Volatile anaesthetic actions on norepinephrine-induced contraction of small splanchnic resistance arteries

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The aim of this study was to investigate volatile anaesthetic action on small splanchnic resistance arteries. Employing isometric tension recording, we studied the effects of clinically relevant concentrations (0.25–1.25 minimum alveolar concentration (MAC)) of isoflurane, sevoflurane and enflurane on contractions induced by norepinephrine (NE), a sympathetic neurotransmitter, in the rabbit small mesenteric artery. Rhythmic oscillations were observed in contractile responses to NE. Both isoflurane (≥0.25 MAC, 0.5% (≈0.11 mM)) and sevoflurane (≥0.75 MAC, 2.8% (≈0.38 mM)) inhibited the NE (10 μM)-induced contraction with concomitant inhibition of average amplitude of the oscillations. Only enflurane (≥0.25 MAC, 0.7% (≈0.20 mM)) generated vasoconstriction superimposed on the NE-induced contraction; however, the vasoconstriction was transient and was followed by vasorelaxation. Concurrently, enflurane (≥0.25 MAC) strongly inhibited the average amplitude of the oscillations; higher concentrations (≥1.0 MAC) of enflurane completely eliminated the oscillations. The frequency of the NE-induced oscillations was less affected by the anaesthetics. The observed vasodilator action of these anaesthetics in small resistance arteries may contribute to their hypotensive effects in vivo. The potent inhibition of the rhythmic oscillations also may play a role in volatile anaesthetic-induced alterations in cardiovascular homeostasis.

Cette étude avait pour objectif de rechercher les effets de certains anesthésiques volatils sur les petites artères splanchniques de résistance. En enregistrant la tension isométrique, les auteurs ont étudié les effets de concentrations efficaces en clinique (0,25–1,25 MAC d'isoflurane, de sévoflurane et d'enflurane) sur les contractions induites par le neurotransmetteur sympathique norépinéphrine (NE) sur la petite artère mésentérique du lapin. En réponse à la NE, on a observé des oscillations rythmiques contractiles. L'isoflurane (≥0,25 MAC, 0,5% (≈0,11 mM)) et le sévoflurane (≥0,75 MAC, 2,8% (≈0,38 mM)) inhibaient la contraction induite par la NE (10μM) avec une inhibition simultanée de l'amplitude moyenne des oscillations. Seul l'enflurane (≥0,25 MAC, 0,7% (≈0,20 mM)) a provoqué une vasoconstriction superposée à la contraction induite par la NE; cependant, cette vasoconstriction était transitoire et suivie de vasorelaxation. Simultanément, l'enflurane (≥0,25 MAC) a inhibé vigoureusement l'amplitude moyenne des oscillations; les concentrations plus élevées (≥1,0 MAC) d'enflurane ont éliminé complètement les oscillations. La fréquence des oscillations induites par la NE a été moins affectée par les anesthésiques. L'activité vasodilatatrice observée avec ces anesthésiques sur les petites artères de résistance peut contribuer à leurs effets hypotensifs in vivo. La puissante inhibition des oscillations rythmiques peut aussi jouer un rôle dans les altérations de l'homéostase cardiovasculaire induites par les anesthésiques.

Key words

ANAESTHETICS, VOLATILE: isoflurane, sevoflurane, enflurane;
MUSCLE, SMOOTH, VASCULAR;
ENDOTHELIUM, VASCULAR;
OSCILLATIONS;
ENDOTHELIUM-DERIVED RELAXING FACTOR;
VASOMOTION, ARTERY, RESISTANCE.

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Volatile anaesthetics are known to affect cardiovascular stability by causing changes in vascular tone and reactivity, cardiac function and reflexes that detect alterations and initiate adjustments in the cardiovascular system.[1-3] The overall impact of volatile anaesthetics is a decrease in mean arterial pressure.[2] The vasodilatory and cardiac depressant effects of anaesthetics have been proposed to be due either to direct actions of anaesthetics on cardiovascular tissues or to decreased sympathetic nervous system tone.[2,3]

Until recently, the effects of volatile anaesthetics on vascular smooth muscle have been investigated mainly in large conductance arteries such as the aorta,[4-13] where they have been shown to have direct vasodilating or vasoconstricting actions.[5-7,10-13] However, small arteries and arterioles are known to be the major sites of regulation of vascular resistance and thus of tissue perfusion and of arterial blood pressure.[14-16] These small vessels have been suggested to be quite different from large conductance arteries in many of their properties, including density or proximity of neuronal innervation,[17] nature of adrenergic receptors,[18] calcium mobilization processes,[19] characteristics of endothelium-mediated vascular responses,[20] and sensitivity or response to pharmacological agents including volatile anaesthetics.[21-24] This indicates the need to investigate further volatile anaesthetic actions in small resistance arteries. In this study, small splanchnic resistance arteries were used to study the effects of three clinically important volatile anaesthetics, isoflurane, sevoflurane and enflurane on contractions induced by norepinephrine (NE), a neurotransmitter that plays a central role in sympathetic maintenance of vascular tone *in vivo*.[14,15] The effects of volatile anaesthetics on small splanchnic arteries may be of particular clinical importance. Intraoperative maintenance of splanchnic blood flow in seriously ill surgical patients could be a critical factor in preventing bacterial translocation across the gut wall due to splanchnic ischaemia.[25-29] Nevertheless, less is known about direct volatile anaesthetic actions on small splanchnic arteries which have major effects on the regulation of splanchnic blood flow.[15,16]

The isolated splanchnic resistance arteries we used in this study represented rhythmic oscillations in contractile response to NE. The similar oscillatory contractile activity, often referred to as vasomotion and regarded as a feature of small vessels, has been demonstrated in a number of vascular beds *in vivo*.[30-36] This emergence of vasomotion provided us with an opportunity for the first time, to demonstrate the effects of these volatile anaesthetics on the arterial vasomotion, which has been proposed to play an important physiological role in regulating vascular resistance without disturbing tissue perfusion or oxygen delivery to tissue, or in maintaining normal tissue fluid balance through reduction of net fluid filtration into tissue.[37,38]

Methods

Tissue preparation and recording of mechanical activity

After receiving institutional approval for this animal study, male albino rabbits (2.0–2.5 kg) were given sodium pentobarbital (40 mg · kg^{-1} *iv*) and exsanguinated. The mesentery in the jejunal region was immediately placed in a dissecting chamber filled with preoxygenated Krebs-bicarbonate solution, and the mesenteric artery was rapidly excised. The distal portion of the third- or fourth-order branches (0.15–0.25 mm in outside diameter), known to contribute substantially to vascular resistance,[16] were used for the experiments. Under a binocular microscope, the surrounding connective and fat tissues were carefully removed from the vessel. The vessel was then carefully cut open lengthwise, and transverse strips (400–600 μm long and 80–120 μm wide) were prepared from the "vascular sheet." Both ends of the strip were tied with two thin silk threads and, for isometric tension measurement, the strip was fixed between one end of a chamber (0.9 ml capacity) and an L-shaped glass rod connected to a strain gauge transducer (UL-2 type, Shinko Co., Tokyo) as previously reported.[39-41] The chamber was mounted on a microscope stage, and the resting tension was adjusted to obtain the maximum contractile response to 40 mM K^+. The solution was changed by perfusing it rapidly from one end while aspirating it simultaneously from the other end. All experiments were performed in the endothelium-intact strips at 35°C. The endothelium-intactness was verified by almost complete (≥90%) relaxation by ACh (1 μM).[41]

Solutions and drugs

The ionic concentrations of the Krebs solution were as follows (mM): NaCl 111.9, KCl 3.7, $MgCl_2$ 1.2, $CaCl_2$ 2.6, $NaHCO_3$ 25.5, KH_3PO 1.2, glucose 11.4. High K^+ solution was prepared by replacing NaCl with KCl isoosmotically. The solution was bubbled with 95% O_2/5% CO_2 (pH 7.3–7.4, 35°C).

Norepinephrine HCl (NE) and acetylcholine (ACh) were obtained from Sigma Chemical Co., St. Louis, Mo U.S.A. Isoflurane and enflurane were obtained from Dainabot Co., Tokyo, Japan, and sevoflurane was obtained from Kodama Pharmaceutical Co., Osaka, Japan.

Experimental protocol

The experimental protocol is illustrated in Figure 1. Application of high K^+ (40 mM) was initiated after a 30 min equilibration following the mounting of the strips in the chamber. After obtaining steady contractions, in-

TABLE I The measured concentrations (mM) of isoflurane, sevoflurane and enflurane dissolved in Krebs solution

Anaesthetics	*0.25 MAC*	*0.50 MAC*	*0.75 MAC*	*1.00 MAC*
Isoflurane	0.107 ± 0.005	0.204 ± 0.017	0.298 ± 0.007	0.359 ± 0.009
Sevoflurane	0.128 ± 0.008	0.247 ± 0.042	0.378 ± 0.030	0.497 ± 0.007
Enflurane	0.196 ± 0.030	0.410 ± 0.025	0.587 ± 0.019	0.768 ± 0.009

The one MAC concentrations for isoflurane, sevoflurane and enflurane in the rabbit are 2.0%, 3.7% and 2.8%, respectively.[43,44] The data are expressed as mean ± SEM (n = 4–5). Abbreviation; MAC = minimum alveolar concentration.

duced by high K^+, we first recorded the control contractile response to norepinephrine (NE, 10 μM), in which rhythmic oscillations were normally observed with constant frequency and amplitudes. However, rarely (<10%), the oscillations were not observed or the amplitudes of the oscillations varied (either decreased or increased) during application of NE as we reported recently.[42] The experiments with volatile anaesthetics were performed in such strips where the oscillations were well preserved during application of NE. We studied the effects of isoflurane, sevoflurane and enflurane at concentrations equivalent to 0.25–1.25 minimum alveolar concentration (MAC) in the rabbit on rhythmic contractions induced by NE (10 μM). The one MAC concentrations for isoflurane, sevoflurane and enflurane in the rabbit are 2.0%, 3.7% and 2.8%, respectively.[43,44] The NE was applied to the strips for 11 min with an interval of more than five minutes, and a single concentration of each anaesthetic was applied for five minutes after both oscillations and contraction induced by NE had reached a steady state (three minutes after application of NE) in a random sequence. With this protocol, constant vascular responses to NE were usually obtained for ≈ two–three hours after mounting the strip in a chamber. However, as reported,[42] after this period, the oscillations usually started to decay with concomitant increases in tension, and the strips finally reached another steady state where the ACh-induced relaxation was well preserved, but the oscillations disappeared; this state usually continues for ≈ three–six hours at 35°C. To verify endothelial intactness, application of ACh was made after the experiments with volatile anaesthetics.

Volatile anaesthetics delivery and analysis

The volatile anaesthetics were delivered via calibrated agent-specific vaporizers in line with the O_2/CO_2 equilibration gas mixture aerating the Krebs solution. The anaesthetic concentration in the resulting gas mixture was monitored with a calibrated infrared multi-anaesthetic gas analyzer (Capnomac, Datex, Finland). We confirmed that 15 min was sufficient for the Krebs solution to be equilibrated with the anaesthetics. Therefore, the Krebs solution was equilibrated with the anaesthetics for 15 min prior to introduction into the chamber, which was covered with several pieces of glass plates to prevent the gas from escaping into the atmosphere. The concentrations of the anaesthetics in the solution were determined by gas chromatography (Table I), and these values were within 90% of theoretical values predicted by the partition coefficient of isoflurane and enflurane in Krebs solution[45] or by that of sevoflurane in water (0.36 at 37°C; the partition coefficient of sevoflurane in Krebs solution is not available).

Calculation and statistical analysis

All results were expressed as the mean ± SEM. The n denotes the number of animals (= the number of strips) in the experiments with vascular preparations, while the n denotes the number of samples in gas chromatography experiments.

The concentrations of volatile anaesthetics in the solution were not measured in all experiments. However, since the relationship between the concentrations of volatile anaesthetics in solution and anaesthetic concentrations (vol%) in the gas mixture should be linear, the anaesthetic concentrations on the x-axis (logarithmic) were displayed as vol% for volatile anaesthetic concentration-response relationships. The data points in the concentration-response relationships for anaesthetic effects were fitted according to a four parameter logistic model described by De Lean *et al*,[46] and the EC_{50} or IC_{50} values (concentrations which produce 50% of the maximal response or 50% inhibition of the maximal response, respectively) were derived from these fits; the four parameters included the maximal and minimal responses, the EC_{50} or IC_{50} values and the slope of the curve.

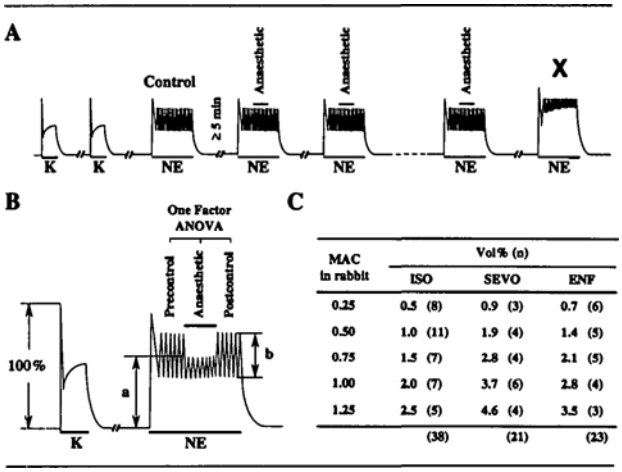
The anaesthetic-induced inhibitions of the average amplitude and frequency of the oscillations were expressed as percent changes from those of the oscillations before exposure to the anaesthetics. The amplitude was measured from the top to the bottom of the oscillations, excluding any erratic peaks. The oscillations were analyzed

after enlargement of the original recordings, when necessary. When the oscillations were completely eliminated by the anaesthetics, both average amplitude and frequency of the oscillations were defined as zero. For the analysis of the volatile anaesthetic effects on NE contractions, the magnitude of the NE contraction was defined as the amplitude from the zero line (resting tension level before application of NE) to the middle of the oscillation, also excluding any erratic peaks. Enflurane-induced vasoconstriction and transient vasorelaxation after washout of enflurane were expressed as relative tension of the NE-induced phasic contraction (100%).

Statistical analysis was made by analysis of variance (ANOVA), which was followed by Scheffé's F test, when necessary. It was not always possible to examine the effects of all concentrations of all anaesthetics in the same strip because of the rundown of the NE-induced oscillations as described above; the number of each experiment to examine the effect of each concentration of each anaesthetic on the NE-induced contraction was shown in Figure 1-C. In other words, in this study, repeated measures experiments could not be designed to compare the effects on the NE-induced contraction or oscillations among the volatile anaesthetics or among the concentrations within each anaesthetic. Therefore, two-factor (concentration, anaesthetic) ANOVA with repeated measures or one-factor (concentration) ANOVA with repeated measures was not used to compare the effects among the anaesthetics or among the concentrations within each anaesthetic, respectively. In this study, the anaesthetic effects on the NE-induced contraction or oscillations were analyzed by comparing the value in the presence of each concentration of each anaesthetic with the precontrol or postcontrol value using a one-factor (treatment (precontrol, anaesthetic, postcontrol); Figure 1-B) ANOVA with repeated measures and thereby determining significance of the effect of each concentration of each anaesthetic on the NE-induced contraction or oscillations. A factorial (anaesthetic (isoflurane, enflurane, sevoflurane)) ANOVA was used to compare the effects on the NE-induced contraction or oscillations among the volatile anaesthetics at each MAC concentration. A level of $P < 0.05$ was considered significant. All the statistical analyses were performed on a computer (Powerbook 170®, Apple, Cupertino, CA, U.S.A.) using Statview® (Abacus Concepts, Inc., Berkeley, CA, U.S.A.).

Results

Volatile anaesthetic effects were examined in 17 vascular strips from 17 rabbits, in which NE was applied 82 times (Figure 1-C). The frequency and average amplitude of the precontrol NE-induced oscillations before application



MAC in rabbit	Vol% (n)		
	ISO	SEVO	ENF
0.25	0.5 (8)	0.9 (3)	0.7 (6)
0.50	1.0 (11)	1.9 (4)	1.4 (5)
0.75	1.5 (7)	2.8 (4)	2.1 (5)
1.00	2.0 (7)	3.7 (6)	2.8 (4)
1.25	2.5 (5)	4.6 (4)	3.5 (3)
	(38)	(21)	(23)

FIGURE 1 Study protocol and data analyses: **A:** Experimental protocol. After recording steady contraction induced by 40 mM K^+, experiments to examine the volatile anaesthetic effects on the NE-induced contraction were performed during the period where the NE-induced oscillations were well-preserved (before the rundown of the NE oscillations). **B:** Data analyses. A single concentration of each anaesthetic was applied after both oscillations and contraction induced by NE had reached a steady state. The magnitude of the NE contraction was defined as the amplitude from the zero line to the middle of the oscillation (a). The amplitude of the oscillations (b) was expressed as relative tension of the 40 mM K^+-induced phasic contraction (100%). **C:** Concentrations of volatile anaesthetics tested in this study and the number of each experiment. The number in each parenthesis indicates the number of each experiment. Abbreviations: ANOVA, analysis of variance; K, 40 mM K^+; NE, norepinephrine; MAC, minimum alveolar concentration; ISO, isoflurane; SEVO, sevoflurane; ENF, enflurane.

of the anaesthetics (Figure 1-B) were 3.19 ± 0.07 cycles per minute and 0.31 ± 0.02 times the 40 mM K^+-induced phasic contraction, respectively (82 observations, $n = 17$).

Vasodilating and vasoconstricting action of the volatile anaesthetics

All volatile anaesthetics affected the magnitude of the NE-induced contraction, which was defined as the amplitude from the baseline to the middle of the oscillation (Figure 1-B). Both isoflurane and sevoflurane inhibited the magnitude of the NE-induced contraction (Figures 2-BC and 3). When the effect of each concentration of isoflurane or sevoflurane on the magnitude of the NE-induced contraction was assessed with the one-factor (treatment (precontrol, anaesthetic, postcontrol)) ANOVA with repeated measures, vasorelaxation was observed with isoflurane and sevoflurane at concentrations above 0.25 and 0.75 MAC (≥0.25 and 0.75 MAC), respectively ($P < 0.05$).

Only enflurane enhanced the magnitude of the NE-induced contraction; however, the enflurane-induced va-

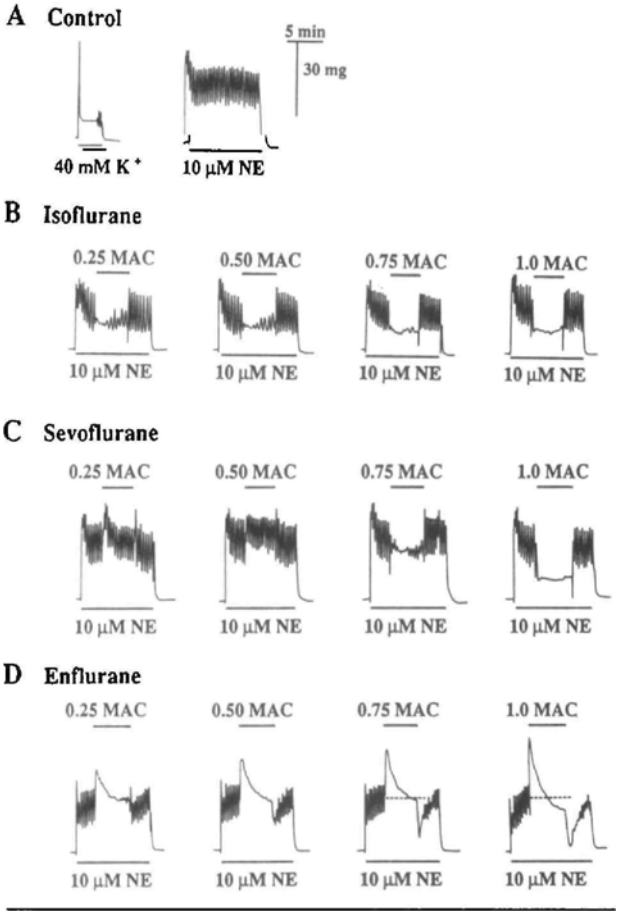


FIGURE 2 Effects of volatile anaesthetics on NE (10 μM)-induced rhythmic contractions in the endothelium-intact strips. A: Control. Vertical and horizontal calibrations represent 30 mg and 5 min, respectively. B–D: Examples of effects of isoflurane (B), sevoflurane (C), and enflurane (D) on NE-induced oscillatory contractions. The anaesthetic effect on the oscillations were analyzed after enlargement of the original data, when necessary. All data in this figure were recorded in the same strip; the enflurane-induced vasorelaxation following its transient vasoconstriction was relatively small in this strip.

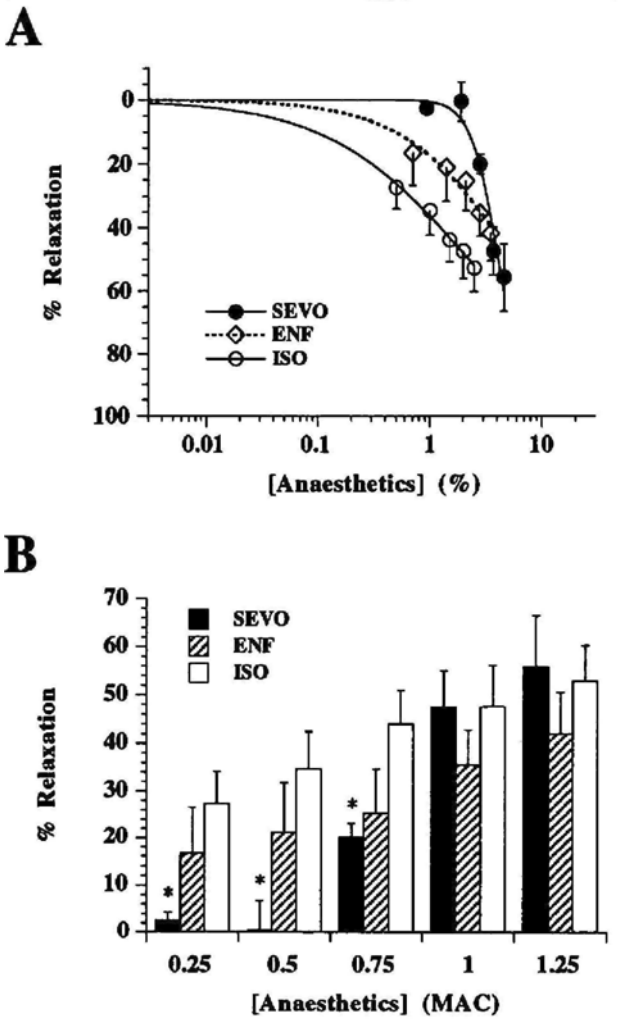


FIGURE 3 Effects of isoflurane (open circles and open bars), sevoflurane (closed circles and black bars) and enflurane (open squares and hatched bars) on NE (μM)-induced contractions. The anaesthetic-induced inhibition of the NE-induced contraction was expressed as percent changes (% relaxation) from the magnitude of NE contraction before exposure to anaesthetics. A: the IC_{50} (50% inhibitory concentration) values for the inhibitory effects of isoflurane, sevoflurane and enflurane on the NE-induced contractions were 6.55, 4.37 and 6.88%, respectively. B: Comparison of the inhibitory effects of volatile anaesthetics on the NE contraction at equi-MAC concentration. *$P < 0.05$ vs isoflurane.

soconstriction superimposed on the NE contraction was transient and it was followed by vasorelaxation (Figures 2-D, 3 and 4). When the vasoconstricting or vasorelaxing effect of each concentration of enflurane was assessed using the one-factor (treatment (precontrol, anaesthetic, postcontrol)) ANOVA with repeated measures, vasoconstriction and vasorelaxation were observed both at concentrations above 0.25 MAC (≥0.25 MAC) ($P < 0.05$).

Finally, when the vasorelaxing effects of those three anaesthetics were compared with the factorial (anaesthetic (isoflurane, enflurane, sevoflurane)) ANOVA at each MAC concentration, the obtained rank order potency was isoflurane ≥ enflurane > sevoflurane at 0.25–0.75 MAC concentrations; no differences were observed in the vasorelaxing effects between isoflurane and enflurane at these MAC concentrations (Figure 3-B). However, no differences were observed in the vasorelaxing effects among these three anaesthetics at higher MAC (≥0.75 MAC) concentrations (Figure 3-B).

Volatile anaesthetic effects on the average amplitude of NE-induced oscillations

In addition to the above-described vasodilator or vaso-

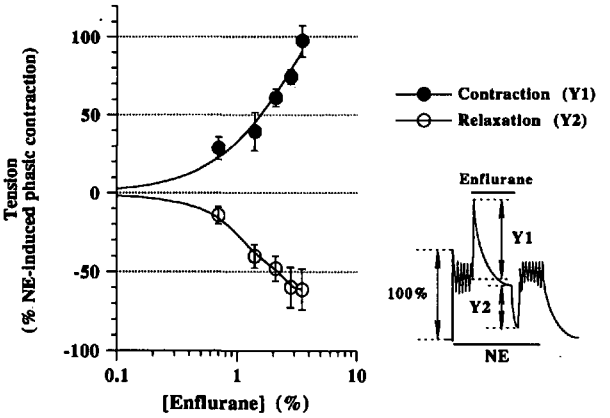


FIGURE 4 Enflurane-induced vasoconstriction (open circles, Y1) and transient vasorelaxation after washout of enflurane (closed circles, Y2) in the presence of NE (10 μM). The EC_{50} (50% effective concentration) values for the vasoconstricting action of enflurane was 3.57%. All concentrations (0.7–3.5%, 0.25–1.25 MAC) of enflurane caused vasoconstriction superimposed on the NE contraction ($P <$ 0.05), and caused transient vasorelaxation after washout of enflurane in the presence of NE ($P < 0.05$).

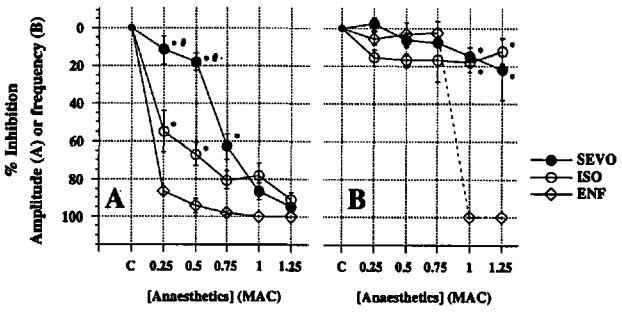


FIGURE 5 Effects of isoflurane (open circles), sevoflurane (closed circles) and enflurane (open squares) on the average amplitude (A) and frequency (B) of the NE-induced oscillations. Since the apparent periodic activity could hardly be identified in the presence of higher MAC concentrations (≥1.00 MAC) of enflurane even after enlargement of the original data in all the strips tested, both frequency and average amplitude of the oscillations after exposure to the high concentrations (1 and 1.25 MAC) of enflurane were plotted at zero (limitation of this way to analyze the oscillations was discussed in the text). Sevoflurane, isoflurane and enflurane at concentrations above 0.25 MAC (≥0.25 MAC) inhibited the amplitude of the oscillations. However, the frequency was less affected by the anaesthetics: inhibition was observed with isoflurane (≥0.25 MAC) and enflurane (≥1 MAC). Please see details regarding the statistical data analysis in the text. $*P < 0.05$ vs enflurane at each concentration. $\#P < 0.05$ vs isoflurane at each concentration.

constricting action, all these volatile anaesthetics strongly inhibited the average amplitude of the NE-induced oscillations (Figures 2 and 5). When the effect of each concentration of each anaesthetic on the average amplitude was assessed using the one-factor (treatment (precontrol, anaesthetic, postcontrol)) ANOVA with repeated measures, inhibition was observed with sevoflurane, isoflurane and enflurane at all concentrations above 0.25 MAC (≥0.25 MAC) ($P < 0.05$). In addition, when the inhibitory effects of the three anaesthetics on the average amplitude were compared with the factorial (anaesthetic (isoflurane, enflurane, sevoflurane)) ANOVA at each MAC concentration, the rank order potency was enflurane > isoflurane > sevoflurane (Figure 5-A).

Volatile anaesthetic effects on the frequency of the NE-induced oscillations

In spite of their potent inhibition of the average amplitude of the NE-induced oscillations, the volatile anaesthetics only modestly inhibited the frequency of the NE-induced oscillations, at most by ≈20% except with higher concentrations of enflurane (≥1 MAC) which almost completely abolished the oscillations (Figures 2 and 5). When the effect of each concentration of each anaesthetic on the frequency was assessed with the one-factor (treatment (precontrol, anaesthetic, postcontrol)) ANOVA with repeated measures, inhibition was observed with isoflurane and enflurane at concentrations above 0.25 and 1 MAC (≥0.25 and 1 MAC), respectively ($P < 0.05$). However, no inhibition was observed with sevoflurane at the concentrations tested. In addition, no consistent rank order potency was observed among the volatile anaesthetic effects on the frequency of the NE-induced oscillations when compared with the factorial (anaesthetic (isoflurane, enflurane, sevoflurane)) ANOVA at each MAC concentration.

Reversibility of the volatile anaesthetic effects on the NE-induced oscillations and contraction

The above-described inhibitory effects of isoflurane and sevoflurane on either oscillations or contraction induced by NE were quickly reversed after washout of these anaesthetics (Figure 2-BC). In other words, when the effect of each concentration of either anaesthetic on the average amplitude or frequency of the NE-induced oscillations or the magnitude of the NE-induced contraction was assessed with the one-factor (treatment (precontrol, anaesthetic, postcontrol)) ANOVA with repeated measures, no differences were observed between the precontrol and postcontrol values regarding all these variables.

Only the enflurane-induced inhibition of the NE oscillations was sustained after washout of enflurane (Figure 2-D). If the effect of each concentration of enflurane on the average amplitude or frequency of the NE-induced

TABLE II Prolonged inhibition of the NE-induced oscillations by enflurane

	0.25 MAC	*0.50 MAC*	*0.75 MAC*	*1.00 MAC*	*1.25 MAC*
Amplitude	28.5 ± 3.6*	33.0 ± 7.6*	43.6 ± 7.2*	48.4 ± 4.0*	43.7 ± 5.3*
Frequency	−10.5 ± 1.4*	−1.7 ± 8.8	−5.6 ± 10.4	−1.3 ± 10.6	5.2 ± 4.3

The one MAC concentration for enflurane in rabbit is 2.8%.[43] The average amplitude and frequency of the oscillations after washout of enflurane were expressed as percent changes from those of the oscillations before exposure to enflurane (precontrol value = 100%).
*$P < 0.05$ vs precontrol.

oscillations was assessed with the one factor (treatment (precontrol, anaesthetic, postcontrol)) ANOVA with repeated measures, as shown in Table II, the postcontrol values were still inhibited compared with the precontrol values. In addition, a transient decrease in tension was observed after washout of enflurane (Figures 2D and 4). The larger the enflurane-induced contraction, the larger the transient decrease in tension and the more sustained the inhibition of the oscillations after washout of enflurane (Figures 2D and 4). However, both oscillations and contractions induced by NE were almost completely restored on the next application of NE.

Discussion

Vasodilating action of volatile anaesthetics

Vascular tone is essentially under dual control, centrally through the nervous system and locally in the tissues by the environmental conditions in the immediate vicinity of the blood vessels.[47,48] The relative importance of these two control mechanisms varies among tissues.[47,49] Unlike vital organ circulation, the neural control mechanism predominates in the splanchnic circulation, particularly in the absence of ingestion or digestion.[15,47,49] The splanchnic resistance vessels are heavily innervated by sympathetic vasoconstrictor nerves, and the sympathetic nervous system plays a major role in the neural control.[15,47,49,50] The vasoconstrictor regions in the vasomotor centre are always active, producing a continuous repetitive firing of sympathetic vasoconstrictor nerves that sustains a partial contractile state of splanchnic vessels (i.e., vasomotor tone) and thereby allowing them to serve as resistance vessels.[47,48] Increases in sympathetic activity in response to emergencies (e.g., haemorrhage) cause pronounced vasoconstriction in the splanchnic vascular beds and subsequent increases in vascular resistance, thereby shifting blood flow from the temporarily less important splanchnic circulation to the vital organs.[15,47,49,50] Therefore, by being subject to the neural regulation by sympathetic nervous system, the splanchnic vessels contribute to overall cardiovascular control both as a site of adjustable arteriolar resistance and as an important blood reservoir.[15,49,50] Thus, it is important to understand factors influencing vascular tone of the splanchnic resistance vessels in order for us to sustain the proper rate of blood flow not only in the vital organs but also in the splanchnic region during anaesthesia. Although a number of factors influence vascular tone of the splanchnic vessels, direct volatile anaesthetic action on the splanchnic resistance vessels, particularly in the presence of NE (a sympathetic neurotransmitter), is one of the major factors during anaesthesia.

The present study has demonstrated that clinically relevant (subanaesthetic or anaesthetic) concentrations of isoflurane, enflurane and sevoflurane inhibited the magnitude of the NE contraction in the small mesenteric artery, a representative for the splanchnic resistance vessels. Only enflurane caused vasoconstriction superimposed on the NE contraction; however, it was transient and followed by vasorelaxation. Therefore, it seems to be unlikely that these anaesthetics interfere with splanchnic blood flow because of the direct action on vascular smooth muscle tissues. However, the observed vasorelaxing action of these anaesthetics in the resistance vessels would contribute to their hypotensive effects *in vivo*, which could threaten the blood flow to vital organs. On consideration of possible species difference in characteristics between the rabbit and human vessels, further investigations with the human vessels would be necessary to elucidate this issue.

We recently reported that all these anaesthetics inhibit the ACh-induced endothelium-dependent relaxation in the same artery,[41] suggesting that these anaesthetics inhibit the endothelium-derived relaxing factor (EDRF) pathway in this resistance artery. In addition, we have demonstrated dramatic enhancement (≈2–3 times) of the NE contraction of the same artery after exposure to inhibitors of the EDRF pathway,[39,41,42] indicating considerable involvement of the EDRF pathway in the generation of the NE contraction in this artery. Therefore, it is conceivable that these anaesthetics enhance the NE-induced contraction as a result of inhibition of the EDRF pathway. Nevertheless, these anaesthetics inhibited the NE contraction, suggesting that the vasodilating action

of these anaesthetics is at least in part independent of EDRF. The direct vasodilating action of these anaesthetics is probably dominant over their inhibitory action on the EDRF pathway in the presence of NE.

Previous studies have suggested considerable differences in vascular responses to volatile anaesthetics between large conductance and small resistance coronary arteries both *in vivo* and *in vitro*.[21,23,24] Until now, only a few studies have examined the effect of isoflurane, sevoflurane or enflurane on α-adrenergic agonist-induced contractions in large conductance arteries proximal to small mesenteric resistance artery in the presence of intact endothelium.[6,7,10] Low concentrations (≤ 2%) of isoflurane have been shown to cause vasoconstriction in rat aorta precontracted with phenylephrine (PHE, 1 μM), while high concentrations (≥3%) of isoflurane caused vasodilation.[7] This contrasts with our data where isoflurane (0.5–2.5%) produced vasodilatation without initial vasoconstriction in the splanchnic resistance arteries. Sevoflurane (2.3 and 4.6%) has been shown to inhibit PHE (0.1 μM–1 mM)-induced, but not NE (0.1–100 μM)-induced, contractions in large canine mesenteric arteries;[10] this again contrasts with our results in small mesenteric arteries where sevoflurane (2.9–4.8%) inhibited NE contractions. Studies examining the effect of enflurane on α-adrenergic agonist-induced contractions in conductance vessels have produced variable results. Enflurane (0.5–5.0%) has been shown to cause vasoconstriction without vasodilatation in the presence of PHE (1 μM) in rat aorta,[7] while only vasodilation was described with enflurane (1.7–5.0%) in the presence of PHE (0.01–1 mM) or NE (3–100 μM) in large canine mesenteric arteries.[6] Our results show that enflurane (0.7–3.5%) causes transient vasoconstriction followed by vasorelaxation in the small splanchnic resistance arteries. These considerable differences observed in vascular responses to anaesthetics could be due to regional differences (large conductance artery vs small resistance artery), species difference (rat or canine vs rabbit) or the difference in agents used to percontract the vessels (NE vs PHE). In spite of rather high anaesthetic concentrations used in previous studies, the maximal inhibitions of NE contractions by these anaesthetics previously observed in aorta or large mesenteric artery were rather small (up to ≈20%)[6,7,10] compared with those observed in small mesenteric arteries (≈40–60%; Figure 3), suggesting that these anaesthetics may preferentially relax small resistance arteries.

Volatile anaesthetic-induced inhibition of the NE-induced oscillations

This study first demonstrates that subanaesthetic concentrations of isoflurane, enflurane and sevoflurane strongly inhibit the rhythmic oscillations in contractile responses to NE observed in small splanchnic resistance artery. The rhythmic oscillatory contractile activity has been demonstrated in a number of *in vitro* vascular preparations.[42,51–53] Correspondingly, rhythmic oscillatory changes in vessel diameter, blood flow or blood pressure have also been documented in a number of *in vivo* vascular beds such as cat mesenteric arterioles or human skin vessels.[30–36] The functional importance of the oscillatory contractile activity of blood vessels, often referred to as vasomotion and regarded as a feature of small vessels, does not appear to be fully understood. However, the vasomotion has been proposed to provide mechanisms beneficial for tissue perfusion or oxygen delivery to tissue, i.e., mechanisms by which the vascular resistance can be effectively regulated without causing continuous tissue hypoxia by at least intermittently supplying blood flow or oxygen to the peripheral tissue.[37,38] In support of this, oscillations in oxygen tension were also demonstrated at frequencies similar to those of the vascular oscillations.[54] Other suggested physiological roles of vasomotion are minimization of fluid filtration into extravascular space by reducing hydrostatic pressure and enhancement of lymphatic drainage through the pumping action of closely adjacent arterioles.[37,38] The observed potent inhibition of the oscillatory vasomotion by the volatile anaesthetics may result in alteration of vascular homeostasis such as fine regulation of tissue blood flow or vascular permeability.

The concentration-response relationships for the inhibitory effect of enflurane on the frequency of oscillations were unexpected; complete inhibition suddenly appeared at higher concentrations (≥1.00 MAC) of enflurane (Figure 5B). This is presumably because of the method used to analyze the oscillations with which both amplitude and frequency of the oscillations were defined as zero when the oscillations were completely abolished. The amplitude of oscillations was obviously much more sensitive to all these volatile anaesthetics than their frequency (Figure 3). Therefore, we believe that the NE-induced oscillations disappeared probably due to strong inhibition of the amplitude of oscillations by enflurane, but not due to inhibition of the frequency of oscillations. Probably, the volatile anaesthetics preferentially inhibit some peripheral processes involved in the generation of the oscillations (e.g., gap junctional transmission) rather than pacemaker activity pivotal for the generation of the oscillations.

It has recently been suggested that rhythmic oscillations in contractile responses to α-adrenergic agonists are mediated through EDRF (or NO) in hamster aorta or rat mesenteric artery.[51,52] In support of this, we recently demonstrated that the NE-induced oscillations in this artery were eliminated by various inhibitors of EDRF path-

way or endothelial denudation.[42] Various volatile anaesthetics have been shown to inhibit the EDRF-mediated vasorelaxation in a number of vascular preparations including this resistance artery.[4,8-12,41] Although the precise mechanism(s) by which the volatile anaesthetics inhibit EDRF-mediated relaxation is still a matter of discussion, the anaesthetics may inhibit the EDRF pathway as a result of inhibition of EDRF synthesis and/or release, inactivation of EDRF and/or inhibition of guanylate cyclase in vascular smooth muscle cells.[4,8-12,42] Since EDRF is thought to be involved in the generation of vascular oscillatory behaviour,[42,51,52] the observed volatile anaesthetic-induced inhibition of arterial oscillations may result from inhibition of the EDRF pathway.

Volatile anaesthetics inhibit NE-induced oscillations in rabbit mesenteric veins;[55] however, the oscillations in the veins were not inhibited by LNNA (50 μM) and therefore have been proposed to be independent of EDRF.[55] The volatile anaesthetics may inhibit both the EDRF-dependent and -independent oscillations by inhibiting some common process involved in generation of both kinds of oscillations (e.g., gap junctional transmission between smooth muscle cells or periodic release of Ca^{++} from intracellular store).

The rhythmic contractions of multicellular preparations must require considerable coordination of activity among the vascular smooth muscle cells; such coordination may involve cell-to-cell coupling via gap junctions between vascular smooth muscle cells. In fact, octanol, known to disrupt gap junction communication,[56] was recently described to inhibit the phenylephrine-induced oscillations in aorta.[51] In addition, halothane and enflurane have been demonstrated to strongly inhibit gap junction transmission in cardiac cells.[57] Thus, the observed inhibitory effects of volatile anaesthetics on the oscillations could also be explained by inhibition of gap junction transmission, known to exist in vascular smooth muscle cells.

Enflurane-induced vasoconstriction

Recent studies have suggested that enflurane causes vasoconstriction by releasing Ca^{++} from intracellular store in large conductance arteries.[58,59] In support of this, we have recently shown that enflurane generates ryanodine-sensitive transient vasoconstriction either in the presence or absence of extracellular Ca^{++} in the endothelium-denuded strips from the same rabbit artery or rat mesenteric artery.[41,60] Therefore, the observed enflurane-induced vasoconstriction is probably, at least in part, due to Ca^{++} release from intracellular Ca^{++} stores in vascular smooth muscles cells. The observed transient decrease in tension after washout of enflurane (Figures 2D and 4) may reflect refilling of Ca^{++} into the store which had been depleted by enflurane. Furthermore, the prolonged enflurane-induced inhibition of the rhythmic oscillations (Figure 2D and Table II) may be due to the time required to refill ryanodine-sensitive intracellular Ca^{++} stores of endothelial cells;[61] endothelial intracellular Ca^{++} stores are believed to play an important role in the regulation of endothelial function.[61,62] Decreases in Ca^{++} availability for EDRF production or release might cause prolongation of enflurane-induced inhibition of the EDRF-mediated oscillations.

Conclusions

Isoflurane, sevoflurane and enflurane at clinically relevant concentrations all inhibited NE-induced contractions and oscillations in small splanchnic resistance arteries. The observed vasodilating action of volatile anaesthetics may contribute to their hypotensive effects *in vivo*. The potent inhibition of oscillations may also play a role in volatile anaesthetic-induced alterations of cardiovascular homeostasis. Comparison of our data with previous findings in large conductance arteries suggest that the volatile anaesthetics may preferentially relax small resistance arteries. This is the first investigation to demonstrate the effects of subanaesthetic (0.25–0.75 MAC) concentrations of three currently used volatile anaesthetics isoflurane, enflurane and sevoflurane on rhythmic contractions induced by the sympathetic neurotransmitter, NE, in small splanchnic resistance artery that plays a critical role in the regulation of splanchnic blood flow.

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References

1 *Marshall BE, Longnecker DE.* General anesthetics. *In*: Gilman AG, Rall TW, Nies AS, Taylor P (Eds.). The Pharmacological Basis of Therapeutics, 6th ed. New York: Pergamon Press, 1990: 285–310.

2 *Pavlin EG, Su JY.* Cardiopulmonary pharmacology. *In*: Miller RD (Ed.). Anesthesia, 3rd ed. New York: Churchill Livingstone Inc., 1990: 105–34.

3 *Stevens WC, Kingston HGG.* Inhalational anesthesia. *In*: Barash PG, Cullen BF, Stoelting RK (Eds.). Clinical Anesthesia, 2nd ed. Philadelphia: J.B. Lippincott Company, 1992: 439–65.

4 *Muldoon SM, Hart JL, Bowen KA, Freas W.* Attenuation of endothelium-mediated vasodilation by halothane. Anesthesiology 1988; 68: 31–7.

5 *Blaise G, Sill JC, Nugent M, Van Dyke AR, Vanhoutte PM.* Isoflurane causes endothelium-dependent inhibition of contractile responses of canine coronary arteries. Anesthesiology 1987; 67: 513–7.

6 *Kobayashi Y, Yoshida K-I, Noguchi M, Wakasugi Y, Itoh H, Okabe E.* Effects of enflurane on contractile reactivity in isolated canine mesenteric arteries and veins. Anesth Analg 1990; 70: 530–6.

7 *Stone DJ, Johns RA.* Endothelium-dependent effects of halothane, enflurane, and isoflurane on isolated rat aortic vascular rings. Anesthesiology 1989; 71: 126–32.

8 *Uggeri MJ, Proctor GJ, Jones RA.* Halothane, enflurane, and isoflurane attenuate both receptor- and non-receptor-mediated EDRF production in rat thoracic aorta. Anesthesiology 1992; 76: 1012–7.

9 *Blaise G, To Q, Parent M, Lagarde B, Asenjo F, Sauvé R.* Does halothane interfere with the release, action, or stability of endothelium-derived relaxing factor/nitric oxide? Anesthesiology 1994; 80: 417–26.

10 *Yoshida K, Okabe E.* Selective impairment of endothelium-dependent relaxation by sevoflurane: oxygen free radical participation. Anesthesiology 1992; 76: 440–7.

11 *Toda H, Nakamura K, Hatano Y, Nishiwada M, Kakuyama M, Mori K.* Halothane and isoflurane inhibit endothelium-dependent relaxation elicited by acetylcholine. Anesth Analg 1992; 75: 198–203.

12 *Hart JL, Jing M, Bina S, Freas W, Van Dyke RA, Muldoon SM.* Effects of halothane on EDRF/cGMP-mediated smooth muscle relaxations. Anesthesiology 1993; 79: 323–31.

13 *Brendel JK, Jones AR.* Isoflurane does not vasodilate rat thoracic aortic rings by endothelium-derived relaxing factor or other cyclic GMP-mediated mechanisms. Anesthesiology 1992; 77: 126–31.

14 *Guyton AC.* Textbook of Medical Physiology, 6th ed. Philadelphia: W.B. Saunders Company, 1981: 219–31.

15 *Stephenson RB.* The splanchnic circulation. *In*: Patton HD, Fuchs AH, Hille B, Scher AM, Steiner R (Eds.). Textbook of Physiology, 21st ed. Philadelphia: J.B. Lippincott Company, 1989: 911–23.

16 *Christensen KL, Mulvany MJ.* Mesenteric arcade arteries contribute substantially to vascular resistance in conscious rats. J Vasc Res 1993; 30: 73–9.

17 *Rowan RA, Bevan JA.* Quantitative ultrastructural measurement of innervation density, neurotransmitter vesicles and neuromuscular cleft width in the rabbit central ear artery and its main side branch. Blood Vessels 1987; 24: 181–91.

18 *Bevan JA, Laher I, Rowan R.* Some implications of the intrasynaptic norepinephrine concentrations in resistance arteries. Blood Vessels 1987; 24: 137–40.

19 *Cauvin C, Lukeman S, Cameron J, Hwang O, van Breemen C.* Differences in norepinephrine activation and diltiazem inhibition of calcium channels in isolated aorta and mesenteric resistance vessels. Circ Res 1985; 56: 822–8.

20 *Nagao T, Illiano SC, Vanhoutte PM.* Heterogeneous distribution of endothelium-dependent relaxations resistant to N^G-nitro-L-arginine in rats. Am J Physiol 1992; 263: H1090–4.

21 *Sill JC, Bove AA, Nugent M, Blaise GA, Dewey JD, Grabau C.* Effects of isoflurane on coronary arteries and coronary arterioles in the intact dog. Anesthesiology 1987; 66: 273–9.

22 *Cauvin C, Weir SW, Bühler FR.* Differences in Ca^{2+} handling along the arterial tree: an update including studies in human mesenteric resistance vessels. J Cardiovasc Pharmacol 1988; 12: S10–5.

23 *Hatano Y, Nakamura K, Yakushiji T, Nishiwada M, Mori K.* Comparison of the direct effects of halothane and isoflurane on large and small coronary arteries isolated from dogs. Anesthesiology 1990; 73: 513–7.

24 *Park KW, Dai HB, Lowenstein E, Darvish A, Sellke FW.* Heterogeneous vasomotor responses of rabbit coronary microvessels to isoflurane. Anesthesiology 1994; 81: 1190–7.

25 *Haglund U.* Systemic mediators released from the gut in critical illness. Crit Care Med 1993; 21: S15–8.

26 *Meakins JL, Marshall JC.* The gut as the motor of multiple system organ failure. *In*: Marston A, Bulkley GB, Fiddian-Green RG, Haglund UH (Eds.). Splanchnic Ishemia and Multiple Organ Failure. St. Louis: CV Mosby Co., 1989: 339–48.

27 *Gianotti L, Alexander JW, Fukushima R, Childress CP.* Translocation of Candida albicans is related to the blood flow of individual intestinal villi. Circulatory Shock 1993; 40: 250–7.

28 *Landow L.* Splanchnic lactate production in cardiac surgery patients. Crit Care Med 1993; 21: S84–91.

29 *Ford EG, Baisden CE, Matteson BS, Picone AL.* Sepsis after coronary bypass grafting: evidence for loss of the gut mucosal barrier. Ann Thorac Surg 1991; 52: 514–7.

30 *Strucl M, Peterec D, Finderle Z, Maver J.* Pressure sensitivity of flow oscillations in postocclusive reactive skin hyperemia. Am J Physiol 1994; 266: H1762–8.

31 *Pyykkö I, Gemne G, Kolari P, Starck J, Ilmarinen R, Aalto H.* Vasomotor oscillation in vibration-induced white finger. Scand J Work Environ Health 1986; 12: 389–94.

32 *Nakae I, Quan L, Sugimoto Y, Tsutamoto T, Kinoshita M.* Glibenclamide-induced oscillations of canine coronary artery is independent of myocardial ischemia. J Cardiovasc Pharmacol 1994; 23: 473–9.

33 *Colantuoni A, Bertuglia S, Intaglietta M.* Quantitation of

rhythmic diameter changes in arterial microcirculation. Am J Physiol 1984; 246: H508–17.

34 *Bouskela E, Wiederhielm CA.* Microvascular myogenic reaction in the wing of the intact unanesthetized bat. Am J Physiol 1979; 237: H59–65.

35 *Meyer J-U, Borgström P, Lindbom L, Intaglietta M.* Vasomotion patterns in skeletal muscle arterioles during changes in arterial pressure. Microvasc Res 1988; 35: 193–203.

36 *Johnson PC, Wayland H.* Regulation of blood flow in single capillaries. Am J Physiol 1967; 212: 1405–15.

37 *Intaglietta M.* Vasomotion as normal microvascular activity and a reaction to impaired homeostasis. *In*: Intaglietta M (Ed.). Vasomotion and Flow Modulation in the Microcirculation. Progress in Applied Microcirculation. Basel: Krager, 1989; vol 15: 1–9.

38 *Secomb TW, Intaglietta M, Gross JF.* Effects of vasomotion on microcirculatory mass transport. *In*: Intaglietta M (Ed.). Vasomotion and Flow Modulation in the Microcirculation. Progress in Applied Microcirculation. Basel: Krager, 1989; vol 15: 49–61.

39 *Akata T, Yoshitake J, Nakashima M, Itoh T.* Effects of protamine on vascular smooth muscle of rabbit mesenteric artery. Anesthesiology 1991; 75: 833–46.

40 *Akata T, Kodama K, Takahashi S.* Effects of heparin on the vasodilator action of protamine in the rabbit mesenteric artery. Br J Pharmacol 1993; 109: 1247–53.

41 *Akata T, Nakashima M, Kodama K, Boyle WA III, Takahashi S.* Effects of volatile anesthetics on acetylcholine-induced relaxation in the rabbit mesenteric resistance artery. Anesthesiology 1995; 82: 188–204.

42 *Akata T, Kodama K, Takahashi S.* Role of endothelium in oscillatory contractile responses to various receptor agonists in isolated small mesenteric and epicardial coronary arteries. Jpn J Pharmacol 1995; 68: 331–43.

43 *Drummond JC.* MAC for halothane, enflurane, and isoflurane in the New Zealand white rabbit: and a test for the validity of MAC determinations. Anesthesiology 1985; 62: 336–8.

44 *Scheller MS, Saidman LJ, Partridge BL.* MAC of sevoflurane in humans and the New Zealand white rabbit. Can J Anaesth 1988; 35: 153–6.

45 *Renzi F, Waud BE.* Partition coefficients of volatile anesthetics in Krebs' solution. Anesthesiology 1977; 47: 62–3.

46 *De Lean A, Munson PJ, Rodbard D.* Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. Am J Physiol 1978; 235: E97–102.

47 *Berne RM, Levy MN.* The peripheral circulation and its control. *In*: Berne RM, Levy MN (Eds.). Physiology, 2nd ed. St. Louis: The C.V. Mosby Company, 1988; 508–24.

48 *Weems WA, Downey JM.* Regulation of arterial pressure. *In*: Johnson LR (Ed.). Essential Medical Physiology, New York: Raven Press, 1992: 195–203.

49 *Weisbrodt NW, Downey JM.* Regulation of regional blood flow. *In*: Johnson LR (Ed.)., Essential Medical Physiology. New York: Raven Press, 1992: 213–20.

50 *Berne RM, Levy MN.* Special circulations. *In*: Berne RM, Levy MN (Eds.). Physiology, 2nd ed. St. Louis: The C.V. Mosby Company, 1988; 540–60.

51 *Jackson WF, Mülsch A, Brusse R.* Rhythmic smooth muscle activity in hamster aortas is mediated by continuous release of NO from the endothelium. Am J Physiol 1991; 260: H248–53.

52 *Gustafsson H, Mulvany MJ, Nilsson H.* Rhythmic contractions of isolated small arteries from rat: influence of the endothelium. Acta Physiol Scand 1993; 148: 153–63.

53 *Chemtob S, Inayatulla A, Varma DR.* Eicosanoid-dependent and endothelium-independent oscillations of rat aorta. J Vasc Res 1992; 29: 270–80.

54 *Gaylarde PM, Sarkany I.* Periodic skin blood flow (Letter). N Engl J Med 1985; 312: 1194–5.

55 *Stadnicka A, Flynn NM, Bosnjak ZJ, Kampine JP.* Enflurane, halothane, and isoflurane attenuate contractile responses to exogenous and endogenous norepinephrine in isolated small mesenteric veins of the rabbit. Anesthesiology 1993; 78: 326–34.

56 *Spray DC, Burt JM.* Structure-activity relations of the cardiac gap junction channel. Am J Physiol 1990; 258: C195–205.

57 *Burt JM, Spray DC.* Volatile anesthetics block intracellular communication between neonatal rat myocardial cells. Circ Res 1989; 65: 829–37.

58 *Kakuyama M, Hatano Y, Nakamura K, et al.* Halothane and enflurane constrict canine mesenteric arteries by releasing Ca^{2+} from intracellular Ca^{2+} stores. Anesthesiology 1994; 80: 1120–7.

59 *Su JY, Chang YI, Tang LJ.* Mechanisms of action of enflurane on vascular smooth muscle. Anesthesiology 1994; 81: 700–9.

60 *Akata T, Boyle WA III.* Volatile anesthetic actions on contractile proteins in membrane-permeabilized small mesenteric arteries. Anesthesiology 1995; 82: 700–12.

61 *Ziegelstein RC, Spurgeon HA, Pili R, et al.* A functional ryanodine-sensitive intracellular Ca^{2+} store is present in vascular endothelial cells. Circ Res 1994; 74: 151–6.

62 *Nilius B.* Ion channels and regulation of transmembrane Ca^{2+} influx in endothelium. *In*: Sperelakis N, Kuriyama H (Eds.). Ion Channels of Vascular Smooth Muscle Cells and Endothelial Cells. New York: Elsevier Science Publishing Co., Inc., 1991: 317–25.