Effects of Volatile Anesthetics on Cardiac Metabolism in the Low-pressure Perfused Rat Heart

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Effects of halothane, enflurane and isoflurane on the myocardial metabolism were studied in the rat heart-lung preparation. Hearts were perfused at a low perfusion pressure (SBP 50 mmHg, DBP 30 mmHg) with succinate or glutamate as substrates. Thirty minutes after the perfusion, intramyocardial ATP, pyruvate, lactate and glycogen were measured enzymatically. Although there was no significant difference in ATP levels of hearts with either substrate, and whether or not volatile anesthetics were present, 1% halothane and 1.5% isoflurane reduced the L/P ratio when succinate was substrate (24.46 ± 4.81, 17.68 ± 9.10 vs 39.82 ± 10.83), and 2% enflurane decreased it when glutamate was substrate (22.25 ± 10.99 vs 38.44 ± 6.55). The glycogen levels in volatile anesthetics groups were lower than control when succinate was substrate. The improvement of energy demand-supply balance by inhalation anesthetics may be stronger than their inhibition of electron transport in mitochondria under certain ischemic conditions.
(Key words: volatile-halothane, enflurane, isoflurane, metabolism)

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It is well known that halothane inhibits mitochondrial respiration at the level of nicotinamide adenine dinucleotide-reduced (NADH) dehydrogenase in the liver[1–5], brain[4] and heart[2,6]. The other volatile anesthetics also inhibit electron transport[3,7,8]. Halothane-induced inhibition of electron transport has been studied with several substrates[1,4,9]. The sensitivity to inhibition by halothane was greater with NAD-linked glutamate than with non-linked succinate as substrate. However, these data were obtained from the studies in the isolated mitochondria. Effects of volatile anesthetics on the energy metabolism in the global heart have not been previously studied.

The aim of the present study was to determine whether different anesthetics and substrates affect the myocardial metabolism in qualitatively similar ways. Metabolic variety by different anesthetics may be more prominent in ischemic perfusion than in normal one. The low blood pressure induces reduced coronary perfusion and myocardial ischemia[10,11]. So, we selected the low pressure perfusion using a heart-lung preparation which was easy to make the myocardial energy demand constant and to measure the metabolic contents[12].

Materials and Methods

The techniques used in this study were identical to those used in the prior study[12]. In brief, male Wistar rats (300–330g) were

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Table 1. Intramyocardial ATP Contents (μ mole/g dry weight)

Substrates	Control (n = 8)	1% halothane (n = 8)	2% enflurane (n = 6)	1.5% isoflurane (n = 6)
Succinate	18.72±1.25	19.19±0.94	18.57±1.25	19.08±1.67
Glutamate	19.25±1.10	18.95±1.71	18.96±1.21	18.71±0.99

Table 2. Intramyocardial L/P Ratio

Substrates	Control (n = 8)	1% halothane (n = 8)	2% enflurane (n = 6)	1.5% isoflurane (n = 6)
Succinate	39.82±10.83	24.46±4.81$^{\sharp}$	30.32± 7.52	17.68± 9.10$^{\sharp}$
Glutamate	38.44± 6.55	31.23±5.09	22.25±10.99$^{\sharp}$	28.19±11.37

$^{\sharp}P$ <0.005 vs control

Table 3. Intramyocardial Glycogen Contents (μ mole/g dry weight)

Substrates	Control (n = 8)	1% halothane (n = 8)	2% enflurane (n = 6)	1.5% isoflurane (n = 6)
Succinate	99.24±17.44	62.27± 7.81$^{\sharp}$	70.69±14.73$^{\sharp\sharp}$	76.74± 7.76$^{\sharp\sharp\sharp}$
Glutamate	80.54±14.26	66.11±14.61	67.89±13.83	73.35±14.30

$^{\sharp}P$ <0.005, $^{\sharp\sharp}P$ <0.01, $^{\sharp\sharp\sharp}P$ <0.05 vs control

anesthetized with 50 mg/kg of pentobarbital intraperitoneally. A tracheostomy was performed, and constant volume (1.5 ml) intermittent positive pressure ventilation was instituted at a rate of 20 breaths/min with 100% oxygen (control groups: each substrate group; n = 8), 1% halothane (halothane groups: each group; n = 8), 2% enflurane (enflurane groups: each group; n = 6) or 1.5% isoflurane (isoflurane groups: each group; n = 6) in oxygen. The chest was opened and flooded with icecold saline and the heart was arrested during the preparation. Cannulae were inserted into the aorta and the superior and inferior venae cavae. A pacing wire was placed on the right atrium. A heart lung preparation was perfused with perfusate blood (25 ml), containing red blood cells which were collected from another rat and Krebs Ringer bicarbonate buffer, and its hematocrit and pH were 25 per cent and 7.4, respectively. Succinate or glutamate of 11 mM/L was added to the perfusate blood. The perfusate blood pumped from the aorta, passing through a pneumatic resistance, was collected in a reservoir that was warmed at 37°C throughout the experiment by means of a water jacket and then returned to the inferior vena cava. No other organs except heart and lung were perfused.

All hearts were perfused at a heart rate of 300 beats/min, a cardiac output of 20 ml/min and systolic and diastolic pressures of 50 and 30 mmHg, respectively. Thirty minutes after the start of perfusion, hearts were freeze-clamped by liquid nitrogen. Subsequently, the heart tissue was freeze-dried for 6 days. A part of the freeze-dried sample was extracted with perchloric acid and centrifuged at 3000 xg. Adenosine triphosphate (ATP), pyruvate and lactate were determined spectrophotometrically by standard techniques according to Bergmeyer[13]. Another piece of freeze-dried sample was placed in 30% KOH and digested in a boiling water bath. Tissue glycogen was extracted, hydrolyzed and assayed as glucose equivalents[14].

Data reported in this experiment are mean ± SD values. A two way analysis

of variance was used to determine the significant difference. A probability of $P < 0.05$ was regarded as statistically significant.

Results

There were no significant differences in ATP contents between the 4 groups with both substrates (table 1). Intramyocardial lactate/pyruvate (L/P) ratios in halothane and isoflurane groups were significantly lower than that in control group when succinate was sustrate, and L/P ration in enflurane group was lower than that in control group when glutamate was substrate (table 2). Glycogen levels in three anesthetic groups were more reduced than that in control when succinate was substrate (table 3).

Discussion

All hearts were perfused at a heart rate of 300 beats/min and a perfusion pressure of 50 mmHg. Although this heart rate is not so high for the rat[15], the low perfusion pressure might induce reduced coronary flow and myocardial ischemia. In this condition, there were no significant differences in ATP levels between the 4 groups with both substrates. However, halothane and isoflurane improved the intramyocardial L/P ratios that reflect oxidation-reduction state when succinate was substrate, and enflurane reduced it when glutamate was substrate. It is well known that halothane produces a dose related depression of myocardial function accompanied by decreases in myocardial oxygen consumption and coronary blood flow[16–18]. Therefore, halothane has been thought either dangerous because it lowers perfusion pressure[16,19] or beneficial because it lessens myocardial oxygen demand[20–22]. Enflurane and isoflurane also decrease myocardial oxygen consumption (MVO_2)[23,24]. However, the rate pressure product (RPP) was constant in this experiment. Although the RPP does not always correlate with the MVO_2[11,25,26], it is likely that the MVO_2 in all groups should be same because the cardiac output was also constant. Therefore, the improvement of the L/P ratio by these volatile anesthetics may not be due to their depressant effects, but due to their direct pharmacologic property. If volatile anesthetics had only the metabolic effect as the pharmacologic property, L/P ratio that correlates with NADH oxidation would have increased. One possible explanation is that diastolic intramyocardial tissue pressure was probably lower with anesthetics and that the coronary blood flow would increase. However, it is only isoflurane that has been reported to have a coronary vasodilating effect[27].

Halothane and isoflurane more reduced the L/P ratios in succinate group than those in glutamate one. Since the L/P ratio is concerned with NADH redox level, halothane or isoflurane-induced inhibition of the NADH oxidation might influence the L/P ratio when glutamate was substrate. However, enflurane decreased the L/P ratio when glutamate was substrate. We don't know exactly the cause of this difference. It may be due to the fact that their NADH effects are quantitatively different[8].

Volatile anesthetics decreased the glycogen contents in the myocardium when succinate was substrate, but did not reduce them significantly when glutamate was substrate. Since the cardiac work with or without anesthetics was almost same, it seems unlikely that the glycogenolysis in inhaled anesthetic groups might be due to the increased MVO_2. Biebuyck et al.[5] have shown that halothane stimulates the glycolysis and inhibits the gluconeogenesis in the perfused liver of rats. Although this report is not concerned with the cardiac metabolism, it appears possible that succinate would be easier to be metabolized as energy source than glutamate, and that volatile anesthetics would increase the utilization of glucose instead of succinate as substrate.

The deterioration in myocardial oxidation-reduction status that is related to the ability of volatile anesthetics to inhibit the electron transport chain in mitochondria was not observed in this experiment. However, we have observed the different result that halothane and enflurane had increased

the intramyocardial lactate contents after postischemic reperfusion in the same rat's preparation[12]. The improvement of energy demand-supply balance by inhalation anesthetics may be stronger than their inhibition of electron transport in mitochondria under certain ischemic situations. This hypothesis agrees with the reported data showing that with a normal level of myocardial perfusion, halothane causes deterioration in energy balance and, on the contrary, in severely hypoperfused myocardium, it improves energy balance[28]. It is concluded that the volatile anesthetics have beneficial effects in this low-pressure perfusion model but differ in their ability to cause the metabolic improvement.

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