Do enflurane and isoflurane interfere with the release, action, or stability of endothelium-derived relaxing factors?

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Purpose: The volatile anaesthetics enflurane and isoflurane inhibit the endothelium dependent-relaxation in some *in vitro* preparations. To determine their site of action on the endothelium-derived relaxing factor/nitric oxide (EDRF/NO) pathway, experiments were conducted in a bioassay system.

Method: Continuously perfused cultured bovine aortic endothelial cells (BAEC) were the source of EDRF/NO while a phenylephrine-precontracted denuded rabbit aortic ring, directly superfused by the BAEC effluent served to detect EDRF/NO. The effect of basal and bradykinin (Bk)-stimulated EDRF/NO release on vascular tension was measured. The effect of 4% enflurane or 2% isoflurane on EDRF/NO-induced relaxation was determined.

Results: Enflurane added to the perfusate either upstream or downstream in relation to BAEC attenuated the relaxation induced by Bk at low concentrations. On the other hand, isoflurane, added either upstream or downstream to BAEC, potentiated the relaxation induced by the basal release of EDRF but attenuated the relaxation induced by the Bk stimulated release of EDRF. Neither enflurane nor isoflurane attenuated the relaxation induced by sodium nitroprusside (SNP), an NO donor.

Conclusion: Enflurane decreases the stability of EDRF/NO released after Bk stimulation while isoflurane can have opposite effects depending on whether the relaxation results from basal or Bk-stimulated release of endothelial derived relaxing factor(s). Isoflurane increases the stability or action of the basal relaxing factor, decreases the stability of the Bk-stimulated relaxing factor (which is probably NO).

Objectif : Les anesthésiques volatils enflurane et isolurane inhibent la relaxation dépendante de l'endothélium induite dans des préparations vasculaires *in vitro*. Des expériences ont été conduites dans un système Bioassay pour déterminer leur site d'action sur la cascade de réaction induite par le facteur relaxant dérivé de l'endothélium/monoxyde d'azote (EDRF/NO).

Méthode : Des cultures de cellules endothéliales aortiques bovines (CEAB) perfusées en continu constituaient la source de l'EDRF/NO; un anneau aortique de lapin dénudé, préalablement contracté par de la phényléphrine, directement superfusé par l'effluent de CEAB servait à détecter l'EDRF/NO. Nous avons mesuré la relaxation induite soit par la libération basale ou par la libération stimulée par la Bk de facteurs endothéliaux en l'absence et en présence de 4% d'enflurane et de 3% d'isoflurane.

Résultats : L'enflurane ajouté dans le système Bioassay en amont ou en aval relativement aux CEAB atténuait la relaxation induite par Bk à basse concentration. d'autre part, l'isoflurane ajouté en amont ou en aval des CEAB, potentialisait la relaxation basale de l'EDRF mais atténuait la relaxation induite par la libération d'EDRF provoquée par la stimulation de Bk induite par la libération par la stimulation de Bk. Ni l'enflurane ni l'isoflurane n'atténuait la relaxation induite par le nitroprussiate de sodium (SNP) ou un donneur de NO.

Conclusion : L'enflurane diminue la stabilité de l'EDRF/NO libérée après la stimulation par la Bk; l'isoflurane peut avoir un effet opposé selon que la relaxation résulte de la libération basale ou stimulée par la Bk de l'EDRF. L'isoflurane augmente la stabilité ou l'activité du facteur relaxant de base et diminue la stabilité du facteur de relaxation stimulé par le Bk (qui est vraisemblablement le NO).

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ENFLURANE and isoflurane are widely used volatile anaesthetics. These agents have a direct and an indirect effect on arterial and arteriolar tone which is maintained by a complex interaction between competing vasoconstrictors and vasodilators.[1,2] The important role of the endothelium in the control of vascular tone has recently been emphasized. Endothelial cells release several vasodilators, including the endothelium-derived relaxing factor/nitric oxide (EDRF/NO) synthesized from L-arginine by NO synthase.[3] When stimulated with agonists such as acetylcholine (Ach), bradykinin (Bk), ADP, or physical stimuli such as shear-stress, endothelial cells respond by producing NO. Nitric oxide can also be produced in the adventitia by non-adrenergic non-cholinergic nerves.[4,5] Nitric oxide stimulates soluble guanylate cyclase and increases cGMP which in turn activates the cGMP-dependent protein kinases that induce relaxation. Several organ chamber studies have shown that volatile anaesthetics, such as enflurane and isoflurane interfere with the relaxation induced by endothelial and nonendothelial-dependent substances. If most of the publications have shown an inhibition of the EDRF/NO relaxation by enflurane, the effect of isoflurane on endothelium-dependent relaxation is more controversial. We have shown that the isoflurane-induced reduction of the contractile response of isolated canine coronary artery rings to prostaglandin F_2 alpha, 5-hydroxytryptamine, and phenylephrine (P-E) is endothelium-dependent.[6] Park *et al.* have shown that the isoflurane-induced relaxation of conductance coronary arteries is endothelial and partially EDRF/NO-dependent.[7] Greenblatt *et al.* have shown that the decrease in vascular resistance induced by isoflurane is reversed *in vivo* by N^G-Mono-Methyl-L-Arginine (L-NMMA), a blocker of NO synthesis.[8] However, several authors have also shown that isoflurane reduces the endothelial-dependent relaxation to receptor and non-receptor dependent agonists.[9–11]

The NO metabolic pathway is complex, and the mechanism of interference by volatile anaesthetics remains unclear. Several sites of action are possible: decreased NO production in endothelial cells; decreased NO action on soluble guanylate cyclase; decreased cGMP production or cGMP dependent-kinases action; decreased NO stability and half-life. We have shown that halothane decreases the EDRF/NO metabolic pathway by lowering the stability of NO.[12] To determine the mechanism of action of enflurane and isoflurane on the EDRF/NO metabolic pathway, we used a bioassay system in which rabbit aortic rings lacking endothelium were perfused by a saline solution having previously passed through cultured endothelial cells (superfusion by the effluent of the endothelial cells). The anaesthetics were then added to the perfusate either upstream or downstream of the endothelial cells in order to treat either both the endothelial cells and the vascular ring (to see an effect on the production of NO) or only the vascular ring (to see an effect on the action or stability of NO). Such a study is important to determine whether the normal functions of endothelial cells are diminished by the anaesthetics since the latter might affect vasomotor tone by sensitizing smooth muscle fibres to vasoconstrictors. Such localized effects could have real repercussions.

Methods

The protocol was approved by the Research and Animal Welfare Committee of our institution; the animals were treated according to the guidelines set by the Canadian Council for Animal Care.

Experiments were performed in a bioassay system as previously described.[12] Briefly, cultured bovine aortic endothelial cells (BAEC) seeded on microcarrier beads were used as the donors of EDRF. A precontracted rabbit aortic ring without endothelium was used as the EDRF detector. Endothelial cells and denuded rings were continuously perfused with an oxygenated and carbonated (O_2 95%, CO_2 5%) Krebs-Ringer (K-R) solution kept at 37°C. Indomethacin (10^{-5} M) was added to K-R solution to block the synthesis of prostaglandins.

Endothelial cell preparation

Microcarrier beads, endothelial cells, and all solutions, including the serum obtained from the Physiology Department of the Université de Montréal, were prepared in a sterile environment and kept at 37°C. The cell medium was replaced every two days with fresh DMEM (Dulbecco's modified Eagle medium) solution supplemented with newborn calf serum (NCS), either 10% or 20%.

The presence of factor VIII-related antigen was tested to confirm the endothelial nature of the cells. The cells were then subcultured in 75 cm^2 flasks (No. 3024, Falcon, Oxnard, CA, USA) with 15 ml DMEM (No. 430- 1600eb, Gibco, Grand Island, NY, USA), supplemented with 3.7 $g \cdot l^{-1}$ $NaHCO_3$, 100 $U \cdot ml^{-1}$ penicillin, 100 $\mu g \cdot ml^{-1}$ streptomycin (No 600-6010, Gibco) and 10% NCS (No. 230-6010, Gibco), and incubated under CO_2 5%. Confluent cells were removed from the flasks by trypsinization (trypsin 0.05% and EDTA 0.02%), resuspended in the culture medium, and mixed with collagen-coated microcarrier beads (Cytodex 3, Pharmacia). The cell/bead suspension was placed in a 100 ml spinner bottle and stirred, first intermittently for seven hours to allow optimal seeding of cells onto the beads, then continu-

ously to avoid bead aggregation. The cell confluency on the beads (80 cells per bead), which usually occurred after three to four days, was verified by microscopy. The cells were then ready to be perfused in the bioassay system.

The perfusion system (Figure 1)

Two 3-ml glass cylinders filled with either the microcarrier beads or BAEC seeded on beads were continuously perfused with 4 $ml \cdot min^{-1}$ of oxygenated and carbonated (O_2 95%, CO_2 5%) K-R solution containing 10^{-5} M indomethacin at 37°C. A denuded rabbit aortic ring was suspended between two L-shaped hooks, one of which was connected to a metallic support and the other connected to an isometric force transducer. The transducer was linked to a recorder in order to measure the tension imposed on or generated by the vascular tissue. The isometric force transducer and support were fixed to the same metallic frame to facilitate the vascular ring's displacement. The ring was perfused either by the effluent of the microcarrier beads (direct line), or superfused by the effluent of microcarrier beads covered with endothelial cells (superfusion line).

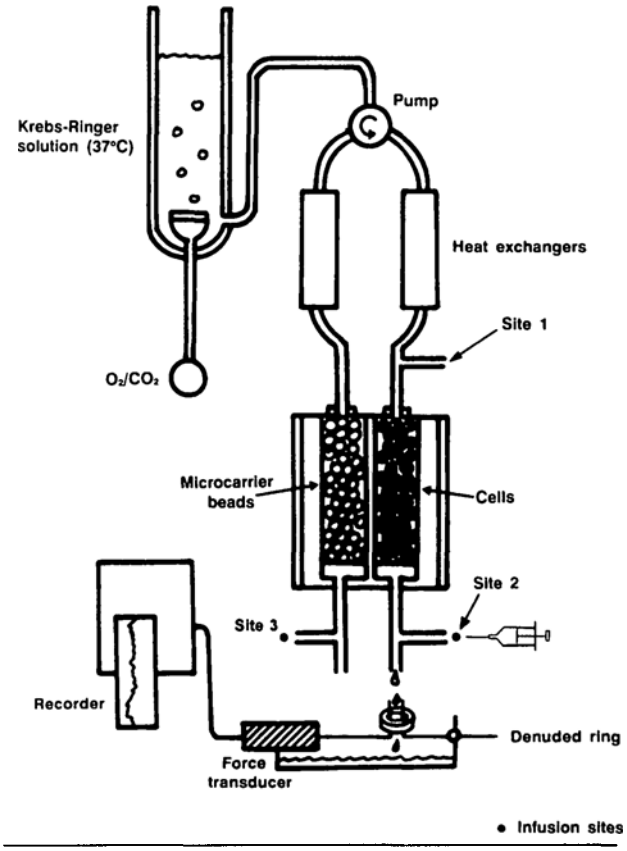

FIGURE 1 The Bioassay System consists of two columns: one packed with microcarrier beads, the other packed with endothelial cells seeded on microcarrier beads. Directly under these two columns, a vascular denuded ring can be placed, moved, and perfused by either the effluent of the beads (direct line) or the effluent of the cells (superfusion line). The solution that passes through the system is an oxygenated and carbonated Krebs-Ringer solution. Medications can be added to the perfusate or to the superfusion line at site 1 (upstream to the perfusion of the endothelial cells) or at site 2 (downstream to the perfusion of the endothelial cells) or to the direct line at site 3.

Vascular ring preparation and perfusion

New-Zealand white rabbits weighing between 1.5 and 2 kg were sacrificed with 1 $ml \cdot kg^{-1}$ of T61® anaesthesia solution (Hoechst Canada, Inc.). The abdominal aorta was removed by laparotomy, cleaned of fat and fibrotic tissue and cut into 5mm rings. The endothelium was mechanically removed by rolling each ring on a wet paper towel with the tip of a small surgical forceps. Each ring was suspended between two L-shaped hooks and first perfused by the effluent of the microcarrier beads for 30 min to allow recovery from surgical and ischemic stress. It was then progressively stretched by steps of 2 g up to 10 g. This tension, which gives the maximal active response to 20 mM KCl (data not shown), was considered to be the optimal passive tension (OPT) for isolated rabbit aortic rings. The OPT was kept at this level for the duration of the experiment. The ring was then contracted with 3×10^{-7} M phenylephrine (P-E) which induces a stable contraction of half the maximal tension induced by higher concentrations of P-E (EC 50). After the plateau was reached, 10^{-9} M bradykinin (Bk) was added to the perfusate (at site 3, figure 1) to verify the removal of the endothelium from the vascular ring. Any ring relaxing to Bk while being directly perfused was discarded. Bradykinin was then discontinued and the holding tray was moved under BAEC column. The relaxation induced by the basal release of EDRF/NO was recorded and measured: EDRF/NO production was then stimulated by different doses of Bk added to the perfusate upstream of the perfusion of the endothelial cells (site 1, figure 1), and the resulting relaxation was recorded and measured.

Addition of the anaesthetics to the perfusate

Based on a saline partition coefficient of 0.74 for enflurane and of 0,59 for isoflurane, 115 µl enflurane or 86 µl isoflurane were added to 50 ml K-R in a gas-tight glass syringe to prepare a concentrated solutions of containing 2995.75 $\mu g \cdot ml^{-1}$ (16.2 mM) enflurane or 2236.34 $g \cdot ml^{-1}$ (12.2 mM) isoflurane.[13,14] These solu-

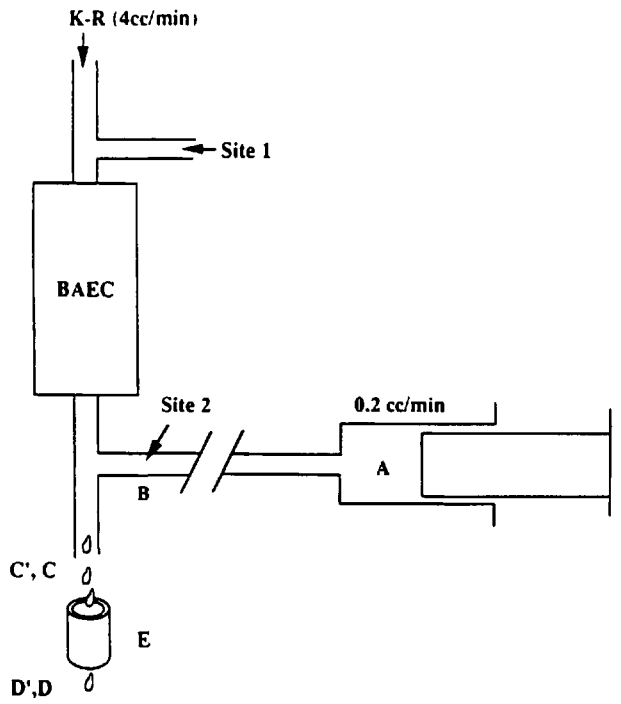

FIGURE 2 Sites of collection of anaesthetic samples in the Bioassay System.

(A) Syringe, (B) Syringe plus tip, © Before the aortic ring with perfusion at site 2, (D) After the aortic ring with perfusion at site 2, (C') Before the aortic ring with perfusion at site 1, (D') After the aortic ring with the perfusion at site 1.

tions correspond to salines equilibrated with enflurane 53% or isoflurane 52% (in the gas phase). Each anaesthetic was added to K-R and stirred with a magnet for 30 min and then perfused (Figure 1) by an automatic pump into the bioassay system at site 1 (upstream of BAEC) or at site 2 (downstream of BAEC and upstream of the aortic ring) at a rate of 0.4 ml·min^{-1} to yield a calculated concentration of 4.8% for enflurane (diluted 11 times) or at a rate of 0.2 ml·min^{-1} to yield final concentrations of 2.5% for isoflurane (diluted 21 times). Compared with the basal flow in the bioassay (0.2 or 0.4 *vs* 4 ml·min^{-1}), these added low flows coming from the automatic syringe had minimal diluting effect on the P-E or EDRF/NO concentrations (data not shown). Furthermore, the negligible increases in total flow rates were insufficient to increase shear-stress on the BAEC and did not increase their EDRF/NO secretion. The concentration of the anaesthetics in the bioassay system were measured using an electron capture detector (ECD) gas chromatograph. Samples were taken at different levels of the perfusion system (Figure 2). In order to measure and compare the relaxation starting from the same tension level in the control and the anaesthetic-treated rings, a matched tension was performed in the anaesthetic treated groups. This was achieved by slightly decreasing the rate of P-E infused into the system until a tension level similar to the one prior to anaesthetic addition was obtained (Figures 3A and 3B). As enflurane 2% did not affect the tension generated by the vascular rings in any of the experimental protocols only the data obtained from experiments performed with enflurane 4% and isoflurane 2% are presented.

Organ chamber experiments

These experiments were conducted to verify that the anaesthetics do not interfere with the NO activation of soluble guanylate cyclase, the formation of cGMP or the relaxation induced by the activation of cGMP-dependent protein kinases.[15] Denuded rabbit aortic rings prepared as above were suspended between two metallic stirrups and introduced into organ chambers filled with oxygenated and carbonated K-R solution kept at 37°C. The lower stirrup was fixed to the bottom of the organ chamber while the upper stirrup was connected to an isometric force transducer. The transducer itself was connected to a recorder to measure the tension imposed on or generated by the vascular rings. The rings were precontracted as described above and organ chambers were either left untreated or were treated with enflurane 4% or isoflurane 2% added to the gas mixture bubbling the saline solution. Volatile anaesthetics were added to the gas mixture using a calibrated vaporizer and their concentrations verified with a gas analyzer (Datex, Ultima). Fifteen minutes after anaesthetic introduction, the relaxation induced by increasing the sodium nitroprusside (SNP) concentration from 10^{-10} to 10^{-4} M was measured in the control and treated chambers.

Calculation and statistics

Phenylephrine-induced contractions are presented in grams and expressed as mean ± SEM. The relaxations are expressed as percentages compared with the tension induced by phenylephrine. In experiments where the effect of enflurane or isoflurane was measured, 100% represented the tension induced by phenylephrine either before the introduction of the anaesthetic or after the tension matching to the level prior to the anaesthetic addition. Anaesthetic-treated ring tension were compared with control ring tensions. Data are expressed as mean ± SEM; Student paired t tests were used to analyze the results, the significant level was set at 5%.

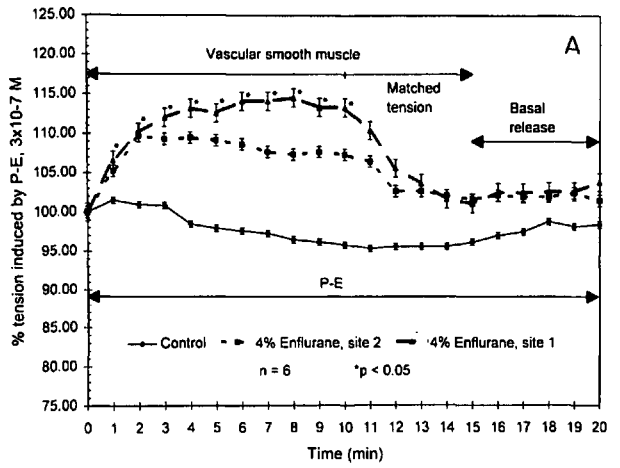

FIGURE 3A Effect of A enflurane 4% (n = 6) and B isoflurane 2% (n = 5), perfused at site 1 or at site 2, on the relaxation induced by the basal release of EDRF/NO.

Basal release in the figure refers to the rings being placed under the BAEC column.

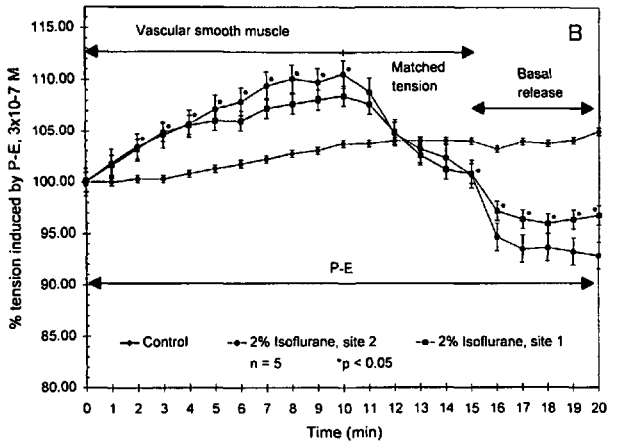

FIGURE 3B

Results

The concentrations of anaesthetics measured in the K-R solution of the syringes were 46.6 ± 1.6% for enflurane and 53.2 ± 2.9% for isoflurane. Anaesthetic concentrations were well maintained throughout the perfusion system (Table I, Figure 2). Phenylephrine (3×10^{-7}M) induced a stable contraction in denuded rabbit aortic rings. The tensions recorded for the control, enflurane, and isoflurane groups were 8.88 ± 0.54 g, 7.34 ± 0.89 g, and 9.74 ± 0.80 g respectively (*P*:NS). The absence of endothelium was demonstrated by the lack of relaxation to bradykinin while the rings were directly perfused by the effluent of the microcarrier beads (perfusion of Bk at site 3, figure 1). Enflurane or isoflurane, added to the perfusate at the plateau of P-E-induced contraction, increased the vascular tension by 14.5 ± 0.5% and 5.8 ± 0.5% respectively. After performing a matched tension, moving the vascular rings from direct-line perfusion to endothelial superfusion induced weak transient reductions in tension due to the basal release of EDRF/NO. Bradykinin (from 10^{-10} M to 3×10^{-8} M) added to the perfusate upstream of the endothelial cells (at site 1) induced further release of EDRF/NO and further relaxation of the vascular ring. We did not observe an effect an effect of enflurane 2% on the tension generated by the vascular rings (data not shown).

Effect of enflurane and isoflurane on the relaxation induced by the basal release of EDRF/NO

Four percent enflurane added to the perfusate at site 1 or 2 did not affect the relaxation induced by the basal release of EDRF/NO (Figure 3A). The residual tension for the relaxation induced by the basal release of EDRF/NO in the control, and in preparations treated with enflurane 4% added at site 1 or at site 2 corresponded to 98.4 ± 2.9%, 103.8 ± 1.4%, and 101.5 ± 2.9% respectively.

On the other hand, results presented in Figure 3B show that isoflurane 2% potentiated the relaxation

TABLE I The concentration of enflurane and isoflurane at various levels of the Bioassay System.

	Sites of Sample Collection					
	A	*B*	*C*	*D*	*C'*	*D'*
Enflurane (4%)	46.6 ± 1.6 n = 16	37.3 ± 3.2 n = 12	4.65 ± 0.45 n = 12	4.08 ± 0.43 n = 12	4.08 ± 0.56 n = 4	3.72 ± 0.47 n = 4
Isoflurane (2%)	53.2 ± 2.9 n = 10	42.4 ± 2.8 n = 10	2.03 ± 0.09 n = 15	1.78 ± 0.09 n = 15	1.83 ± 0.07 n = 5	1.64 ± 0.05 n = 5

induced by the basal release of EDRF/NO; this effect was observed whether the drug was added at site 1 or at site 2. The residual tension for the relaxation induced by the basal release of endothelial derived relaxing factor(s) was 105.1 ± 0.9% in control preparations, 96.8 ± 3.0% in preparations treated by isoflurane at site 1 and 92.9 ± 5.6% in preparations treated by isoflurane at site 2.

Effect of enflurane or isoflurane on relaxation induced by increasing concentrations of Bk (from 10^{-10} to 3×10^{-8} M)
Enflurane, 4%, added to the perfusate at site 2 had an inhibitory effect on the relaxation induced by Bk: the residual tension was 38.1 ± 6.3% in control rings and 53.2 ± 2.2% in the enflurane treated rings (Figure 4A). Treatment at site 1 only reduced the relaxation for low concentrations of Bk (from 3×10^{-10} to 3×10^{-9} M). At higher concentrations of Bk (from 10^{-8} to 3×10^{-8} M) the relaxation in control and enflurane 4% treated rings was similar. Isoflurane, 2%, added to the perfusate at site one or at site two attenuated the relaxation induced by low concentrations of Bk (10^{-10} to 10^{-9} M, Figure 4B). Furthermore, we observed a difference between the control and isoflurane treated groups for the relaxation induced by Bk at 10^{-9} M. In this case, the residual tensions were (61.5 ± 6.3%) for the control group, (86.3 ± 5.7%) for the isoflurane treated group at site 1, and (83.6 ± 6.3%) for the isoflurane group treated at site 2.

Organ chamber experiments: neither enflurane nor isoflurane had an effect on the relaxation induced by increasing concentrations of SNP in phenylephrine precontracted denuded rabbit aortic rings (Figure 5).

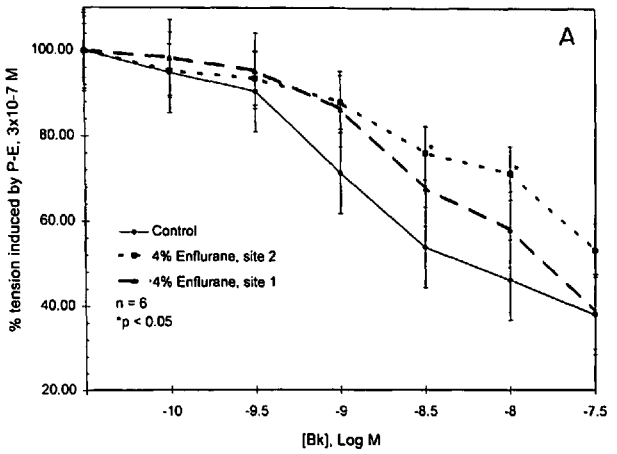

FIGURE 4A Effect of A enflurane 4% (n = 6) and B isoflurane 2% (n = 5) on the relaxation induced by BAEC release of EDRF/NO with increasing concentrations of bradykinin.

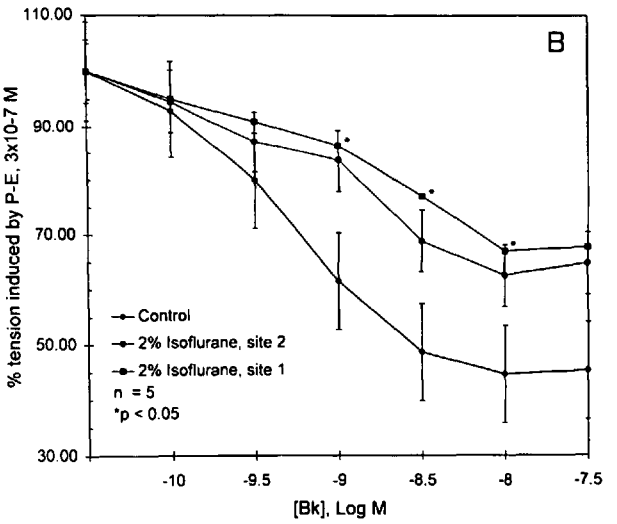

FIGURE 4B

Discussion

Both enflurane and isoflurane, when added to the perfusate at the plateau of phenylephrine-induced contraction, increased the tension to a level which was stable until the next step of the experiment. It had previously been shown that these anaesthetics have a biphasic effect on the tension of contracted rings.[16] This effect is characterized by an increase in tension followed by a decrease. It is possible that they increase intracellular free Ca^{++} by potentiating Ca^{++} release from intracellular stores.[17] Smooth muscle cells have two separate sarcoplasmic reticulum (SR) Ca^{++} stores: one is released by IP_3 and caffeine (ryanodine-sensitive channel) and the other is released by IP3 alone.[18,19] Which of the two SR pools is released by the volatile anaesthetic agents is not known. It has been shown that these agents release Ca^{++} from the SR rayonidine-sensitive channel in skeletal muscles[20,21] and in some vascular smooth muscle cells.[22] However, in pituitary cells, volatile anaesthetics induce efflux from the IP3-gated store.[17] The ensuing relaxation induced by these agents would be due to inhibition of Ca^{++} entry through Ca^{++} channels and a secondary reduction of Ca^{++} stores.[23,24] Vascular smooth muscle contraction induced by alpha-1 agonist stimulation is biphasic: an initial fast contraction due principally to Ca^{++} release is followed by a sustained contraction due to Ca^{++} influx.[25,26] The initial fast contraction is mediated through the activation of phospholipase C with production of IP3 and the diacylglycerol cascade while the plateau is maintained through Ca^{++} entry.

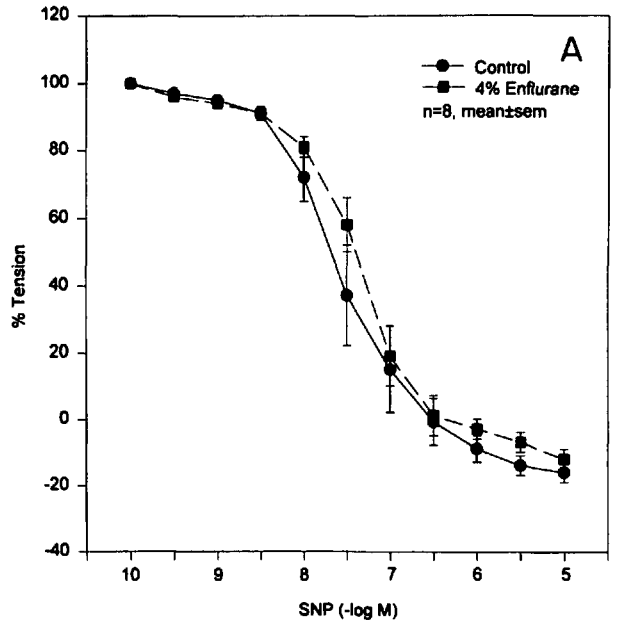

FIGURE 5 Organ chamber experiments. Effect of A enflurane 4% (n =8) or B isoflurane 2% (n = 8) on the relaxation induced by increasing concentrations of sodium nitroprusside.

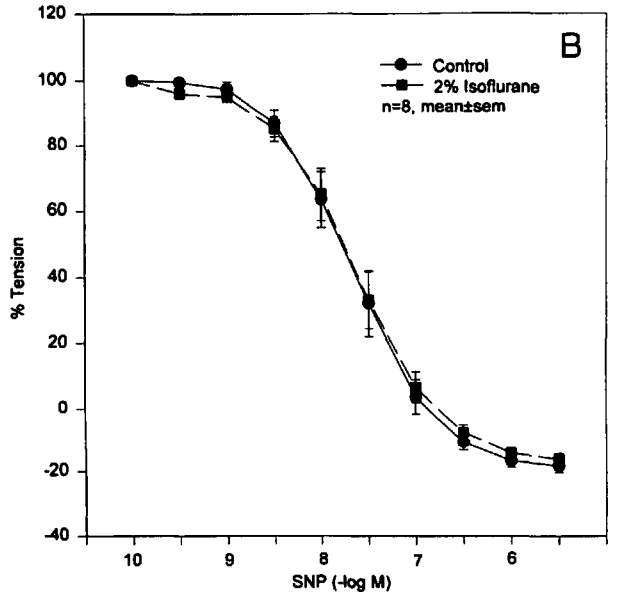

FIGURE 5B

Endothelial cells release several vasodilators such as EDRF/NO, prostacyclin, and endothelium-derived hyperpolarising factor (EDHF).[2] The preparations were treated with indomethacin to block prostaglandin synthesis. We have previously shown that the relaxation induced by the effluent of BAEC is suppressed at the lowest concentrations (10^{-10} to 10^{-9} M) of Bk in the presence of N^G-Mo-no-Methyl-L-Arginine (L-NMMA), a blocker of NO synthase.[12] However, we had also shown that L-NMMA cannot completely abolish the relaxation induced by BAEC stimulation at higher concentrations of Bk (3×10^{-9} to 3×10^{-8} M), suggesting that either L- NMMA does not completely suppress NO synthase activity or that another relaxing factor such as EDHF is released by BAEC stimulated at higher concentrations of Bk. Indeed, it has been demonstrated that different concentrations of acetylcholine can stimulate the release of either NO or EDHF from the endothelial cells[27] and that, in a bioassay system, the nature of the relaxing factor released in basal conditions is different from the relaxing factor released after stimulation of the endothelial cells by Bk.[28,29] The exact nature of EDHF is not known, but recent experimental evidence suggests that EDHF is a cytochrome P450-derived arachidonic acid metabolite.[30–32] Nitric oxide itself could hyperpolarize the cells in some preparations as suggested recently by Bolotina *et al.*[33] The exact role of cell membrane hyperpolarization in vasorelaxation in this experimental setting has not been established, but it could be involved in the relaxation induced by high concentration of Bk.

Unlike experiments performed in organ chambers, the biossay system makes it possible either to treat simultaneously the endothelial cells and smooth muscle cells or to treat only the smooth muscles cells by the anaesthetics. Thus the system allows us to differentiate, in the same experiment, the effects of anaesthetics on these two different types of cells. Furthermore, it was possible to explore the effects of the volatile agents on the relaxation induced by the basal release of EDRF/NO. We also showed that it was possible to prepare concentrated solutions and maintain stable concentrations of these anaesthetics in an open bioassay system.

Our data are in agreement with previously published data showing that enflurane and isoflurane, despite being vasodilators *in vivo*, can ultimately attenuate the Bk stimulated endothelium-dependent relaxation.[9,10]

Despite a very good response to Bk stimulation, endothelial cells released quite low levels of basal EDRF/NO in these experiments. There could be at least two explanations for this observation: 1) that the Bk stimulated release of the vasodilator was better preserved than the basal release in the cultured bovine aortic endothelial cells, and 2) that, because of the low viscosity of the K-R solution, the shear-stress in the perfusion system was low and the stimulus for the basal release of the vasodilator was consequently small.

Neither enflurane nor isoflurane attenuated the relaxation induced by the basal release of EDRF/NO. Isoflurane even potentiated this relaxation. Both the addition of isoflurane, at site 1 (to the perfusate) or at site 2 (to the effluent) of BAEC, potentiated the relaxation induced by the basal release of vasorelaxing substance(s). This suggests that isoflurane increases the stability (half-life) or potentiates the action of the vasodilator(s) released by the endothelial cells in the basal perfusion conditions. The effect of isoflurane on relaxation induced by the basal release of vasodilators had not previously been studied.

We failed to detect any effect of enflurane 2% on the relaxation induced by the effluent of BAEC stimulated by Bk (data not shown). This is in agreement with a previous publication which showed that enflurane 2% does not interfere with endothelium-dependent relaxation.[9] On the other hand, enflurane 4% added to the perfusate either at site 1 or at site 2, attenuated the relaxation induced by Bk. Enflurane displayed a greater effect when added downstream of BAEC, particularly at higher concentrations of Bk. Enflurane was thus a weak agent especially when considering its effect on EDRF/NO pathways.

Two percent isoflurane added either to the perfusate or to the effluent of BAEC attenuated the relaxation induced by the increasing concentration of Bk. It had already been shown that isoflurane can attenuate the response to endothelium-dependent relaxing substances such as acetylcholine or Bk.[9–11] Our data agree with these observations. However, we show here that isoflurane does not interfere with the synthesis or the release of EDRF/NO but rather with its stability or its action. The effect of enflurane and isoflurane on the relaxation induced by NO donors such as SNP or trinitroglycerine has been studied and most authors were unable to demonstrate an effect of enflurane and isoflurane under these conditions.[9–11] Our results also show that SNP-induced relaxation is not attenuated by these anaesthetics. This strongly suggests that they do not interfere with the action of NO on the cGMP pathway as recently shown, nor do they interfere with the relaxation induced by the cGMP pathway itself.[15,34] Since these anaesthetics do not decrease the production of NO nor do they attenuate the action of NO donors, the reduction of endothelium-dependent relaxation has to be due to an anaesthetic-induced decrease in NO stability. We have already shown that the same mechanism was involved in the interaction between halothane and EDRF/NO. Scavenging of the superoxide anions with superoxide dismutase suppresses the effect of halothane on the EDRF/NO pathway.[35] This suggests that the interaction between anaesthetics and NO is mediated through superoxide anion production by endothelial cells.

How can isoflurane potentiate the relaxation induced by the basal release of endothelium-derived relaxing substances and inhibit the relaxation following the treatment with Bk? It has been shown that the aortic endothelial cells can release several vasodilators and that the vasodilator(s) released in basal conditions is (are) different from the vasodilator(s) released following the stimulation of the endothelial cells by different agonists.[2,28,29] In a bioassay system, using either dog coronary arteries or cultured porcine endothelial cells as source of EDRF, the action of the vasodilator released in basal perfusion conditions is suppressed by ouabain.[28,29] In contrast, ouabain has no effect on the relaxation induced by the vasodilator released by Bk which is presumed to be NO. Our data suggest that isoflurane can increase the stability or the action of one of the vasodilators and decrease the stability of the other. Continuous release of basal relaxing factors from the endothelium maintains a low arterial pressure. We do not know if the potentiation of the relaxation induced by this (these) relaxing factor(s) contribute to the vasodilation induced by the anaesthetic.

We conclude that, similar to halothane,[12] enflurane and isoflurane interfere with the stability of EDRF/NO. We do not yet know if they also potentiate the interaction between NO and the superoxide anion produced by the endothelial cells.

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