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Enflurane Enhances Postischemic Functional Recovery in the Isolated Rat Heart

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Enflurane is a direct myocardial depressant and may act as a myocardial protective agent during ischemia. The authors studied the effects of enflurane on myocardial high-energy phosphates and tolerance to ischemia in the normothermic, isolated rat heart. After isolation and perfusion with Krebs–Henseleit buffer, the hearts were perfused with either buffer (control) or buffer gassed with 2% enflurane for 10 minutes. Thereafter, hearts were made globally ischemic and elapsed times to initiation of ischemic contracture (IC) were determined. ATP and creatine phosphate (CP) were measured at the conclusion of control and enflurane administration and at IC. Ten hearts per group were reperfused with buffer following IC for 20 min; peak pressure and ATP and CP were determined. Administration of 2% enflurane significantly decreased peak pressure by 20% but did not alter baseline high-energy phosphate levels nor did it prolong time to IC. However, enflurane-treated hearts exhibited significantly greater ($P < 0.01$) recovery of function as defined by per cent return of peak pressure (67% ± 3%) when compared with those hearts not treated with enflurane preischemically (44% ± 5%). Also, enflurane-treated hearts had significantly higher ($P < 0.01$) ATP levels at the conclusion of reperfusion than hearts not perfused with enflurane (12.2 ± .8 μmol/g dry weight *vs.* 9.0 ± 0.8 μmol/g dry weight). These findings suggest that enflurane administered prior to an ischemic interval enhances postischemic myocardial recovery. (Key words: Anesthetics, volatile: enflurane. Heart: ischemia, recovery.)

DURING THE PAST TWO DECADES, the halogenated inhalational anesthetics have enjoyed great popularity in cardiac surgery used either alone or in conjunction with various narcotics. This trend had been primarily due to the drugs' relative safety, ease of titration, and predictability of action. Recently, numerous investigators have explored the effects of enflurane on myocardial function, metabolism, and on the systemic vasculature.[1-7] There is sufficient evidence that enflurane exerts a direct, dose-dependent, myocardial inodepressive effect in isolated cardiac muscle preparations,[4] the intact hearts of experimental animals,[1,5] and in humans.[6] Additionally, clinical studies have revealed marked decreases in mean arterial pressure and in systemic vascular resistance in patients exposed to enflurane in the operating room.[7]

An important consideration for enflurane as a cardiac anesthetic may rest in its ability to reduce global myocardial oxygen consumption. Yusa and Obara demonstrated that enflurane decreases myocardial oxygen extraction rates in dogs.[2] Smith, *et al.* have reported that enflurane actually may improve the oxygen availability/consumption ratio in the acutely ischemic myocardium.[3] These apparent attributes may be of importance during periods of reduced myocardial oxygen supply, (*i.e.*, ischemic arrest during cardiopulmonary bypass) and may prove important in preventing ischemic injury or improving the extent of post ischemic myocardial recovery. This study was designed to test the hypothesis that enflurane might act as a myocardial protective agent. The effects of the administration of enflurane on the preischemic and postischemic myocardium were investigated in the normothermic, isolated rat heart model, using ischemic contracture as a physiologic index of myocardial injury.[8] High-energy phosphate stores, considered a sensitive determinant of myocardial preservation,[9,10] also were measured in order to correlate metabolic activity with mechanical function both prior to ischemia and following buffer reperfusion.

Material and Methods

PREPARATION

Male Sprague–Dawley rats (350–450 g) that had been maintained on a standard diet were used in this experiment. Hearts were excised from rats anesthetized with 40 mg sodium pentobarbital intraperitoneally and immediately placed in 20 ml iced physiologic saline that contained 5 units/ml sodium heparin. Upon cessation of contractions, the hearts were mounted on the stainless steel cannula of the Langendorff apparatus, and retrograde aortic perfusion at a constant pressure of 65 cm H_2O was initiated with a modified Krebs–Henseleit bicarbonate buffer (KHB). The concentrations (mM) of the buffer constituents were: NaCl 118, KCl 4.7, $CaCl_2$ 2.5 plus 0.5 to balance the ethylenediaminetetraacetate (EDTA), $MgSO_4 \cdot 7H_2O$ 1.2,

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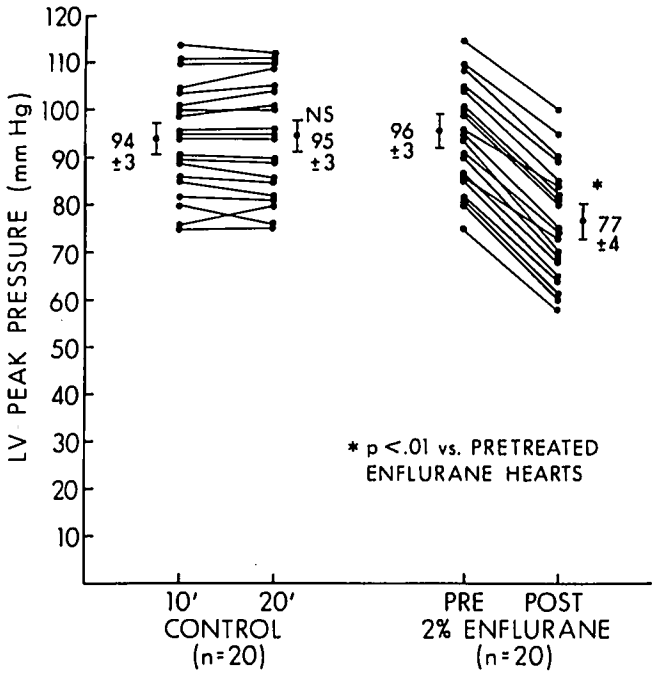

FIG. 1. This illustrates peak-pressure data for control hearts and hearts prior to and after enflurane administration. Each line represents one heart. The circles are the mean ± standard error. Comparisons within groups to baseline pressures demonstrated no changes with buffer perfusion in the control group but a significant decrease ($P < 0.01$) in peak pressure following enflurane.

KH_2PO_4 1.2, $NaHCO_3$ 25, NaEDTA 0.5, and glucose 11.1. The perfusate was bubbled with a mixture of 95% O_2 and 5% CO_2, which produced a P_{O_2} range of 400–500 mmHg, a P_{CO_2} range of 36–42 mmHg, and a *p*H of 7.38–7.46. Temperature was maintained at 37° C throughout the experiment by means of a water jacket that surrounded all reservoirs, tubing, and the heart itself.

PROTOCOL

All hearts were perfused initially for a 10-min stabilization period. During this time, a balloon-tipped, fluid-filled catheter connected to a pressure transducer was inserted through a left atriotomy into the left ventricle. This allowed us to monitor intracavity pressure continuously. Saline was injected into the balloon until left ventricular end-diastolic pressure reached 0–2 mmHg, and pacing wires were placed on the right atrium. Left ventricular peak pressure and coronary flow rates were monitored at a heart rate of 300 beats/min.

During the preischemic treatment period, the hearts were perfused for 10 min with either Krebs–Henseleit buffer (control) or with KHB from a separate chamber that had been saturated with 2% enflurane in 95% O_2 + 5% CO_2 delivered by an enflurane vaporizer (Harris Lakes Incorporated) that had been calibrated with an EMMA gas analyzer. Afterwards, they were made globally ischemic at 37° C by cross-clamping the aortic perfusion line and the time to onset of ischemic contracture was determined. The onset of contracture was defined as a 2 mmHg elevation in left ventricular pressure. Both control and enflurane groups were reperfused for 20 min with Krebs–Henseleit buffer immediately after reaching contracture initiation. Peak pressure and coronary artery flow rate in these hearts were measured at the conclusion of reperfusion and functional recovery was calculated as per cent return of peak pressure to pretreatment levels.

In a parallel series of experiments, high-energy phosphate levels were determined in control and enflurane-treated hearts prior to global ischemia, at the initiation of ischemic contracture, and, in the reperfused hearts, at the conclusion of reperfusion. Hearts were frozen rapidly between precooled, stainless steel, Wollenberger tongs and immediately submerged in liquid nitrogen. Aliquots of tissue were taken for preparation of perchloric acid extract as described by Lowry and Passonneau.[11] ATP and creatine phosphate (CP) determinations were performed by the enzymatic assay of Lamprect *et al.*[12] Values were derived and are expressed as micromoles per gram of dry weight.

Values are expressed as mean ± standard error of the mean. Significant differences were determined by paired Student's *t* test for the functional recovery data within each reperfusion group and by unpaired Student's *t* test for all other data.

Results

Marked physiologic effects were recorded after 10 min of 2% enflurane administration to the isolated rat heart. Left ventricular peak pressure was decreased significantly by 20% from a mean pressure of 96 mmHg to 77 mmHg in enflurane-perfused hearts, while there was no effect in controls (fig. 1). Table 1 lists preischemic ATP and CP levels, preischemic coronary artery flow rate, times to initiation of ischemic contracture, and high-energy phosphate levels at contracture initiation for both the control and enflurane-treated groups. There were no significant differences in any of these variables between control hearts and enflurane-treated hearts.

The effects of preischemic enflurane administration on subsequent myocardial recovery are shown in table 2. Peak pressure in the normally perfused heart is referenced to peak pressure following 20 min of KHB reperfusion in both groups. Enflurane-treated hearts generated 67 ± 3% of their pretreatment left ventricu-

TABLE 1. Effect of Enflurane on Preischemic High-Energy Phosphate Levels, Coronary Artery Flow, and Time to Ischemic Contracture

	Preischemic ATP (μmol/g dry wt)	Preischemic CP (μmol/g dry wt)	Preischemic Coronary Artery Flow (ml/min)	Time to IC (min)	ATP at IC (μmol/g dry wt)	CP at IC (μmol/g dry wt)
Control	22.3 ± 1.0	22.9 ± 1.9	10.4 ± 0.6	12.7 ± 0.5	8.7 ± 0.5	3.8 ± 0.8
	NS	NS	NS	NS	NS	NS
Enflurane	22.5 ± 1.0	24.0 ± 2.4	10.6 ± 0.3	12.8 ± 0.4	9.2 ± 0.6	3.9 ± 0.7

n = 10 for all groups. NS = no statistical difference by Student's unpaired *t* test.

lar pressure, a value significantly greater ($P < 0.01$) than the 44 ± 5% recovery obtained in control hearts. Both ATP and CP levels at the conclusion of reperfusion were significantly greater ($P < 0.01$) in those hearts administered enflurane preischemically when compared with high-energy phosphate levels in control hearts. Additionally, the coronary artery flow rate following reperfusion was equivalent between both the control and enflurane pretreated hearts.

Discussion

Improved technology during the past quarter century has provided the method with which to unravel the pathophysiology of ischemic heart disease. In 1960, Danforth *et al.*[13] reported that after 15 min of anoxia, the canine myocardium exhibited reduced glycogen levels and ATP concentrations, as well as a concomitant increase in lactic acid levels. Reperfusion of that ischemic myocardium resulted in restoration of ATP levels. Meanwhile, Jennings and associates[14] investigated the electrophysiologic and structural alterations associated with severe regional ischemia followed by reperfusion. Twenty minutes of circumflex artery occlusion produced morphologic evidence of subendocardial necrosis and the electrocardiographic changes associated with ischemic injury. Furthermore, a high incidence of ventricular fibrillation was recorded upon release of the coronary occlusion to the affected myocardium. Subsequent studies have created a more detailed understanding of the subcellular events that occur during myocardial ischemia and reperfusion. Briefly, ischemic injury results in cellular membrane derrangements, metabolic dysfunction, electrolyte imbalance, and cell swelling. Reperfusion of the myocardium following severe ischemia appears to induce a massive, sudden extension of this injury at the subcellular level with myofibril and sarcolemma disruption and explosive cell swelling. This is followed by mitochondrial rupture with aerobic metabolic inhibition and the appearance of intramitochondrial calcium phosphate granules.[15]

Jennings and Ganote[16] showed that severe ischemia followed by reperfusion led to a significant cellular uptake of calcium. From this they postulated that reperfusion or reoxygenation damage may be attributable to uncontrolled oxygen dependent calcium fluxes. Subsequent studies investigating the connection between reoxygenation and sudden mitochondrial calcium uptake suggested that electron transport within the mitochondrium might be responsible for triggering this phenomenon.[15,17] It appears evident that a number of aspects of reperfusion injury may be explained by the cell's inability to regulate calcium homeostasis and to maintain a normal pattern of ion distribution.

In 1977, Hearse, *et al.* used an isolated rat heart model to demonstrate that the onset of ischemic contracture correlated with intracellular energy metabolism and in particular with a specific cellular ATP content.[8] They also showed that the time required to reach the onset of ischemic contracture was prolonged by interventions that either augmented myocardial energy stores or reduced myocardial energy demands.[8,18] Using this same model, these manipulations have demonstrated that there is improvement in functional and metabolic recovery in the postischemic myocardium as

TABLE 2. Functional and Metabolic Recovery following Postischemic Reperfusion

	Preischemic Peak Pressure (mmHg)	Postreperfusion Peak Pressure (mmHg)	Per Cent Recovery Peak Pressure	ATP (μmol/g dry wt)	CP (μmol/g dry wt)	Coronary Artery Flow (ml/min)
Control	96 ± 3	42 ± 5	44 ± 5	9.0 ± 0.8	14.5 ± 1.6	4.8 ± 0.4
	NS	*	*	†	†	NS
Enflurane	100 ± 3	67 ± 3	67 ± 3	12.2 ± 0.9	22.4 ± 1.6	5.3 ± 0.4

n = 10 for all groups. NS = no statistical difference.
* $P < 0.01$ by paired Student's *t* test.
† $P < 0.01$ by unpaired Student's *t* test.

measured by restoration of peak systolic pressure, aortic flow rate, and high-energy phosphate stores.[9,19] These investigations and others have indicated that ischemic contracture is a reliable marker of myocardial ischemic injury and can be used to appropriately evaluate myocardial tolerance to ischemia.

We, therefore, elected to use the isolated Langendorff rat heart preparation that was independent of vascular and reflex changes to elaborate on the direct myocardial effects of enflurane and its potential myocardial protective qualities in the preischemic and postischemic states. Studies from our laboratory have shown that, in the isolated rat heart, at matched heart rates, peak pressure correlates well with dP/dt and contractile state.[20] Administration of 2% enflurane via passive retrograde aortic perfusion resulted in a significant 20% decrease in left ventricular generated peak pressure. We hypothesized that this depressant dose of enflurane would reduce myocardial oxygen demands and would decrease high-energy phosphate utilization both prior to and during ischemia, thus favorably affecting myocardial tolerance to ischemia. Time to ischemic contracture and high-energy phosphate stores prior to ischemia and at ischemic contracture were statistically equivalent in both control and enflurane-treated groups. These findings suggest that for a given period of ischemia, equivalent injury ensued irrespective of the preischemic administration of enflurane.

However, reperfusion for 20 min with oxygenated, bicarbonate buffer after having reached ischemic contracture showed that those hearts administered enflurane preischemically demonstrated greater recovery of myocardial function when compared with hearts not perfused with enflurane preischemically. This was manifest by a significant difference in percent return of peak pressure to pretreatment levels ($67 \pm 3\%$ *vs.* $44 \pm 5\%$ in controls), which corresponded to a higher inotropic state and more effective myocardial performance. It is not likely that this finding is related to an effect of enflurane on coronary vascular resistance, as there was no difference in postreperfusion coronary artery flow rate between groups (table 2). Similarly, control and enflurane groups possessed equivalent energy stores both preischemically and up to the onset of ischemic contracture. However, during the reperfusion period, changes in cellular metabolism must have occurred in the enflurane hearts since, at the conclusion of the reperfusion period, enflurane pretreated hearts possessed higher ATP and CP levels than did control hearts. This increase in high-energy phosphates strongly suggests that enflurane enhances postischemic metabolic recovery and may ameliorate the extent of postischemic reperfusion injury.

There have been a number of investigations directed at enflurane's mode of action at the subcellular level. Lynch *et al.* demonstrated that enflurane at concentrations of 2% or greater depressed action potentials mediated by inward calcium current through the voltage-dependent slow channels.[21] Recently, Blanck and Thompson reported that enflurane stimulated calcium uptake by the sarcoplasmic reticulum *in vitro*.[22] This effect was most pronounced at low ATP concentrations, similar to those found during severe myocardial ischemia. Interpretation of these data implies that enflurane could reduce calcium flux into the myocyte or lower the cytoplasmic concentration of calcium by enhancing its transport into the sarcoplasmic reticulum. By either mechanism, less intracellular calcium would be available for uptake by the mitochondria. As a result, mitochondrial function would be preserved and the calcium-related damage observed during myocardial reperfusion would be diminished.

We conclude that the administration of 2% enflurane to the isolated rat heart prior to a severe ischemic insult enhanced both inotropic and metabolic recovery in the postischemic state. In the aftermath of an ischemic insult, these attributes could play an important role in reestablishing myocyte integrity and long-term myocardial function.

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