

Effects of Halothane on Myocardial High-energy Phosphate Metabolism and Intracellular pH Utilizing ^{31}P NMR Spectroscopy

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Utilizing ^{31}P phosphorus nuclear magnetic resonance (NMR) spectroscopy, the authors tested the two hypotheses that the negative inotropic action of halothane is the result of: 1) myocardial intracellular acidosis, and 2) a decrease in myocardial high-energy phosphates. In isolated, paced, Langendorff-perfused rabbit hearts, halothane (1.5 vol %) dissolved in the coronary perfusate produced a $48 \pm 2\%$ decrease ($P < 0.01$) in left ventricular developed pressure. In contrast, halothane administration had no significant effect on myocardial intracellular pH (7.18 ± 0.04 at control vs. 7.21 ± 0.02 during halothane). Halothane exposure decreased ($P < 0.01$) the forward rate constant of the creatine kinase reaction by $32 \pm 6\%$, as measured using saturation transfer NMR, suggesting a decline in the rate of high-energy phosphate metabolism. This was further indicated by a concomitant decrease ($P < 0.05$) in myocardial oxygen consumption ($20 \pm 5\%$). During the halothane-induced reduction in left ventricular developed pressure, only small decreases in the myocardial steady state concentrations of phosphocreatine ($7 \pm 1\%$; $P < 0.01$) and βATP ($12 \pm 4\%$; $P < 0.05$), and an increase in Pi ($18 \pm 6\%$; $P < 0.05$) were observed. However, similar changes in steady-state high-energy phosphate metabolites were also measured in time-control hearts not exposed to halothane. These results indicate that the negative inotropic action of halothane is not mediated by myocardial intracellular acidosis. Moreover, these findings do not support the concept that the negative inotropic action of halothane is the result of a reduction in myocardial high-energy phosphates. (Key words: Anesthetics, volatile: halothane. Enzymes: creatine kinase. Heart: negative inotropism; oxygen consumption. Isotopes: ^{31}P phosphorus. Measurement techniques: NMR spectroscopy.)

MYOCARDIAL CONTRACTILITY has been demonstrated to be tightly coupled to alterations in myocardial intracellular pH during ischemia.^{1,2} Because halothane has been shown to inhibit mitochondrial respiration and to uncouple oxidative phosphorylation,³⁻⁷ effects which could lead to the generation of hydrogen ions, it is possible that the negative inotropic action of halothane is mediated by or associated with a decrease in myocardial intracellular pH.

The effect of halothane on myocardial high-energy phosphate balance is difficult to predict.⁸ Halothane could decrease myocardial high-energy phosphate stores if it inhibits mitochondrial respiration and/or uncouples oxidative phosphorylation. Alternatively, if halothane decreases myocardial oxygen consumption secondary to its negative inotropic effect, this could put the heart in a favorable energy supply/demand balance and increase high-energy phosphate concentrations.

Utilizing ^{31}P phosphorus nuclear magnetic resonance spectroscopy, the specific objectives of this study were: 1) to determine whether the negative inotropic action of halothane is associated with a change in myocardial intracellular pH; 2) to assess the net effect of halothane on the steady state concentrations of myocardial high-energy phosphates; and 3) to measure the effects of halothane on the forward rate constant for the transfer of phosphate from phosphocreatine to γ ATP ($\text{PCr} + \text{ADP} + \text{H}^+ \rightleftharpoons \text{ATP} + \text{Cr}$). This reaction is catalyzed by creatine kinase, and is important in high-energy phosphate metabolism because phosphocreatine dynamically buffers the energy stores of the heart.⁹

Materials and Methods

EXPERIMENTAL PREPARATION

Twenty-four New Zealand white rabbits weighing 1.5–2.0 kg were heparinized and anesthetized (sodium pentobarbital 35 mg/kg, intra-peritoneal). The hearts were excised rapidly and placed into iced saline (150 mM NaCl). The ascending aorta was cannulated, and the coronary circulation was perfused with a modified Krebs-Ringer bicarbonate buffer containing 117 mM NaCl, 6 mM KCl, 3 mM CaCl_2 , 1 mM MgSO_4 , 16.7 mM glucose, and 0.55 mM EDTA. The free calcium concentration of the perfusate was approximately 2.5 mM. The perfusate was bubbled continuously in a reservoir with a mixture of 95% O_2 and 5% CO_2 . The final perfusate pH was adjusted to 7.40 by adding 24 mM NaHCO_3 , and the perfusate temperature was maintained at 37° C. Following aortic cannulation and the initiation of coronary artery perfusion, aortic valvular competency was verified by the absence of any significant flow through a vent placed in the left ventricular apex. A balloon-tipped catheter (PE 190 tubing) was positioned in the left ventricle *via* the left atrial appendage to measure isovolumic left ventricular pressure.

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The balloon was carefully purged of air and inflated with sufficient fluid to achieve a left ventricular end-diastolic pressure of 10 mmHg. Left ventricular developed pressure was measured as the difference between left ventricular systolic and end-diastolic pressures. In six of the rabbits, an additional catheter was positioned in the right ventricular cavity *via* the pulmonary artery to sample coronary venous effluent for blood gas analysis. Following catheter placements, the hearts were positioned in a 25 mm NMR sample tube, and submerged in the perfusion medium to optimize conditions for NMR signal acquisition. Coronary perfusion pressure was held constant at 110 cm H₂O. Heart rate was also maintained constant at 181 ± 9 beats/min by right ventricular pacing with a saturated KCl wick encased in PE tubing and connected to a Grass-SD9 stimulator. Coronary blood flow was measured by vacuum aspiration of the coronary perfusate overflow, which was then discarded. Arterial and venous O₂ contents were measured with a Lex O₂ Con-TL from samples withdrawn simultaneously and anaerobically from the aortic and right ventricular catheters, respectively. Myocardial O₂ consumption (per gram of heart) was calculated from the product of coronary blood flow and the arterio-venous O₂ content difference. A 30–45-min equilibration period was allowed prior to the accumulation of NMR spectra.

NUCLEAR MAGNETIC RESONANCE METHODS, PROTOCOLS, AND STATISTICAL ANALYSIS

³¹Phosphorus NMR spectra were obtained from a wide-bore superconducting magnet (4.23 tesla) at 72.89 MHz, using a Bruker WH-180 spectrometer. The instrument was operated in the pulsed, Fourier transform mode, and interfaced with a Bruker 1080 computer. The effects of halothane on myocardial intracellular pH and the steady-state contents of high-energy phosphates were assessed in ten rabbits. Data were collected during the relaxation period after 25 microsecond (45°) radio frequency pulses, which were delivered at 2-s intervals. Spectra, 300 pulses, were accumulated over a 10-min period at a 5000 Hz spectral width with a 4k data table. Following acquisition of the control spectrum, each heart was then exposed to halothane by switching to the perfusate reservoir containing halothane. Halothane was bubbled into the perfusate *via* a Foregger vaporizer through which 95% O₂ and 5% CO₂ flowed. The output of the vaporizer was calibrated by ultraviolet spectroscopy. The concentration of halothane in the perfusate was 0.633 ± 0.016 mM ($n = 5$ measurements). This corresponds to 1.54 ± 0.08 ml vapor/100 ml (1.54 vol %) of halothane. This concentration of halothane was chosen because it reproducibly decreased left ventricu-

lar developed pressure by approximately 50%. After 10–15 min of exposure to halothane, when left ventricular developed pressure was constant and reduced by approximately 50% from baseline values, a second 10-min spectral acquisition period was commenced.

To examine possible changes in myocardial intracellular pH or high-energy phosphates as a function of time of perfusion, time control studies were performed in a separate group of eight rabbits. Following the equilibration period, 10-min spectral acquisitions were obtained during the control period and after 60 min of perfusion with the non-halothane-containing coronary perfusate. Estimates of steady-state tissue contents of phosphocreatine, β ATP, and Pi were obtained by planimetric measurement of the areas (square inches) under their individual spectral peaks and above the computer generated baseline with a Hewlett-Packard electronic digitizer. Planimetry was performed by a technician who was unaware of whether the spectra were obtained at baseline, during halothane administration, or from time-control hearts. In six additional rabbits, the effects of halothane on the unidirectional rate constant for the transfer of phosphate from phosphocreatine to γ ATP (forward rate constant of creatine kinase reaction) was measured using saturation transfer ³¹P NMR spectroscopy as previously described.^{10,11}

Intracellular pH was calculated from the chemical shift (ζ_0) of the Pi peak by the equation:

$$pH_i = pK - \log \frac{\zeta_0 - \zeta_B}{\zeta_A - \zeta_0}$$

To minimize the effects of tissue inhomogeneity, the chemical shifts were measured relative to the resonance of phosphocreatine, which, because of its low pK (4.6), is largely insensitive to pH changes above 6.0. As extensively documented,² constants used in this equation are $pK = 6.90$, $\zeta_A = 3.29$ ppm, and $\zeta_B = 5.81$ ppm.

All values are presented as mean \pm SEM. Statistically significant effects of halothane or perfusion time on the measured variables were assessed by Student's *t* test for paired comparisons. Statistically significant differences between groups (*i.e.*, halothane-treated *vs.* time controls) were assessed by Student's *t* test for grouped comparisons.¹²

Results

MYOCARDIAL INTRACELLULAR pH

With heart rate held constant by electrical pacing, halothane administration (1.5 vol %) reduced ($P < 0.01$) left ventricular (LV) systolic pressure from 130 ± 4 to 76 ± 3 mmHg, had no effect on LV end-diastolic pressure (13 ± 3 mmHg from a control value of 10 ± 1

mmHg), and thus decreased ($P < 0.01$) LV developed pressure from 120 ± 4 to 63 ± 4 mmHg (fig. 1, upper panel). Coronary blood flow was unchanged during halothane administration (36 ± 2 ml/min vs. 33 ± 2 ml/min, respectively), and myocardial oxygen consumption was reduced ($P < .01$) from 0.052 ± 0.009 to 0.042 ± 0.009 ml \cdot min $^{-1}$ \cdot g $^{-1}$. In contrast to the marked depressant effect of halothane on LV developed pressure, halothane had no detectable effect on myocardial intracellular pH (fig. 1, lower panel).

STEADY-STATE MYOCARDIAL HIGH-ENERGY PHOSPHATES

Halothane-treated Hearts. 31 Phosphorus NMR spectra in an individual rabbit heart during the control period prior to halothane administration (bottom spectrum), and during halothane administration (top spectrum) are illustrated in figure 2. The steady-state concentrations of β ATP, phosphocreatine, and Pi at baseline and during halothane administration are summarized in table 1. During halothane exposure, β ATP was decreased ($P < 0.05$) by 0.17 ± 0.06 from a baseline of 1.27 ± 0.11 in 2 , phosphocreatine was decreased ($P < 0.01$) by 0.17 ± 0.06 from a baseline of 2.39 ± 0.15 in 2 , and Pi was increased ($P < 0.05$) by 0.11 ± 0.05 from a baseline of 0.61 ± 0.06 in 2 . The ratio of phosphocreatine/Pi was decreased ($P < 0.01$) during halothane from a baseline value of 4.26 ± 0.41 to 3.46 ± 0.40 .

Time-control Hearts. To determine whether these observed changes in myocardial high-energy phosphates were simply the result of perfusion time rather than specific effects of halothane administration, time-control studies were performed with normal (*i.e.*, non-halothane containing) coronary perfusate. As summarized in table 1, after 60 min of perfusion with non-halothane-containing coronary perfusate, β ATP was decreased ($P < 0.05$) by 0.11 ± 0.05 from a baseline of 1.55 ± 0.10 in 2 , phosphocreatine was decreased ($P < 0.05$) by 0.27 ± 0.14 from a baseline of 2.39 ± 0.11 in 2 , and Pi was slightly but not significantly increased from baseline. The changes in β ATP, phosphocreatine, and Pi in this time control series were not significantly different ($P > 0.2$) from the changes observed during halothane administration (table 1).

CREATINE KINASE REACTION

Utilizing saturation transfer NMR, halothane was observed to decrease ($P < 0.01$) the forward rate constant for the transfer of phosphate from phosphocreatine to γ ATP from a baseline value of 0.63 ± 0.06 to 0.43 ± 0.04 sec $^{-1}$.

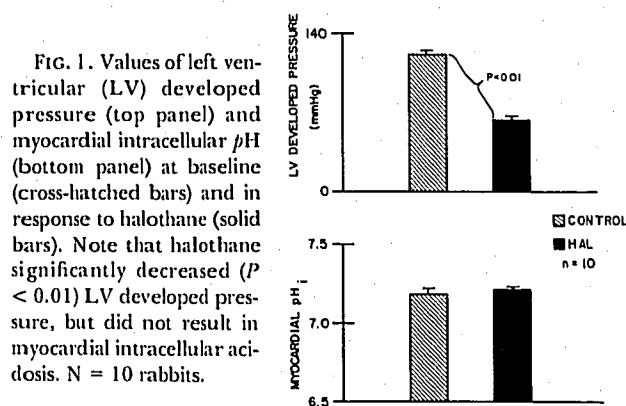


FIG. 1. Values of left ventricular (LV) developed pressure (top panel) and myocardial intracellular pH (bottom panel) at baseline (cross-hatched bars) and in response to halothane (solid bars). Note that halothane significantly decreased ($P < 0.01$) LV developed pressure, but did not result in myocardial intracellular acidosis. N = 10 rabbits.

Discussion

Halothane in high concentrations is known to depress myocardial function both in humans¹³ and in experimental animals.¹⁴ Although halothane has been demonstrated to cause a direct depression of cardiac contractility,¹⁴ the mechanism responsible for this negative inotropic action has not been clearly established.

In the present study, 1.5 vol % halothane markedly reduced LV developed pressure, but was not associated with a concomitant change in myocardial intracellular

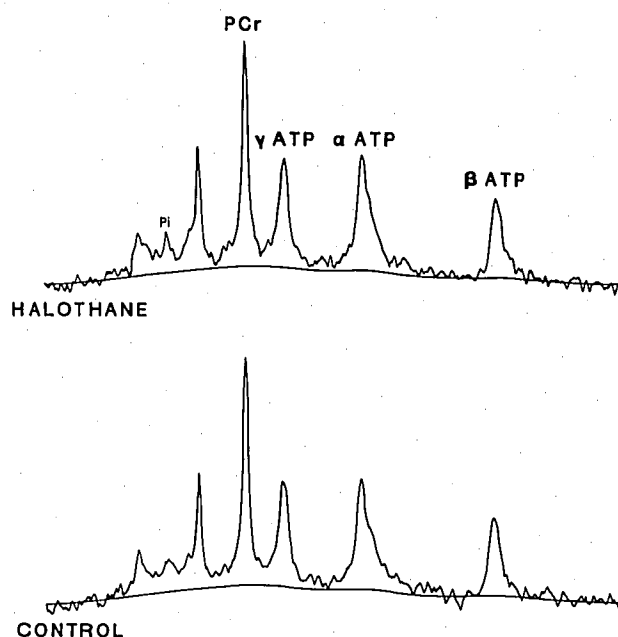


FIG. 2. 31 Phosphorus NMR spectra in an individual rabbit heart prior to halothane (control) and during halothane administration (halothane). Estimates of steady-state concentrations of phosphocreatine (PCr), β ATP, and Pi were obtained by planimetric measurement of the areas under the individual spectral peaks and above the computer generated baseline.

TABLE 1. Effects of Halothane and Perfusion Time on Myocardial High-energy Phosphates

	Baseline	Halothane	Δ	Baseline	60 min Perfusion	Δ
β ATP (in ²)	1.27 \pm 0.11	1.10 \pm 0.09	-0.17 \pm 0.06†	1.55 \pm 0.10	1.44 \pm 0.10	-0.11 \pm 0.05†
PCr (in ²)	2.39 \pm 0.15	2.22 \pm 0.15	-0.17 \pm 0.06*	2.39 \pm 0.11	2.12 \pm 0.15	-0.27 \pm 0.14†
Pi (in ²)	0.61 \pm 0.06	0.72 \pm 0.09	0.11 \pm 0.05†	0.43 \pm 0.13	0.48 \pm 0.06	0.05 \pm 0.11

Symbols (* $P < 0.01$; † $P < 0.05$) indicate significant changes (Δ) from baseline values in response to halothane (1.5 vol %) or perfusion time. No significant differences ($P > 0.2$) in the changes in β ATP, PCr, and

Pi in the halothane group ($n = 10$) versus the time control group (perfusion time of 60 min; $n = 8$) were noted. Values are mean \pm SE.

pH. This clear dissociation indicates that myocardial intracellular acidosis does not mediate the negative inotropic action of halothane. Moreover, although halothane administration appeared to decrease the steady-state concentrations of phosphocreatine and ATP, and to simultaneously increase Pi, these changes were similar in magnitude to the changes that occurred in control hearts perfused for the same length of time. Thus, these results do not support the hypothesis that the negative inotropic action of halothane is mediated by a specific reduction in the tissue concentration of myocardial high-energy phosphates.

It is important to note that the steady-state concentrations of phosphocreatine and ATP provide little information concerning the potential effects of halothane on the rate of turnover of these metabolites, which has been estimated to occur approximately four times per minute in control hearts.⁹ Results from our saturation transfer studies provide direct evidence that halothane reduces the rate of high-energy phosphate exchange. Because the rate of exchange of high-energy phosphates has been shown to be directly correlated with myocardial performance,^{11,15} the decrease in the forward rate constant for the creatine kinase reaction during halothane is likely to be secondary to the concomitant reduction in LV developed pressure.

In contrast to previous studies in closed-chest dogs,¹⁶ coronary blood flow was maintained during halothane despite a 20% decrease in myocardial oxygen consumption. Thus, halothane appears to have caused coronary vasodilation in this Langendorff-perfused rabbit heart preparation, in which heart rate was held constant and no external work was performed. It is also interesting that, in contrast to other experimental models,¹⁶ the halothane-induced decrease in LV developed pressure was proportionately greater than the decrease in myocardial oxygen consumption (48% vs. 20%, respectively). This is surprising because the energy requirements of muscle contraction normally account for 90% of high-energy phosphate metabolism in the heart, and there is usually a very close relationship between myocardial performance and the rate of oxygen consumption.^{2,17} The fact that this was not observed suggests

that mitochondrial function, in particular oxygen utilization, may be altered during halothane.

The phosphocreatine/Pi ratio is considered to be an index of the balance between energy supply and utilization.^{18,19} If the balance between supply and demand had been maintained during the depressed state induced by halothane, then little change would occur in this ratio. Alternatively, if the halothane-induced depression in contractility was exclusive of an energy production defect, one might expect to see an increase in the phosphocreatine/Pi ratio, as observed in KCl arrested hearts. We observed that, although halothane was associated with a 19% decrease in the phosphocreatine/Pi ratio, a similar decrease (18%) was observed in the time-control hearts not exposed to halothane. These results imply that the halothane-induced decrease in LV developed pressure is unlikely to be due to an imbalance between energy supply and demand.

In contrast to the results of the present study, in which halothane administration was associated with a decrease in myocardial high-energy phosphates, Döring²⁰ noted that higher concentrations of halothane sufficient to induce severe cardiac failure were associated with increases in the ratio of phosphocreatine/Pi and ATP/ADP. As noted by Döring, these changes in high-energy phosphates induced by toxic doses of halothane probably indicated a reduction in high-energy phosphate utilization secondary to severe cardiac depression. In a preliminary report,[†] Dedrick and Allen noted that the ratio of phosphocreatine/ATP was unchanged during halothane exposure in rat hearts. The ratio of phosphocreatine/ATP was also unchanged (1.97 ± 0.12 vs. 2.08 ± 0.12) during halothane in the present study, providing additional evidence that changes in the steady-state concentrations of high-energy phosphates do not appear to mediate the profound reduction in LV developed pressure during halothane.

In summary, in the isolated, paced Langendorff-perfused rabbit heart, halothane markedly reduced LV de-

† Dedrick D, Allen PD: NMR studies of myocardial high-energy phosphates and sodium as mediators of negative inotropic effects of volatile anesthetics (abstract). ANESTHESIOLOGY 59:A23, 1983.

veloped pressure but did not result in myocardial intracellular acidosis. A specific halothane-induced reduction in the steady-state concentrations of high-energy phosphates also was not observed. Although halothane was associated with a significant decrease in the forward rate constant of the creatine kinase reaction, this effect is likely a result of (rather than a cause of) the concomitant decrease in myocardial performance. Thus, our results indicate that the negative inotropic action of halothane is not mediated either by intracellular acidosis or by an abnormality in high-energy phosphate metabolism.

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