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Isoflurane Causes Endothelium-dependent Inhibition of Contractile Responses of Canine Coronary Arteries

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The authors sought to determine if isoflurane would attenuate effects of three different types of vasoconstrictors on isolated segments of canine epicardial coronary arteries removed from healthy dogs. As the endothelium has a major role in regulating epicardial coronary artery tone, and as it modulates the effect of many vasoactive substances, experiments were conducted both on normal rings and on rings whose endothelium had been mechanically removed. In addition, the endothelium is thought to be damaged in human atherosclerosis. Rings were suspended in organ chambers filled with modified Krebs-Ringer bicarbonate solution, aerated with 95% oxygen and 5% carbon dioxide, and connected to strain gauges for the measurement of isometric tension. Isoflurane 2.3% (1.5 MAC in the dog) was added to the aerating gas mixture in half the preparations, while the other rings served as control. The vasoconstrictors serotonin, phenylephrine, or prostaglandin $F_{2\alpha}$ were added in increasing concentrations to the bath solution. In the presence of endothelium, vasoconstrictor evoked contractions were attenuated by isoflurane. Maximal tension generated by prostaglandin $F_{2\alpha}$ in untreated rings was 114 ± 18% (mean ± SEM) of a reference contraction, while, following isoflurane, it was 46 ± 8% ($P < 0.005$). In the absence of endothelium, isoflurane attenuated neither prostaglandin $F_{2\alpha}$ nor serotonin evoked contraction, and had decreased effectiveness against phenylephrine mediated contraction ($P < 0.001$). It is concluded that isoflurane attenuates vasoconstrictor-evoked contraction of isolated canine epicardial coronary arteries, and that this effect is mediated by the endothelium. (Key words: Anesthetics, volatile: isoflurane. Arteries: coronary; endothelium. Pharmacology: phenylephrine; prostaglandin $F_{2\alpha}$, serotonin; vasoconstrictors.)

ISOFLURANE IS A VASODILATOR with effects upon both the peripheral and coronary circulations.[1–5] Although it is a potent dilator of canine coronary arterioles, isoflurane does not have a direct dilating effect on epicardial coronary arteries.[3] However, whether or not it attenuates increases in epicardial coronary artery tone when these vessels are exposed to vasoconstrictors is unknown. The purpose of this experiment was to determine if isoflurane would attenuate contraction of isolated canine epicardial coronary rings evoked by three different types of vasoconstrictors.

The coronary vasculature in humans and animals, in simplified terms, consists of large proximal epicardial coronary arteries and smaller distal intramyocardial arteries and arterioles.[6–8] Coronary blood flow is regulated by constriction and dilatation of the small distal intramyocardial arterioles, while the large proximal vessels serve principally to conduct blood between the aorta and the arteriolar bed. Normally, although the arterioles are highly vasoactive, the epicardial vessels do not constrict and dilate to a major degree. In healthy animals and humans, basal epicardial coronary artery tone is thought to be low, and vessels normally function in a relatively relaxed state.[8] However, in animals following endothelial damage and in humans following atherosclerosis, normal regulation of proximal coronary tone is disrupted, and epicardial vessels become particularly sensitive to vasoconstrictor stimuli and may respond with abnormally intense constriction.[9–13] Mechanisms responsible for elevation of coronary tone in humans are not known, although endothelial damage combined with local exposure to endogenous vasoconstrictor substances are probably the two most essential components.[9,14] The endothelium is thought to be vital to preservation of normal epicardial coronary tone, as it both protects the underlying smooth muscle from circulating vasoconstrictors and synthesizes dilator substances. Its damage or loss may result in the local aggregation of blood elements, such as platelets and white blood cells, that then release vasoconstrictor substances, including serotonin and prostaglandins.[9,14] In the absence of endothelium, these substances have ready access to the underlying smooth muscle, where they may produce coronary constriction.

In these experiments, both normal rings and rings denuded of endothelium were investigated. Denuded vessels were used both to permit assessment of the role of the endothelium in mediating the effect of isoflurane and to mimic the vessel in coronary artery disease. Experimentally induced ring contraction was evoked by prostaglandin $F_{2\alpha}$, serotonin, and phenylephrine, each chosen to represent a different class of constrictor.

Materials and Methods

The experiments were performed on left circumflex and proximal left anterior descending coronary arteries taken from mongrel dogs of either sex (18–28 kg) anes-

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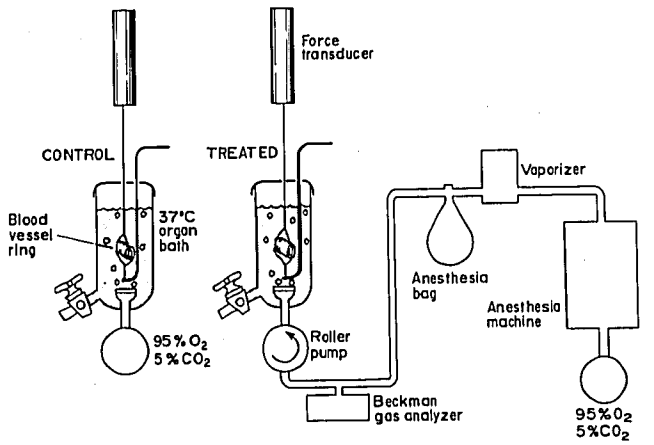

FIG. 1. Schematic diagram of the experimental methods demonstrating anesthetic delivery and analysis system and isolated coronary rings in organ chambers. Vasoconstrictors were added to the bath solution. Isoflurane concentration in the bath solution was measured using gas chromatography.

thetized with sodium pentobarbital (30 mg/kg iv). The blood vessels were studied in modified Krebs-Ringer bicarbonate solution (control solution) of the following millimolar composition: NaCl 118.3, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, Calcium disodium EDTA 0.026, glucose 11.1. The rings (4–5 mm long) were cleaned of fat and loose connective tissue, with special care being taken not to touch the luminal surface, and then immersed in cold control solution. In approximately half of the preparations, the endothelium was mechanically removed. To do so, the bent tips of a watchmaker's forceps were inserted into their lumen, and the endothelial cell layer was removed by gently rolling the preparation back and forth for 15 s over a filter paper soaked with Krebs-Ringer bicarbonate solution. The rings were suspended in organ chambers filled with 25 ml of control solution at 37° C, and gassed with 95% O_2, 5% CO_2 (*p*H 7.44–7.49). The preparations were connected to a strain gauge (Gould UTC 2), and isometric tension was recorded. The rings were progressively stretched to optimal tension (approximately 11 gm) determined by repeated stimulations with KCl (2 10^{-2} M). Maximal contraction (100%) was measured after stimulation with KCl (4 10^{-2} M), and was used as a reference contraction. The presence or absence of functional endothelium was confirmed by demonstrating the response to acetylcholine (10^{-6} M) in rings contracted with KCl (4 10^{-2} M).[15] (Rings precontracted with KCl and then challenged with acetylcholine (10^{-6}) respond with transient partial relaxation.[16] Denuded rings do not relax.) The rings were allowed to equilibrate for 30 min before experimentation.

Isoflurane 2.3% (1.5 MAC in the dog), delivered by a vaporizor, was added to the mixture gassing the bath solution (fig. 1). The concentration of the anesthetic was adjusted using an infrared Beckman LB II gas analyzer. Isoflurane has a low solubility in Krebs-Ringer solution. To ensure equilibration between the aerating gas carrying 2.3% isoflurane and the bath solution, a high gas flow (at least 150 ml/min) was bubbled through the bath solution and covers were placed over the organ chambers to prevent the aerating gas from immediately escaping into the atmosphere. Preliminary experiments demonstrated that isoflurane releases vasoactive substances from either rubber or teflon connections; therefore, the use of these materials was avoided. The concentration of isoflurane in the bath solution was measured by gas chromatography.[17] Reproducible amounts of isoflurane were achieved in the bath solution. After introduction of isoflurane, the blood vessels were allowed to equilibrate for 45 min. They were then exposed to increasing concentrations of either phenylephrine, serotonin, or prostaglandin $F_{2\alpha}$.

To determine if the isoflurane effect was mediated *via* receptors on the vascular endothelium, a group of rings with endothelium were incubated for 45 min with various receptor blockers. These drugs were: phentolamine (10^{-6} M) to block alpha-adrenoceptors, propranolol (3×10^{-6} M) to block beta-adrenoceptors, atropine (10^{-5} M) to block muscarinic receptors, pyrilamine plus cimetidine (10^{-5} M) to block H_1- and H_2-histaminergic receptors, methiothepin (10^{-5} M) to block serotonergic receptors, and indomethacin (10^{-5} M) to inhibit cyclooxygenase. The effect of these receptor blockers on the isoflurane effect was tested by bubbling isoflurane through the bath solution, adding increasing concentrations of $PGF_{2\alpha}$, and measuring the contractile response.

DRUGS

The following drugs were used: Acetylcholine chloride (Sigma Chemical Co., St. Louis, MO); atropine (Sigma); cimetidine (Smith Klein and French Labs, Philadelphia, PA); ergonovine maleate (Sigma); 5-hydroxytrytamine (serotonin, Sigma); indomethacin (Sigma); isoflurane (Anaquest, Madison, WI); methiothepine (Hoffman LaRoche, Nutley, NJ); phentolamine mesylate (Ciba Geigy); prazosin HCl (Pfizer Inc., Brooklyn, NY); prostaglandin $F_{2\alpha}$ (Sigma); propranolol (Sigma); and hydrochloride pyrilamine maleate (Merck Sharp and Dohme Research Lab, Rahway, NY). The drugs were dissolved in distilled water, except for indomethacin, which was dissolved in Na_2CO_3 (5 10^{-3} M) and sonicated before use. Isoflurane was added to the aerating gas mixture from a vaporizor.

CALCULATION AND STATISTICS

Agonists and antagonists quantities are expressed as the negative logarithm of the final molar concentration in the bath solution. Concentrations of isoflurane are expressed as percentage of the gas mixtures. Contrac-

tile responses are given as percentage of the contraction obtained in the individual preparations with KCl (4 $\times 10^{-2}$ M) (reference contraction). Paired rings from the same artery were studied in parallel in the presence or absence of isoflurane (fig. 1).

The results are expressed as the mean ± SEM, and n equals the number of rings removed from different dogs. Statistical analysis was performed in the following manner. To determine if isoflurane attenuated ring contraction, the integrated areas under the constrictor evoked dose-response curves were compared before and after isoflurane treatment using analysis of variance with two sample paired *t* tests. To determine if effect of isoflurane on phenylephrine-evoked contraction was decreased following removal of endothelium, the difference between the integrated areas under the dose-response curves were compared between rings with and without endothelium using unpaired *t* testing. Values were considered significant when P was less than 0.05.

Results

Isoflurane had no effect on the tension generated by coronary rings contracted with KCl solution (from 10 to 80 mM) (fig. 2). Isoflurane did not alter tension generated by unstimulated rings.

Prostaglandin $F_{2\alpha}$ (10^{-8} to 10^{-4} M) evoked concentration-dependent contractions of rings both with and without endothelium. In rings with endothelium, maximal tension generated by untreated control rings was 114 ± 18% (mean ± SEM) of the potassium-induced reference contraction. Maximal tension generated by isoflurane treated rings was 46 ± 8%. Comparison of integrated areas under dose-response curves demonstrated that isoflurane treatment resulted in significant depression of tension generation, $P < 0.005$ (fig. 3). In rings without endothelium, maximal tension in control and treated rings were 120 ± 8% *versus* 130 ± 18%, respectively, and there was no significant difference between the areas under the dose-response curves. In the absence of endothelium, isoflurane had no effect on ring contraction (fig. 3).

The presence of inhibitors (phentolamine, propranolol, atropine, pyrilamine plus cimetidine, methiothepin, and indomethacin) did not significantly alter the contractions evoked by prostaglandin $F_{2\alpha}$. In the presence of the inhibitors, isoflurane caused a significant depression of the response which was comparable to that observed in control solution.

Phenylephrine (10^{-8} to 10^{-5} M) evoked concentration-dependent contractions in rings both with and without endothelium. In rings with endothelium, maximal tension generated by untreated rings was 23 ± 6% of the reference contraction, and, in the isoflurane treatment group, maximal tension was 5 ± 2%. Comparison of integrated areas under the dose-response curves demonstrated that isoflurane significantly depressed tension generation, $P < 0.05$ (fig. 4). In rings without endothelium, maximal control tension was 32 ± 5% and, following isoflurane, it was 18 ± 2%. Comparison of areas under the curves demonstrated that isoflurane depressed contraction despite the absence of endothelium, $P < 0.005$ (fig. 4). However, degree of contractile depression in the rings without endothelium was less than that produced in rings with endothelium ($P < 0.001$).

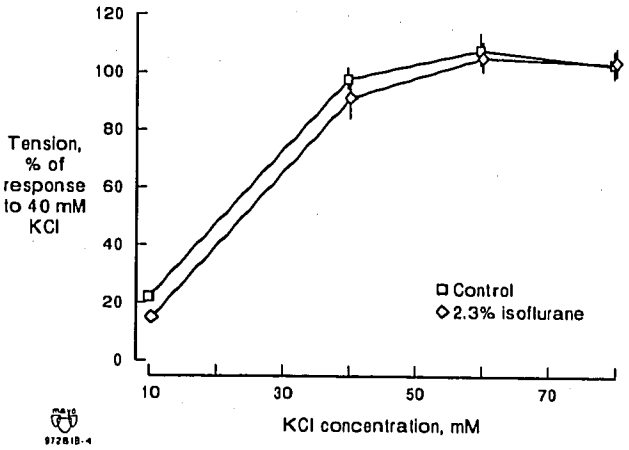

FIG. 2. Potassium concentration-ring tension response curves. Responses of rings treated with 2.3% isoflurane and untreated rings exposed to increasing concentrations of KCl (10–80 mM) are shown. All rings had endothelium. Contractions are expressed as a percentage of the previous contractile response to a standard 40 mM KCl challenge. Potassium-evoked ring contraction was not altered by isoflurane. Data are mean ± SEM (n = 6 rings per group).

Serotonin (10^{-6} to 10^{-4} M) evoked a typical biphasic response in both rings with and without endothelium.[18]

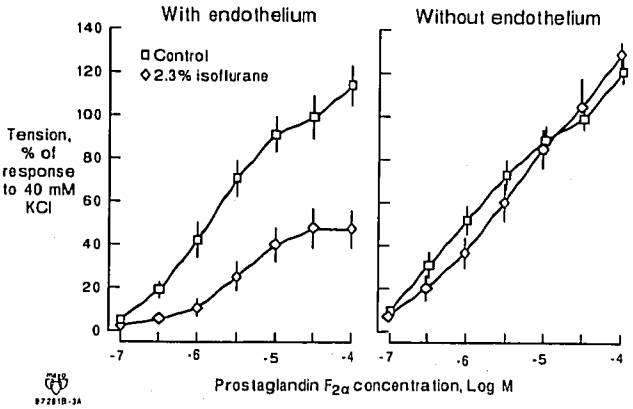

FIG. 3. Cumulative concentration response relationships of rings with and without endothelium exposed to increasing concentrations of prostaglandin $F_{2\alpha}$ (10^{-8} M to 10^{-4} M). Contractions are expressed as a percentage of the previous contractile response to a standard 40 mM KCl challenge. Prostaglandin $F_{2\alpha}$ evoked concentration-dependent contraction of rings both with and without endothelium. Comparison of integrated areas under the dose-response curves demonstrated that isoflurane depressed contraction in rings with endothelium, $P < 0.05$, but not in rings without endothelium. Data are mean ± SEM (n = 6).

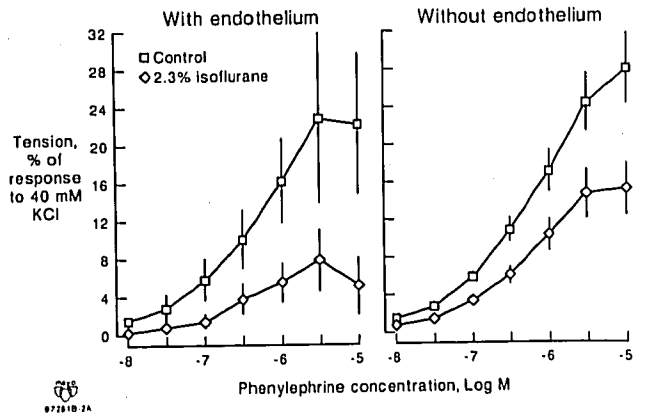

FIG. 4. Cumulative concentration response relationship of rings with and without endothelium exposed to increasing concentrations of phenylephrine (10^{-8} to 10^{-5} M). Contractions are expressed as a percentage of the previous contractile response to a standard 40 mM KCl challenge. Phenylephrine evoked concentration-dependent contraction in rings both with and without endothelium. Comparison of integrated areas under the dose-response curves demonstrated that isoflurane significantly depressed tension generation in both rings with endothelium, $P < 0.05$, and rings without endothelium, $P < 0.005$. Magnitude of the depression of contraction was decreased in the absence of endothelium, $P < 0.001$. Data are mean ± SEM (n = 6).

Maximal tension in control rings with endothelium was 21 ± 5% and, following isoflurane treatment, it was 10 ± 6%. Area under the dose-response curve was depressed by isoflurane, $P < 0.01$ (fig. 5). Maximal tension in control rings without endothelium was 48 ± 7% and, following isoflurane, it was 53 ± 10%. Areas under the dose-response curves were not different from one another, indicating absence of isoflurane effect following removal of the endothelium (fig. 5).

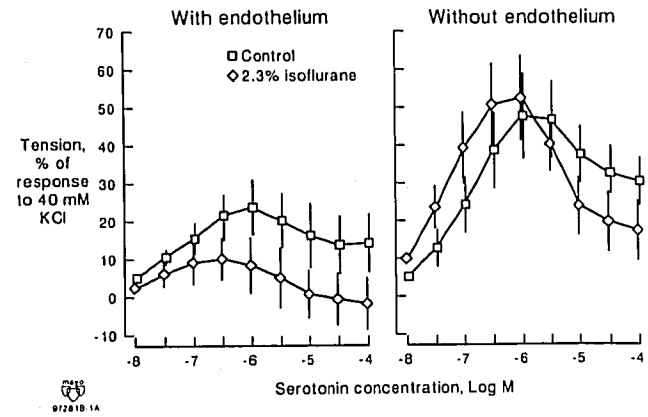

FIG. 5. Cumulative concentration response relationships of rings with and without endothelium exposed to increasing concentrations of serotonin (10^{-8} to 10^{-4} M). Contractions are expressed as a percentage of the previous contractile response to a standard 40 mM KCl challenge. Serotonin evoked biphasic response in rings both with and without endothelium. Comparison of integrated areas under the dose-response curves demonstrated that isoflurane-depressed contraction in rings with endothelium, $P < 0.01$, but not in rings without endothelium. Data are mean ± SEM (n = 6).

Discussion

This study demonstrates that isoflurane attenuates vasoconstrictor-induced canine coronary ring contraction, and that the effect is endothelium dependent. In the presence of endothelial cells, isoflurane is a potent inhibitor of contractions of coronary vascular smooth muscle. In the absence of endothelial cells, it no longer attenuates contractions evoked by $PGF_{2\alpha}$ and serotonin, and has decreased effect on the response to phenylephrine. Results also demonstrate that isoflurane has no direct non-selective depressing effects on epicardial coronary vascular smooth muscle.

Isoflurane did not alter basal tension of the canine coronary artery. This is not surprising, as in control conditions, this vessel exhibits little spontaneous tone. The fact that isoflurane did not depress contractions evoked by either potassium ions or by prostaglandin $F_{2\alpha}$ in preparations without endothelium demonstrates that isoflurane lacks non-selective inhibitory effects on vascular smooth muscle cells. In particular, the data with potassium suggests that isoflurane does not interfere with the entry of calcium ions.[19] The only direct effect of isoflurane on vascular smooth muscle obvious from the present experiments could be a moderate alpha-adrenergic inhibitory effect. This interpretation is based on the observation that the anesthetic agent depressed contractions evoked by the alpha-adrenergic agonist phenylephrine[20] in rings of coronary arteries without endothelium. The facts that isoflurane does not affect responses of de-endothelialized preparations to prostaglandin $F_{2\alpha}$, and that a mixture of autonomic inhibitors does not significantly alter its inhibitory effect on rings with endothelium, rule out the possibility that isoflurane may cause the release of either norepinephrine, acetylcholine, histamine, serotonin, or metabolites of cyclo-oxygenase from tissue stores.[21,22]

The mechanism of isoflurane's action is not known, although it appears to act *via* an endothelium-dependent mechanism. The endothelium is a major regulator of epicardial coronary tone. When stimulated by many vasoactive substances, such as bradykinin, adenosin diphosphate, and acetylcholine, endothelial cells liberate one or more vasoactive substances that cause relaxation of the underlying smooth muscle.[14,23] Prostacyclin is one of these dilators,[14] and endothelium-derived relaxing factor (EDRF), an as yet unidentified substance that produces marked dilatation, is another.[11,23] Isoflurane relaxation is not due to accelerated release of prostacyclin, as pretreatment with indomethacin did not prevent isoflurane effect.[22,24] (Indomethacin inhibits cyclo-oxygenase, the enzyme responsible for generating prostaglandins from arachidonic acid.) It seems more likely to assume that isoflurane either causes the release of EDRF or facilitates its action on vascular smooth mus-

cle. It is also possible that the peripheral vasodilating action of isoflurane is mediated *via* increased EDRF release; however, for the moment, this suggestion is speculative.

In humans with coronary artery disease, the resistance coronary stenoses pose to flow is proportional to the fourth power of the radius. For this reason, even small changes in vessel diameter can have marked effects on coronary blood flow. Drugs that attenuate coronary constriction may, therefore, be of therapeutic value in patients with coronary artery disease. These data demonstrate that, in the presence of normally functioning endothelium, isoflurane attenuates coronary constriction. However, it would be premature to extrapolate directly from responses of isolated vessels in an experimental setting to patients with coronary artery disease. Intact canine vessels may not behave in the same way as human coronary arteries, although endothelium-dependent ring relaxation has been demonstrated in many vertebrate[25] species, including primates with coronary atherosclerosis.[12] In addition, abnormal responses to the endothelium-dependent dilator acetylcholine in patients with coronary artery disease may represent a defect in endothelial vasodilator function.[13] Vasoconstrictors used in this experiment may not necessarily resemble those responsible for constriction in humans, although the three constrictors were chosen to resemble those thought to be associated with human coronary constriction. The concentration of isoflurane found effective in these experiments was 2.3%, a concentration that would have marked cardiovascular depressant effects in humans. Despite an unwillingness to extrapolate to humans, it is possible that isoflurane will have an effect upon epicardial coronary constriction in patients providing that the endothelium is relatively intact.

In conclusion, the major findings are that isoflurane attenuates vasoconstrictor-evoked contraction of isolated canine coronary arteries, and this effect is mediated by the endothelium.

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