

Differential Protective Effects of Halothane and Isoflurane Against Hypoxic and Reoxygenation Injury in the Isolated Guinea Pig Heart

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The authors investigated the effects of halothane (HAL) and isoflurane (ISO) on cardiac depression produced by global hypoxia and the recovery of function following reoxygenation in isolated guinea pig hearts perfused with Krebs' solution at constant pressure. Isovolumetric left ventricular systolic (LVSP) and end-diastolic pressures (LVEDP) were measured by placing a saline filled, latex balloon into the left ventricle. Bipolar electrodes were placed in the right atrium and right ventricle for measurement of heart rate (HR), atrioventricular conduction time (AVCT), and determination of the incidence and severity of dysrhythmias occurring during hypoxia and reoxygenation. Hearts were divided into three groups: control ($n = 20$), halothane ($n = 12$), and isoflurane ($n = 13$). All hearts were exposed in sequence to oxygenated perfusate (P_{O_2} , 530 mmHg), moderately hypoxic perfusate (P_{O_2} , 91 mmHg) for 30 min, and then to oxygenated perfusate for 40 min. Halothane (1%, 0.4 mM) or isoflurane (1.5%, 0.5 mM) were administered 10 min before hypoxia, during hypoxia, and during the first 10 min of reoxygenation. Exposure to halothane and isoflurane before hypoxia produced a 14 and 11% decrease in heart rate, a 32 and 23% increase in AVCT, and a 47 and 28% decrease in LVSP (all $P \leq 0.001$) for halothane and isoflurane, respectively, and no significant change in LVEDP. During hypoxia, HR decreased and AVCT increased similarly in both groups. Left ventricular systolic pressure (LVSP) decreased sharply with a narrowing of the prehypoxic differences among the groups. In the control and isoflurane groups, LVEDP increased during hypoxia but remained unchanged in the halothane group. After 40 min of reoxygenation, LVEDP in the control group (11 ± 3 mmHg) was significantly higher than in the halothane group (0.5 ± 1.0 mmHg) but not significantly different from the isoflurane group (5.5 ± 3.0 mmHg). Halothane reduced the incidence of ventricular tachycardia from 80 to 42% and of ventricular fibrillation from 70 to 17% during the 30-min period of hypoxia and during

the first 30 min of reoxygenation, respectively, and produced a threefold increase in the average time the hearts were in sinus rhythm. The results indicate that halothane protects the isolated heart against hypoxic damage as assessed by improved recovery of LVSP, reduced LVEDP, and reduced incidence and duration of dysrhythmias during hypoxia and reoxygenation. The mechanism of protection by halothane could be related to reduced cardiac work before and after hypoxia or possibly to other direct protective myocardial cellular effects. (Key words: Anesthetics, volatile: halothane; isoflurane. Animal: guinea pig. Heart: arrhythmias; electrophysiology; hypoxia; isolated; left ventricular pressure; myocardial protection; perfused; ventricular fibrillation. Hypoxia.)

ARTERIAL HYPOXEMIA AND hypoxic coronary perfusion can occur during the course of general anesthesia. These can result from ventilatory problems such as severe pulmonary edema, aspiration, bronchospasm, improper intubation or endobronchial obstruction, or in association with pulmonary perfusion problems, including deficient hypoxic pulmonary vasoconstriction, ventilation/perfusion mismatch, and right to left intracardiac shunting. Myocardial hypoxia with reoxygenation, like ischemia with reperfusion, is associated with compromised cardiac function and a variety of dysrhythmias. Although the mechanisms underlying myocardial injury under these conditions are multifactorial and not well understood, excess calcium ion influx and its intracellular accumulation appear to be important events leading to a decline of contractile function and the development of arrhythmias. Intracellular calcium may increase because of an increase in sarcolemmal permeability to calcium,¹ enhanced release of intracellularly stored calcium, decreased capacity of sarcoplasmic reticulum for calcium binding,² and impaired sodium/calcium exchange.³ Intracellular calcium accumulation in the hypoxic heart is preceded by a decline in peak developed tension and an increase in resting tension resulting from a depletion in high-energy phosphate reserves. Intracellular accumulation of free radicals,⁴ hydrogen and sodium ions, and depletion of potassium ions also occurs during hypoxia.⁵

Volatile anesthetics, like calcium entry blockers and beta blockers, depress myocardial function but are not known to block specific receptors altering automaticity, conduction, or contractility of the heart. Calcium entry blockers, beta blockers, and steroids⁶ have been found to protect cellular ultrastructure and mitochondrial function

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during hypoxia.⁷ Because of its potent negative inotropic effects, the volatile anesthetic halothane is often used to limit myocardial oxygen demand and thereby to reduce the occurrence of ischemic episodes in patients undergoing myocardial revascularization.⁸ Halothane and isoflurane have been shown to decrease both slow (Ca^{2+})⁹ and fast (Na^+)¹⁰ ionic channel fluxes and to reduce the transient rise of myoplasmic calcium during myocardial contraction.^{11,12} In addition, under abnormal conditions, halothane depresses calcium accumulation following ischemia or calcium paradox.^{13,14} Halothane also decreases the incidence of dysrhythmias following ischemia and reperfusion in dogs¹⁵ and rats.¹⁶ In addition, halothane and isoflurane improve recovery of contractile function of "stunned" myocardium,¹⁷ and halothane reduces myocardial infarct size in dogs.¹⁸ These effects may be beneficial for the ischemic or hypoxic myocardium. Isoflurane decreases myocardial contractility to a smaller degree than halothane in intact hearts and in isolated animal and human myocardium.¹⁹ The negative inotropic effects of isoflurane, but not halothane and enflurane, may be, in part, due to a decrease in responsiveness of contractile proteins to calcium.²⁰ Little is known about the potential myocardial protective effects of volatile anesthetics during hypoxia and reoxygenation. This study compares changes in cardiac rhythm and contractile function caused by global cardiac hypoxia and reoxygenation with and without exposure to halothane and isoflurane.

Methods

The isolated Langendorff heart preparation was selected for study so that extrinsic mechanical, humoral, and autonomic nervous system influences could be avoided. With prior approval of the institutional Animal Care Committee, 45 albino English short-haired guinea pigs (weight, 400–600 g) were injected intraperitoneally with 10 mg ketamine and 1000 U heparin and were decapitated when unresponsive to noxious stimulation. After thoracotomy, the inferior and superior vena cavae were cut, and the aorta was cannulated distal to the aortic valve. The heart was immediately perfused retrograde through the aorta and was excised. The perfusate, a modified Krebs' solution, had the following composition (in millimolar concentrations): Na^+ , 137; K^+ , 4.5; Mg^{2+} , 1.2; Ca^{2+} , 2.5; Cl^- , 134; HCO_3^- , 15.5; H_2PO_4^- , 1.2; glucose, 11.5; mannitol, 16; EDTA, 0.05; and insulin, 5 U/l. During control periods (normoxia), the solution was equilibrated with a 97% O_2 /3% CO_2 gas mixture at a flow rate of 3 l/min. At oxygen tensions above 400 mmHg, myocardial high energy phosphate levels are maintained²¹ and lactate is not produced in isolated hearts perfused with crystalloid solution. Global hypoxia was induced by switching to perfusate equilibrated with 97% N_2 /3% CO_2 . Inflow $p\text{H}$,

P_{CO_2} , and P_{O_2} were determined with a blood gas analyzer (Radiometer® ABL-2, Medtron, Chicago, IL). Each heart was perfused with filtered (5 μm) nonrecirculating solution and submerged in a bath of perfusate solution. The perfusate solution and bath temperature were maintained at $36.3 \pm 0.1^\circ \text{C}$ using a thermostatically controlled water circulating system. Perfusion pressure was maintained at 55 mmHg by a 75-cm high-fluid column with an overflow pump (Masterflex 7520, Cole Palmer Inst. Co., Chicago, IL), and was measured at the aortic root with a pressure transducer.

Systolic and diastolic left ventricular pressure (LVSP and LVEDP) were measured isovolumetrically with a transducer connected to a thin, saline-filled latex balloon inserted into the left ventricle through the mitral valve from a cut in the left atrium. Balloon volume was adjusted to maintain an LVEDP of 0 mmHg during the initial control period.

Two pairs of bipolar electrodes (Teflon® coated silver, 125 μm diameter, Cooner Wire Company, Chatsworth, CA) were placed in each heart to monitor intracardiac electrograms from which spontaneous sinoatrial rate and atrioventricular conduction time (AVCT) were measured as reported previously.²² The two electrode signals were amplified 100–1000-fold, filtered at frequencies below 1 Hz and above 10 KHz, and were displayed continuously on a polygraph (MT 9500R, Astro-Med Corp., West Warwick, RI) and on an image storing oscilloscope (5A26, 5113, Tektronix, Inc., Rolling Meadows, IL). Sinus cycle length was measured and spontaneous HR was calculated from the right atrial beat-to-beat interval; AVCT was determined from the superior right atrial signal to the right ventricular pulmonary conus signal. Atrial and ventricular electrograms were audibly amplified. Electrograms, LVSP, LVEDP, perfusion pressure, and a calibrated 50-ms square wave pulse signal were intermittently tape recorded (Vetter D1, A.R. Vetter Co., Rebersburg, PA) at 38 cm/s for later playback at 5.4 cm/s. Electrogram intervals were measured automatically online by digital timer systems that allowed instantaneous beat-to-beat interval and rate analyses. Atrioventricular dissociation was defined by asynchrony in atrial and ventricular electrogram activity that persisted for at least 1 min. Premature ventricular excitation (PVE) was defined as five or more ventricular extrasystoles during 1 min or three or more consecutive extrasystoles. Ventricular tachycardia (VT) was defined by the presence of uniform or multiform ventricular waveforms and a faster ventricular than atrial rate. Ventricular fibrillation (VF) was defined by the presence of erratic activity in the ventricular electrogram and by the absence of pressure generation by the left ventricle.

Anesthetics were administered by switching to perfusate equilibrated with 1% halothane *via* a Draeger® (Draegerwerk, Lubeck, Germany) vaporizer or 1.5% isoflurane

via a Fluotec® (Cyprane, Keighley, England) vaporizer. Delivered halothane and isoflurane fractions were periodically checked for accuracy by mass spectrometry (Perkin-Elmer, Medical Gas Analyzer® 1100A, Norwalk, CT). Perfusate was collected at an aortic inflow port into sealed, air-free 1-ml vials, before, during, and after hypoxia for measurement of anesthetic concentrations by gas chromatography as described previously.²² Mean halothane and isoflurane concentrations were 0.40 ± 0.01 mM and 0.50 ± 0.01 mM, respectively. Halothane and isoflurane concentrations did not change before, during, and after hypoxia.

PROTOCOL

Drug-free control measurements were recorded after a 30-min period of stabilization. Only hearts with no more than two single premature atrial or ventricular beats per min and a LVSP higher than 70 mmHg during the initial control period were used in this study. Following a 10-min initial control period for all groups and a 10-min exposure to halothane or isoflurane in these groups, each heart was perfused for 30 min with hypoxic perfusate and then for 40 min with oxygenated perfusate. In the halothane and isoflurane groups, the anesthetics were continued during hypoxia and for the first 10 min of reoxygenation. Intracoronary lidocaine (0.5 mg) was administered to all hearts 30 min after reoxygenation to assess conversion of dysrhythmias and recovery of LVSP and LVEDP. Experiments were terminated 10 min after administration of lidocaine. Measurements were made during the last minute of the initial stabilization period, after a 10-min exposure to normoxic perfusate (with or without anesthetics), and just before initiating hypoxic perfusion at 5, 15, and 30 min of hypoxia, and at 10 and 40 min of reoxygenation. Variables recorded at each time period were as follows: spontaneous HR, AVCT, LVSP and LVEDP, and inflow pH, P_{CO_2} , and P_{O_2} . The incidence of and type of atrial and ventricular dysrhythmias were monitored continuously.

Statistical differences in the time-dependent changes among the three groups were determined by two-way analysis of variance and Fisher's least significant difference tests. The incidence of dysrhythmias was evaluated by chi-squared tests and their duration by unpaired *t* tests. Mean values were considered significant at $P \leq 0.05$. All data are expressed as mean \pm SEM.

Results

There were no significant differences among the three groups in perfusate P_{CO_2} (30.1 ± 0.9 mmHg) and pH (7.38 ± 0.01) during normoxia or during hypoxia with substitution of 97% N₂ for 97% O₂. P_{O_2} decreased from 530 ± 20 (control) to 105 ± 3 mmHg at 5 min of hypoxia and to 91 ± 4 mmHg at 30 min of hypoxia. There was no significant difference in P_{O_2} among the halothane, isoflurane, and control groups during the control period or during hypoxia.

EFFECTS ON HEART RATE AND ATRIOVENTRICULAR CONDUCTION TIME

Initial control HR and AVCT were not different among the three groups (table 1). During normoxia, halothane and isoflurane significantly decreased HR and significantly increased AVCT ($P \leq 0.05$). Atrioventricular conduction time increased sharply on induction of hypoxia in all three groups, while HR decreased only slightly. However, HR continued to decrease throughout the 30-min period of hypoxia such that rates were not different at 30 min of hypoxia. Heart rate was higher in the control group than in the groups given halothane or isoflurane before hypoxia and at 10 min of reoxygenation; there was no difference in HR after 40 minutes of reoxygenation. Because of the small number of hearts in the control group remaining in sinus rhythm at 30 min of hypoxia and at 10 min of reoxygenation, AVCT could not be compared. After conversion of some hearts to sinus rhythm at 40 min of reoxygenation, there were no significant group differ-

TABLE 1. Effects of Halothane and Isoflurane on Atrial Rate and AV Conduction Time Before, During, and After Hypoxia

Variable → Group →	Atrial Rate (beats per min)			AV Time (ms)		
	CON	HAL	ISO	CON	HAL	ISO
Control 1	208 ± 4	203 ± 7	198 ± 6	61 ± 2	62 ± 4	61 ± 3
Control 2	206 ± 7	174 ± 6*†	176 ± 5*†	66 ± 6	83 ± 3*†	75 ± 3*†‡
Hypoxia 5 min	198 ± 6†	172 ± 3*†	177 ± 5†	111 ± 11†	114 ± 6†	100 ± 6†
Hypoxia 30 min	143 ± 11†	122 ± 6†	139 ± 12†	DYSR	119 ± 4†	110 ± 8†
Reoxygenation 10 min	208 ± 8	179 ± 7†	181 ± 5†	DYSR	80 ± 3†	82 ± 5†
Reoxygenation 40 min	214 ± 4	209 ± 7	196 ± 7	66 ± 5	65 ± 2	67 ± 3

Control 1 = values at initial control; control 2 = values after exposure to 1% halothane for 10 min or after a 10-min time control; CON = control (n = 20); HAL = 1% halothane (n = 12); ISO = 1.5% isoflurane (n = 13); AV time = atrioventricular conduction time; DYSR

= majority of the hearts exhibited continuous dysrhythmias.

All data are means \pm standard error of mean.

* $P \leq 0.05$ versus CON group; † $P \leq 0.05$ versus control 1; ‡ $P \leq 0.05$ HAL versus ISO.

ences in HR and AVCT, and no differences in HR and AVCT from the initial control period for any group. Administration of lidocaine to nonfibrillating hearts produced small and transient decreases in HR and LVSP and a small increase in AVCT; all these variables returned to control values within 5 min.

EFFECTS ON CARDIAC RHYTHM

Figure 1 shows temporal changes in cardiac rhythm for individual hearts in each group during the 30-min period of hypoxia and during the 40-min period of reoxygenation. At the onset of hypoxia, AVCT rapidly increased; in several hearts from all groups, this increase progressed to second or third degree AV block which often preceded ventricular tachycardia (VT) or fibrillation (VF). Following reoxygenation for 30 min, lidocaine converted many fibrillating hearts to sinus rhythm, at least temporarily. Halothane and isoflurane caused a significant delay in onset of dysrhythmias during hypoxia ($P \leq 0.05$).

Halothane increased the incidence of third-degree AV block (fig. 2) and isoflurane increased the incidence of

PVEs. Both halothane and isoflurane significantly decreased the incidence of VF. In the halothane and isoflurane groups, the percentage of hearts in VF during 30 min of hypoxia and the first 30 min of reoxygenation was significantly smaller than in the control group. Figure 3 shows that the incidence of VF increased during hypoxia and reoxygenation in the control group. The incidence of VF in the control group was higher at 30 min of reoxygenation (before lidocaine) than at 15 min of hypoxia. In the halothane group, the incidence of VF at 30 min of reoxygenation was significantly lower than in the control group.

Figure 4 shows that hearts in the halothane group and the isoflurane group were in sinus rhythm 2.2 and 1.7 times longer, respectively, than those in the control group ($P \leq 0.01$) during the 30-min period of hypoxia and the 30-min period of reperfusion before lidocaine was given. Isoflurane decreased the duration of third degree AV block, and there were no group differences in the average duration of second degree AV block or of PVEs during these periods. Halothane, but not isoflurane, decreased the duration of VT and VF. Ventricular tachycardia lasted

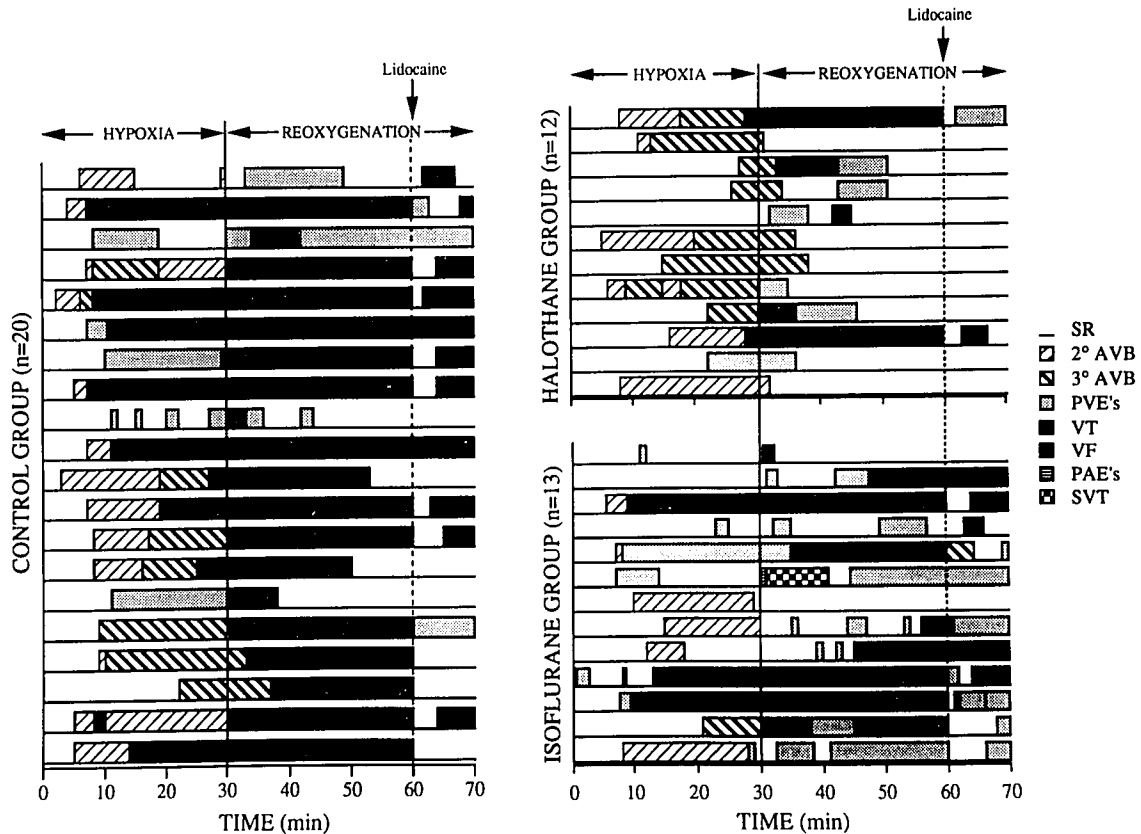


FIG. 1. Temporal changes in cardiac rhythm for control (n = 20), 1% halothane (n = 12), and 1.5% isoflurane (n = 13) groups. Each horizontal line represents the cardiac rhythm of an individual heart during 30 min of hypoxia and during 40 min of reoxygenation. SR = sinus rhythm; 2° AVB = second-degree AV block; 3° AVB = third-degree AV block; PVEs = premature ventricular excitations; VT = ventricular tachycardia; VF = ventricular fibrillation; PAEs = premature atrial excitations; SVT = supraventricular tachycardia.

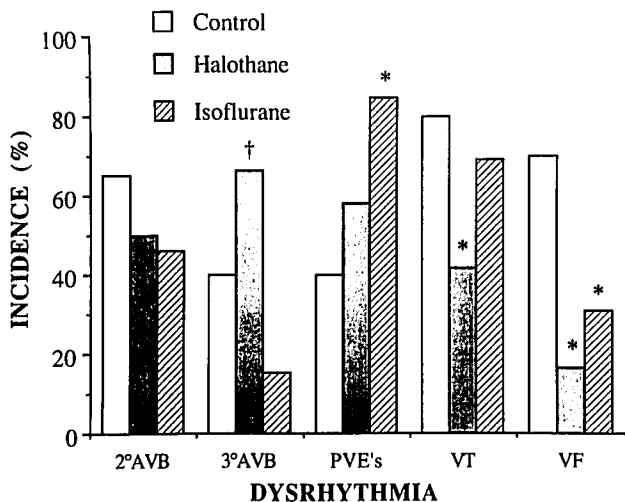


FIG. 2. Incidence of several dysrhythmias during 30 min of hypoxia and during the first 30 min of reoxygenation. Abbreviations are the same as in figure 1. * $P \leq 0.05$ halothane group versus control group; † $P \leq 0.05$ halothane versus isoflurane group.

five times longer and VF lasted six times longer in control group hearts than in halothane group hearts.

EFFECTS ON LEFT VENTRICULAR SYSTOLIC AND DIASTOLIC PRESSURES

Exposure to halothane and isoflurane for 10 min before the onset of hypoxia significantly decreased LVSP by 47 ± 3 and $28 \pm 3\%$, respectively ($P \leq 0.01$). Left ventricular systolic pressure remained unchanged in the control group during the same period (fig. 5). At 5 min of hypoxia, LVSP decreased in all groups, and there was no significant

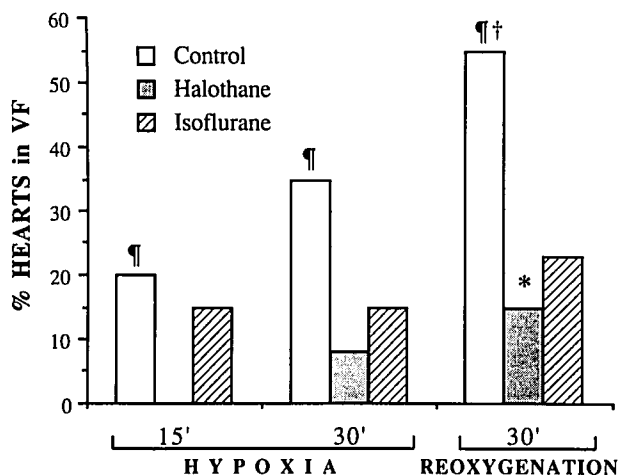


FIG. 3. Incidence of ventricular fibrillation at 15 and 30 min of hypoxia and at 30 min of reoxygenation. * $P \leq 0.01$ versus control group; † $P \leq 0.01$ versus no ventricular fibrillation; ‡ $P \leq 0.01$ versus 15 min of hypoxia.

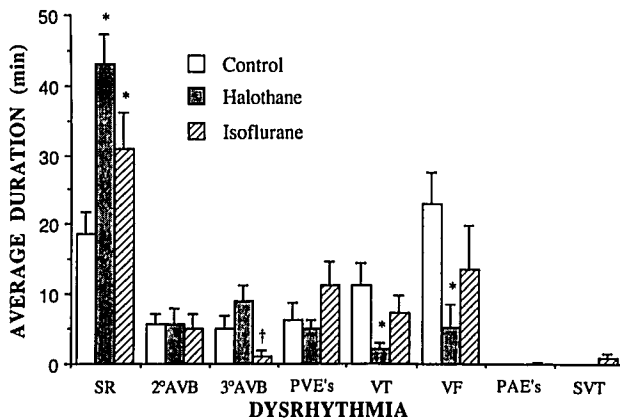


FIG. 4. Average time in a given cardiac rhythm for control, halothane, and isoflurane groups during 30 min of hypoxia and the first 30 min of reoxygenation before lidocaine was given. Abbreviations are the same as in figure 1. * $P \leq 0.05$ halothane group versus control group; † $P \leq 0.05$ halothane versus isoflurane group.

difference in LVSP between the control and the isoflurane groups. In the halothane group, however, LVSP was significantly lower during hypoxia, and a lower diastolic pressure in the halothane group offset the decrease in systolic pressure resulting in no difference among groups in pressure generated during systole. At 10 min of reoxygenation, LVSP increased in all groups with no difference between the control and isoflurane groups; LVSP was lower in the halothane group than in the other groups. At 40 min of reoxygenation, LVSP was significantly higher in the halothane group than in the control group. In no group was LVSP restored to the initial control val-

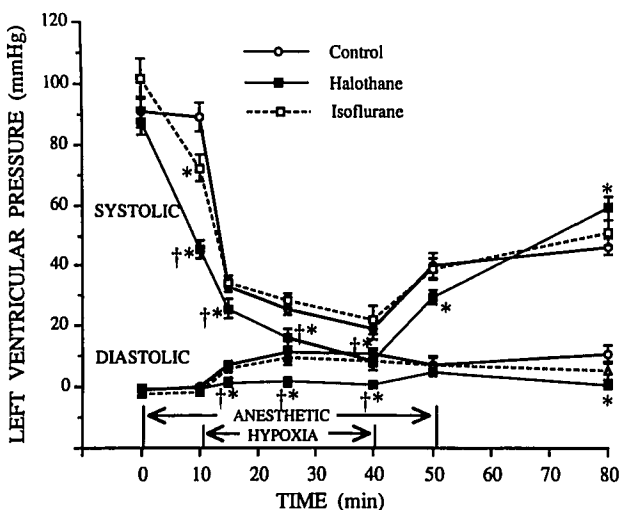


FIG. 5. Changes in systolic and diastolic left ventricular pressures during hypoxia and reoxygenation with or without exposure to halothane or isoflurane. * $P \leq 0.05$ control group; † $P \leq 0.05$ halothane versus isoflurane group.

ues. In a time control group ($n = 9$), *i.e.*, in the absence of experimental maneuvers, LVSP decreased from 96 ± 5 to 89 ± 6 mmHg within 120-min time period, and LVEDP remained unchanged (data not shown).

Exposure to halothane and isoflurane before hypoxia did not change LVEDP from control (set initially at 0 mmHg), and it remained unchanged in the halothane group throughout the hypoxia and reoxygenation. In contrast, after 5 min of hypoxia, LVEDP increased in the control and isoflurane groups and remained elevated throughout the hypoxic period. Reoxygenation slightly, but nonsignificantly, increased LVEDP in the halothane group initially. At 10 min of reoxygenation, there was no significant difference among the groups. LVEDP returned to the prehypoxic control level in the halothane group. In the control and isoflurane groups, diastolic pressure remained elevated after 40 min of reoxygenation.

Discussion

The current study examined the influence of halothane and isoflurane on myocardial injury resulting from global hypoxia and reoxygenation in the isolated heart as assessed by depression of LVSP, elevation of LVEDP, and the type, incidence, and duration of dysrhythmias. The findings indicate that exposure to halothane, but not to isoflurane, before, during, and after a 30-min period of global hypoxia facilitates the recovery of LVSP during reoxygenation and prevents the increase in LVEDP during hypoxia and reoxygenation. Both halothane and isoflurane decreased the incidence of ventricular tachycardia and fibrillation and increased the time in sinus rhythm occurring during hypoxia and reoxygenation; halothane also reduced the duration of ventricular tachycardia and ventricular fibrillation.

Hypoxia contributes importantly to cardiac changes during myocardial ischemia, but hypoxia can occur during adequate myocardial perfusion. As in ischemia, hypoxic perfusion causes a rapid depletion of endogenous reserves of adenosine triphosphatase (ATP) and phosphocreatine. But hypoxia differs from ischemia in that the removal of metabolic byproducts such as hydrogen ion continues and therefore lessens the degree of acidosis.² Also, during hypoxia, normal levels of calcium, substrates for anaerobic energy production, and buffers are delivered. Although calcium is present in the extracellular space during hypoxia, the rate of tissue calcium uptake is unchanged during hypoxia but increased during reoxygenation.²³ Increased mitochondrial calcium uptake is, at least in part, responsible for calcium uptake and impaired recovery of myocardial function after hypoxia.²⁴ Along with other factors, an increase in intracellular calcium concentration may activate membrane-located phospholipases, and other enzyme systems involved in energy production, to cause energy depletion with a resultant decline of contractile

function.²⁵ Early development of contracture during hypoxia is probably due to persistent, calcium-activated, diastolic contraction secondary to impaired calcium re-sequestration by the sarcoplasmic reticulum.² During ischemia, this effect of the decrease in oxygen supply is counteracted by the collapse of coronary vasculature and the development of intracellular acidosis that has been shown to decrease myofibrillar calcium sensitivity.²⁶

Volatile anesthetics are potent negative chronotropic, dromotropic, and inotropic agents.^{12,22,27} Increasing evidence suggests that volatile anesthetics produce negative inotropic effects through mechanisms affecting many steps in the excitation-contraction process. Halothane and isoflurane decrease contractility of papillary muscle and myoplasmic calcium concentration as measured by beat-to-beat changes in bioluminescence of aequorin, an indicator of free intracellular calcium.¹² Using a single ventricular cell clamping technique, it was shown that the decrease in intracellular free calcium, with exposure to halothane and isoflurane, is at least due in part to a decrease in calcium flux through voltage-dependent calcium channels.⁹ Halothane, isoflurane, and enflurane decreased the calcium current to a similar degree. Moreover, halothane was shown to reduce accumulation of calcium following regional myocardial ischemia and after temporary depletion of calcium from the perfusate (calcium paradox), and to improve contractile function with reperfusion in isolated guinea pig heart.^{13,14} All these studies suggest that halothane and isoflurane have at least one effect similar to calcium channel blockers, *i.e.*, attenuation of the slow calcium current through voltage-dependent calcium channels. This is important since alterations of calcium ion homeostasis play an important role in the cascade of events associated with irreversible myocardial injury.

Verapamil has been shown to protect against hypoxic myocardial damage as manifested by a decreased rate of enzyme leakage, preservation of fine ultrastructure, and maintenance of near normal mitochondrial oxidative phosphorylation and Ca^{2+} -accumulating activities.⁷ Perhaps because of similar negative effects of calcium entry blockers and volatile anesthetics on transmembrane calcium flux and contractility, our results suggest that volatile anesthetics also afford protection during and after hypoxia. The finding that halothane had better protective effects than isoflurane on recovery of contractile function may be due to its greater depression of contractility^{12,22,27} and oxygen consumption²⁷ before and after hypoxia.

During hypoxia, halothane prevented the increase in LVEDP observed during hypoxia, whereas isoflurane did not alter development of contracture. Since in this preparation the volume of the balloon (and therefore the volume of the left ventricle) was kept constant, an increase in LVEDP reflected an increase in left ventricular wall stiffness or contracture. The mechanism of early myocardial contracture during hypoxia and its protection by

halothane is not clear. Because calcium does not begin to accumulate until later during hypoxia,²⁴ intracellular redistribution, rather than net uptake of the calcium from extracellular space,^{25,28} is the likely mechanism of the early rise in diastolic pressure (stiffness).²⁹ At physiologic intracellular pH, halothane reduces both the rate of Ca^{2+} sequestration of isolated cardiac sarcoplasmic reticulum (SR) and the capacity of SR vesicles for Ca^{2+} sequestration.³⁰ The decreased amount of Ca^{2+} in SR available for redistribution may be responsible for the lusitropic effect of halothane on EDLVP. In addition to a decreased oxygen supply, increased oxygen demand may also induce development of hypoxic contracture; e.g., tachycardia produced by pacing in the presence of coronary stenosis is believed to develop through this mechanism.²⁶ It has been demonstrated in the same isolated perfused guinea pig model that halothane improves the oxygen supply to demand ratio during hypoxia more by decreasing oxygen extraction than by increasing oxygen supply.²⁷ In contrast, isoflurane improves this ratio more by increasing O_2 delivery and less by decreasing O_2 extraction. These differences may account for the better cardioprotective effects of halothane than of isoflurane.

Before the onset of hypoxia, LVSP decreased in the halothane and isoflurane groups which resulted in reduced oxygen demand.^{27,31} Volatile anesthetics might act to protect against early hypoxia-induced injury by decreasing the cardiac energy expenditure as shown by the differences in LVSP observed early during hypoxia. But this early protective effect may be lost later during hypoxia and early reoxygenation, since there were no differences in the pressure generated; i.e., the LVSP-EDLVP difference was unchanged during these periods by either anesthetic. LVSP was significantly greater in the halothane group at 40 min of reoxygenation. A greater recovery of LVSP in the halothane group versus the isoflurane group could reflect better protection by an improved O_2 supply to demand ratio with halothane than with isoflurane,²⁷ since HR and LVSP, the dynamic determinants of O_2 demand and supply, decreased more with halothane than with isoflurane. Indeed, a protective effect of lowered HR against dysrhythmias occurring during reperfusion in isolated rat hearts has been demonstrated;³² however, the study required a large number of hearts to show a significant beneficial effect of reduced HR. Another study reported that the incidence of dysrhythmias is not related to HR.¹⁶ A direct effect on decreasing the accumulation of calcium during and after hypoxia, as shown for ischemia,^{13,14} is another possibility.

Halothane may have both pro- and antidysrhythmic effects. Disturbances in automaticity and conduction caused by halothane can lead to cardiac dysrhythmias. We found that halothane diminished the changes in the refractory period of ventricular muscle fibers induced by hypercarbia³³ and that halothane decreased the rate of

spontaneous arrhythmias originating in Purkinje's fibers surviving in the ischemic region of canine heart infarcted 24 hours earlier.³⁴

The antidysrhythmic effect observed with halothane, and to a lesser degree with isoflurane, appears to be a consequence not only of the presence of anesthetics during early reoxygenation but also of prior exposure to anesthetics, because these groups exhibited a lower incidence of dysrhythmias 20 min after the discontinuation of halothane. A possible explanation is that anesthetics attenuate damage to the conduction system or to myocardial cells that have a potential for abnormal automaticity secondary to hypoxia and reoxygenation. The control group exhibited a shorter time in sinus rhythm and a longer time in VF. Isoflurane increased the incidence of PVEs, but this probably reflects lesser incidences of more serious VF and VT.

During hypoxia, AVCT increased and HR decreased. In the isolated heart, these changes may be protective in that metabolic demand is decreased. Occurrence of conduction block may reflect increased refractoriness of the AV node. Endogenously released adenosine is a cause for at least part of the increase in AVCT during hypoxia.³⁵ Although the sinoatrial (SA) rate and AV nodal conduction were dramatically altered during hypoxia, the complete recovery of HR and AVCT with reoxygenation is consistent with the relative resistance of nonworking, specialized nodal fibers to structural damage caused by global hypoxia and reoxygenation.³⁶ A similar decrease in HR during hypoxia and recovery during reoxygenation has been observed in studies by Stowe *et al.* of the isolated guinea pig SA node.³⁷

Overall, our *in vitro* study indicates that halothane is more effective than isoflurane in directly ameliorating the degree of dysfunction induced by global hypoxia and reoxygenation under these experimental conditions. Compared to the control group, the halothane group demonstrated improved contractility, increased diastolic compliance, and reduced incidence and duration of serious ventricular dysrhythmias with reoxygenation. The mechanism could be a result of decreased myoplasmic accumulation of calcium during reoxygenation or decreased metabolic requirements for oxygen afforded by halothane before and after reoxygenation. Isoflurane, which is a cardiodepressant agent to a lesser degree than halothane, was not as effective; this suggests that protection may be proportional to the increase in oxygen delivery or to the decrease in oxygen requirements before, during, and after hypoxia. *In vivo* global hypoxia of increasing duration would have more serious sequelae because as hypotension occurs and coronary flow decreases, cardiac ischemia also ensues and death may result from intractable dysrhythmias and cardiac standstill. If hypoxemia occurs during general anesthesia, rapid reoxygenation and cardiopulmonary resuscitation may be necessary

to prevent myocardial damage. However, the relative increase in the O₂ supply-to-demand relationship, or the calcium flux effects, afforded by volatile anesthetics before the onset and during hypoxemia could be beneficial in improving myocardial function with reoxygenation.

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