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Volatile anaesthetics restore bradykinin and serotonin-induced coronary vasodilation after blocking nitric oxide synthase: lack of anaesthetic effects on K_{ATP} channels and prostaglandin pathways

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Summary

Background and objective Volatile anaesthetic effects on altering tone after blocking nitric oxide synthase, cyclo-oxygenase-prostaglandin synthase and K_{ATP} channel pathways are controversial. We examined in isolated guinea pig hearts whether anaesthetics alter bradykinin and 5-hydroxytryptamine-induced effects on coronary flow and percentage oxygen extraction after blocking these pathways.

Methods Before and during exposure to sevoflurane, halothane or isoflurane, hearts were infused with 10^{-13}–10^{-8} M bradykinin, or 10^{-8}–10^{-6} M 5-hydroxytryptamine (serotonin), with either L-NAME, indomethacin, or glibenclamide. Bradykinin or 5-hydroxytryptamine alone increased flow and decreased percentage oxygen extraction in a concentration-dependent manner; these effects were largely blocked by L-NAME (nitro-L-arginine methylester), which also decreased basal flow and increased basal percentage oxygen extraction.

Results The anaesthetics restored bradykinin and 5-hydroxytryptamine-induced increases in flow or decreases in percentage oxygen extraction after inhibition by L-NAME. Indomethacin or glibenclamide alone had little effect on basal flow and percentage oxygen extraction. The anaesthetics restored bradykinin and 5-hydroxytryptamine-induced increases in flow or decreases in percentage oxygen extraction after inhibition by L-NAME. Indomethacin or glibenclamide alone had little effect on basal flow and percentage oxygen extraction. Drug-induced increases in flow and decreases in percentage oxygen extraction in the absence or presence of glibenclamide or indomethacin were not altered at either of the two concentrations of anaesthetics.

Conclusions Endothelium-dependent vasodilatation is not affected by blocking prostaglandin release or K_{ATP} channels in the intact heart even in the presence of an anaesthetic. However, the diminished responses to vasodilators after nitric oxide synthase inhibition is largely restored or enhanced by anaesthetics.

Keywords: ANAESTHETICS, GENERAL, ANAESTHETICS, INHALATION, halothane, isoflurane, sevoflurane; BLOOD CIRCULATION, coronary circulation; ENZYME INHIBITORS, N^G-nitroarginine methyl ester; ENZYME INHIBITORS, CYCLOOXYGENASE INHIBITORS, indomethacin; ENDOTHELIUM, VASCULAR; HAEMODYNAMICS, vasodilatation; HEART, myocardium; oxygen; RODENTIA, guinea pigs; SULFONYLUREA COMPOUNDS, glyburide; TRYPTAMINES, SEROTONIN, 5-hydroxytryptamine; NEUROTRANSMITTERS AND NEUROTRANSMITTER AGENTS, NEUROTRANSMITTERS, biogenic amine neurotransmitters, serotonin; NEUROTRANSMITTERS, NEUROPEPTIDES, bradykinin; VASODILATOR AGENTS.

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Introduction

Release of nitric oxide (NO) and prostaglandins (PG) by the vascular endothelium and ATP-regulated K^+ channel opening in vascular smooth muscle has been

demonstrated to contribute importantly to coronary vasodilation elicited by a variety of physiological and pharmacological stimuli. There are conflicting reports whether volatile anaesthetics alter endothelium-dependent vasodilation by the nitric oxide synthase (NOS) [1–8] or cyclo-oxygenase (COX)-PG synthase (PGS) pathways [9–12]. Most studies have been undertaken *in vitro* in endothelial/vessel strip or coculture preparations in the absence and presence of COX [9,10] or NOS antagonism [1,2,13–20]. Anaesthetics also have been proposed to cause vasodilation, at least in part, by opening sarcolemmal K_{ATP} channels [21–26]. We reported recently that volatile anaesthetics did not interfere with bradykinin (BK)-induced coronary flow increases, NO release and L-citrulline production in the isolated guinea pig heart [27]. There remained the possibility that anaesthetics alter endothelium-mediated vascular responses after inhibiting NOS or COX or blocking K_{ATP} channels in the same model. To test this we examined further whether the anaesthetics sevoflurane (SEV), isoflurane (ISO) and halothane (HAL) alter bradykinin or serotonin [5-hydroxytryptamine (5-HT)]-induced increases in coronary artery flow and decreases in percentage oxygen extraction in guinea pig hearts after blocking K_{ATP} channels, or after inhibiting basal and stimulated release of nitric oxide or prostaglandin. Drugs used to test this were glibenclamide (GLIB) to block K_{ATP} channels, nitro-L-arginine methylester (L-NAME) to inhibit NOS and indomethacin (INDO) to inhibit the COX-PGS pathway.

Methods

Langendorff heart preparation

The investigation conformed to the US National Institutes of Health (NIH publication No. 85–23, revised 1995) laboratory animal care and use standards and was approved by the Medical College of Wisconsin animal studies committee. Description of the surgical preparation for this model has been reported in detail previously [27–29]. Hearts were harvested from albino English short-haired guinea pigs (250–300 g). Each heart was perfused in retrograde fashion through the aorta at 55 mmHg with a 95% oxygenated, modified Krebs–Ringer's (KR) solution as described previously [27–29]. Coronary sinus effluent was collected by placing a small, gas impermeable cannula into the right ventricle through the pulmonary artery after ligating the superior and inferior venae cavae. Coronary outflow (coronary sinus) PO_2 was measured continuously on-line with a miniature thermostabile Clark O_2 electrode (Instech Laboratories, Model 203B, Plymouth Meeting, PA, USA). Perfusate, bath and O_2 electrode temperatures were maintained tightly at 37.2 ± 0.1°C using a thermostatically controlled water circulator to jacketed glass tubing, bath and aluminum heat exchangers. Coronary inflow and effluent pH and PO_2 and PCO_2 were measured during each manoeuvre off-line at 37°C with an intermittently self-calibrating gas analyser. Isovolumetric left ventricular pressure, coronary flow, percentage oxygen extraction and spontaneous heart rate were measured continuously as described previously [27–29]. Percentage oxygen extraction was utilized as an indicator of vascular tone independent of cardiac metabolism with the assumption that a change in oxygen need is met by a change in oxygen delivery in the absence of ischaemia. Percentage oxygen extraction was calculated as the inflow and outflow tension difference multiplied by 100 and divided by the inflow PO_2. Inflow perfusate O_2 tension was kept constant at 0.7 kPa above atmospheric pressure. Heart rate, inflow and outflow oxygen (kPa), coronary flow, isovolumetric systolic and diastolic left ventricular pressures (kPa) were displayed continuously an eight-channel chart recorder. Adenosine (0.2 mL of 200 μM stock solution) was injected as a bolus to measure maximal coronary flow after the heart had initially stabilized. Adenosine was given again at the end of each experiment to observe any change in maximum flow reserve.

Anaesthetics were delivered by calibrated agent specific vaporizers and anaesthetic concentrations were measured in the effluent by gas chromatography as described previously [28,29]. Anaesthetics were delivered at two concentrations approximating 1–2 MAC (minimum alveolar concentration). Coronary sinus anaesthetic concentrations for sevoflurane ($n = 47$) were 0.31 ± 0.03 mM and 0.63 ± 0.04 mM, which, respectively, gave concentrations of 2.2 ± 0.2 and 4.5 ± 0.3 Vol.%, or approximately 1.0 and 2.2 MAC. For the low and high concentrations of isoflurane ($n = 15$) the corresponding values were 0.20 ± 0.02 and 0.35 ± 0.03 mM, 0.9 ± 0.01 and 1.6 ± 0.0 Vol.% and 1.3 MAC; values for halothane ($n = 16$) were

0.19 ± 0.02 and 0.38 ± 0.01 mM, 0.06 ± 0.0 and 1.3 ± 0.2 Vol.%, or 0.8 and 1.6 MAC.

Protocol

The hypothesis was to test whether anaesthetics influence vasodilatory responses to endothelium dependent drugs after blocking one of three pathways that modulate vascular tone. After baseline control values were re-established, 84 hearts were randomized into three series of studies identified by the three blockers-glibenclamide (4 μM), L-NAME (100 μM) and indomethacin (100 μM). Before and during blockade in the presence or absence of an anaesthetic, these hearts was infused with random ordered concentrations of 10^{-12} bradykinin [27,29] or 10^{-8}–10^{-6} M 5-hydroxytryptamine (5-HT) [29,30] for 2 min at each concentration with a 5 min intervening control period. After administering a given blocker, each heart was exposed to two randomized concentrations of halothane, isoflurane or sevoflurane. Thus, a range of bradykinin or 5-HT responses were tested 3–4 times in each heart. Because of the potential for long-lasting effects of glibenclamide, L-NAME and indomethacin, step increases in bradykinin or 5-HT were conducted first in the absence of these blockers. Responses to bradykinin or 5-HT alone did not change over time. Anaesthetic effects on endothelium-dependent vasodilation were not routinely conducted in the absence of blockers because we have reported previously that anaesthetics do not alter bradykinin-induced vasodilatory responses in this model [27] and because each experiment comprised three treatments and a control, each with 14 measurements, and lasted approximately 240 min. Ancillary experiments to test bradykinin and 5-hydroxytryptamine responses, also in the presence of an anaesthetic, confirmed results of the earlier study.

Statistical analysis

All data are expressed as means ± standard errors of the mean (SEM). Each heart served as its own control for either glibenclamide, L-NAME or indomethacin and two concentrations of one anaesthetic, as well as for the repeated effects of either bradykinin or 5-HT. No comparisons were made among different groups, i.e. different anaesthetics, treatments, or vasodilators. Effects of glibenclamide, L-NAME or indomethacin vs. no treatment (control), either anaesthetic concentration vs. control or glibenclamide, L-NAME or indomethacin, and effects of bradykinin or 5-hydroxytryptamine, on coronary flow, percentage oxygen extraction and isovolumetric left ventricular pressure (Figs 1–3), were compared by Tukey's comparison of means tests after ANOVA for repeated measures (Super Anova 1.11® software for Macintosh®, Abacus Concepts, Inc, Berkeley, CA, USA).

Concentration response curves to bradykinin or 5-hydroxytryptamine in the presence or absence of glibenclamide, L-NAME, or indomethacin, and in the presence or absence of sevoflurane, isoflurane, or halothane, were analysed by logarithmic regression (Statview®, Abacus Concepts, Calabasas, CA, USA) in the form of coronary flow, or percentage O_2 extraction, $= m\ x + B$ (where m = slope, B = Y-intercept at a given log concentration x = bradykinin or 5-HT and r^2 = the regression coefficient). In the equations, bradykinin values are in nM and 5-hydroxytryptamine values are in μM. Regression coefficients and slope and intercept confidence intervals were plotted using multivariate scattergrams. Regression curves were compared by posteriori tests for differences among a set of regression coefficients by the simultaneous test procedure. Differences among means, regression coefficients, slopes and intercepts were considered statistically significant when $P = 0.05$.

Results

Coronary flow at least doubled in each series after the initial bolus adenosine injection and the flow increase to that elicited initially was statistically similar ($P < 0.05$) to that after the final adenosine injection, respectively, for the glibenclamide series (Fig. 1a–c), 17.2 ± 0.8 vs. 16.3 ± 1.2 mL g^{-1}min^{-1}, and for the indomethacin series (Fig. 3a–c), 16.3 ± 0.8 vs. 14.8 ± 0.9 mL g^{-1}min^{-1}, but not for the L-NAME series (Fig. 2a–c), 14.0 ± 0.8 and 10.4 ± 0.8 mL g^{-1}min^{-1} ($P > 0.1$). The three figures display coronary flow, percentage oxygen extraction and isovolumetric left ventricular pressure data for each of the three series treated with bradykinin and sevoflurane only. These data include responses to a vasodilator with and without a blocking agent alone and with two concentrations of an anaesthetic. Other results not displayed

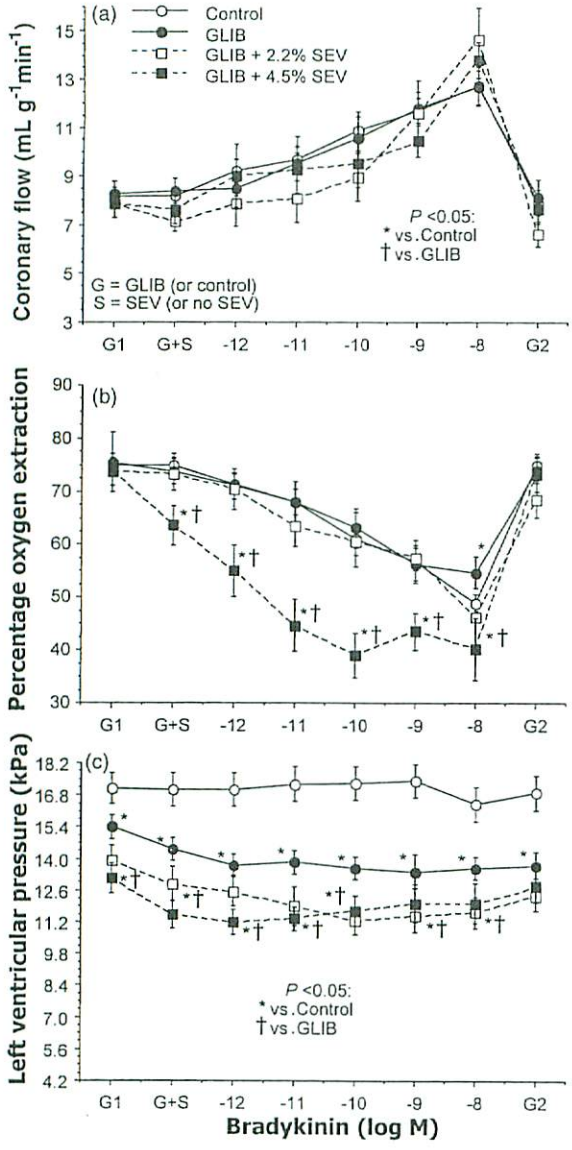

Fig. 1. (a, b, c) Cardiac effects of 4 μM glibenclamide (GLIB) in the absence and presence of sevoflurane (SEV) on responses to increasing concentrations of bradykinin (BK). Bradykinin responses were measured first without treatment (Control) and repeated in the presence of glibenclamide alone, glibenclamide plus low sevoflurane (2.2%) and glibenclamide plus high sevoflurane (4.5%). Anaesthetic concentrations were randomized. See text and Table 1a-g for bradykinin slope analyses. Glibenclamide with high sevoflurane reduced percentage oxygen extraction (%O_2E) more than glibenclamide alone or with low sevoflurane, but bradykinin-induced changes in coronary flow and percentage oxygen extraction slopes were not altered significantly by glibenclamide, or by glibenclamide with sevoflurane. Left ventricular pressure (LVP) decreased slowly but more so in the presence of sevoflurane. G1, first control; G2, second control.

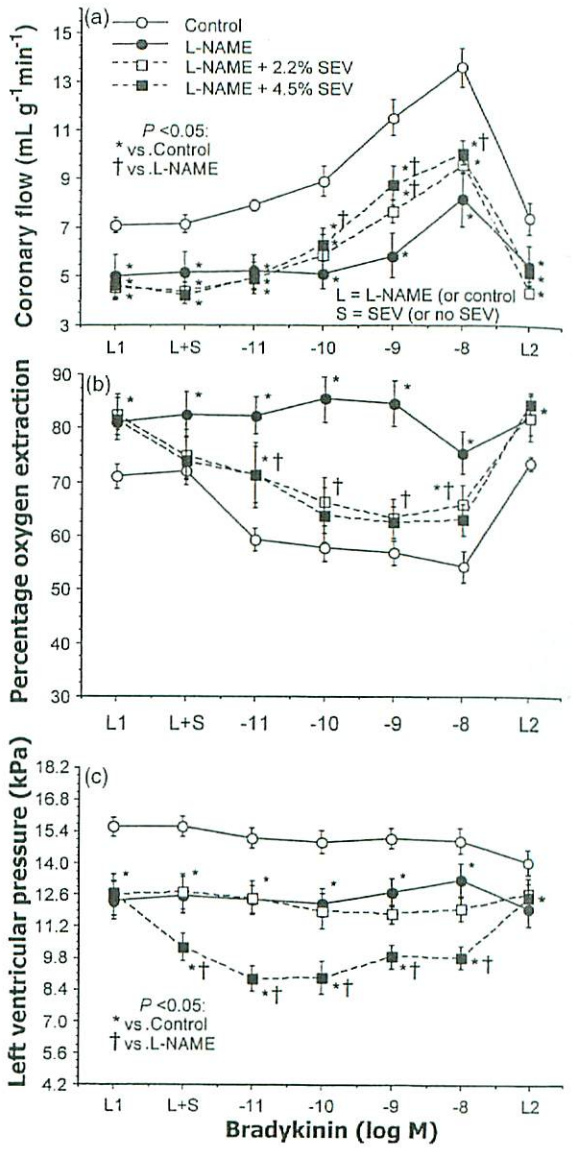

Fig. 2. (a, b, c) Cardiac effects of 100 μM L-NAME in the absence and presence of sevoflurane (SEV) on responses to increasing concentrations of bradykinin. Bradykinin responses were measured first without treatment (Control) and repeated in the presence of L-NAME alone, L-NAME plus low sevoflurane (2.2%) and L-NAME plus high sevoflurane (4.5%). L-NAME alone decreased coronary flow and increased percentage oxygen extraction (%O_2E), and markedly blunted the bradykinin-induced increase in coronary flow and decrease in percentage oxygen extraction. However, in the presence of sevoflurane, these responses were improved. Isovolumetric left ventricular pressure decreased slowly but more so in the presence of sevoflurane. L1, first control; L2, second control.

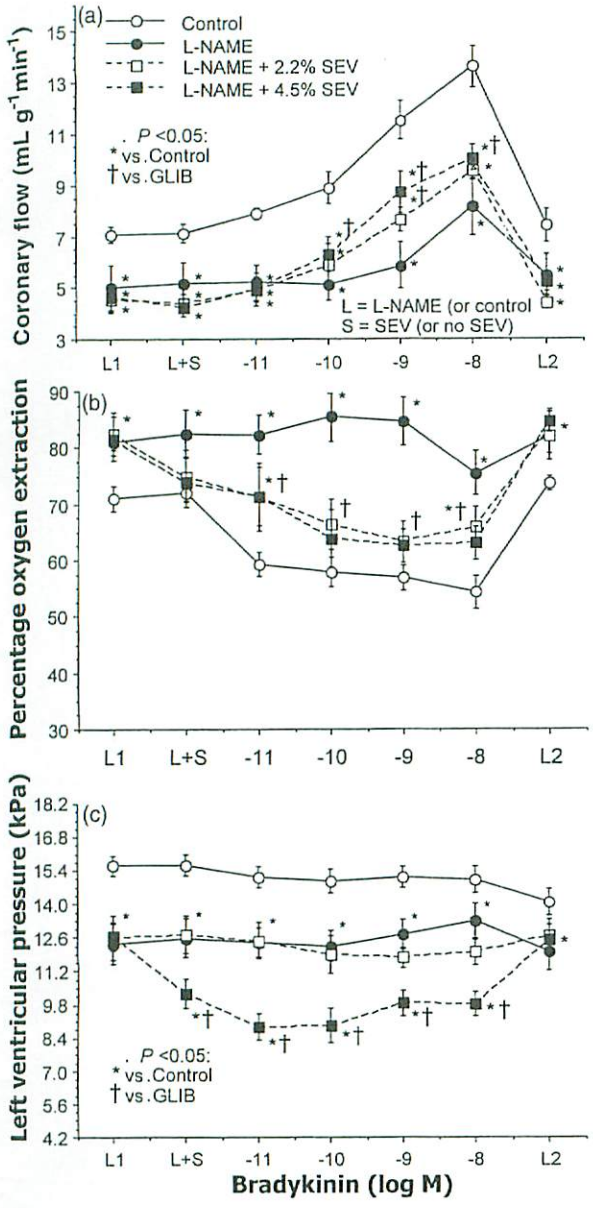

Fig. 3. (a, b, c) Cardiac effects of 100 μM indomethacin (INDO) in the absence and presence of sevoflurane (SEV) on responses to increasing concentrations of bradykinin. Bradykinin responses were measured first without treatment (Control) and repeated in the presence of indomethacin alone, indomethacin plus low sevoflurane (2.2%) and indomethacin plus high sevoflurane (4.5%). Indomethacin alone increased coronary flow and decreased percentage oxygen extraction. High sevoflurane reduced percentage oxygen extraction more than indomethacin alone or with low sevoflurane, but bradykinin-induced changes in coronary flow and percentage oxygen extraction slopes were not altered significantly by indomethacin, or by indomethacin with sevoflurane. Isovolumetric left ventricular pressure decreased slowly but more so in the presence of sevoflurane. I1, first control; I2, second control.

graphically derive from additional glibenclamide and L-NAME series data (Table 1d–g) in which halothane or isoflurane was substituted for sevoflurane and 5-hydroxytryptamine was substituted for bradykinin. Table 1a–g summarizes regression analyses of bradykinin or 5-HT responses in all eight study groups before or after inhibition with glibenclamide, L-NAME, or indomethacin and two concentrations of one of the three anaesthetics.

Figures 1a, 2a and 3a show effects of sevoflurane on bradykinin-induced increases in coronary flow for the glibenclamide, L-NAME and indomethacin series. Glibenclamide (Fig. 1a) alone and sevoflurane + glibenclamide did not change coronary flow. The concentration dependent rise (slope) in flow elicited by 10^{-12}–10^{-8} M bradykinin (Table 1a) was not altered significantly by glibenclamide or by low and high sevoflurane + glibenclamide. L-NAME (Fig. 2a) alone decreased flow but sevoflurane + L-NAME did not further change flow. The rise in coronary flow by 10^{-11}–10^{-8} M bradykinin (Table 1b) was shifted significantly downward (lower Y-intercept) by L-NAME and the slope was not different from zero. However, the bradykinin slope was again significantly increased in the presence of low or high sevoflurane + L-NAME, whereas the Y-intercept remained lower. Indomethacin (Fig. 3a) slightly increased flow but low and high sevoflurane + indomethacin did not further change flow. The rise in coronary flow by 10^{-13}–10^{-8} M bradykinin (Table 1c) was not altered significantly by indomethacin or by low and high sevoflurane + indomethacin.

Figures 1b, 2b and 3b show effects of sevoflurane on bradykinin-induced decreases in percentage O_2 extraction for each series. Glibenclamide (Fig. 1b) alone did not change percentage oxygen extraction, but high sevoflurane + glibenclamide did decrease it. The concentration-dependent fall (negative slope) in percentage O_2 extraction by 10^{-12}–10^{-8} M bradykinin (Table 1a) was not altered significantly by glibenclamide or by low or high sevoflurane + glibenclamide, but the Y-intercept was decreased by high sevoflurane + glibenclamide. L-NAME (Fig. 2b) alone increased percentage oxygen extraction, but low and high sevoflurane + L-NAME decreased the extraction back to control values. The slope fall in the percentage oxygen extraction by 10^{-11}–10^{-8} M bradykinin (Table 1b) was not different from zero whereas the

Table 1. Regression analyses of blockers with and without volatile anaesthetics on responses to bradykinin (BK) and 5-hydroxytryptamine (HT) in isolated hearts.

Table 1a. $n=15$; Fig. 1.

Condition	Coronary flow	Percentage O_2 extraction
Control	0.7logBK + 11.3; r^2 0.47*	−2.3logBK + 63.2; r^2 0.39*
GLIB	0.7logBK + 11.2; r^2 0.48*	−2.2logBK + 63.1; r^2 0.42*
GLIB + 2.2% SEV	1.7logBK + 11.8; r^2 0.42*	−2.9logBK + 55.6; r^2 0.41*
GLIB + 4.5% SEV	1.0logBK + 11.4; r^2 0.38*	−3.5logBK + 40.9; r^2 0.47*§

Table 1b. $n=16$; Fig. 2.

Condition	Coronary flow	Percentage O_2 extraction
Control	1.0logBK + 11.3; r^2 0.41*	−3.4logBK + 56.6; r^2 0.44*
L-NAME	0.5logBK + 6.2; r^2 0.18 †§	−0.4logBK + 84.2; r^2 0.11 †§
L-NAME + 2.2% SEV	0.8logBK + 7.6; r^2 0.41*‡§	−2.2logBK + 65.7; r^2 0.41*‡
L-NAME + 4.5% SEV	0.9logBK + 8.1; r^2 0.42*‡§	−2.6logBK + 63.1; r^2 0.36*‡

Table 1c. $n=15$; Fig. 3.

Condition	Coronary flow	Percentage O_2 extraction
Control	0.7logBK + 11.3; r^2 0.46*	−2.9logBK + 53.7; r^2 0.46*
INDO	0.4logBK + 10.6; r^2 0.38*	−2.1logBK + 50.6; r^2 0.44*
INDO + 2.2% SEV	0.5logBK + 11.6; r^2 0.42*	−1.6logBK + 44.5; r^2 0.43*
INDO + 4.5% SEV	0.4logBK + 11.3; r^2 0.41*	−2.1logBK + 46.4; r^2 0.44*

Table 1d. $n=7$; no figure.

Condition	Coronary flow	Percentage O_2 extraction
Control	0.6logHT + 10.3; r^2 0.47*	−3.3logHT + 66.0; r^2 0.44*
GLIB + 2.2% SEV	0.5logHT + 9.6; r^2 0.39*	−3.6logHT + 56.4; r^2 0.48*
GLIB + 4.5% SEV	0.8logHT + 11.3; r^2 0.49*	−5.4logHT + 45.8; r^2 0.46*§

Table 1e. $n=8$; no figure.

Condition	Coronary flow	Percentage O_2 extraction
Control	0.4logHT + 8.0; r^2 0.43*	−3.1logHT + 60.1; r^2 0.49*
L-NAME + 2.2% SEV	0.6logHT + 7.3; r^2 0.47*	−7.2logHT + 58.5; r^2 0.48*†
L-NAME + 4.5% SEV	1.0logHT + 7.1; r^2 0.47*†§	−13.4logHT + 33.1; r^2 0.47*†§

Table 1f. $n=11$; no figure.

Condition	Coronary flow	Percentage O_2 extraction
Control	1.5logBK + 10.5; r^2 0.46*	−3.2logBK + 54.2; r^2 0.48*
L-NAME + 0.6% HAL	1.5logBK + 7.1; r^2 0.47*§	−9.3logBK + 59.8; r^2 0.45*†
L-NAME + 1.3% HAL	1.6logBK + 7.1; r^2 0.46*§	−9.3logBK + 64.7; r^2 0.46*†

Table 1g. $n=12$; no figure.

Condition	Coronary flow	Percentage O_2 extraction
Control	0.8logBK + 9.6; r^2 0.43*	−3.8logBK + 57.5; r^2 0.41*
L-NAME + 0.9% ISO	0.8logBK + 7.7; r^2 0.39*§	−7.8logBK + 66.6; r^2 0.48*†
L-NAME + 1.6% ISO	0.7logBK + 7.3; r^2 0.40*§	−9.3logBK + 60.4; r^2 0.46*†

Equation form: $Y = m\,x + B$. For $P<0.05$: *significance of slope; †any treatment slope (m) vs. Control slope; ‡either anaesthetic treatment slope (m) vs. GLIB, L-NAME, or INDO slopes; §any treatment intercept (B) vs. Control intercept. See text for abbreviations.

slope was shifted significantly upward (higher Y-intercept) after L-NAME. However, in the presence of low or high sevoflurane + L-NAME, respectively, the slopes and Y-intercepts for percentage oxygen extraction were restored to control values. Indomethacin (Fig. 3b) slightly decreased the percentage oxygen extraction but low and high sevoflurane + indomethacin did not further decrease it compared with indomethacin alone. The slope fall in the percentage oxygen extraction by 10^{-13}–10^{-8} M bradykinin (Table 1c) was not altered significantly by indomethacin alone or by low and high sevoflurane + indomethacin.

Figures 1c, 2c and 3c show a gradual 17–23% fall in isovolumetric left ventricular pressure over 240 min for each series, irrespective of drug treatment. Isovolumetric left ventricular pressure was not apparently decreased by glibenclamide independent of the time effect (Fig. 1c); low and high sevoflurane equivalently decreased isovolumetric left ventricular pressure and bradykinin had no additional effect on it. Isovolumetric left ventricular pressure was decreased after L-NAME (Fig. 2c), *per se*, and more so after high sevoflurane, but bradykinin had no additional effect on it. Isovolumetric left ventricular pressure was not apparently decreased by indomethacin independent of the time effect (Fig. 3c); low and high sevoflurane equivalently decreased isovolumetric left ventricular pressure, and bradykinin had no additional effect.

Four additional experimental groups of the glibenclamide and L-NAME series were conducted but not displayed graphically. Table 1d–g displays the following relationships when 10^{-8}–10^{-6} M 5-hydroxytryptamine was given in place of 10^{-11}–10^{-8} M bradykinin, or when 0.8 and 1.6% halothane, or 0.9 and 1.6% isoflurane, was administered in place of sevoflurane. In glibenclamide + 5-hydroxytryptamine + sevoflurane hearts (Table 1d), glibenclamide alone did not alter coronary flow or percentage oxygen extraction. Glibenclamide + low and high sevoflurane did not alter the 5-hydroxytryptamine-induced increase in the coronary flow slope or the 5-hydroxytryptamine-induced decrease in the percentage oxygen extraction slope, but glibenclamide + high sevoflurane decreased the Y-intercept. In L-NAME + 5-HT + sevoflurane hearts (Table 1e), L-NAME alone significantly decreased coronary flow by 1.5 ± 0.1 mL g^{-1} min^{-1} and increased the percentage oxygen extraction by $15 \pm 3\%$ ($P = 0.05$). L-NAME + low sevoflurane did not alter the coronary flow slope but increased the percentage oxygen extraction slope compared with their control, whereas L-NAME + high sevoflurane increased both the coronary flow and percentage oxygen extraction slopes and reduced the Y-intercepts.

In L-NAME + bradykinin + halothane hearts (Table 1f), L-NAME alone decreased coronary flow by 2.1 ± 0.2 mL g^{-1} min^{-1} and increased the percentage oxygen extraction by $22 \pm 3\%$ ($P = 0.05$). L-NAME + low and high halothane did not alter the slopes for coronary flow compared with their control but decreased the Y-intercepts, whereas L-NAME + high halothane increased the slopes for the percentage oxygen extraction and did not alter the Y-intercepts. In L-NAME + bradykinin + isoflurane hearts (Table 1g), L-NAME alone decreased coronary flow by 2.2 ± 0.2 mL g^{-1} min^{-1} and increased the percentage oxygen extraction by $18 \pm 3\%$ ($P = 0.05$). L-NAME + low and high isoflurane did not alter the slopes for coronary flow compared with their control but decreased the Y-intercepts, whereas L-NAME + high isoflurane increased the slopes for the percentage oxygen extraction and did not alter the Y-intercepts.

Discussion

The purpose of this study was to determine whether volatile anaesthetics alter endothelium-dependent coronary vascular tone after NOS or COX, prostaglandin synthase (PGS) inhibition, and to determine whether they alter this tone after K_{ATP} channel blockade. The results demonstrate that both bradykinin and 5-hydroxytryptamine increased coronary flow and decreased percentage oxygen extraction, generally in a concentration-dependent manner in this model. Important to the aims of this study was the negative finding that sevoflurane in the presence of glibenclamide or indomethacin (Figs 1,3 and Table 1a, c) did not alter the bradykinin-induced increases in the positive coronary flow slope and the negative slope for the percentage oxygen extraction. This was also found for 5-hydroxytryptamine responses in the glibenclamide + sevoflurane group (Table 1d). As may be expected, L-NAME alone attenuated basal coronary flow and increased basal percentage oxygen extraction and greatly attenuated the coronary flow and the percentage oxygen extraction slope responses to bradykinin; only 1 nM bradykinin increased flow and

reduced the percentage oxygen extraction (Fig. 2). The most interesting finding for all subsets of the L-NAME series (Fig. 2 and Table 1b, e–g) was that the high, or both low and high, concentrations of sevoflurane, halothane and isoflurane either restored, or accentuated, the bradykinin or 5-HT-induced effects on flow and percentage oxygen extraction slopes even though flow remained lower after L-NAME.

Anaesthetic effects on products of nitric oxide synthase and cyclo-oxygenase prostaglandin synthase

The results from the L-NAME series suggest that anaesthetics not only do not inhibit NOS, but also that they in large part restore or augment the reduction in bradykinin and 5-HT-induced vasodilation caused by NOS inhibition. Possibilities for these unexpected results are that (a) anaesthetics elicit greater endothelium-mediated vasodilation by augmenting release of nitric oxide to overcome the blocking effect of L-NAME (i.e. by upregulation), (b) they interfere with the blocking effect of L-NAME, e.g. by reducing the effectiveness of L-NAME, (c) they enhance nitric oxide effects on soluble guanylyl cyclase, e.g. by reducing cGMP metabolism by phosphodiesterases, (d) they shift the response of bradykinin and 5-hydroxytryptamine from a NOS-dependent endothelial dependent pathway to a non NOS-dependent pathway, possibly a endothelium derived hyperpolarizing factor, or (e) they exert a more effective vasorelaxant effect via vascular receptors or intracellular messengers due to the relative vasoconstrictor effect of L-NAME caused by NOS inhibition. For example, NOS inhibition is known to alleviate the inhibition of endothelium derived hyperpolarizing factor release by nitric oxide [31], and to increase adenosine production [32]. In any case, reduced bradykinin or 5-hydroxytryptamine-induced coronary vascular responsiveness in the intact heart after NOS inhibition can, at least in part, be overridden by a volatile anaesthetic. By whatever means these anaesthetics interfere with NOS inhibition, whether an effect of nitric oxide on soluble gyanylyl cyclase or cGMP, endothelium-derived hyperpolarizing factor, or intracellular signalling, the net effect is improved vascular responsiveness in the intact, L-NAME-blocked, coronary circulation.

Volatile anaesthetics differentially depresses mechanical activity and energy demands, so it was very important to measure not only coronary flow, but also the percentage oxygen extraction, to control for the metabolic and mechanical component of coronary flow autoregulation [28]. Blocking the endogenous vasodilatory effect of nitric oxide by L-NAME increases the percentage oxygen extraction as well as decreases coronary flow in a nearly inversely proportional manner, but the percentage oxygen extraction is probably a better measure of vascular tone in the intact heart. Similar to coronary flow after L-NAME, the anaesthetics restored or enhanced the responses to bradykinin and 5-hydroxytryptamine induced decreases in percentage oxygen extraction after L-NAME.

In a recent article, we reported that perfusion pressure-induced changes in coronary flow were associated with proportional changes in coronary effluent nitric oxide concentration and that these effects were attenuated by L-NAME [29]. In a related study [27], we found a significant correlation between increases in effluent nitric oxide concentration and coronary flow induced by bradykinin, showing a NOS-dependent effect. We showed that anaesthetics did not alter this relationship; moreover, they did not alter bradykinin-induced increases in effluent L-citrulline concentration. The finding of no significant influence of anaesthetics on bradykinin-induced coronary flow, nitric oxide release and L-citrulline concentration suggested that they do not interfere significantly in the pathway between endothelial release of nitric oxide and coronary smooth muscle relaxation. In fact, isoflurane enhanced the bradykinin-induced decrease in the percentage oxygen extraction, which suggested that after normalizing for autoregulatory influence on coronary flow, vascular tone is additively reduced by bradykinin and isoflurane. This present study wherein NOS was inhibited, supports our earlier study and additionally suggests that under NOS inhibition and reduced basal and stimulated release of nitric oxide, volatile anaesthetics may directly or indirectly restore or enhance endothelium-dependent vasodilatory responses.

Coronary flow is locally regulated in part by nitric oxide in the guinea pig heart [33] as we have also observed [29]. Volatile anaesthetics could alter specific endothelial receptor responses (e.g. to thrombin, muscarinic, Ca^{2+} ionophores and BK_2 receptors) [34], that modulate nitric oxide production, or alter effects of nitric oxide on the guanylyl cyclase, cGMP system that

promotes vascular relaxation [5,6]. But several studies suggest anaesthetics do not stimulate release of endothelium-dependent relaxing factors and that they have little or no effect on endothelium-independent vasodilation induced by nitro-vasodilators [1,4,14]. On the other hand, several indirect studies [2,8,16–18,35] suggest anaesthetics modify responses to endothelium-dependent vasodilators. Halothane was reported to inhibit vasodilation induced by nitric oxide and nitroglycerin [20], but halothane did not inhibit measured soluble guanylyl cyclase [36]. More than 2% halothane or 1% isoflurane was seen to depress cGMP formation independent of guanylyl cyclase activation when endothelial and vascular smooth muscle cell co-cultures were treated with bradykinin [2]. In intact dogs treated with L-NAME, it was reported that 1.2% halothane interfered with NOS activity in carotid, mesenteric and renal vascular beds, but not in the coronary vascular bed [35]. One suggestion was that halothane impairs nitric oxide mediated regulation of some vascular beds, but not the coronary vascular bed, because it largely eliminated systemic responses of NOS inhibition by L-NAME in rats [8].

Although anaesthetic-induced vasodilator effects are probably independent of soluble guanylyl cyclase, cGMP-induced vasorelaxation, anaesthetics could attenuate endothelium-dependent relaxation by one factor, e.g. nitric oxide, while enhancing relaxation by another, e.g. endothelium-derived hyperpolarizing factor. If one system is blocked they could alter activation of endothelial receptor sites, modify second messengers, or affect intracellular Ca^{2+} flux in the cascade of events leading to vasodilation. Anaesthetics may reduce endothelial Ca^{2+} entry or release of Ca^{2+} from intracellular stores, or inhibit phospholipase C (PLC) and IP_3-mediated Ca^{2+} release [19]. Anaesthetics have been suggested to attenuate non-receptor-mediated nitric oxide production, an effect that bypasses receptors [16], or by reducing nitric oxide [18] efficacy once released from NOS. Based on a bioassay technique, or with relaxation induced by nitroprusside, it was proposed that halothane attenuates nitric oxide mediated relaxation, not by disrupting endothelial cell release of nitric oxide, but by interfering with nitric oxide stability after endothelial release [18]. In isolated preconstricted rat coronary vessels, isoflurane attenuated relaxation due to acetylcholine, to a calcium ionophore, and to a high concentration of nitroprusside; the interpretation was that isoflurane blocks endothelial Ca^{2+} and guanylyl cyclase [37].

In contrast, there are reports that anaesthetics do not alter vasodilation induced by nitric oxide [1,2,4,14,27]. Isoflurane relaxed isolated cerebral arteries rings independently of the endothelium [14] and isoflurane-induced relaxation of isolated aortic rings was independent of nitric oxide as well as cGMP-mediated mechanisms [1]. Coronary vasodilation induced by isoflurane in open-chest dogs was not affected by L-NAME [4]. Anaesthetics were found not to stimulate or inhibit cGMP production in endothelial-vascular co-cultures suggesting that they did not activate either NOS or guanylyl cyclase [2]. We showed that anaesthetics neither alter nitric oxide release nor change coronary flow, nitric oxide and L-citrulline release induced by bradykinin [27]. The present results agree with many of the above studies that anaesthetics do not significantly decrease relaxation mediated by nitric oxide. Moreover, our results demonstrate that attenuated bradykinin-induced vasodilation by L-NAME is significantly reversed by clinical levels of anaesthetics in the intact heart. A key factor may be the unmasking of another vasodilatory pathway after the relative vasoconstriction caused by L-NAME.

We examined vasodilatory effects of both bradykinin and 5-hydroxytryptamine. In our related study [29], we found that indomethacin did not alter a bradykinin-induced increase in coronary flow or release of nitric oxide, but that L-NAME (given in the presence of indomethacin and tetraethylammonium, a non-specific K^+ channel blocker) inhibited increases in effluent coronary flow and nitric oxide induced by up to 10^{-9} M bradykinin. Although L-NAME completely blocked release of nitric oxide to 10^{-8} M bradykinin, it did not completely block the increase of coronary flow. This suggested the major mechanism of vasodilation by up to 10^{-8} M bradykinin is dependent on release of nitric oxide but that higher bradykinin concentrations exert additional mechanisms of endothelium-induced vasodilation, or a direct effect on vascular smooth muscle via BK_2 receptors [34]. Thus in addition to stimulating endothelial cell Ca^{2+} influx to trigger production of nitric oxide, bradykinin and 5-hydroxytryptamine may also cause vasodilation via prostacyclin production [9,10,34,38] or via production of a endothelium-derived hyperpolarizing factor [39], of which a major class is derived from P450 metabolites of ara-

chidonic acid called epoxyeicosatrienoic acids [40]. This effect is evidenced by smooth muscle cell hyperpolarization via Ca^{2+}-activated K^+ channels in the presence of inhibited prostaglandins and NOS to rule out these two effector pathways [41]. From our study, it appears that low to middle concentrations of bradykinin affect primarily the NOS pathway because L-NAME blocked most of the vasodilatory effect of bradykinin.

In most studies the relative contributions of nitric oxide, prostaglandins, endothelium-derived hyperpolarizing factor and K_{ATP} channel opening to anaesthetic modulated vasodilation were not tested. It is, thus, interesting that indomethacin, an inhibitor of the COX-PGS pathway, did not alter bradykinin-induced vasodilation in this study, and that the presence of a volatile anaesthetic had no effect on this relationship. This suggests anaesthetics do no interfere significantly with the COX-PGS pathway in the intact heart. But it remains possible that anaesthetics stimulate or upregulate the pathway of the epoxyeicosatrienoic acids after NOS inhibition because inhibiting PGS indirectly via COX with indomethacin did not blunt bradykinin-induced vasodilation.

Anaesthetic effects on K_{ATP} channels

In contrast to the findings with L-NAME, and like the findings with indomethacin, glibenclamide did not block vasodilation elicited by bradykinin or 5-hydroxytryptamine in the presence or absence of volatile anaesthetics. This indicates that bradykinin-induced vasodilation in the intact crystalloid perfused heart, whether endothelium dependent or independent, is not mediated by K_{ATP} channel opening in the absence of ischaemia. Moreover, volatile anaesthetics do not significantly affect this pathway.

Similar to the NO pathway, the magnitude and importance of the reported attenuating effect on volatile anaesthetic-induced vasodilation after K_{ATP} channel blockade is controversial. Several studies suggest that anaesthetics produce vasodilation, at least in part, by opening K_{ATP} channels because blocking these channels results in attenuated vasodilation and because dilatation induced by K_{ATP} channel agonists may be reduced by anaesthetics. It was reported initially that glibenclamide reduced halothane-induced vasodilation in tetrodotoxin-arrested crystalloid perfused rat hearts by about 56% [21]. In the regionally perfused swine heart it was demonstrated that glibenclamide abolished vasodilation induced by 1.5% and 3% isoflurane [22]; however, because glibenclamide alone decreased regional blood flow while decreasing regional oxygen consumption, the region of perfusion may have been ischaemic so that endogenous K_{ATP} channels were opened. In dogs, glibenclamide blunted increases in coronary blood flow induced by isoflurane, sevoflurane and enflurane [23]. But in another study [42], sevoflurane-induced increases in coronary collateral blood flow in dogs were not blocked by glibenclamide. In pig isolated, preconstricted coronary vessels, glibenclamide attenuated isoflurane-induced vasodilation but this effect waned over time [24]. In the pulmonary circulation of intact dogs, the attenuating effect of isoflurane on hypoxic pulmonary vasoconstriction was abolished by indomethacin [11]. In preconstricted canine pulmonary vascular rings, isoflurane attenuated relaxation of rings induced by both bradykinin and by a K_{ATP} channel opener, but not by a nitric oxide donor; but it was suggested that isoflurane interfered with a synergistic interaction between nitric oxide and prostaglandin, possibly via K_{ATP} channels [10]. It is possible that anaesthetic effects on these pathways differ between the pulmonary and coronary circulations.

It is important to know whether volatile anaesthetics inhibit, modify, or enhance coronary vasodilation in patients who are taking drugs that affect the above pathways, or who have abnormal vascular endothelial function. This study in an intact animal heart model suggests that volatile anaesthetics not only do not attenuate coronary vasodilatation induced by two endothelium-dependent vasodilators after K_{ATP} channel blockade, or COX-PGS inhibition, but that anaesthetics also abrogate the reduced responsiveness of these vasodilators after coronary vasoconstriction caused by NOS inhibition.

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