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The Effect of Halothane on the Steady-state Levels of High-energy Phosphates in the Neonatal Heart

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The neonatal heart is especially vulnerable to cardiac depression caused by halothane. The cause of this sensitivity is uncertain. Isolated, perfused, isovolumic, contracting neonatal rabbit hearts were studied using ^{31}P NMR to determine whether changes in concentrations of high energy phosphates (HEP) or intracellular *p*H mediated the effects of halothane. Steady-state HEP concentrations and intracellular *p*H were unaffected, despite profound reductions of mechanical performance. The data also suggest that halothane, in the concentrations studied, does not cause significant uncoupling of oxidative phosphorylation. (Key words: Anesthetics, volatile: halothane. Heart: high-energy phosphates. Neonate.)

BRADYCARDIA AND HYPOTENSION occur frequently when halothane is used for induction of anesthesia in the newborn and older infant. While the exact cause of the cardiovascular depression is unknown, there is evidence that the immature myocardium is especially sensitive to halothane.[1] We postulate that halothane may cause depression of the myocardium of the newborn by altering high-energy phosphate levels and by increasing cytosolic inorganic phosphorus.

Previous work suggests that halothane may decrease high energy phosphates (HEP) in the adult isolated perfused rabbit heart.‡ The same study reported an increase in the inorganic phosphate concentration during halothane exposure. Halothane was found to produce no change in the creatine phosphate to adenosine triphosphate (ATP) ratio of adult rat heart; the effect on inorganic phosphorus was not specifically mentioned.§

Inorganic phosphorus (Pi) has been found to modulate myocardial contractile force.[2] The negative inotropic effects of halothane could be mediated by increasing Pi concentration. Myocardial depression could also occur as a result of decreased sarcoplasmic reticulum (SR) calcium uptake.[3] Decreased ATP concentrations in the presence of halothane has been shown to reduce the rate of SR calcium uptake.

Inhibition of mitochondrial respiration may result in decreased ATP concentration unless ATP consumption is reduced proportionately. This is also true if oxidative phosphorylation is uncoupled, *i.e.*, P/O ratio reduced, if oxygen consumption is unchanged at a given work load. Halothane has been found to inhibit State III respiration by 33% at 0.7 mM in adult beef heart mitochondria,[4] and, at higher concentrations, submitochondrial particles.[5] Heart mitochondria appear to be less susceptible to this effect than liver mitochondria.[4,6] Halothane concentrations of greater than 2 mM are required to produce changes in adenosine diphosphate (ADP)/O ratios.[4] In adult heart, inhibition of ADP stimulated mitochondrial respiration probably occurs in the range of clinically used concentrations, while frank uncoupling, that is, a reduction in the ADP/O or P/O ratio, may occur at higher halothane concentrations than routinely used.

The lipid composition of the cardiac mitochondria of the newborn is distinctly different from the adult.[7] Halothane could affect the newborn myocardium in a different manner than the adult due to the elevated neutral lipid content of the newborn mitochondrial membrane.

We investigated the effects of halothane, in concentrations sufficient to produce significant mechanical depression, on the steady-state HEP levels and intracellular *p*H of the newborn rabbit heart using ^{31}P nuclear magnetic resonance (NMR), a non-invasive technique for measuring phosphorus containing metabolites in an intact beating heart. Oxygen consumption measure-

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‡ Murray PA, Blank TJJ, Rogers MC, Jacobus WE. Halothane-induced abnormalities of myocardial high energy phosphate metabolism. ANESTHESIOLOGY 59:A63, 1983

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§ 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

ments were made and these data were used in conjunction with the NMR data to assess the degree of uncoupling of oxidative phosphorylation.

Methods

New Zealand white rabbits aged 1–4 days were obtained from a single supplier. The animals weighed from 55–85 grams. The wet heart weight (ww) ranged from .320–.479 grams. The animals were anesthetized with pentobarbital 50 mg/kg and systemically heparinized; 300 U/kg. The hearts were rapidly excised and placed in iced buffer. The aorta was cannulated and perfused with Krebs-Henseleit buffer equilibrated with 95% O_2/5% CO_2. The buffer composition is as follows (mM/L) NaCl 118, KCl 4.7, $CaCl_2$ 2.0, $MgSO_4$ 1.2, Na_2 EDTA 0.5, $NaHCO_3$ 25, and glucose 11.0 mM. The *p*H of the buffer ranged from 7.39–7.43. During the halothane exposure phase of the experiments, the perfusion medium was equilibrated with halothane vapor from a calibrated vaporizor using a bubble oxygenator device. The hearts were perfused at a mean rate of 16.2 cc min $^{-1} \cdot g_{ww}^{-1}$ at a temperature of 37.5° C. A left ventricular drain was placed *via* the left atrium. Then a latex, saline-filled, isovolumic balloon was placed in the left ventricle and secured. The left ventricular pressure tracing was recorded continuously using a Hewlett Packard recorder (Model No. 7754A). The preparation described above had been previously found to be mechanically and biochemically stable for a period of 3 h. The time required for completion of the halothane experiments was less than 1 h.

Six unpaced hearts were used for the NMR experiments. Each heart was subjected to the same protocol. Only one initial workload was used, and all six hearts were set to this value by adjusting the volume of the LV balloon. The mechanical performance, or workload, is defined in terms of the rate pressure product, RPP, which is the heart rate (HR) times the developed pressure (systolic pressure minus the diastolic pressure).

NMR Spectra

Spectra were obtained from a Nicolet spectrometer using a 12-mm probe tuned for ^{31}P in wide-bore 8.4T Oxford magnet. All ^{31}P spectra were acquired using the pulse Fourier transform mode with quadrature phase detection. NMR parameters included a tip angle of 60°, a 2-s interpulse delay time, a spectral width of 4000 Hz, and a 2K data block size. One hundred twenty-eight acquisitions were summed and apodized using a 20-Hz line broadening to enhance signal to noise ratio.

Control spectra were taken at 5-min intervals (×2), followed by a period during which the hearts were exposed to halothane in sufficient concentration (avg. 1.5%) to produce 50% depression of baseline mechanical performance. After the mechanical performance had stabilized, two spectra, 5 min apart, were obtained to assess the effects of halothane on the steady-state HEP levels. The halothane was turned off and allowed to wash out. Spectra were acquired during the washout phase as mechanical performance returned to baseline.

Intracellular concentrations of creatine phosphate (CrP), ATP, and Pi were determined using the areas of the resonance peaks for those species. Intracellular *p*H was read from a standard curve using the chemical shift of Pi relative to CrP. Free ADP concentration was calculated using the equilibrium constant for the creatine kinase reaction at a *p*H of 0.1 below that measured by NMR.[8] Total creatine was determined using the method of Kammermeier.[9]

Oxygen consumption measurements were made on a group of hearts perfused under conditions identical to those used in the NMR experiments. A total of six hearts were used, each following the same protocol. The oxygen tension of the aortic perfusate and PA effluent were measured with a Clark type electrode calibrated at 37.5° C. Coronary flow was held constant using an infusion pump.

Oxygen tension measurements were made at three different workloads allowing a period of 5 min after each change in workload. The heart was then exposed to halothane (1–1.5%), and oxygen tension measurements were made at two workloads. The workloads were chosen so that the final data represented three measurements for each of six workloads under control conditions and three for each of four workloads during halothane exposure. The data points were used to derive regression equations for oxygen consumption *versus* workload.

Data were analyzed using Wilcoxon's rank test, with each heart serving as its own control. A P value of .05 was taken as significant.

Results

All data obtained during the NMR experiments are summarized on table 1. Typical spectra and mechanical performance strip charts are shown in figures 1 and 2, respectively. Halothane consistently produced a 50% reduction in mechanical performance at concentrations of 1.5%. This was manifested as a decrease in systolic pressure and a small (2–5 mmHg) increase in diastolic pressure. The time from peak pressure to diastolic pressure was unaltered by halothane (data not shown). HR was not significantly affected.

The steady-state levels of ATP, Pi, and CrP do not change from control values when the neonatal hearts

TABLE 1. NMR Measured Values—Steady-state High-energy Phosphate Levels (Mean + SEM)

	ATP (μ moles g^{-1})	CrP (μ moles g^{-1})	Pi (μ moles g^{-1})	Intracellular pH
Control (RPP = 11,000) mmHg min^{-1}	21 + 2	7.13 + 0.30	8.95 + .56	7.17 + 0.02
Halothane (RPP = 5,000) mmHg min^{-1}	21 + 2 (NS)	7.13 + .42 (NS)	8.95 + .56 (NS)	7.17 + 0.02 (NS)
Washout (RPP = 10,500) mmHg min^{-1}	21 + 2 (NS)	7.05 + .37 (NS)	8.48 + .60 (NS)	7.16 + 0.02 (NS)

NS = No significant change from control; RPP = Rate pressure product (mmHg min^{-1}); product of HR × (developed systolic pressure − diastolic pressure).

are depressed with halothane. The ATP concentration in these hearts is approximately 5 mM (assumes 0.8 ml H_2O/1.0 gm heart). The measured intracellular pH did not change during mechanical depression of the myocardium by halothane. Further, the ratio of CrP:(CrP + Pi) remained stable throughout the experiments, indicating that the preparation was biochemically stable. The CrP content was found to be approximately one-third the ATP content. This is characteristic of the isolated glucose substrate perfused neonatal rabbit heart.

The oxygen consumption data are summarized in table 2. A linear regression equation relating oxygen consumption to workload was derived from the individual data points. Oxygen consumption was found to vary linearly with workload for both groups of hearts. The regression equation is minute consumption of oxygen (MVO_2) (u mole $g_{DW}^{-1} \cdot min^{-1}$) = 0.000683 × RPP + 5.19 (r^2 = .91) for control hearts and MVO_2 = .000733 × RRP + 5.22 (r^2 = .89) for hearts exposed to halothane (DW = dry weight of the heart). At a workload (RPP) of 5,000, the predicted MVO_2 for the control and halothane groups are 8.6 u mole $g_{DW}^{-1} \cdot min^{-1}$ and 8.9 u mole $g_{DW}^{-1} \cdot min^{-1}$ respectively. These values are not significantly different.

Calculated values are listed in table 3. The mean values of ATP production, calculated assuming a P:O ratio of 3, and free ADP content are given for the control, halothane exposure, and washout periods. ATP production was found to decrease markedly during halothane exposure, as did workload, despite a constant free ADP content.

Discussion

The spectra shown in figure 1 are different than expected if one is familiar with ^{31}P-NMR spectra of adult heart preparations. The creatine phosphate content is lower than ATP. This finding has been noted consistently in our metabolic studies of the neonatal rabbit heart. The low CrP content is due in part to the low total creatine content (16–20 u moles g_{DW}^{-1} *vs.* greater than 50 u moles g_{DW}^{-1} for the adult). As CrP cannot exceed total creatine, the CrP/ATP ratio must be less than 1. The CrP-to-ATP ratio is a major determinant of the free intracellular ADP concentration. The majority

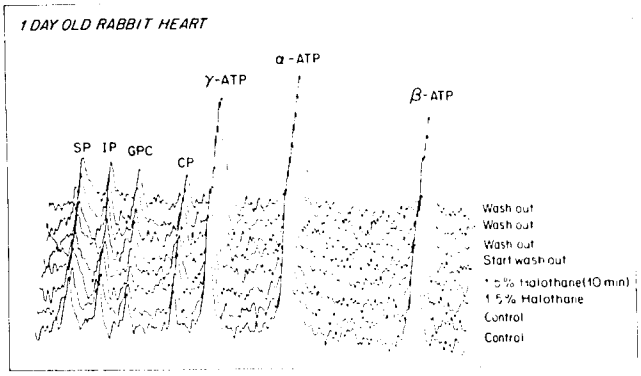

FIG. 1. The phosphate resonances observed by ^{31}P-NMR are shown. The sequence of spectra cover the control, halothane, and washout periods. During halothane exposure, the mechanical performance was significantly depressed (fig. 2). However, the resonance areas of the HEP's are unchanged. The resonances are SP = sugar phosphates and phosphomonoesters: IP = inorganic phosphorus: GPC = glycerol phosphocholine: CrP = creatine phosphate: ATP = has three resonances: gamma, alpha, and beta.

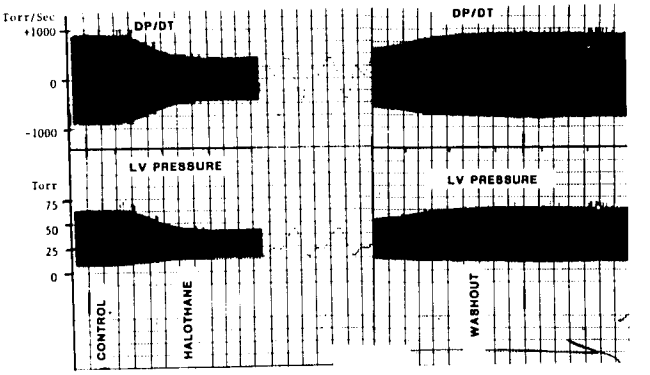

FIG. 2. The mechanical performance of the heart for which the ^{31}P NMR spectra are shown in figure 1. The onset of mechanical depression appears rapidly during halothane exposure. The full exposure period is not shown. The terminal washout phase is shown and full recovery of function is noted. The control LV pressure is 62/7.

TABLE 2. Oxygen Consumption Data (Mean Values)

Control*			Halothane Exposure†		
n‡	Workload (mmHg −min^{-1})	MV O_2§ (μ mole O_2 min^{-1}·g_{DW}^{-1})	n‡	Workload (mmHg −min^{-1})	MV O_2§ (μ mole O_2 min^{-1}·g_{DW}^{-1})
3	12,600	14.1	3	8,900	12.2
3	11,300	13.3	3	6,000	8.9
3	9,000	10.4	3	4,000	8.5
3	8,200	10.2	3	2,700	7.5
3	5,000	9.1			
3	4,700	8.5			

Dry heart weight = 0.063 ± .003 g.
* Mean HR = 219 ± 14; Coronary flow = 6.0 cc/min.
† Mean HR = 215 ± 8.7; Coronary flow = 6.0 cc/min.
‡ Number of data points of each workload.
§ Error of measurements is ± 5%.

of intracellular ADP is bound, direct measurement of ADP yields values 20 times greater than the free concentration.[8] Free ADP concentration can influence state III respiration if it is near its Km value (20–30 mM).[10] In all cases shown in table 3, the calculated free ADP is well above the Km for mitochondrial respiration, and, thus, free ADP is probably not a controlling influence on the rate of ATP synthesis. The phosphorylation potential (ATP)/(ADP) (Pi) is also unchanged by halothane: as a result, the expected free energy of hydrolysis of ATP does not change during halothane exposure.

In spite of marked depression of mechanical performance of the left ventricle by halothane in these isolated neonatal rabbit hearts, intracellular concentrations of HEP, Pi, and intracellular *p*H remained unchanged. The availability of energy in the form of ATP is a function of the flux through the creatine kinase reaction.[11] The flux through the reaction has been reported to be decreased by halothane.‡ However, creatine kinase flux is a function of cardiac work; the reported decrease in flux was exactly that expected for the decrease in cardiac work caused by halothane.[12] Thus, the flux-workload relationship is unaltered by halothane. It is unlikely that the negative inotropic effects of halothane are mediated by a decrease in energy availability at the contractile apparatus.

Blank and Thompson had found that, at a *p*H of 7.3, halothane had no effect on SR calcium uptake, but, at a *p*H of 6.9, the plateau phase was affected.[3] The intracellular *p*H measured in these isovolumic preparations was 7.17 during the control period and during halothane exposure. Impaired SR calcium uptake during halothane exposure cannot be excluded, but the decrease would be less than that expected if halothane also produced a significant decrease in intracellular *p*H.

TABLE 3. Calculated Values (Mean Values)

Conditions	MVO_2* (μ mole O_2 min^{-1}·g_{DW}^{-1})	ADP† (μ mole g_{DW}^{-1}) mM	Phosphorylation Potential‡
Control	12.7	.268 (.067)	3.7×10^4
Halothane	8.9	.268 (.067)	3.7×10^4
Washout	12.4	.268 (.067)	3.7×10^4

* From regression equations, using RPP values from table 1.
† Using K eq of 141 (see ref. 7), and free creatine concentration of 3.2 mM (free creatine = total creatine − CrP; error is ±20%).
‡ Equal to (ATP)/ADP (Pi).

Various indices of cardiac work have been correlated with oxygen consumption.[13] Pressure development, or the tension time index, was found to be the major determinant of the non-zero work oxygen consumption in isolated perfused hearts.[14] Previous studies in our laboratory have found that the HR times developed pressure is linearly related to oxygen consumption.[13] Other indices, such as stroke work and dp/dt, have been found to be unreliable predictors of myocardial oxygen consumption.[11] We found that oxygen consumption was linearly related to workload, as defined by HR × developed pressure for the neonatal rabbit heart during control conditions and halothane exposure. The regression equations are not different over the range of physiologically achievable workloads. This observation, in conjunction with the NMR data, suggests that there is no significant uncoupling of oxidative phosphorylation with the concentration of halothane studied.

Oxygen consumption is related to ATP production by the P:O ratio, which is generally accepted to be 3 for NAD linked substrate.[15] The constant ATP content observed during halothane exposure means that ATP production and consumption are equal. If uncoupling of oxidative phosphorylation occurs, the P:O ratio decreases. The oxygen consumption needed to support the ATP demands would be greater than expected in the normally coupled state (control conditions). This was not observed; rather, the measured oxygen consumption at a given workload is not significantly different during halothane exposure from that measured under control conditions.

We conclude that the negative inotropic effects of halothane are mediated by mechanisms which operate

at a normal intracellular pH and HEP concentrations. The observed depression of mechanical performance is not caused by an increase in inorganic phosphate concentration, and there does not appear to be any significant uncoupling of oxidative phosphorylation in hearts exposed to halothane at the concentrations studied.

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References

1. Krane EJ, Su JY. Comparison of halothane induced depression in newborn and adult rabbit ventricle (abstract). ANESTHESIOLOGY 65:A437, 1986
2. Kusuoka H, Weisfeldt ML, Zweier J, Jacobus WE, Marban E. Mechanisms of early contractile failure during hypoxia in intact ferret heart: Evidence for modulation of maximal Ca^{+2}-activated force by inorganic phosphate. Circ Res 59:270–282, 1986
3. Blank TJJ, Thompson M. Calcium transport by cardiac sarcoplasmic reticulum: Modulation of halothane action by substrate concentration and pH. Anesth Analg 60:390–4, 1981
4. Harris RA, Munroe J, Farmer B, Kim KC, Jenkins P. Action of halothane upon mitochondrial respiration. Arch Biochem Biophys 142:435–444, 1971
5. Grist EM, Baum H. A possible mechanism for the halothane-induced inhibition of mitochondrial respiration binding of endogenous calcium to NADH dehydrogenase. FEBS Lett 48:41–44, 1974
6. Hall GM, Kirtland SJ, Baum H. The inhibition of mitochondrial respiration by inhalational anesthetic agents. Br J Anaesth 45:1005–1009, 1973
7. Nagatomo T, Hattori K, Ikeda M, Shimada K. Lipid composition of sarcolemma, mitochondria and sarcoplasmic reticulum from newborn and adult rabbit cardiac muscle. Biochem Med 23:108–118, 1980
8. Veech RL, Lawson JWR, Cornell NW, Krebs HA. Cytosolic phosphorylation potential. J Biol Chem 6538–6547, 1979
9. Kammermeier H. Microassay of free and total creatine from tissue extracts by combination of chromatographic and fluorometric methods. Anal Biochem 56:341–345, 1973
10. Chance B, Williams GR. Respiratory enzymes in oxidative phosphorylation. 1. Kinetics of oxygen utilization. J Biol Chem 217:383–393, 1955
11. Ingwall JS. Changes in creatine kinase system during the transition from compensated to uncompensated hypertrophy in the spontaneously hypertensive rat, Perspectives in Cardiovascular Research, Vol. 8. Edited by Tarazi RC, Dunbar JB. New York, Raven Press, 1983, pp 145–155
12. Bittl JA, Ingwall JS. Reaction rates of creatine kinase and ATP synthesis in the isolated rat heart. J Biol Chem 260:3512–3517, 1985
13. Gibbs CL. Cardiac energetics. Physiol Rev 58:174–254, 1978
14. Neely JR, Liebermeister H, Battersby EJ, Morgan HE. Effect of pressure development on oxygen consumption by isolated rat heart. Am J Physiol 212:804–814, 1967
15. Lemasters JJ. The ATP-to-oxygen stoichiometries of oxidative phosphorylation by rat liver mitochondria. J Biol Chem 259:3058–3063, 1984