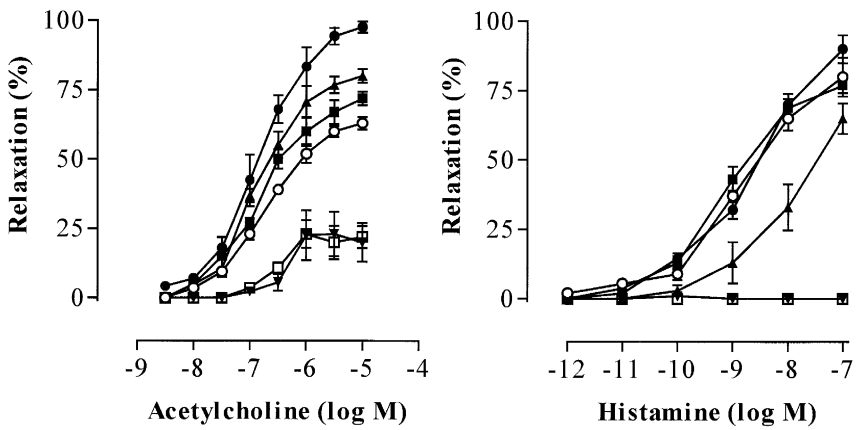

Fig. 1. Effects of halothane (2%) and inhibitors of cyclooxygenase and NO synthase on endothelial-dependent relaxations of human isolated pulmonary arteries produced by acetylcholine and histamine. Intact preparations were precontracted with noradrenaline (0.3–1 μM; ~ 70%) and then a cumulative concentration-response curve was obtained for acetylcholine (left panel) and histamine (right panel) in the absence (control tissues; ●) or presence of indomethacin (2.8 μM; ▲); L-NOARG (100 μM; ○), indomethacin and L-NOARG (▼), halothane (2%; ■), or the combination of indomethacin, L-NOARG and halothane (□). Relaxation is expressed as percent inhibition of noradrenaline-induced contraction. Data are means ± S.E.M. of 5 experiments.

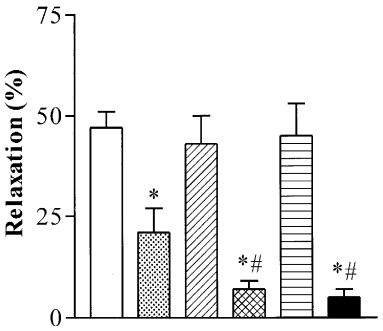

Fig. 2. Effects of halothane (2%) and inhibitors of cyclooxygenase and NO synthase on endothelial-dependent relaxations of human isolated pulmonary arteries produced by anti-human immunoglobulin E. Intact preparations were precontracted with noradrenaline (0.3–1 μM; ~70%) and then challenged with anti-human immunoglobulin E (1:1000 dilution) in the absence (control tissues; open columns) or presence of indomethacin (2.8 μM; stippled columns); L-NOARG (100 μM; hatched columns), indomethacin and L-NOARG (cross-hatched columns), halothane (2%; horizontally striped columns), or the combination of indomethacin, L-NOARG and halothane (solid columns). Relaxation is expressed as percent inhibition of noradrenaline-induced contraction. Data are means ± S.E.M. of 5 experiments. * $P < 0.05$ vs. control; # $P < 0.05$ vs. indomethacin.

hibitors, the relaxation to acetylcholine was markedly reduced (Fig. 1).

Indomethacin (2.8 μM) significantly reduced the histamine-induced relaxation (Fig. 1; pD_2 and E_{max} values shown in Table 1) while L-NOARG (100 μM) did not significantly inhibit the relaxation produced by histamine. However, L-NOARG inhibited completely the relaxation induced by histamine in indomethacin-treated tissues (Fig. 1). Indomethacin (2.8 μM), but not L-NOARG (100 μM), significantly reduced the relaxation elicited by anti-human IgE, and the combination of L-NOARG and indomethacin virtually abolished the relaxation induced by anti-human IgE (Fig. 2). Incubation with D-NOARG (100 μM) did not alter the relaxant responses to either acetylcholine or histamine (data not shown).

Halothane (2%) significantly inhibited the relaxation produced by acetylcholine without altering the relaxation to histamine and anti-human IgE (Figs. 1 and 2). Halothane (2%) had no significant effect on the responses to acetylcholine, histamine or anti-human IgE obtained in tissues treated with the combination of indomethacin and L-NOARG (Figs. 1 and 2).

Forskolin, sodium nitroprusside and cromakalim each produced endothelium-independent concentration-related relaxations of human pulmonary arteries (Fig. 3). The potency and efficacy of these relaxants were similar to those reported by other groups (Table 2; Ellis and Müller-Schweinitzer, 1991; Rabe et al., 1994). Halothane (2%) did not alter the concentration–response curve to forskolin, sodium nitroprusside or cromakalim (Fig. 3, Table 2).

Table 1
Endothelial-dependent relaxation of human isolated pulmonary arteries to acetylcholine and histamine in the absence and presence of halothane (2%) and of the enzyme inhibitors L-NOARG (100 μM) and indomethacin (IND; 2.8 μM)

	Acetylcholine		Histamine	
	E_{max} (%)	pD_2	E_{max} (%)	pD_2
Control	97.6 ± 2.0	6.84 ± 0.03	90.3 ± 5.1	8.66 ± 0.14
L-NOARG	63.0 ± 2.2 [a]	6.72 ± 0.03	80.1 ± 6.9	8.87 ± 0.09
IND	80.1 ± 2.5	6.89 ± 0.05	65.0 ± 5.4 [a]	7.94 ± 0.13 [a]
L-NOARG + IND	23.3 ± 8.2 [a,b]	NC	0	NC
Halothane	72.0 ± 2.5 [a]	6.83 ± 0.05	77.1 ± 2.8	8.90 ± 0.06
L-NOARG + IND + halothane	23.0 ± 5.1 [a,b]	NC	0	NC

Relaxation was elicited in intact preparations submaximally precontracted with noradrenaline. The maximal relaxation (E_{max}) is expressed as percentage of inhibition of the contraction to noradrenaline, and apparent pD_2 values are $-\log EC_{50}$. Data are mean ± S.E.M. of 5 experiments. NC is not calculated.
[a] $P < 0.05$ compared to control values.
[b] $P < 0.05$ compared to IND and L-NOARG values.

Table 2
Apparent pD_2 (i.e., $-\log EC_{50}$) values and maximal effects (E_{max}) of forskolin, sodium nitroprusside (NaNP) and cromakalim in human isolated pulmonary arteries, in the absence and presence of halothane (2%)

	Control tissues		Halothane-treated tissues	
	pD_2	E_{max}	pD_2	E_{max}
Forskolin	6.37 ± 0.07	95.2 ± 5.0	6.45 ± 0.04	94.8 ± 4.3
NaNP	7.37 ± 0.08	86.1 ± 4.2	7.46 ± 0.09	89.0 ± 5.9
Cromakalim	6.56 ± 0.07	65.0 ± 3.9	6.69 ± 0.06	63.3 ± 5.8

E_{max} values are expressed as percent inhibition of the contraction to noradrenaline. Data are mean ± S.E.M of 5 experiments in each group. Data in halothane-treated tissues did not significantly differ from the corresponding control values.

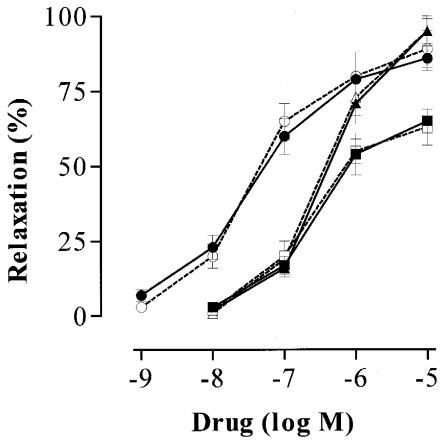

Fig. 3. Cumulative concentration-response curves for the relaxant effects of forskolin (△, ▲), sodium nitroprusside (○, ●) and cromakalim (□, ■) in human isolated pulmonary arteries, in the absence (closed symbols) or presence (open symbols) of halothane (2%). Relaxant responses were produced in intact preparations precontracted with noradrenaline (0.3–1 μM). Ordinate indicates relaxation as percent inhibition of noradrenaline contraction. Abscissa represents drug concentration as log M. Data are means ± S.E.M. of 5 experiments.

3.2. Mediator release

The wet weight of the intact arterial rings was 51 ± 12 mg ($n = 10$). Production of prostaglandin I_2 by human isolated intact pulmonary arterial rings was 0.31 ± 0.04 ng mg^{-1} tissue ($n = 5$) after 30 min incubation period in PSS. These levels were significantly increased following incubation with halothane 2% (0.50 ± 0.06 ng mg^{-1} tissue; $n = 5$, $P < 0.05$ vs. control tissues).

4. Discussion

The present study demonstrates that a clinically relevant concentration of halothane (2%) significantly attenuated the endothelium-dependent relaxation produced by acetylcholine in human isolated pulmonary artery. This result confirms a similar finding for halothane in rat and rabbit aortae, and canine femoral and carotid arteries (Muldoon et al., 1988; Toda et al., 1992), and extends this observation to a human isolated blood vessel in which the effect of halothane had not been previously reported.

Acetylcholine-induced relaxation is produced by activation of muscarinic M_3 and M_1 receptors in endothelial cells (Norel et al., 1996) which subsequently leads to the formation of inositol 1,4,5-trisphosphate and intracellular Ca^{2+} release. The increase in $[Ca^{2+}]_i$ activates the constitutive endothelial NO synthase and the formed NO diffuses to activate soluble guanylyl cyclase in smooth muscle cells thus producing cyclic GMP and subsequently relaxation. Removal of endothelium resulted in suppression of the vascular relaxation to acetylcholine; however, pretreatment with inhibitors of NO synthesis resulted only in a partial reduction of the relaxation to acetylcholine (Greenberg et al., 1987; Crawley et al., 1990; Dinh Xuan et al., 1990; Norel et al., 1996; this study). We found that halothane (2%) reduced the endothelial-dependent relaxation to acetylcholine to an extent close to that produced by the NO synthesis inhibitor L-NOARG (100 μM). Sodium nitroprusside directly activates guanylyl cyclase in vascular smooth muscle and produces an endothelium-independent relaxation (Rabe et al., 1994). Halothane (2%) was ineffective to attenuate relaxation to sodium nitroprusside thus indicating that the inhibitory effect of this anaesthetic is not exerted on guanylyl cyclase in vascular smooth muscle but rather on an earlier stage involving NO production by endothelial cells or NO diffusion to its target. The precise site of action for halothane cannot be ascertained in this study but recent work in cultured bovine aortic endothelial cells shows that halothane (2%) modifies NO half-life or its activated redox form (Blaise et al., 1994) whilst it failed to alter the activity of endothelial NO synthase (Rengasamy et al., 1995).

Endothelial-derived vasodilator prostaglandins contribute to acetylcholine-induced relaxation in a number of arteries (Furchgott, 1983). However, pretreatment with indomethacin in a concentration that effectively inhibits cyclooxygenase in human pulmonary arteries (Ortiz et al., 1993) did not significantly alter the relaxation elicited by acetylcholine in this preparation (Greenberg et al., 1987; this study) or produced only a minor inhibition (Norel et al., 1996). Since prostaglandins seem not decisive in acetylcholine-induced relaxation of human pulmonary arteries, the inhibitory effect of halothane against relaxation to acetylcholine is unlikely to be explained by inhibition of the production of vasodilator prostaglandins by pulmonary artery endothelial cells. In fact, we found that halothane (2%) enhanced the basal release of prostaglandin I_2 by human pulmonary artery. This finding is consistent with results obtained in cultured bovine pulmonary artery endothelial cells where halothane (1%) enhances the production of prostaglandin I_2 and other eicosanoids although the mechanism underlying this effect is uncertain (Barnes et al., 1992).

Prostaglandin I_2 relaxes human pulmonary arteries (Haye-Legrand et al., 1987; Ellis and Müller-Schweinitzer, 1991); hence, halothane-induced release of prostaglandin I_2 may result in vasodilatation as reported for this anaesthetic in other vascular smooth muscle preparations (Stone and Johns, 1989). However, halothane did not produce inhibition of baseline tension in human isolated pulmonary arterial rings but this preparation is known to have little or none inherent tone (Greenberg et al., 1987) and therefore any inhibitory effect of halothane on resting tone would be hardly detected. Precontraction level induced by noradrenaline in human pulmonary artery was not significantly reduced in halothane-treated strips as found for phenylephrine (0.3 μM)-induced contractions in rat isolated aorta (Toda et al., 1992). Differences in the level of contraction, vascular bed, and animal species may explain this discrepancy. In addition, the lack of per se effects for halothane observed in the present study may result from a balance between vasodilator and vasoconstrictor effects, the latter derived from interference with the inhibitory effects produced by the basal release of NO (Crawley et al., 1990).

Another mechanism involved in the endothelium-dependent relaxation to acetylcholine is the release of EDHF which may activate K_{ATP} as well as other K^+ channels (Standen et al., 1989; Garland et al., 1995). The contribution of this mechanism to the relaxation produced by acetylcholine in human pulmonary arteries has not yet been explored. Results from Norel et al. (1996) and the present study indicate that the combination of both inhibitors (i.e. cyclooxygenase and NO synthase inhibitors) virtually abolished the relaxation to acetylcholine but a residual response remained. This residual response was not sensitive to halothane (2%). On the other hand, halothane (2%) did not antagonize the relaxation elicited by cromakalim, thus suggesting that inhibition of K_{ATP} channels is not involved in the inhibitory effect of halothane against relaxation to acetylcholine. However, the participation of

other K^+ channels in the effects of halothane cannot be ruled out since this anaesthetic has been shown to interfere with the opening of large conductance Ca^{2+}-dependent K^+ channels in vascular smooth muscle and other tissues (Hong et al., 1994).

The effect of halothane on endothelium-dependent relaxants other than acetylcholine has been scarcely studied (Toda et al., 1992). We found that halothane (2%) was not inhibiting the endothelium-dependent relaxation of human pulmonary artery produced by histamine and anti-human IgE. The endothelial-dependent relaxation to histamine and anti-human IgE is well maintained in L-NOARG (100 μM)-treated preparations but was significantly reduced in indomethacin (2.8 μM)-treated tissues and abolished when both enzyme inhibitors were present (Ortiz et al., 1992, 1993; this study). The relaxation of human pulmonary artery to histamine was due to activation of endothelial H_1 receptors (Ortiz et al., 1992). The relaxation by anti-human IgE was associated with release of histamine and prostaglandin I_2, the latter acting through the cyclic AMP pathway (Ortiz et al., 1993). The lack of inhibitory effect of halothane on the relaxation produced by histamine and anti-human IgE further supports the notion that this anaesthetic is unlikely to exert its effects by interfering with the synthesis, release or action of vasodilator cyclooxygenase products and is consistent with the NO pathway as the site of action for halothane. This part of the study was completed by exploring the ability of halothane to interfere with the vasodilatation produced by agents such as forskolin which directly activate adenylyl cyclase in vascular smooth muscle cells (Rabe et al., 1994). As expected, halothane (2%) did not alter the concentration-related relaxation produced by forskolin in human pulmonary arteries.

In conclusion, the volatile anaesthetic halothane (2%) inhibits the endothelial-dependent relaxation to acetylcholine observed in human isolated pulmonary artery. This effect appears related to interference with the NO pathway but not of vasodilator cyclooxygenase products. Although this effect was observed at clinically relevant concentrations, the complex balance existing in vivo makes difficult to extrapolate these results to pulmonary haemodynamics in the clinical setting.

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