Anesthesiology
69:17–23, 1988

Myocardial Tolerance to Total Ischemia in the Dog Anesthetized with Halothane or Isoflurane

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Myocardial tolerance to total ischemia was compared in animals anesthetized with halothane or isoflurane by measuring the time required for development of cardiac rigor in the absence of coronary circulation or wall stress. Sixteen dogs, eight in each group, were anesthetized with equally potent inspired concentrations of either halothane (2 MAC) or isoflurane (2 MAC), intubated, and ventilated. Thirty minutes later, the heart was rapidly excised. A left ventricular slab was prepared and maintained at 37° C. A portion of each slab was placed in a compressibility gauge that detects rigor onset by an abrupt increase in resistance to tissue deformation. Subendocardial tissue pressure was continuously measured in a second slab using needle-tipped Millar pressure transducers. A third slab was used for intermittent tissue sampling and HPLC assay of high-energy nucleotide levels. There were no differences in pre-ischemic heart rate, mean arterial pressure, glucose, lactate, P_{O_2}, P_{CO_2}, *p*H, plasma epinephrine, or norepinephrine levels between the two groups. The onset of rigor as measured by the compressibility gauge was delayed in the halothane group (68 ± 7.2 *vs.* 60 ± 5.0 min; $P < .05$). Tissue ATP and ADP levels declined throughout the period of ischemia, with a trend towards preservation in the halothane group. The data show that myocardial tolerance to total normothermic ischemia is improved in animals anesthetized with halothane compared to isoflurane, independent of the effects on hemodynamics or collateral coronary circulation. (Key words: Anesthetics, volatile: halothane; isoflurane. Heart: myocardial ischemia; myocardial protection.)

CONTROVERSY EXISTS OVER the relative influence of halothane and isoflurane on myocardial ischemia. Recent clinical and laboratory data have shown that regional myocardial ischemia may occur more frequently with isoflurane, presumably because of different effects on hemodynamic determinants of myocardial oxygen supply and demand and alterations of collateral coronary circulation.[1–5] It is also possible that isoflurane and halothane exert different metabolic influences that may affect myocardial tolerance to ischemia. A great deal of work has demonstrated that hypothermia and cardioplegic arrest influence ischemic myocardial metabolism in a manner that improves tolerance to global ischemia during cardiac surgery.[6–9] Little, however, has been done to investigate the influence of anesthetic drugs on myocardial tolerance to total ischemia independent of their effects on loading conditions or collateral coronary blood flow.

The purpose of this study was to compare myocardial tolerance to total ischemia in animals anesthetized with either halothane or isoflurane. Tolerance to ischemia was measured by the time required for development of cardiac rigor mortis in the absence of coronary perfusion or myocardial wall stress in a well-established model.[10] Previous work has shown that the onset of cardiac rigor as measured in this model correlates well with ultrastructural evidence of irreversible cellular injury and myocardial ATP depletion.[10] Myocardial metabolism of high-energy nucleotides during ischemia was also compared in animals anesthetized with the two inhalation agents.

Materials and Methods

Following approval of the Institutional Animal Care and Use Committee, 16 healthy mongrel dogs, weighing 14–25 kg, were randomly assigned to one of two groups. General anesthesia was induced by inhalation of halothane with oxygen in group 1 or isoflurane with oxygen in group 2. Anesthesia was maintained with equally potent inspired concentrations of halothane 1.8% (2 MAC)[11] or isoflurane 2.6% (2 MAC)[12] in the respective groups (see discussion regarding MAC multiples). Anesthetic concentrations were attained using calibrated vaporizors. All animals had their tracheas intubated, and they were paralyzed with vecuronium 0.1 mg/kg iv, and ventilated with 100% oxygen using the Air Shields Ventimeter® Ventilator and a semi-closed breathing circuit. Ventilation was adjusted to maintain normocarbia. The surface electrocardiogram and femoral artery blood pressure were continuously monitored using a Hewlett-Packard 78308A system with H.P. 1290A transducers and a Gould 2800 multichannel recorder. All dogs received a continuous intravenous infusion of lactated Ringers solution totaling 10–15 ml/kg.

Fifteen minutes after tracheal intubation, a median sternotomy and pericardial incision were performed to expose the heart in all animals. Anesthesia was main-

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Received from the Departments of Anesthesiology and Surgery, Duke University Medical Center, Durham, North Carolina. Accepted for publication February 9, 1988. Supported by a grant from Anaquest of Madison, Wisconsin.

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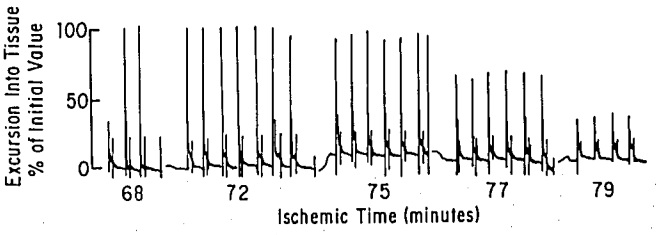

FIG. 1. Footplate excursion into a left ventricular tissue slab resulting from an intermittently applied compressive force of constant magnitude. Footplate excursion is displayed as a percentage of the initial value at 3 min of ischemia. The onset of rigor, detected by a reduction in footplate excursion to less than 90% of initial excursion, occurred after 75 min of ischemia in this example.

tained for an additional 15 min, after which time blood samples were drawn for determination of Pa_{O_2}, Pa_{CO_2}, *p*H, hematocrit, serum lactate, serum glucose, and plasma norepinephrine and epinephrine levels in all dogs. Thirty minutes after induction of anesthesia, the heart was rapidly excised to create uniform total ischemia, and a left ventricular free wall slab was prepared and divided as previously described.[10] Each portion of the left ventricular slab was encased in polyethylene and incubated in a water bath to maintain myocardial temperature at 37° C for the duration of total ischemia.

A portion of each left ventricular slab was immediately placed in a specially designed tissue compressibility gauge. This gauge has been extensively used in this laboratory, and has been found to accurately identify the onset of cardiac rigor.[10] The gauge functions by measuring the compressibility of myocardium when subjected to an intermittently applied compressive force of 30 mmHg over the surface of two opposing footplates. Compressibility was determined by measuring the distance of footplate excursion into the tissue. Rigor onset was determined by an abrupt increase in resistance to tissue deformation defined by a reduction of footplate excursion to less than 90% of the initial value (fig. 1). A second portion of slab was instrumented with a Millar SPR-230 5 Fr Mikro-tip® catheter continuously transduced to measure tissue pressure in the subendocardial region. Onset of ischemic contracture was defined as the time at which subendocardial tissue pressure reached its peak (fig. 2).

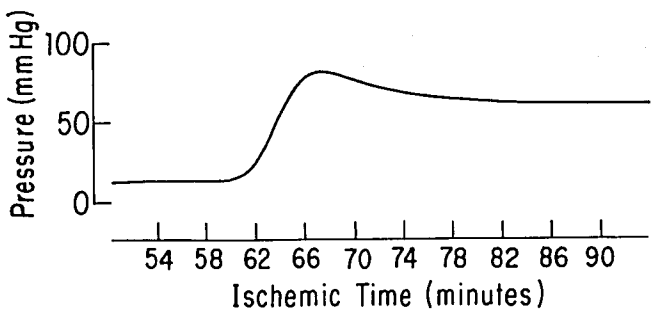

FIG. 2. Left ventricular subendocardial tissue pressure as a function of ischemic time. Onset of ischemic contracture defined as the time of peak pressure occurred at 67 min in this example.

A third slab was used for tissue sampling. Subepicardial and subendocardial tissue samples were taken at 3 min after excision and then at 15-min intervals. Each sample was rapidly weighed on a Cahn Model DTL microbalance, placed in cold 3.6% perchloric acid, and homogenized with a cold Tri-R teflon pestle. The homogenate was extracted for 30 min in an ice bath and was cold centrifuged at 850 g for 20 min in an International Equipment Company Centra-7R centrifuge. The supernant was neutralized to *p*H 6.8 with a potassium hydroxide/potassium carbonate buffer. High-energy nucleotides were analyzed by high-performance liquid chromatography as previously described.[13] Tissue lactate was measured using a standard enzymatic assay[14] in a Perkin-Elmer Lambda-5 spectrophotometer.

All blood samples were immediately stored on ice. Determination of P_{O_2}, P_{CO_2}, *p*H, hematocrit, lactate, and glucose were made within 20 min by standard clinical laboratory methods. Samples for catecholamine assays were cold centrifuged at 850 g for 15 min. Serum was stored at −70° C. Catecholamine levels were assayed using alumina adsorption and a high-performance liquid chromatography electrochemical detector.[15] The minimum detectable levels for the assay are 40 pg/ml of norepinephrine and 50 pg/ml of epinephrine. The coefficient of variation is 9%.

Statistical comparisons of individual parameters between groups were made using one-way analysis of variance. Linear regression analysis was performed to study the relation between hemodynamic parameters, catecholamine levels, and time to ischemic contracture. Between-group comparisons of time-dependent declines in high-energy nucleotides were made by analysis of variance with repeated measures of the log of tissue levels using the BMDP statistical software program (University of California). Standard deviations of high-energy nucleotides were proportional to the magnitude of means at each sampling time. Logarithmic transformation was used to stabilize the variance of the means over time. Results are reported as mean ± standard deviation. *P* values less than 0.05 were considered statistically significant.

Results

PREISCHEMIC FACTORS

There were no statistically significant differences in heart rate or mean arterial pressure between the two groups immediately prior to cardiac excision (table 1).

TABLE 1. Pre-ischemic Factors

	Group 1: Halothane	Group 2: Isoflurane
HR (beats·min^{-1})	98 ± 17	108 ± 11
MAP (mmHg)	82 ± 13	75 ± 14
Glucose (mg/dl)	118 ± 18	124 ± 12
Lactate (mg/dl)	2.7 ± 0.2	3.0 ± 1.2
Hematocrit	35.8 ± 6.2	32.1 ± 4.3
P_{O_2} (mmHg)	505 ± 47	511 ± 38
P_{CO_2} (mmHg)	33.2 ± 3.5	32.9 ± 3.4
*p*H	7.42 ± .04	7.39 ± .04
Norepinephrine (pg/ml)	190 ± 84	259 ± 160
Epinephrine (pg/ml)	1050 ± 360	1070 ± 327

Results are mean ± standard deviation. N = 8 in each group. No significant difference in any factor between groups.

Serum glucose and lactate, hematocrit, arterial P_{O_2}, P_{CO_2}, and *p*H, and plasma catecholamine levels were also similar for both groups (table 1).

ISCHEMIC CONTRACTURE

The time required for development of cardiac rigor as measured by the compressibility gauge was significantly greater in the halothane group than in the isoflurane group (68 ± 7.2 *vs.* 60 ± 5.0 min; $P < .05$, table 2). In only one dog receiving isoflurane was the onset of rigor prolonged to the average time for the halothane group. The average reduction of footplate excursion into tissue as a function of ischemic time is shown in figure 3.

The earlier onset of ischemic contracture in subendocardial slabs determined by continuous tissue pressure measurement with Millar needle probes correlated well with the compressibility gauge (r = .81, $P < .01$), and tended to be prolonged in slabs from dogs receiving halothane compared to those receiving isoflurane (64 ± 6.9 *vs.* 57 ± 6.8 min; $P = .08$).

There was a negative correlation between the time of rigor onset and the pre-ischemic heart rate in both groups (r = −.71; $P < .005$; fig. 4). There was no correlation between any other pre-ischemic parameter and the time to onset of rigor.

TABLE 2. Time to Onset of Rigor (Min)

Halothane	Isoflurane
72	68
68	58
65	60
64	56
80	53
70	58
55	61
70	66
68 ± 7.2*	60 ± 5.0

* $P < 0.05$, halothane *vs.* isoflurane.

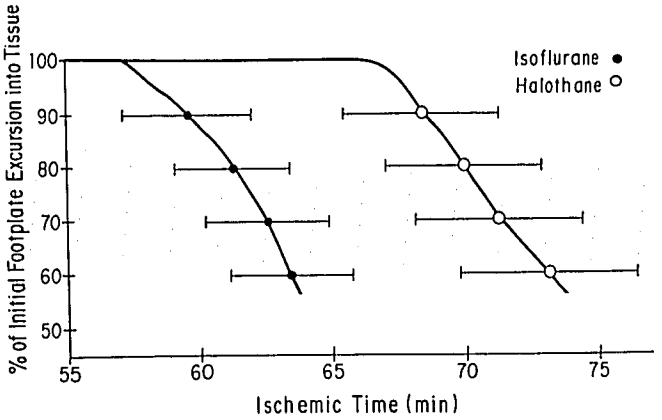

FIG. 3. Average excursion of footplates into tissue as a function of time when subjected to an intermittent force of constant magnitude. Mean ± SEM.

ISCHEMIC MYOCARDIAL METABOLISM

Analysis of subendocardial and subepicardial tissue assays of high-energy nucleotides revealed a decline in concentrations of ATP and ADP over time of ischemia in both groups (table 3). Initial ATP and ADP tissue levels were virtually identical (table 3) at 3 min of total ischemia in both groups. This suggests that intracellular energy stores were similar for both groups immediately prior to ischemia. As high-energy phosphates became depleted during total ischemia, time-dependent levels of ATP were greater in the halothane group in both subendocardial specimens ($P < .01$), as shown in figure

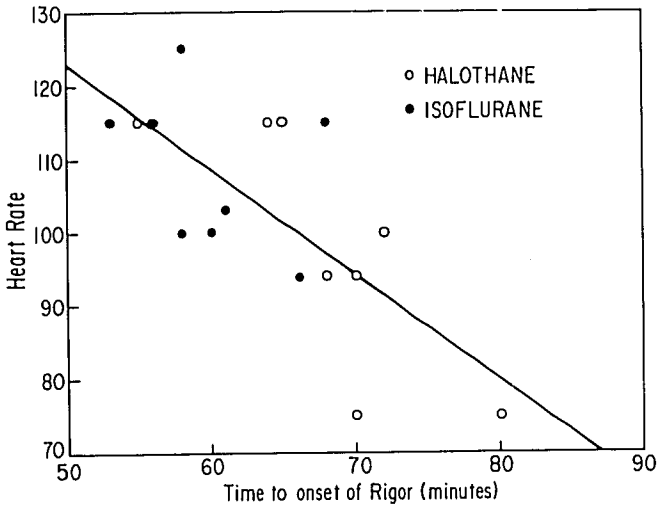

FIG. 4. Pre-ischemic heart rate *versus* time to the onset of cardiac rigor. A negative correlation between pre-ischemic heart rate and time to rigor was observed (y = −1.42x + 194; r = 0.71; $P < .005$).

TABLE 3. High-energy Nucleotide and Lactate Levels (μmol/gm Wet Weight)

		Ischemic Time (Min)					
		3	15	30	45	60	75
ATP subendocardium*	H	4.36 ± .96	2.84 ± .47	2.22 ± .58	1.39 ± .37	0.70 ± .27	0.18 ± .07
	I	4.46 ± .85	3.30 ± .54	1.77 ± .40	1.20 ± .25	0.43 ± .26	0.11 ± .02
ADP subendocardium†	H	2.10 ± .74	1.91 ± .51	1.39 ± .40	1.28 ± .27	1.03 ± .25	0.51 ± .11
	I	2.12 ± .76	1.56 ± .39	1.53 ± .32	1.29 ± .21	0.77 ± .24	0.41 ± .03
ATP subepicardium*	H	4.31 ± .98	2.85 ± .67	2.25 ± .67	1.36 ± .50	0.66 ± .34	0.22 ± .15
	I	4.55 ± .65	2.81 ± .64	2.08 ± .43	1.06 ± .24	0.33 ± .17	0.11 ± .02
ADP subepicardium	H	2.22 ± .82	1.97 ± .56	1.47 ± .32	1.33 ± .28	0.94 ± .29	0.59 ± .18
	I	2.17 ± .51	2.12 ± .39	1.59 ± .44	1.25 ± .15	0.79 ± .25	0.45 ± .06
Lactate subendocardium	H	7.17 ± 4.5	13.3 ± 4.2	26.9 ± 7.1	39.4 ± 7.0	50.5 ± 6.1	63.7 ± 6.9
	I	5.88 ± 2.7	16.0 ± 5.8	26.4 ± 10	43.2 ± 8.7	54.3 ± 6.5	59.5 ± 5.0
Lactate subepicardium	H	3.88 ± 3.6	14.7 ± 5.5	24.1 ± 6.3	37.0 ± 8.0	51.9 ± 8.8	60.8 ± 9.1
	I	5.75 ± 3.2	15.3 ± 6.5	27.5 ± 5.7	40.6 ± 7.9	53.3 ± 6.4	58.9 ± 8.5

Mean ± standard deviation. H = halothane; I = isoflurane.

* $P < .01$, H *vs.* I.
† $P < .05$, H *vs.* I.

5, and subepicardial specimens ($P < .01$). Subendocardial ADP levels also tended to be preserved in the halothane group ($P < .05$). There was no statistically significant difference in the decline of subepicardial ADP levels between groups. There was a progressive increase over time in tissue lactate levels in specimens from all dogs (fig. 6). There were no significant differences in lactate levels between specimens from the halothane and isoflurane groups at any time during ischemia.

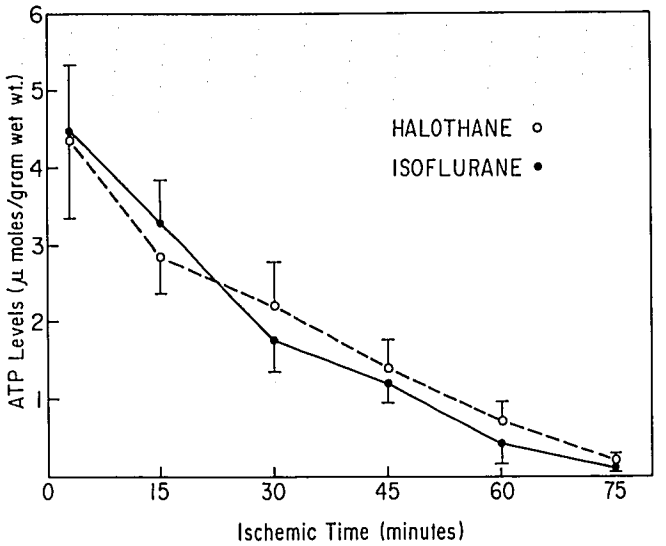

FIG. 5. Subendocardial tissue levels of ATP as a function of ischemic time. There was a small but statistically significant trend of ATP preservation in the halothane group compared to the isoflurane group ($P < .01$, analysis of variance with repeated measures of log ATP levels). Mean ± SD.

Discussion

Acute myocardial ischemia initiates a complex series of initially reversible and subsequently irreversible processes of cellular injury. The metabolic changes during ischemia lead to structural changes in myofibrils culminating in cardiac rigor mortis.[16] The development of cardiac rigor is closely associated with the depletion of high-energy nucleotides and the onset of cell death.[10,17] The detection of cardiac rigor, therefore, has been used as an experimental means of identifying the onset of irreversible injury. Our model uses a tissue compressibility gauge that identifies an increase in myocardial stiffness, which has been shown to correlate with both a rise in intracavitary balloon pressure in intact hearts, as well as microscopic ultrastructural evidence of irreversible cell injury.[10] This model allows adjacent myocardial tissue sampling for metabolic studies in the absence of myocardial wall stress or coronary blood flow.

Jennings *et al.*[18] have shown that total ischemia *in vitro* and severe ischemia *in vivo* result in virtually identical qualitative changes in high-energy phosphate metabolism. The decline in tissue levels of high-energy phosphates following the onset of total ischemia is the result of differences in the rates of production and utilization. The production of new high-energy phosphates is almost solely dependent on anaerobic glycolysis with a yield of 1.5 μmol of high-energy phosphate per μmol of lactate generated from glucose entering glycolysis from glycogen.[18] Initially, there is a period of rapid anearobic glycolysis, which slows as ischemic time progresses. The glycolytic production of ATP is, however, unable to keep up with utilization, and ATP levels are depleted over time. The rate of glycolysis appears to be regulated

by high-energy phosphate demand mediated by relative concentrations of ATP, ADP, and AMP. Anaerobic glycolysis has been shown to cease when ATP levels drop to a critically low level, even in the presence of significant quantities of glycogen substrate.[18] Cell death is temporally associated both with depletion of high-energy phosphates to critically low levels and cessation of anaerobic glycolysis. In the present study, dogs anesthetized with halothane exhibited greater time-dependent ATP levels than those anesthetized with isoflurane. Since lactate accumulation was similar, it appears that the rate of high-energy phosphate utilization was decreased in the halothane group.

Metabolic changes occur more slowly with total ischemia *in vitro* than with severe *in vivo* ischemia, presumably because of continued electrical stimulation and mechanical activity *in vivo*. Since tissue is excised to create total ischemia in our model, it is not subjected to mechanical load, and is electrically quiescent shortly after excision. This is similar to clinical conditions encountered during cardiac surgery in which the heart is globally ischemic and empty while the aorta is cross clamped. In clinical practice, however, cold cardioplegic solution is used to produce electrical arrest and hypothermia is maintained to further reduce the rate of ischemic metabolism. Whether the addition of hypothermia and potassium-based cardioplegia would exaggerate or diminish the difference in tolerance to total ischemia between the two groups of the present study is unknown.

The final event that causes irreversible injury and ischemic contracture remains unclear, but sarcolemmal disruption has been implicated.[19,20] Sarcolemmal disruption may result from a calcium-activated protease that degrades cytoskeletal support of the sarcolemma.[21] The onset of ischemic contracture can be delayed by various interventions that reduce myocardial energy demands or increase energy supply.[22] In addition to hypothermia and electrical arrest, beta-adrenergic blocking drugs and calcium entry blockers have had protective effects[23-25] in several experimental preparations of total ischemia. Treatment with large doses of propranolol increased the time to ischemic contracture from 63 to 79 min in pentobarbital-anesthetized dogs using the present model.[26] Inhalation anesthetic drugs, like beta adrenergic blockers and calcium entry blockers, affect myocardial Ca^{++} metabolism and, therefore, might be expected to influence myocardial tolerance to total ischemia. Enflurane has been shown to improve post-ischemic function in isolated perfused rat hearts.[27] Halothane was observed to exert a residual protective effect on globally ischemic myocardium as evidenced by decreased isoenzyme release.[28] In models

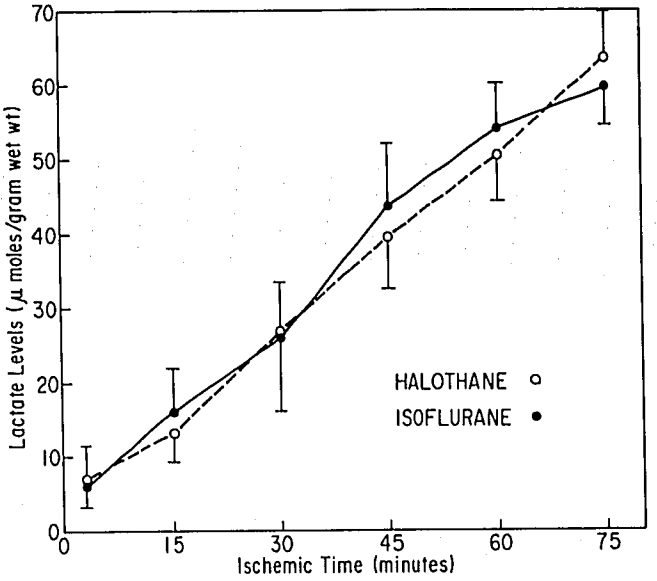

FIG. 6. Subendocardial tissue lactate accumulation during ischemic time. There was no difference between groups. Mean ± SD.

of regional myocardial ischemia, halothane has been shown to reduce ST-segment elevation in dogs[29] and decrease the frequency of dysrhythmias[30] and lower the mortality rate[30] in rats following coronary artery ligation. Infarct size was decreased in dogs anesthetized with halothane after left anterior descending coronary artery ligation.[31] Isoflurane increased the myocardial work required to produce ischemic ECG changes in humans with coronary artery disease compared to awake patients, although no other anesthetic was tested.[32]

The present data show a significant prolongation in time to onset of cardiac rigor in dogs anesthetized with equally potent inspired concentrations of halothane compared to isoflurane during total ischemia. The administration of equally potent inspired concentrations of halothane and isoflurane over equal periods of time should have resulted in a slightly more potent blood concentration of isoflurane in terms of MAC multiples because of its lower solubility. This small difference could have baised the investigation in favor of isoflurane if anesthetic influence on tolerance to total ischemia is dose dependent, as are most drug effects. The results, however, indicate that tolerance to total ischemia was greater in hearts from dogs receiving halothane. The dogs in the two groups did not exhibit differences in pre-ischemic heart rate, mean arterial pressure, glucose, lactate, hematocrit, *p*H, or catecholamine levels prior to initiation of total ischemia. There is evidence that elevated levels of norepinephrine have detrimental effects on ischemic myocardium.[33,34] Hyper-

glycemia has been implicated in worsening the effects of cerebral ischemia. The normal arterial *p*H and lactate levels found suggest that adequate cardiac performance persisted in all anesthetized animals prior to ischemia. The two groups of dogs were very similar with respect to these parameters prior to ischemia. It has recently been demonstrated that, during regional myocardial ischemia, halothane preserves function in collateral-dependent zones to a greater extent than does isoflurane.[4] This is presumably due to the detrimental effects of coronary steal associated with isoflurane.[4,5] Our results indicate that halothane, when compared to isoflurane, may also improve myocardial tolerance to total ischemia independent of effects on systemic hemodynamics or collateral coronary flow. The influence of halothane on subcellular membrane systems could contribute to this observation.

Previous studies suggest that both halothane and isoflurane alter cellular uptake and release of Ca^{++}.[35,36] Isoflurane and halothane may regulate intracellular Ca^{++} activity by different mechanisms.[37] Anesthetic modulation of myocardial cellular calcium metabolism is, however, controversial. The work of Komai and Rusy[38,39] suggests that the negative inotropic action of isoflurane is due primarily to inhibition of the influx of extracellular Ca^{++} whereas halothane also decreases the availability of intracellular Ca^{++} from sarcoplasmic reticulum. During total ischemia, in the absence of collateral extracellular perfusion, the intracellular regulation of Ca^{++} by halothane may offer a greater protective effect than inhibition of extracellular Ca^{++} influx by isoflurane.

Finally, we observed that the onset of rigor was delayed in dogs that exhibited lower pre-ischemic heart rates in both groups. It is unlikely, however, that differences in pre-ischemic heart rates account for the differences in tolerance to total ischemia between the halothane and isoflurane groups. Tachycardia adversely influences tolerance to ischemia by increasing the metabolic demands relative to substrate supply only in the presence of a limited blood or oxygen supply. In healthy dogs without a limited blood supply (no coronary disease), it is extremely unlikely that heart rates within normal limits (75–125 bpm) would have any detrimental effects on the balance between myocardial oxygen supply and demand. The finding of nearly identical initial myocardial ATP and ADP levels (table 3) for both groups suggests there was no pre-ischemic hemodynamic factor affecting myocardial oxygen supply and demand that resulted in cellular energy depletion in the isoflurane group compared to the halothane group. Previous work from this laboratory has failed to demonstrate any difference in myocardial high-energy phosphate levels or subsequent tolerance to total ischemia between pentobarbital anesthetized dogs paced at a rate of 242 bpm compared to those with an unpaced rate of 151 bpm.[40] Unpublished work has also shown no effect of bradycardia produced by SA node destruction. The time to rigor onset was found not to correlate with any other pre-ischemic parameter measured.

The results of this investigation suggest that myocardial tolerance to total ischemia in the absence of coronary perfusion or wall stress is greater during halothane than isoflurane when administered in equally potent inspired concentrations. In addition to its previously described benefits during incomplete regional myocardial ischemia, halothane probably influences cellular metabolism in a way that renders myocardial tissue less vulnerable to injury during complete ischemia than does isoflurane.

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