Anesthesiology
73:100–108, 1990

Isoflurane and Halothane Attenuate Coronary Artery Constriction Evoked by Serotonin in Isolated Porcine Vessels and in Intact Pigs

T. M. Witzeling, M.D., J. C. Sill, M.B.B.S.,† J. M. Hughes, M.D.,‡*
G. A. Blaise, M.D.,§ M. Nugent, M.D.,† D. K. Rorie, M.D.¶

Serotonin is a vasoconstrictor thought to cause coronary artery constriction in humans. The purpose of this study was to determine if isoflurane and halothane each attenuate coronary artery constriction evoked by serotonin in pigs. Both *in vitro* and *in vivo* experimental methods were used. Isolated coronary arteries with and without endothelium were studied in organ chambers in the presence and absence of 2.5% concentrations of the anesthetics. In intact pigs serotonin was infused directly into the left anterior descending coronary arteries to induce constriction. The vasodilator effects of 0.5%, 1.25%, and 2.0% isoflurane and halothane were determined using quantitative angiography. Contractile responses of isolated coronary arteries were depressed by the two anesthetics. Maximum contractile responses to serotonin were as follows: rings with endothelium 45 ± 5% untreated *versus* 29 ± 5% with isoflurane 2.5% (difference between dose–response curves, $P < 0.01$) and without endothelium 67 ± 5% untreated *versus* 51 ± 6% with isoflurane 2.5% ($P < 0.001$); with endothelium 52 ± 7% untreated *versus* 28 ± 7% with halothane 2.5% ($P < 0.001$) and without endothelium 65 ± 5% untreated *versus* 40 ± 6% with halothane 2.5% ($P < 0.001$). In intact pigs isoflurane and halothane dilated constricted coronary arteries with and without endothelium at all anesthetic concentrations tested, including concentrations as low as 0.5%. Isoflurane 1.25% increased diameter of vessels with endothelium from 1.5 ± 0.1 mm to 1.7 ± 0.1 mm ($P < 0.02$) and halothane 1.25% increased diameter from 1.6 ± 0.1 mm to 1.7 ± 0.1 mm ($P < 0.01$). In vessels without endothelium isoflurane 1.25% increased diameter from 1.8 ± 0.1 mm to 2.0 ± 0.1 mm ($P < 0.01$) and halothane 1.25% increased diameter from 1.9 ± 0.1 mm to 2.0 ± 0.2 mm ($P < 0.01$). Results demonstrate that isoflurane and halothane attenuate contractile responses evoked by serotonin in pig coronary arteries studied both *in vitro* and *in vivo*. (Key words: Anesthetics, volatile: halothane; isoflurane. Arteries: coronary; endothelium. Pharmacology: serotonin.)

SEROTONIN is an endogenous vasoconstrictor. It is thought to cause coronary artery constriction in human beings with stable coronary disease, unstable ischemic syndromes, and myocardial infarction.[1–4] Isoflurane and halothane are vasodilators. Whether they attenuate coronary artery constriction caused by serotonin is not known, and comprised the purpose of this experiment.

The investigation had three aims. The first was to determine if isoflurane and halothane each attenuated contraction evoked by serotonin in pig coronary arteries studied *in vitro*. Previous work has shown isoflurane to attenuate serotonin-evoked contractions of isolated dog coronary arteries.[5] A similar effect in porcine vessels would exclude a species-dependent isoflurane effect. The effects of halothane are unknown.

The second aim was to determine if isoflurane and halothane each dilate coronary arteries preconstricted with serotonin in intact animals. Despite a number of reports concerning isoflurane and halothane effects on isolated conductance vessels from various vascular beds and various species,[5–8] little data exist demonstrating *in vivo* effects of the anesthetics in intact animals.

A third aim concerned isoflurane, halothane, and the coronary artery endothelium. In coronary arteries the endothelium has a major role in regulating tone and thus vessel diameter and does so by synthesizing and releasing constricting and relaxing factors.[9,10] Preliminary evidence obtained using isolated dog coronary arteries has suggested that isoflurane does not have a direct relaxant effect on coronary arteries but attenuates contractile responses by an endothelium-dependent mechanism.[5] The effects of halothane on contractile responses to serotonin are unknown. Therefore, whether isoflurane and halothane have a direct coronary effect or an effect that is endothelium-dependent was the final aim of this investigation. This final question was of particular importance because the coronary endothelium is thought to be dysfunctional in human beings with coronary disease,[10,11] decreasing responsiveness to endothelium–dependent dilators.

Methods

Institutional Animal Care Committee approval was obtained and experiments performed in a humane manner using young adult Durrock and Yorkshire pigs obtained from nearby farms.

Isolated Coronary Artery Experiments

Twelve 35-kg pigs were premedicated with intramuscular (im) ketamine 1 g, anesthetized with intravenous (iv) pentobarbital 35 mg/kg, and their hearts were ex-

* Instructor in Anesthesiology, Mayo Clinic.
† Associate Professor of Anesthesiology, Mayo Clinic.
‡ Fellow in Cardiovascular Anesthesiology, Mayo Graduate School of Medicine.
§ Associate Professor of Anesthesiology, University of Montreal.
¶ Professor of Anesthesiology, Mayo Clinic.

Received from the Departments of Anesthesiology, Mayo Clinic, Rochester, Minnesota, and the University of Montreal, Montreal, Quebec, Canada. Accepted for publication February 12, 1990. Supported by the B. B. Sankey Award (IARS), Burroughs Wellcome Fellowship (ASA), and NIH Grant Nos. HL 38668 and GM 41797.

Address reprint requests to Dr. Sill: Department of Anesthesiology, Mayo Clinic, 200 First Street SW, Rochester, Minnesota 55905.

cised. Left anterior descending and circumflex coronary arteries were dissected free, cut into rings 5 mm long, and cleaned of surrounding fat and connective tissue. Special care was taken to avoid touching the endothelium-lined luminal surface. Eight rings were obtained from each pig. The endothelium was deliberately removed in four rings obtained from each heart by gently rubbing the luminal surface with a wooden implement. The rings were suspended in organ chambers filled with aerated (95% O_2–5% CO_2) modified Krebs-Ringer bicarbonate solution at 37° C at *p*H 7.40 and of the following composition (mM): NaCl 118; KCl 4.7; $CaCl_2$ 2.5; $MgSO_4$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 25; and glucose 4.1. The rings were attached to strain gauges (Grass FT03) and isometric tension recorded (Gould electrostatic TA 2000).

The optimal point on the length tension curve was obtained for each ring by varying passive tension between 8 and 12.5 g and determining at which passive tension the greatest response to 20 mM KCl occurred.[12] Each ring was then maintained at its optimal passive tension. Maximum tension developed following a standard 80 mM KCl challenge, and this response was used as a reference. Contractile responses evoked by serotonin during the experiment were compared with this reference contraction.

Presence or absence of endothelial function was tested by observing the relaxation response of rings precontracted with 20 mM KCl to a single 10^{-7} M dose of bradykinin added to all the chambers.[12] (Bradykinin is a useful test for endothelial function. It causes smooth muscle relaxation by evoking release of relaxing factors from the endothelium.) A 70–80% relaxation was obtained in rings with endothelium, and no response was observed in rings without endothelium. Occasionally rings designated as having endothelium failed to relax appropriately and were replaced with rings removed from the same artery of the same pig heart.

Isoflurane (Ohio) or halothane (Ayerst) was delivered to one-half the organ chambers by vaporizers (Ohio Medical). The other chambers were not treated with an anesthetic, and the rings served as controls. Concentration of the anesthetics in the gas phase entering each chamber was continuously monitored using an infrared analyzer (Beckman LB2) calibrated using commercially available tanks containing known concentrations of isoflurane or halothane (Scott Medical Products). Concentrations of the anesthetics in the chamber fluid were monitored by removing 2-ml aliquots of Krebs-Ringer solution, extracting the anesthetic in hexane and measuring the concentrations using gas chromatography with electron capture (Hewlett Packard 5880).

Protocol

The effects of 2.5% isoflurane upon quiescent ring tension and upon contractions evoked by serotonin were investigated in rings of coronary arteries with and without endothelium obtained from five pig hearts to determine if isoflurane depressed the contractile response. Four rings from each heart were exposed to the anesthetic and the other four rings served as control. Isoflurane was administered for 60 min while quiescent tension was measured. Sequentially increasing doses of serotonin were added by pipette to each organ chamber to achieve final concentrations ranging from 10^{-9} M to 10^{-4} M, and tension generated by the rings was measured. Indomethacin was added to one-half the chambers to produce a 10^{-5} M concentration. (Contractions evoked by serotonin are biphasic, higher concentrations failing to induce further contraction or causing relaxation.[12,13]) The same protocol was used in seven pig hearts in separate experiments to determine the effects of 2.5% halothane on contractions evoked by serotonin.

Drugs

Serotonin (creatinine sulphate complex) and bradykinin were obtained from Sigma Chemical Company (St. Louis, Missouri) and were prepared and diluted in Krebs-Ringer bicarbonate solution.

Intact Pig Experiments

Computer-assisted quantitative coronary angiography was used to determine the effects of 0.5%, 1.25%, and 2.0% concentrations of isoflurane and halothane on left anterior descending coronary arteries preconstricted by constant serotonin infusion. Twenty-one 40-kg pigs received preanesthetic medication consisting of 1 g im ketamine (Ketaset, Aveco) and were anesthetized with iv fentanyl 10 g (USPC Inc.) ketamine 500 mg, and thiopental 80 mg (Abbott). Following tracheal intubation their lungs were mechanically ventilated (Harvard Respirator) with oxygen and air, and the adequacy of ventilation was ascertained by repeatedly measuring arterial blood gas values (Instrumentation Laboratories). Anesthesia was maintained with fentanyl 1–2 mg/kg and ketamine approximately 100 mg/kg infused intravenously during the course of the experiment. Anticoagulation was provided by heparin (Elkins-Sinn) 125 units $\cdot kg^{-1} \cdot h^{-1}$.

A 4.5-Fr left coronary artery guide catheter (constructed by the investigators) was advanced using fluoroscopy through a sheath introducer (Cordis) in the left carotid artery to the ostium of the left main coronary artery. Its placement was accompanied by a single dose of iv lidocaine 50 mg (Abbott) and three or four 50-mg iv doses of esmolol (DuPont) to prevent fatal ventricular arrhythmias. Care was taken to ensure that at least 1 h elapsed between administration of these drugs and angiographic measurements. A 1.0-mm gold-tipped coronary infusion catheter (Ducor Angiographic, Cordis) was

inserted over a guide wire (USCI 0.014 in.) through the guide catheter and into the proximal 1 cm of the left anterior descending coronary artery. This catheter was used to infuse serotonin. Coronary artery pressures were measured (Statham transducers) using the guide catheter, systemic pressures were measured *via* the carotid introducer catheter, and both pressure waveforms were continuously displayed. Animals were warmed to maintain normal temperature, urine output was monitored, and iv fluid was provided.

Coronary angiograms were obtained during the injection of 7 ml of meglomine diatrizoale (Renografin 76) *via* the guide catheter; cassette-type x-ray film (Kodak T-Mat L Diagnostic) was exposed at 110 kV, 300 mA for 6 ms (Picker GX 850 and General Electric 300) gated to mid-diastole using an R-wave-triggered time delayed switch (Mayo Engineering). The opacified edges of the coronary artery lumens were manually traced and digitized using a computer[14,15] (PDP 11/34 Digital Equipment). The program calculated luminal diameter at 1-mm intervals. Three scans of each vessel were performed and the results averaged. Angiograms, with their identification numbers obscured, were shuffled prior to analysis to prevent observer bias.

Protocol

1. Ten intact pigs were studied. Serotonin was infused at 3 μg/min *via* the intracoronary catheter for 45 min to establish constriction. Isoflurane or halothane (0.5%, 1.25%, or 2.0% end-tidal concentrations) were administered to determine whether the anesthetics dilated constricted coronary arteries. Coronary angiograms were performed prior to and following serotonin infusion and at each concentration of the anesthetic. Twenty minutes was allowed for wash-in of the anesthetic and then an angiogram performed. The three anesthetic concentrations were not administered in order of increasing magnitude but according to a randomized schedule generated by a computer. End-tidal anesthetic concentrations were measured in the endotracheal tubes using a gas analyzer (Beckman LB2). Forty-five minutes was allowed for anesthetic wash-out (end-tidal concentration < 0.1% being confirmed) and a final angiogram obtained during continued serotonin infusion to demonstrate vessel reconstriction. The second anesthetic was administered according to the same protocol. (Whether isoflurane or halothane was studied first was determined according to a random number sequence generated by a computer.)

Eight pigs received both isoflurane and halothane. Two pigs died of ventricular fibrillation. Two replacement pigs were used and received only one anesthetic.

The effects of iv nitroglycerin (DuPont) on preconstricted coronary arteries was studied in six pigs following completion of the isoflurane/halothane phase of the protocol. (Seven pigs were scheduled to be studied, but one died of ventricular fibrillation.) The purpose was to demonstrate vessel responsiveness to a standard, direct-acting vasodilator with a known mechanism of action[16] and proven clinical usefulness.[17] Serotonin infusion was continued at 3 μg/min and nitroglycerin was administered intravenously first at 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 10 min and then at 2.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 10 min. Angiograms were performed before and during nitroglycerin infusion. The nitroglycerin study was always performed as the final phase of the experiment because nitroglycerin can have a prolonged vasodilator effect.

2. Eleven intact pigs with coronary arteries mechanically denuded of endothelium were investigated using a virtually identical protocol to that used to study pigs with undamaged vessels. The purpose was to determine whether isoflurane and halothane each dilated preconstricted coronary arteries without endothelium.

The coronary endothelium was removed by passing a 2.0-Fr coronary angioplasty catheter (Gruntzig Dilica, USCI) into the left anterior descending coronary artery *via* the guide catheter and gently withdrawing it five times. (Light and scanning electron microscopy have been used to demonstrate that this method removes endothelium but leaves the underlying vascular smooth muscle intact.[15]) In a study performed concurrently with this study, the technique was shown to abolish the normal vasodilator response to bradykinin yet leave the constrictor response to acetylcholine intact.[18] (Bradykinin stimulates endothelium-dependent relaxing factor (EDRF) and prostacyclin release from endothelial cells resulting in smooth muscle relaxation.[19] Acetylcholine causes direct coronary constriction in pigs, whether the endothelium is present, by stimulation of smooth muscle cholinergic receptors.[18,20])

Angiograms were performed prior to and 30 min following removal of endothelium. Serotonin was infused at 3 μg/min, constriction induced, and isoflurane or halothane administered (0.5%, 1.25%, and 2.0% end-tidal concentrations) according to the protocol used in pigs with undamaged arteries. Seven pigs received both isoflurane and halothane and four additional pigs received either isoflurane or halothane. Four pigs died due to fatal ventricular arrhythmias of unknown etiology.

Nitroglycerin administered intravenously at 0.5 and 2.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$ was studied upon completion of the isoflurane/halothane protocol to demonstrate the effect in vessels denuded of endothelium.[16,17] Intracoronary serotonin infusion was continued and angiograms performed before and at the end of the 10-min infusion of each concentration of nitroglycerin. Seven pigs were scheduled to be studied, but one died during halothane administration (ventricular fibrillation) and one died im-

mediately on discontinuation of halothane (ventricular fibrillation).

Eighteen pigs were killed at the end of the experiment while still anesthetized with an iv overdose of barbiturate.

The coronary arteries of two other animals were examined to ascertain that the endothelium had a normal appearance in undamaged vessels and had been effectively removed in denuded arteries. Two percent glutaraldehyde and 1% para formaldehyde in 0.1 M cacodylate (*p*H 7.25) at a pressure of 100 mmHg was perfused in retrograde fashion for 15 min to allow fixation of the arteries *in situ.* Vessels were removed and ring sections were stained with hematoxylin and eosin and with Heidenhain Weigert-van Geison stain. Stained specimens were examined using light microscopy. Fixed segments were also coated with carbon and gold–palladium alloy and studied with scanning electron microscopy (ETEC Autoscan).[12,15,18] Specimens (light and electron microscopy) were taken randomly from each coronary artery and were evaluated blindly by a consultant pathologist skilled in assessment of coronary artery pathology.

Statistical Analysis

Organ chamber experiments: In all experiments, n equals the number of animals from which rings were removed. Data are expressed as mean ± SEM. Statistical evaluation was performed by comparing integrated areas under the dose–response curves using analysis of variance.

Intact animal experiments: Results are reported as mean ± SEM. Coronary dimensions are expressed as the absolute vessel diameter and as percent changes. Diameters were measured between two fixed anatomic points along the length of the vessel. Statistical analysis was performed by *t* testing for paired on unpaired data with appropriate consideration for multiple comparisons and by analysis of variance. A P value < 0.05 was considered statistically significant except when multiple comparison considerations were applied.

Results

Isolated Coronary Artery Experiments

The absolute tension in grams generated in response to a standard 80-mM KCl challenge is given in table 1.

Table 1. Absolute Tension (g) Following KCl (80 mM) in Isolated Porcine Coronary Artery Rings

	Pre-isoflurane	Control	Pre-halothane	Control
Endothelium present	12.4 ± 0.6	12.2 ± 0.4	14.9 ± 0.5	12.0 ± 0.5
Endothelium absent	10.1 ± 0.5	9.9 ± 0.4	9.8 ± 0.3	10.2 ± 0.3

Values are mean ± SEM (isoflurane and control, n = 20 pairs of rings; halothane and control, n = 28 pairs of rings).

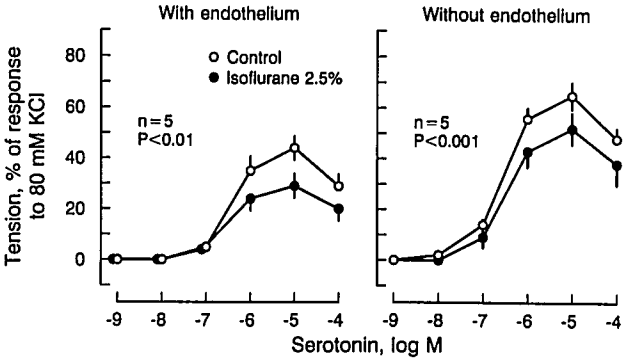

FIG. 1. Cumulative concentration response relationships of rings with and without endothelium exposed to increasing concentrations of serotonin (10^{-9} to 10^{-4} M). Percent contraction in the presence and absence of isoflurane 2.5% is shown. Contractions are expressed as a percentage of a response to 80 mM KCl. Analysis of variance demonstrated statistically significant differences in responses between rings treated with isoflurane 2.5% *versus* untreated rings. Data are mean ± SEM. Twenty pairs of rings from five hearts were used.

Serotonin (10^{-9} to 10^{-4} M) evoked contractions in all rings. The dose–response relationships obtained were typical of those established in the past by other investigators.[12,13,21] (More marked contraction occurred in rings without endothelium, an observation that is believed to indicate that serotonin evokes EDRF production in rings with endothelium, resulting in a lesser degree of contraction.[21]) Maximal tension occurred at approximately 10^{-5} M serotonin. Greater serotonin concentrations did not evoke further contraction. (The presence of low affinity serotonin receptors on smooth muscle linked to relaxation may explain this effect.[13])

Isoflurane 2.5% had no effect on resting ring tension but attenuated the contractile response evoked by serotonin. Contraction was depressed in rings both with and without endothelium (fig. 1). Maximum tension generated by rings with endothelium (no isoflurane) was 45 ± 5% of a standard KCl-induced reference contraction and was decreased to 29 ± 5% by isoflurane treatment (difference between dose–response curves, $P < 0.01$). The maximum tension generated by rings without endothelium (no isoflurane) was 67 ± 5% and decreased to 51 ± 6% during isoflurane administration (difference between curves $P < 0.001$).

Halothane 2.5% had no effect on resting ring tension but attenuated contraction evoked by serotonin in rings both with and without endothelium (fig. 2). The maximum tension generated by rings with endothelium (no halothane) was 52 ± 7% of a standard KCl-induced reference contraction and was decreased to 28 ± 7% by halothane treatment (difference between dose–response curves, $P < 0.001$). The maximum tension generated by rings without endothelium (no halothane) was 65 ± 5%

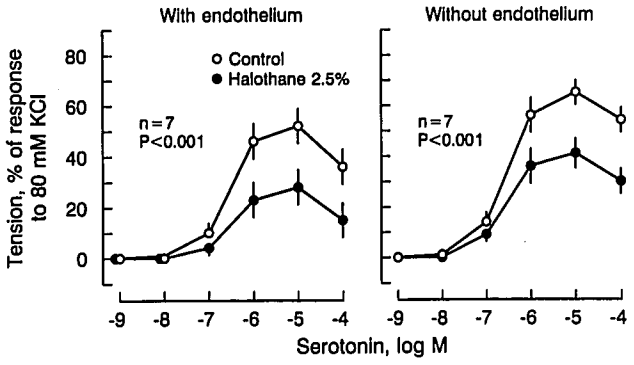

FIG. 2. Cumulative concentration response relationships of rings with and without endothelium exposed to increasing concentrations of serotonin (10^{-9} to 10^{-4} M). Percent contraction in the presence and absence of halothane 2.5% is shown. Contractions are expressed as a percentage of a response to 80 mM KCl. Analysis of variance demonstrated statistically significant differences in responses between rings treated with halothane 2.5% *versus* untreated rings. Data are mean ± SEM. Twenty-eight pairs of rings from seven hearts were used.

and decreased to 40 ± 6% during halothane administration (difference between curves, $P < 0.001$).

Angiography Experiments in Intact Pigs

With Endothelium

Prior to isoflurane administration, serotonin infusion induced coronary artery constriction. Vessel diameter decreased from 1.9 ± 0.1 mm to 1.5 ± 0.1 mm ($P < 0.01$). Following isoflurane administration the vessels dilated, with the diameter increasing from 1.5 ± 0.1 mm to 1.7 ± 0.1 mm at 0.5% ($P < 0.02$), to 1.7 ± 0.1 mm at 1.25% ($P < 0.01$), and to 1.9 ± 0.1 mm at 2% isoflurane ($P < 0.001$). On discontinuation of isoflurane but continued serotonin infusion, coronary arteries reconstricted to 1.6 ± 0.1 mm (fig. 3).

Prior to halothane administration, serotonin infusion caused coronary artery constriction, with the vessel diameter decreasing from 2.0 ± 0.1 mm to 1.6 ± 0.1 mm ($P < 0.01$). Following halothane administration the vessels dilated, with the diameter increasing from 1.6 ± 0.1 mm to 1.65 ± 0.1 mm at 0.5% ($P < 0.02$), to 1.7 ± 0.1 mm at 1.25% ($P < 0.01$), and to 1.8 ± 0.1 mm at 2% halothane ($P < 0.001$). The vessels reconstricted, with the diameter decreasing to 1.6 ± 0.2 mm on discontinuation of halothane (fig. 3).

Without Endothelium

Prior to isoflurane administration, serotonin infusion caused constriction of coronary arteries without endothelium. The vessel diameter decreased from 2.2 ± 0.1 mm to 1.8 ± 0.1 mm ($P < 0.01$). Following administration of isoflurane the vessels dilated, with the diameter increasing from 1.8 ± 0.1 mm to 1.9 ± 0.1 mm at 0.5% ($P < 0.001$), to 2.0 ± 0.2 mm at 1.25% ($P < 0.01$), and to 2.05 ± 0.1 mm at 2.0% isoflurane ($P < 0.001$). The vessels reconstricted on discontinuation of isoflurane, with the coronary diameter decreasing to 1.8 ± 0.1 mm (fig. 4).

Prior to halothane administration, serotonin infusion induced coronary constriction, with the vessel diameter decreasing from 2.2 ± 0.1 mm to 1.9 ± 0.1 mm ($P < 0.001$). Following halothane administration dilatation occurred, with the diameter increasing from 1.9 ± 0.1 mm to 2.0 ± 0.1 mm at 0.5% ($P < 0.01$), to 2.0 ± 0.1 mm at 1.25% ($P < 0.01$), and to 2.1 ± 0.2 mm at 2% halothane ($P < 0.02$). Reconstriction occurred on discontinuation of halothane, with the vessel diameter decreasing to 1.8 ± 0.1 mm (fig. 4).

Nitroglycerin

Preconstricted vessel diameters (with endothelium) were 1.5 ± 0.1 mm prior to iv nitroglycerin infusion, remained statistically unchanged at 1.6 ± 0.1 mm during nitroglycerin infusion at 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$, and dilated to 2.0 ± 0.1 mm during infusion of nitroglycerin at 2.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$. Preconstricted vessel diameters (without endothelium) were 1.9 ± 0.2 mm prior to nitroglycerin, increased to 2.0 ± 0.2 mm at 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (*P*

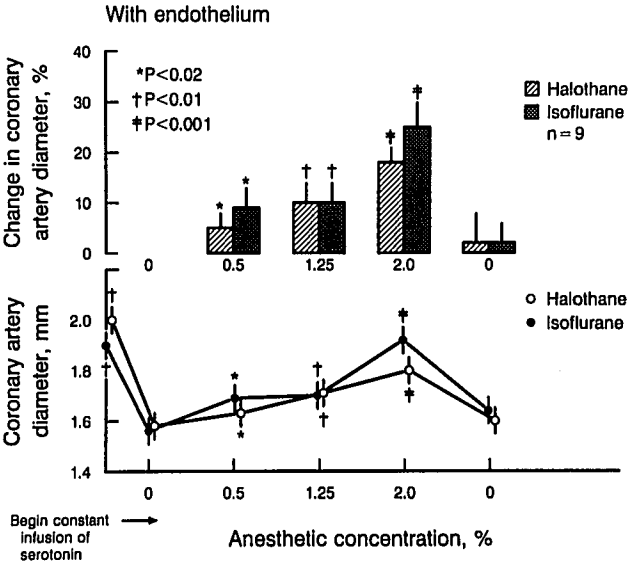

FIG. 3. Responses of left anterior descending coronary arteries in intact pigs. Undamaged vessels are shown. Serotonin was infused *via* an intracoronary catheter at 3 μg/min. Isoflurane or halothane was then administered in three concentrations (0.5%, 1.25%, 2.0%). Responses are presented as actual vessel diameters (mm) or as percent change from vessel diameter prior to anesthetic administration. Statistically significant data points are indicated. Analysis of variance demonstrated significant anesthetic effect. (Percent change: isoflurane $P < 0.001$, halothane $P < 0.001$; actual diameter: isoflurane $P < 0.001$, halothane $P < 0.001$.) Values are mean ± SEM.

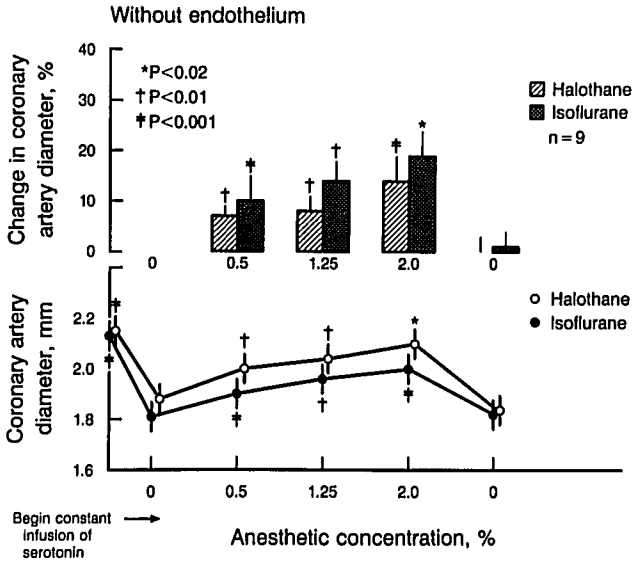

FIG. 4. Responses of left anterior descending coronary arteries in intact pigs. Vessels denuded of endothelium are shown. Serotonin was infused *via* an intracoronary catheter at 3 μg/min. Isoflurane or halothane was then administered in three concentrations (0.5%, 1.25%, 2.0%). Responses are presented as actual vessel diameters (mm) or as percent change from vessel diameter prior to anesthetic administration. Statistically significant data points are indicated. Analysis of variance demonstrated significant anesthetic effect. (Percent change: isoflurane $P < 0.001$, halothane $P < 0.001$; actual diameter: isoflurane $P < 0.001$, halothane $P < 0.001$.) Values are mean ± SEM.

< 0.02), and increased to 2.1 ± 0.2 mm at nitroglycerin 2.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$ ($P < 0.02$) (fig. 5).

Hemodynamic Data

Coronary artery systolic and diastolic pressures and heart rate measured before, during, and after isoflurane and halothane administration are shown in table 2.

Histology

Examination of normal coronary artery segments by light microscopy demonstrated prominent endothelial cell nuclei on the vessels' luminal surfaces, indicating the presence of endothelial cells. In contrast, endothelial cells were absent in specimens from damaged arteries. However, the lamina propria was not ruptured and the muscularis mucosa was intact, indicating that the damage had been superficial. Deep vessel wall injury had not occurred. Scanning electron microscopy of the undamaged specimens showed intact endothelium. Segments that had undergone the denuding procedure had lost the endothelium, exposing the lamina propria. Platelets and blood cell aggregates were seen attached to the underlying internal elastic membrane (fig. 6).

Discussion

Serotonin is a naturally occurring vasoconstrictor amine released by platelets. It is thought to be a major cause of coronary artery constriction both in unstable ischemic syndromes, such as unstable angina and myocardial infarction and in patients with stable coronary artery disease.[1-4] This investigation employed *in vitro* and *in vivo* experimental methods to determine whether isoflurane and halothane each attenuate coronary artery contraction evoked by serotonin. Results from both experimental models demonstrate attenuation of contraction. This study therefore provides the first documentation that anesthetic effects on coronary arteries observed *in vitro* can be reproduced in intact animals studied using *in vivo* methods.

Studies were performed using isolated vessels to provide controlled conditions for assessing vascular responses independent from the many reflex mechanisms that exist in intact animals. In this preparation serotonin interacts with receptors on vascular smooth muscle that mediate contraction.[13] In rings with endothelium the response is attenuated because serotonin also activates receptors on endothelial cells resulting in EDRF release and a lesser degree of contraction.[21] However, the net effect in pig coronary arteries (both *in vivo* and *in vitro*) is contraction.[12,21] In the current experiments isoflurane 2.5% and halothane 2.5% attenuated contraction of rings both with and without endothelium.

Current observations conflict with previous findings obtained from dog coronary arteries when isoflurane was

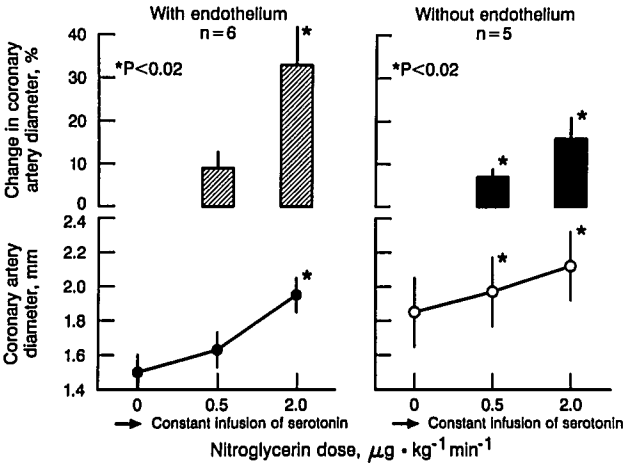

FIG. 5. Responses of left anterior descending coronary arteries in intact pigs. Vessels with and without endothelium are shown. Serotonin was infused *via* an intracoronary catheter at 3 μg/min. Nitroglycerin was administered intravenously in two concentrations: 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ and 2.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$. Responses are presented as actual vessel diameters (mm) or as percent change from vessel diameter prior to nitroglycerin infusion. Statistically significant data points are shown. Values are mean ± SEM.

TABLE 2. Heart Rate and Coronary Artery Pressures during Angiography in Intact Pigs

	Serotonin	Serotonin + Isoflurane 0.5%	Serotonin + Isoflurane 1.25%	Serotonin + Isoflurane 2.0%	Serotonin
Heart rate	104 ± 9	107 ± 8	103 ± 7	100 ± 5	98 ± 13
Systolic coronary artery pressure	115 ± 7	114 ± 8	112 ± 8	111 ± 9	124 ± 10
Diastolic coronary artery pressure	82 ± 6	78 ± 6	73 ± 6	72 ± 5	82 ± 9
	Serotonin	Serotonin + Halothane 0.5%	Serotonin + Halothane 1.25%	Serotonin + Halothane 2.0%	Serotonin
Heart rate	96 ± 14	104 ± 14	101 ± 10	97 ± 9	99 ± 13
Systolic coronary artery pressure	105 ± 6	94 ± 7	90 ± 6	75 ± 8	117 ± 12
Diastolic coronary artery pressure	78 ± 4	68 ± 4	55 ± 7	48 ± 8	82 ± 11

Values are mean ± SEM (isoflurane study, n = 9; halothane study, n = 9).

seen to induce endothelium-dependent attenuation of contractions induced by serotonin.[5] However, considerable differences are known to exist between dog and pig endothelium-dependent responses. Acetylcholine, for example, relaxes dog coronary arteries by evoking EDRF release.[22] In the pig, in contrast, EDRF release does not occur and the net effect is contraction.[22] Similar examples of differences in endothelial regulation occur in other species, including the lower vertebrates. Bullfrog and cayman precontracted aortic rings relax in response to acetylcholine, whereas those of trout and turtles contract.[23] It is possible that species differences may account, at least in part, for the divergence in results obtained in pigs *versus* dogs.

Whether porcine or canine coronary arteries better resemble those of human beings is not known. In response to acetylcholine, coronary artery strips isolated from human beings within 2.5 h of death contract[24] as do those

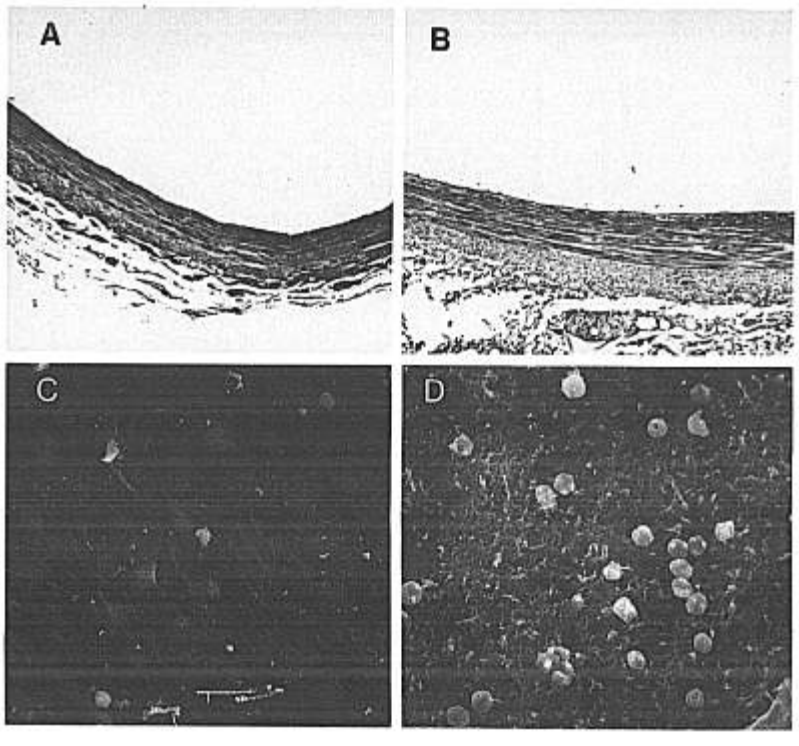

FIG. 6. Histologic appearance of a normal left anterior descending coronary artery and a coronary artery following balloon removal of the endothelium. *A.* Hematoxylin and eosin stain of intact porcine coronary artery showing presence of endothelial cells (original magnification ×160). *B.* Hematoxylin and eosin stain of denuded porcine coronary artery showing absence of endothelial cells but presence of undisturbed internal elastic membrane and underlying smooth muscle (original magnification ×160). *C* and *D.* Scanning electron microscopy of the luminal surface of the arteries demonstrating presence of endothelial cells in figure 6*C* (original magnification ×1,000). *D.* The denuding procedure has resulted in absence of endothelial cells; platelets and blood cell aggregates are seen attached to the underlying internal elastic membrane (original magnification ×1,000).

of the pig.[20] Canine vessels relax.[22] However, no firm conclusions can currently be drawn concerning the most appropriate species for use in scientific experiments.

Porcine coronary arteries precontracted with prostaglandin U44069 have been shown to relax in response to halothane and isoflurane in a concentration-dependent manner.[6] The role of the endothelium in this particular experiment has not been reported. It has been suggested that isoflurane and halothane stimulate the release of a vasodilating prostanoid from the endothelium of rat thoracic aortic rings.[7] In the current experiment although indomethacin was added to one-half the rings, unfortunately, conclusions cannot be drawn concerning prostanoid synthesis because indomethacin was added following anesthetic administration when its effects on anesthetic induced prostanoid synthesis would have been limited.

The mechanism of anesthetic effects on contraction is unknown. In vascular smooth muscle potential sites of anesthetic action range from cell membrane signaling events to intracellular messenger systems to myofilament interaction. In saponin-skinned rabbit aortic strips, halothane decreases tension development by depleting the sarcoplasmic reticulum of Ca^{2+}.[25] In platelets higher concentrations of the anesthetics have been associated with activation of adenylate cyclase and increased intracellular levels of cyclic adenosine monophosphate (cAMP).[26] In vascular smooth muscle cyclic AMP mediates relaxation. Whether isoflurane and halothane potentiate relaxation processes or directly inhibit contraction is not known.

Results from the angiography experiments extend from organ chambers to intact animals the observations concerning the coronary artery relaxant effects of isoflurane and halothane. Results confirm that *in vivo* isoflurane and halothane attenuate coronary constriction evoked by serotonin. Anesthetic concentrations as low as 0.5% had a vasodilating effect. The mechanism underlying anesthetic effect in intact pigs is not known. In intact animals serotonin exerts a direct contractile effect on coronary arteries by activation of smooth muscle receptors but in addition also has indirect constrictor actions.[27] It stimulates noradrenaline release from adrenergic nerves and release of vasoconstricting neuropeptides from peptidergic nerves accompanying coronary arteries.[27] Serotonin is also thought to augment contractile responses to threshold and subthreshold concentrations of other vasoconstrictor agents.[28] Whether isoflurane and halothane both have direct effects upon vascular smooth muscle and upon indirect constrictor effects of serotonin, such as neurotransmitter release, is not known. However, halothane has been shown to inhibit noradrenaline release from adrenergic nerve endings of isolated canine saphenous veins.[29]

A number of confounding factors may have complicated collection and interpretation of data in the angiography experiments. Fentanyl and ketamine were used as background anesthetics and lidocaine and esmolol were used at the beginning of the procedure to prevent fatal arrhythmias. It is possible that background iv anesthesia altered coronary responses. This problem was addressed in separate experiments. Fentanyl was investigated using isolated canine coronary arteries and intact pigs anesthetized with ketamine and was shown to have minimal effects on coronary artery tone and dimensions.[30] In another study ketamine had minimal relaxant effects on isolated pig coronary artery rings precontracted with prostaglandin $F_{2\alpha}$ at organ chamber concentrations corresponding to plasma concentrations of ketamine used in anesthetizing intact pigs (unpublished data).

Esmolol and lidocaine were given during initial insertion of coronary catheters. However, a period of 60 min always elapsed between administration of the antiarrhythmics and collection of data. Pigs, unlike dogs, are prone to fatal arrhythmias during coronary artery manipulations, and esmolol had a marked effect in reducing mortality. (Esmolol's beneficial effect was not documented statistically; nevertheless, this information may be of interest to investigators who use pigs in cardiovascular research.)

Serotonin is a vasoconstrictor normally present in the bloodstream in only minute concentrations. However, in patients with coronary artery disease, atherosclerotic plaques stimulate platelets to release serotonin, resulting in locally high concentrations of the amine.[1-4] Serotonin is also released by platelets during coronary thrombosis. In both stable coronary artery disease and during myocardial infarction serotonin may be a cause of coronary constriction.[2] The results of the current experiment demonstrate that isoflurane and halothane attenuate contractile responses evoked by serotonin in pig coronary arteries studied both *in vitro* and *in vivo*. However, whether such effects would occur in patients with coronary artery disease is unknown. The results do not imply that isoflurane and halothane should be relied upon to reverse coronary constriction in human beings or that the two anesthetics should be considered as a substitute for nitroglycerin in patients with coronary disease undergoing surgery.

References

1. Bolli R: Potential role of serotonin in dynamic coronary stenosis. J Am Coll Cardiol 14:460–461, 1989
2. Willerson JT, Golino P, Eidt J, Campbell WB, Buja LM: Specific platelet mediators and unstable coronary artery lesions: Experimental evidence and potential clinical implications. Circulation 80:198–205, 1989
3. Rubanyi GM, Frye RL, Holmes DR, Vanhoutte PM: Vasoconstrictor activity of coronary sinus plasma from patients with coronary artery disease. J Am Coll Cardiol 9:1243–1249, 1987
4. Van den Berg EK, Schmitz JM, Benedict CR, Malloy CR, Willerson JT, Dehmer GJ: Transcardiac serotonin concentration is in-

creased in selected patients with limiting angina and complex coronary lesion morphology. Circulation 79:116–124, 1989
5. Blaise G, Sill JC, Nugent M, Van Dyke RA, Vanhoutte PM: Isoflurane causes endothelium-dependent inhibition of contractile responses of canine coronary arteries. ANESTHESIOLOGY 67: 513–517, 1987
6. Bollen BA, Tinker JH, Hermsmeyer K: Halothane relaxes previously constricted isolated porcine coronary artery segments more than isoflurane. ANESTHESIOLOGY 66:748–752, 1987
7. Stone DJ, Johns RA: Endothelium-dependent effects of halothane, enflurane, and isoflurane on isolated rat aortic vascular rings. ANESTHESIOLOGY 71:126–132, 1989
8. Muldoon SM, Hart JL, Bowen KA, Freas W: Attenuation of endothelium-mediated vasodilation by halothane. ANESTHESIOLOGY 68:31–37, 1988
9. Furchgott RF: Role of endothelium in responses of vascular smooth muscle. Circ Res 53:557–573, 1983
10. Vanhoutte PM: Vascular physiology: The end of the quest? Nature 327:459–460, 1987
11. Vanhoutte PM, Shimokawa H: Endothelium-derived relaxing factor and coronary vasospasm. Circulation 80:1–9, 1989
12. Shimokawa H, Aarhus LL, Vanhoutte PM: Porcine coronary arteries with regenerated endothelium have a reduced endothelium-dependent responsiveness to aggregating platelets and serotonin. Circ Res 61:256–270, 1987
13. Houston DS, Vanhoutte PM: Comparison of serotonergic receptor subtypes on the smooth muscle and endothelium of the canine coronary artery. J Pharmacol Exp Ther 244:1–10, 1988
14. Miller WL, Bove AA: Differential H_1- and H_2-receptor-mediated histamine responses of canine epicardial conductance and distal resistance coronary vessels. Circ Res 62:226–232, 1988
15. Brum J, Sufan Q, Lane G, Bove AA: Increased vasoconstrictor activity of proximal coronary arteries with endothelial damage in intact dogs. Circulation 70:1066–1073, 1984
16. Gruetter CA, Gruetter DY, Lyon JE, Kadowitz PJ, Ignarro LJ: Relationship between cyclic guanosine 3′:5′-monophosphate formation and relaxation of coronary arterial smooth muscle by glyceryl trinitrate, nitroprusside, nitrite, and nitric oxide: Effects of methylene blue and methemoglobin. J Pharmacol Exp Ther 219:181–186, 1981
17. Gage JE, Hess OM, Murakami T, Ritter M, Grimm J, Krayenbuehl HP: Vasoconstriction of stenotic coronary arteries during dynamic exercise in patients with classic angina pectoris: Reversibility by nitroglycerin. Circulation 73:865–876, 1986
18. Penny WJ, Chesebro JH, Heras M, Badimon L, Fuster V: In vivo identification of normal and damaged endothelium by quantitative coronary angiography and infusion of acetylcholine and bradykinin in pigs (abstract). J Am Coll Cardiol 11:29A, 1988
19. Cherry PD, Furchgott RF, Zawadzki JV, Jothianandan D: Role of endothelial cells in relaxation of isolated arteries by bradykinin. Proc Natl Acad Sci USA 79:2106–2110, 1982
20. Graser T, Leisner H, Tiedt N: Absence of role of endothelium in the response of isolated porcine coronary arteries to acetylcholine. Cardiovasc Res 20:299–302, 1986
21. Cocks TM, Angus JA: Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin. Nature 305:627–630, 1983
22. Furchgott RF: Role of endothelium in responses of vascular smooth muscle. Circ Res 53:557–573, 1983
23. Miller VM, Vanhoutte PM: Endothelium-dependent responses in isolated blood vessels of lower vertebrates. Blood Vessels 23: 225–235, 1986
24. Toda N, Okamura T: Endothelium-dependent and -independent responses to vasoactive substances of isolated human coronary arteries. Am J Physiol 257:H988–H995, 1989
25. Su JY, Zhang CC: Intracellular mechanisms of halothane's effect on isolated aortic strips of the rabbit. ANESTHESIOLOGY 71: 409–417, 1989
26. Walter F, Vulliemoz Y, Verosky M, Triner L: Effects of halothane on the cyclic 3′,5′-adenosine monophosphate enzyme system in human platelets. Anesth Analg 59:856–861, 1980
27. Vanhoutte PM: Cardiovascular effects of serotonin. J Cardiovasc Pharmacol 10(suppl):8–11, 1987
28. Flavahan NA, Vanhoutte PM: Threshold phenomena and interactions between receptors. J Cardiovasc Pharmacol 11(suppl): 67–72, 1988
29. Lunn JJ, Rorie DK: Halothane-induced changes in the release and disposition of norepinephrine at adrenergic nerve endings in dog saphenous vein. ANESTHESIOLOGY 61:377–384, 1984
30. Blaise GA, Witzeling TM, Sill JC, Vinay P, Vanhoutte PM: Fentanyl is devoid of major effects on coronary vasoreactivity and myocardial metabolism in experimental animals. ANESTHESIOLOGY 73:535–541, 1990