

Intravenous Emulsified Halogenated Anesthetics Produce Acute and Delayed Preconditioning against Myocardial Infarction in Rabbits

Pascal C. Chiari, M.D., Paul S. Pagel, M.D., Ph.D.,† Katsuya Tanaka, M.D.,* John G. Krolikowski, B.A.,‡ Lynda M. Ludwig, Ph.D.,§ Raul A. Trillo, Jr., M.D., M.B.A.,‖ Navneet Puri, Ph.D.,# Judy R. Kersten, M.D.,** David C. Warltier, M.D., Ph.D.††*

Background: **Preconditioning against myocardial infarction by volatile anesthetics is well known. The authors tested the hypothesis that new emulsified formulations of halogenated anesthetics administered intravenously reduce myocardial infarct size when administered either 1 or 24 h before prolonged ischemia and reperfusion.**

Methods: **Pentobarbital-anesthetized rabbits (n = 39) were instrumented for measurement of hemodynamics and randomly assigned to receive intravenous saline (control), lipid vehicle, or infusions (3.5 ml · kg^{-1} · h^{-1} for 30 min) of emulsified isoflurane (6.9%), enflurane (7.1%), or sevoflurane (7.5%). Infusions were discontinued 30 min before a 30-min coronary occlusion and 3 h of reperfusion. In three additional groups, conscious rabbits (n = 21) received saline, lipid vehicle, or emulsified sevoflurane (7.5%) infusions (3.5 ml · kg^{-1} · h^{-1} for 30 min) 24 h before ischemia and reperfusion. Infarct size was determined using triphenyltetrazolium staining.**

Results: **Lipid vehicle produced transient increases in heart rate, whereas emulsified volatile anesthetics had no effect on hemodynamics before coronary occlusion. Lipid vehicle did not affect infarct size (38 ± 2% of the area at risk; mean ± SEM) as compared with saline control (41 ± 4%). In contrast, emulsified isoflurane, enflurane, and sevoflurane reduced infarct size (20 ± 3%, 20 ± 3%, and 21 ± 2% of the area at risk, respectively; $P < 0.05$). Administration of lipid vehicle or emulsified sevoflurane did not produce sedation or respiratory depression in conscious rabbits. Emulsified sevoflurane (18 ± 2%) but not lipid vehicle (44 ± 2%) reduced infarct size as compared with control in delayed preconditioning experiments.**

Conclusions: **Intravenous emulsified halogenated anesthetics produce acute and delayed preconditioning against myocardial infarction.**

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* Research Fellow in Anesthesiology, ‡ Research Scientist in Anesthesiology, § Graduate Student, ** Professor of Anesthesiology and Pharmacology, Medical College of Wisconsin, Milwaukee, Wisconsin. † Professor and Director of Cardiac Anesthesia, Medical College of Wisconsin and Clement J. Zablocki Veterans Affairs Medical Center, Milwaukee, Wisconsin, and Professor of Biomedical Engineering, Marquette University, Milwaukee, Wisconsin. ‖ Senior Director, Marketing, # Research Scientist, Baxter Healthcare Corporation, New Providence, New Jersey. †† Professor of Anesthesiology, Medicine (Division of Cardiovascular Diseases), and Pharmacology, Medical College of Wisconsin and Clement J. Zablocki Veterans Affairs Medical Center, Milwaukee, Wisconsin; Professor of Biomedical Engineering, Marquette University, Milwaukee, Wisconsin; and Senior Vice Chairman of Anesthesiology, Medical College of Wisconsin, Milwaukee, Wisconsin.

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Address reprint requests to Dr. Warltier: Medical College of Wisconsin, MEB-M4280, 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226. Address electronic mail to: warltier@mcw.edu. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

INHALED halogenated anesthetics have been repeatedly shown to acutely protect myocardium against irreversible ischemic injury. This anesthetic preconditioning is the subject of intense research by multiple groups of investigators and was the focus of a recent issue of ANESTHESIOLOGY. Many of the mechanisms responsible for anesthetic preconditioning have been identified to date.[1–3] Halogenated anesthetics have been administered in solution in a variety of experimental preparations ranging from isolated ventricular or atrial myocytes to Langendorff-prepared hearts, but from a clinical perspective, inhalation of these drugs is the only currently acceptable route of administration. A new formulation of halogenated anesthetics has been developed that incorporates emulsification of these drugs into a lipid vehicle and thus facilitates their intravenous administration *in vivo*. Such a preparation may be clinically useful for anesthetic induction in the future. We tested the hypothesis that intravenous emulsified halogenated anesthetics acutely protect myocardium against infarction when administered before a prolonged coronary artery occlusion and reperfusion in rabbits. Inhaled halogenated anesthetics have also recently been shown to produce delayed preconditioning when administered 24 h before an ischemic stimulus.[4] Therefore, we also tested the hypothesis that intravenous administration of emulsified sevoflurane 24 h before ischemia protects myocardium against infarction.

Materials and Methods

All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal Care and Use Committee of the Medical College of Wisconsin (Milwaukee, Wisconsin). Furthermore, all conformed to the *Guiding Principles in the Care and Use of Animals*[5] of the American Physiologic Society and were in accordance with the *Guide for the Care and Use of Laboratory Animals*.[6]

General Preparation

Male New Zealand white rabbits weighing between 2.5 and 3.0 kg were anesthetized with intravenous so-

Table 1. Composition of Emulsion of the Volatile Anesthetic

Ingredient	Concentration in Emulsion, mg/ml
Soybean oil	200.00
Volatile anesthetic*	80.00†
Glycerin	22.50
Lecithin	24.00
Sterile water for injection	q.s. ad 1 ml

* Isoflurane, enflurane, or sevoflurane. † Target concentrations in emulsion.
q.s. ad = quantity sufficient added.

Table 2. Concentration of Volatile Anesthetics in Emulsion

Anesthetic Agent	Target Concentration in Emulsion, % w/v*	Experimentally Determined Concentration in Emulsion, % w/v*	
		Refrigerated Vials	After Warming of Emulsion (Opened Vial) for 2 h at 37°C
Isoflurane	8	7.1	6.9
Enflurane	8	7.4	7.1
Sevoflurane	8	7.6	7.5

* Values are average of gas chromatography measurement from two separate vials (two injections per vial).
w/v = weight/volume.

dium pentobarbital (30 mg/kg). As previously described,[7] a tracheostomy was performed through a ventral midline incision, and the trachea was cannulated. The rabbits were ventilated with positive pressure using an air–oxygen mixture (fractional inspired oxygen concentration = 0.33). Arterial blood gas tensions and acid–base status were maintained within a normal physiologic range (pH between 7.35 and 7.45, arterial carbon dioxide tension between 25 and 40 mmHg, and arterial oxygen tension between 90 and 150 mmHg) by adjusting respiratory rate or tidal volume. Body temperature was maintained with a heating blanket. Heparin-filled catheters were inserted into the right carotid artery and the left jugular vein for measurement of heart rate and mean arterial blood pressure and for fluid or drug administration, respectively. Maintenance fluids consisted of 0.9% saline ($15\ ml \cdot kg^{-1} \cdot h^{-1}$), which was continued for the duration of the experiment.

A left thoracotomy was performed at the fourth intercostal space, and the heart was suspended in a pericardial cradle. A prominent branch of the left anterior descending coronary artery (LAD) was identified, and a silk ligature was placed around this vessel approximately halfway between the base and the apex for the production of coronary artery occlusion and reperfusion. Intravenous heparin (500 U) was administered immediately before LAD occlusion. Coronary artery occlusion was verified by the presence of epicardial cyanosis and regional dyskinesia in the ischemic zone, and reperfusion was confirmed by observing an epicardial hyperemic response. Hemodynamics were continuously recorded on a polygraph throughout each experiment. End-tidal concentrations of halogenated anesthetics were measured at the tip of the tracheostomy tube with an infrared anesthetic analyzer that was calibrated with known standards before and during experimentation.

Formulation of Emulsified Halogenated Anesthetics

Isoflurane, enflurane, and sevoflurane were dissolved in soy bean oil containing dispersed egg lecithin, which comprised the oil phase of the emulsion. Dissolution of egg lecithin in soybean oil was facilitated with heat. After dissolution of lecithin, the oil phase was then cooled to approximately 10°C before addition of the volatile anesthetic. The aqueous phase of the emulsion contained glycerin, as a tonicity-adjusting agent, dissolved in water. The aqueous phase was cooled to a temperature similar to that of the oil phase (10°C). The oil phase was then added to the aqueous phase while it was stirred vigorously to form the primary emulsion. The primary emulsion was homogenized at high pressure to form the final emulsion. Care was taken during homogenization to maintain the temperature of the emulsion below 20°C, using a heat exchanger. After homogenization, the volatile anesthetic emulsion was stored in glass vials, capped, and refrigerated (2°–5°C) until use. Vials of the emulsion were opened and warmed to 37°C for 2 h before intravenous administration. The amount of anesthetic in the emulsion was determined using gas chromatography. The quantitative composition of emulsion is described in table 1. Concentrations of volatile anesthetics in the emulsion are shown in table 2.

Experimental Protocols

The experimental design is illustrated in figure 1. Baseline hemodynamics and arterial blood gas tensions were

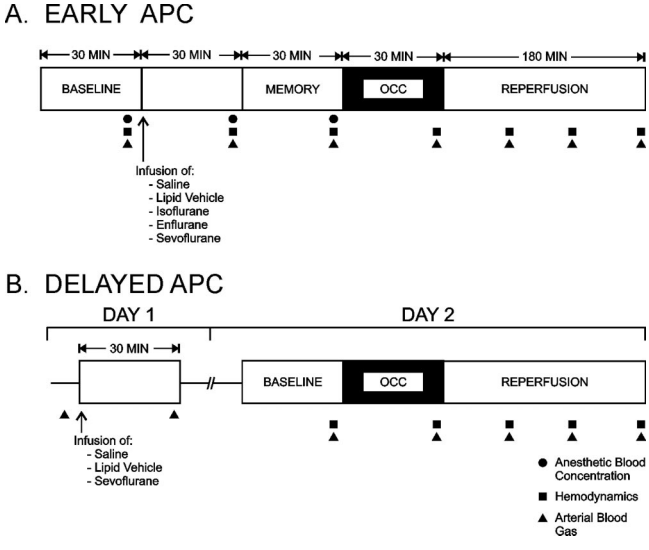

Fig. 1. Schematic diagram depicting the experimental protocols used in early (*A*) and delayed (*B*) anesthetic preconditioning (APC) experiments (see text).

recorded 30 min after instrumentation was completed. In acute preconditioning experiments, rabbits were randomly assigned to receive 0.9% intravenous saline, lipid vehicle, or emulsified isoflurane (6.9%), enflurane (7.1%), or sevoflurane (7.5%) (3.5 ml · kg^{-1} · h^{-1} for 30 min) in five separate experimental groups. Vials of emulsified halogenated anesthetics (Baxter, New Providence, NJ) were stored at 4°C before use. Each vial containing lipid vehicle or an emulsified halogenated agent was warmed in a water bath at 37°C for 2 h before infusion. Intravenous infusions of saline, vehicle, or emulsified halogenated anesthetics were discontinued 30 min (memory period) before LAD occlusion. Arterial blood samples were collected before and during the infusion and immediately before LAD occlusion (at the end of the memory period) for the measurement of blood anesthetic concentration using the flame ionization detection method and Shimadzu GC8A gas chromatograph (Shimadzu, Kyoto, Japan) as previously described.[8] All rabbits underwent a 30-min LAD occlusion followed by 3 h of reperfusion.

In additional experiments designed to examine delayed preconditioning, conscious rabbits were randomly assigned to receive saline, lipid vehicle, or emulsified sevoflurane (7.5%) 24 h before ischemia in three separate groups. Rabbits were restrained within a well-ventilated box, an intravenous catheter was inserted into an ear vein, and saline, lipid vehicle, or emulsified sevoflurane was infused at 3.5 ml · kg^{-1} · h for 30 min. The activity and respiratory rate of each rabbit was monitored before and during the infusion. Arterial blood gas tensions were also obtained from conscious rabbits by sampling from an ear artery immediately before and on completion of the infusion. On the second experimental day, the rabbits were returned to the laboratory and anesthetized with pentobarbital. After instrumentation, rabbits underwent a 30 min LAD occlusion followed by 3 h of reperfusion as described in the previous paragraph.

Determination of Myocardial Infarct Size

Myocardial infarct size was measured as previously described.[9] Briefly, the LAD was reoccluded, and 3 ml patent blue dye was injected intravenously at the completion of each experiment. The left ventricular (LV) area at risk (AAR) for infarction was separated from surrounding normal areas (stained blue), and the two regions were incubated at 37°C for 20 min in 1% 2,3,5-triphenyltetrazolium chloride in 0.1 M phosphate buffer adjusted to pH 7.4. After overnight storage in 10% formaldehyde, infarcted and noninfarcted myocardium within the AAR were carefully separated and weighed. Myocardial infarct size was expressed as a percentage of the AAR. Rabbits that had intractable ventricular fibrillation and those with an AAR less than 15% of total LV mass were excluded from subsequent analysis.

Statistical Analysis

Statistical analysis of data within and between groups was performed with analysis of variance for repeated measures followed by the Student-Newman-Keuls modification of the two-tailed Student *t test*. Changes were considered statistically significant when P was less than 0.05. All data are expressed as mean ± SEM.

Results

Acute Preconditioning Experiments

Forty-five rabbits were instrumented to obtain 39 successful experiments. One rabbit in the isoflurane group was excluded because of technical problems during the surgical preparation. Five rabbits were excluded because intractable ventricular fibrillation occurred during coronary artery occlusion (one saline, two lipid vehicle, one isoflurane, and one enflurane).

Systemic Hemodynamics

There were no differences in baseline hemodynamics between groups (table 3). Infusion of lipid vehicle caused transient increases in heart rate. Infusions of emulsified halogenated anesthetics caused no changes in heart rate or mean arterial pressure. Coronary artery occlusion and reperfusion decreased mean arterial pressure and rate–pressure product in all experimental groups. There were no differences in heart rate, mean arterial pressure, or rate–pressure product between groups during ischemia and reperfusion.

Blood and End-tidal Anesthetic Concentrations

Blood concentrations of isoflurane, enflurane, and sevoflurane measured immediately before completion of the intravenous infusion were 0.15 ± 0.01, 0.31 ± 0.03, and 0.15 ± 0.01 mM, respectively. Blood isoflurane, enflurane, and sevoflurane concentrations were 0.03 ± 0.00, 0.06 ± 0.01, and 0.02 ± 0.00 mM, respectively, at the end of the 30-min memory period. These blood anesthetic concentrations paralleled end-tidal anesthetic concentrations measured during and after intravenous administration of emulsified volatile agents (table 3).

Myocardial Infarct Size

Body weight, LV mass, AAR weight, and the ratio of AAR to LV mass were similar between groups (table 4). Lipid vehicle did not affect myocardial infarct size (38 ± 2% of the LV AAR) as compared with control (41 ± 4%; fig. 2). In contrast, intravenous administration of emulsified isoflurane, enflurane, or sevoflurane significantly reduced infarct size (20 ± 3, 20 ± 3, and 21 ± 2%, respectively).

Delayed Preconditioning Experiments

Twenty-six rabbits were instrumented to obtain 21 successful experiments. One rabbit in the sevoflurane

Table 3. Hemodynamics during Acute Preconditioning Experiments

					Reperfusion		
	Baseline	Drug	Memory	Occlusion	1 h	2 h	3 h
HR, beats/min							
Saline	249 ± 9	244 ± 7	242 ± 6	240 ± 6	224 ± 6*	221 ± 5*	213 ± 6*
Vehicle	235 ± 15	247 ± 10*	250 ± 10*	236 ± 14	232 ± 15	222 ± 14	219 ± 14
Isoflurane	254 ± 11	243 ± 12	238 ± 10	230 ± 10*	216 ± 7*	208 ± 7*	202 ± 6*
Enflurane	248 ± 10	240 ± 11	231 ± 11*	213 ± 9*	216 ± 7*	214 ± 10*	210 ± 7*
Sevoflurane	227 ± 7	235 ± 9	227 ± 11	214 ± 8	206 ± 9*	193 ± 10*	188 ± 9*
MAP, mmHg							
Saline	84 ± 2	83 ± 3	83 ± 3	64 ± 3*	66 ± 4*	68 ± 3*	67 ± 3*
Vehicle	73 ± 4	74 ± 5	70 ± 5	56 ± 6*	58 ± 6*	57 ± 4*	54 ± 4*
Isoflurane	74 ± 6	66 ± 7	73 ± 6	62 ± 4	59 ± 3*	60 ± 3*	66 ± 5
Enflurane	78 ± 4	68 ± 3	70 ± 3	59 ± 6*	65 ± 6*	68 ± 7	75 ± 5
Sevoflurane	92 ± 4	91 ± 4	87 ± 4	64 ± 5*	65 ± 3*	65 ± 3*	65 ± 2*
RPP, $min^{-1} \cdot mmHg \cdot 10^{-3}$							
Saline	23.2 ± 1.1	22.7 ± 1.1	22.6 ± 1.1	17.8 ± 0.9*	16.9 ± 1.2*	16.8 ± 0.9*	16.0 ± 0.8*
Vehicle	19.7 ± 1.5	20.5 ± 1.3	19.9 ± 1.4	15.3 ± 1.6*	15.8 ± 1.6*	15.0 ± 1.3*	13.7 ± 1.1*
Isoflurane	21.4 ± 2.5	19.9 ± 2.7	20.3 ± 2.3	16.8 ± 1.7	14.9 ± 0.9*	14.7 ± 1.0*	15.3 ± 1.1*
Enflurane	22.0 ± 1.9	18.9 ± 1.5*	18.9 ± 1.4*	14.5 ± 1.7*	15.8 ± 1.5*	16.5 ± 1.6*	17.6 ± 1.3*
Sevoflurane	22.9 ± 1.3	23.7 ± 1.7	22.1 ± 1.8	15.7 ± 1.6*	15.2 ± 1.3*	14.0 ± 1.2*	13.8 ± 0.8*
[ET], %							
Saline	—	—	—	—	—	—	—
Vehicle	—	—	—	—	—	—	—
Isoflurane	—	0.31 ± 0.02	0.03 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
Enflurane	—	0.27 ± 0.02	0.05 ± 0.02	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.01
Sevoflurane	—	0.34 ± 0.02	0.07 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.01

Data are presented as mean ± SEM; n = 8 (saline), 7 (vehicle), 8 (isoflurane), 8 (enflurane), 8 (sevoflurane).

* Significantly ($P < 0.05$) different from baseline.

[ET] = end-tidal concentration; HR = heart rate; MAP = mean arterial pressure; RPP = rate–pressure product.

group was excluded as a result of technical problems during instrumentation. Four rabbits were excluded because intractable ventricular fibrillation occurred during coronary occlusion (one saline, one lipid vehicle, and two sevoflurane).

Intravenous administration of saline, lipid vehicle, or emulsified sevoflurane had no apparent effect on the behavior or activity level of conscious rabbits. In particular, emulsified sevoflurane did not produce sedation, induce anesthesia, affect arterial blood gas tensions, or alter respiratory rate (data not shown). Furthermore, no differences in arterial blood gas tensions or respiratory rates were observed between experimental groups.

Hemodynamics and Myocardial Infarct Size

There were no differences in baseline hemodynamics among groups (table 5). Coronary artery occlusion and reperfusion caused similar decreases in heart rate, mean arterial pressure, and rate–pressure product in each experimental group. No differences in body weight, LV mass, AAR weight, or the ratio of AAR to LV mass were observed between groups (table 4). Myocardial infarct sizes were similar (44 ± 2% and 40 ± 4, respectively; fig. 3) in animals receiving either intravenous lipid vehicle or saline infusions 24 h before prolonged ischemia and reperfusion. In contrast, emulsified sevoflurane administered 24 h before coronary occlusion and reperfusion

Table 4. Left Ventricular Area at Risk

	Body Weight, g	LV, g	AAR, g	AAR/LV, %
Acute preconditioning				
Saline	2,838 ± 86	3.29 ± 0.12	1.10 ± 0.17	33 ± 4
Vehicle	2,702 ± 48	3.35 ± 0.17	1.06 ± 0.13	31 ± 3
Isoflurane	2,926 ± 206	3.23 ± 0.25	0.95 ± 0.10	29 ± 2
Enflurane	2,755 ± 49	3.05 ± 0.13	1.11 ± 0.12	36 ± 3
Sevoflurane	2,677 ± 54	2.76 ± 0.09	0.89 ± 0.08	32 ± 3
Delayed preconditioning				
Saline	2,737 ± 47	3.13 ± 0.10	0.90 ± 0.15	28 ± 4
Vehicle	2,838 ± 117	3.27 ± 0.15	1.04 ± 0.13	32 ± 3
Sevoflurane	2,690 ± 114	2.74 ± 0.17	0.74 ± 0.06	27 ± 2

Data are presented as mean ± SEM. Acute preconditioning: n = 8 (saline), 7 (vehicle), 8 (isoflurane), 8 (enflurane), 8 (sevoflurane). Delayed preconditioning: n = 7/group.

AAR = area at risk; LV = left ventricle.

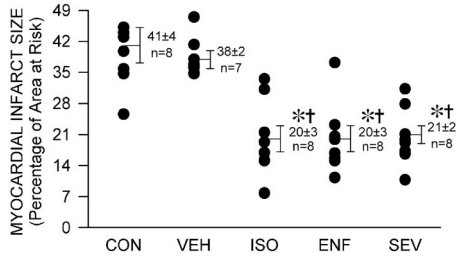

Fig. 2. Myocardial infarct size expressed as a percentage of the left ventricular area at risk for rabbits receiving 0.9% saline (CON, n = 8), lipid vehicle (VEH, n = 7), or emulsified isoflurane (ISO, n = 8), enflurane (ENF, n = 8), or sevoflurane (SEV, n = 8) in acute preconditioning experiments. * Significantly ($P < 0.05$) different from control. † Significantly ($P < 0.05$) different from lipid vehicle.

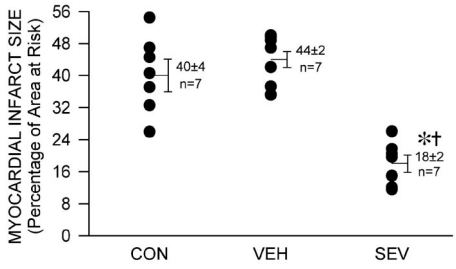

Fig. 3. Myocardial infarct size expressed as a percentage of the left ventricular area at risk for conscious rabbits receiving 0.9% saline (CON, n = 7), lipid vehicle (VEH, n = 7), or emulsified sevoflurane (SEV, n = 7) 24 h before prolonged coronary artery occlusion and reperfusion in delayed preconditioning experiments. * Significantly ($P < 0.05$) different from control. † Significantly ($P < 0.05$) different from lipid vehicle.

reduced infarct size (18 ± 2%) as compared with saline and lipid vehicle controls.

Discussion

Halogenated anesthetics produce protection against myocardial ischemic injury by activating a wide variety of intracellular signaling elements including mitochondrial adenosine triphosphate–dependent potassium channels, adenosine and opioid receptors, intracellular kinases, and reactive oxygen species.[1] Extensive investigation into the mechanisms responsible for anesthetic preconditioning has been conducted in recent years and has revealed that many of these signaling elements and their complex interactions are similar to those implicated in other forms of classic ischemic and pharmacologic preconditioning. Anesthetic preconditioning has been previously demonstrated in a variety of animal models of myocardial ischemia *in vitro* and *in vivo* and has also been shown to occur in humans with coronary artery disease.[10] This important latter observation is particularly exciting because the prevention or reduction of perioperative myocardial ischemia represents a major objective of anesthesiologists caring for patients with coronary artery disease.[11]

The current results confirm and extend many previous findings indicating that halogenated anesthetics acutely protect myocardium against irreversible ischemic injury. However, in the current investigation, emulsified halogenated anesthetics were administered intravenously before prolonged coronary artery occlusion and reperfusion. The results demonstrate that this intravenous route of halogenated anesthetic administration profoundly reduces infarct size *in vivo* to an extent similar to that observed during the inhalational route of administration.[12,13] The blood concentrations of isoflurane, enflurane, and sevoflurane generated during the intravenous infusion of these emulsified agents represent 0.27 ± 0.02, 0.42 ± 0.04, and 0.63 ± 0.04 vol%, respectively, when converted using a standard formula.[14,15] These blood concentrations are also equivalent to minimum alveolar concentration (MAC) values of 0.13 ± 0.01, 0.15 ± 0.01, and 0.17 ± 0.01, respectively.[16,17] Therefore, the current results indicate that relatively small

Table 5. Hemodynamics during Delayed Preconditioning Experiments

			Reperfusion		
	Baseline	Ischemia	1 h	2 h	3 h
HR, beats/min					
Saline	256 ± 12	234 ± 9*	220 ± 8*	218 ± 6*	215 ± 7*
Vehicle	261 ± 11	253 ± 13	231 ± 13*	222 ± 12*	220 ± 12*
Sevoflurane	229 ± 7	216 ± 7*	195 ± 6*	180 ± 6*	175 ± 4*
MAP, mmHg					
Saline	81 ± 3	63 ± 1*	66 ± 2*	67 ± 3*	68 ± 3*
Vehicle	88 ± 5	67 ± 7*	65 ± 5*	67 ± 5*	71 ± 5*
Sevoflurane	88 ± 4	70 ± 3*	69 ± 3*	68 ± 3*	72 ± 4*
RPP, $min^{-1} \cdot mmHg \cdot 10^{-3}$					
Saline	23.8 ± 1.8	17.5 ± 0.8*	17.1 ± 0.3*	16.7 ± 1.1*	16.3 ± 0.7*
Vehicle	25.1 ± 0.8	19.7 ± 2.5*	17.8 ± 1.6*	17.3 ± 1.6*	17.8 ± 1.4*
Sevoflurane	22.5 ± 1.5	17.5 ± 1.2*	15.8 ± 0.9*	14.6 ± 0.7*	14.6 ± 1.0*

Data are presented as mean ± SEM; n = 7/group.

* Significantly ($P < 0.05$) different from baseline.

HR = heart rate; MAP = mean arterial blood pressure; RPP = rate–pressure product.

concentrations of intravenous emulsified halogenated anesthetics are capable of producing cardioprotection without altering systemic hemodynamics in rabbits. Previous investigations have demonstrated that anesthetic preconditioning seems to be dose related. For example, our laboratory demonstrated that higher concentrations of isoflurane cause more pronounced reductions in infarct size during reduced coronary collateral perfusion in canine myocardium.[18] Nevertheless, end-tidal concentrations of isoflurane as low as 0.25 MAC exerted protective effects in dogs.[18] More recently, we demonstrated that 1.0 MAC but not 0.5 MAC isoflurane reduced infarct size in rats *in vivo*.[19] Other investigators have shown that isoflurane- and sevoflurane-induced protection against ischemic injury are dose dependent in Langendorff-prepared guinea pig hearts[20] and isolated rat ventricular myocytes.[21] Whether higher infusion rates of emulsified halogenated anesthetics are capable of producing greater decreases in myocardial injury and whether concomitant hemodynamic effects of higher doses modulate the protective effects of emulsified halogenated anesthetics remain to be determined. The threshold at which lower doses of intravenous emulsified halogenated agents also produce protection also remains to be defined. Additional research will be required to answer these important questions.

We[12] and others[22] have previously demonstrated that inhaled sevoflurane seems to produce a shorter memory period than isoflurane does. A 30-min elimination period after exposure to isoflurane resulted in a substantial reduction in infarct size,[13] but elimination of sevoflurane for a similar or shorter time period markedly reduced the protective effects of this agent in rabbits[22] and dogs.[12] These findings were attributed to differences in the blood-gas solubility coefficients between isoflurane and sevoflurane. In contrast, intravenous emulsified sevoflurane exerted cardioprotective effects after a 30-min elimination period in the current investigation. Concentrations of isoflurane lower than those that have been previously reported[18] also caused cardioprotection in the current investigation. Differences in pharmacokinetics between the intravenous emulsified as compared with the inhaled routes of administration may be responsible for the discrepancy between the current and previous results. For example, intravenous administration of emulsified sevoflurane resulted in a residual end-tidal concentration of 0.07 ± 0.01% at the end of the memory period (table 3). Residual sevoflurane may have contributed to the observed protective effect. Whether the presence of the lipid vehicle enhanced the amount of residual isoflurane, enflurane, or sevoflurane within the myocardium after the memory period or selectively altered the metabolism or elimination of these agents will require additional investigation to determine. Finally, the lipid vehicle may have allowed enhanced delivery of the volatile anesthetic to the site(s) of protective action within the cardiac myocyte.

Delayed ischemic preconditioning is a phenomenon in which protection against myocardial infarction occurs 24–72 h after the initial ischemic stimulus. Like acute ischemic preconditioning, this delayed protective process is characterized by complex signal transduction mediated by a variety of protein kinases,[23–25] nitric oxide synthase,[26] and cyclooxygenase 2.[27] Our laboratory recently demonstrated that isoflurane produces delayed cardioprotection in rabbits[4] but not dogs.[28] In the former experiments,[4] inhaled isoflurane (1.0 MAC) was administered for 2 h on the day before prolonged ischemia and reperfusion were conducted. The protection afforded by this delayed isoflurane exposure was similar in magnitude to that observed during acute preconditioning and was shown to be mediated by cyclooxygenase 2 because the beneficial effect was blocked by the selective cyclooxygenase-2 inhibitor celecoxib.[4] The current results with intravenous emulsified sevoflurane support and extend our previous finding with inhaled isoflurane. Administration of emulsified sevoflurane 24 h before ischemia reduced infarct size to an equivalent degree (18 ± 2% of the LV AAR) as was observed during acute preconditioning experiments (21 ± 2%). Importantly, intravenous emulsified sevoflurane produced this delayed protection independent of any apparent sedative effects or respiratory depression in conscious rabbits while the drug was being administered. These results raise the possibility that intravenous emulsified halogenated agents may be used as therapeutic agents in the management of patients with acute myocardial ischemia or may be capable of evoking delayed pharmacologic preconditioning in the absence of sedation or anesthesia in humans. However, these tantalizing hypotheses will require further study to confirm.

The current results must be interpreted within the constraints of several potential limitations. All groups of rabbits received similar quantities of barbiturates during experimentation. A potential interaction between this baseline barbiturate anesthetic and the emulsified halogenated agents cannot be entirely excluded from the analysis during acute preconditioning experiments. Nevertheless, only rabbits that received emulsified halogenated anesthetics and not lipid vehicle alone demonstrated protection against myocardial infarction in this setting. Myocardial infarct size is determined primarily by the size of the AAR and extent of coronary collateral perfusion. The AARs expressed as a percentage of total LV mass were similar between groups in the current investigation. In addition, rabbits have been shown to have little if any coronary collateral blood flow.[29] Therefore, it seems unlikely that differences in collateral perfusion between groups account for the observed results. However, coronary collateral blood flow was not specifically quantified in the current investigation. The reduc-

tions in myocardial infarct size produced by emulsified isoflurane, enflurane, and sevoflurane occurred independent of changes in the major determinants of myocardial oxygen consumption. Nevertheless, myocardial oxygen consumption was also not directly measured in the current investigation.

In summary, the current results demonstrate that intravenous administration of a new formulation of emulsified isoflurane, enflurane, or sevoflurane produces acute myocardial protection against infarction in rabbits. The current results also indicate that pretreatment of conscious rabbits with emulsified sevoflurane 24 h before prolonged coronary artery occlusion and reperfusion does not cause sedative effects or respiratory depression but protects myocardium against infarction to a degree equivalent to that of exposure 1 h before the ischemic stimulus. The intriguing possibility that small quantities of intravenous emulsified halogenated anesthetic may produce acute or delayed myocardial protection devoid of other cardiovascular actions or sedation deserves future clinical investigation.

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