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Halothane Does not Suppress the Recovery of Depleted ATP Induced by Ischemic Hypoxia in the Canine Liver

Hideo HORIKAWA, Takuya HIGUCHI, Michiko SAKAI, Yuzuru KATO, Tadayoshi KARUBE and Kunio ICHIYANAGI

Department of Anesthesia, Yamagata University School of Medicine, Yamagata 990-23, Japan

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Summary. **Effects of halothane were examined concerning the recovery of reduced ATP content and adenylate energy charge potential (AECP) that had been induced by ischemic hypoxia in the dog liver.**

Tissue hypoxia induced by 15 min occlusion of the hepatic artery and the portal vein resulted in the depletion of ATP and AECP in the liver tissue. Reperfusion for 10 min restored the ATP content and AECP to near the control levels. Two percent halothane did not affect the rate of this restoration.

INTRODUCTION

A number of reports have demonstrated that the state 3 mitochondrial respiration (oxydative phosphorylation) is markedly and dose-dependently inhibited *in vitro* with clinical concentrations of halothane when glutamate is used as a substrate.[1-5]

If this inhibition of the state 3 mitochondrial respiration also occurs *in vivo* as markedly as *in vitro*, there is a possibility that halothane anesthesia may suppress the tissue ATP content. It has already been reported in animal studies, however, that halothane in clinical concentrations does not affect the tissue ATP content.[6,7] This apparent lack of effect by halothane on the tissue ATP content *in vivo* may be partly explained in that, under inhalational anesthesia, suppression of the ATP production may be matched by a proportional decrease in the ATP consumption in the tissue.

The present study was carried out to determine whether the recovery of hypoxia-induced decrease in tissue ATP content is affected by halothane anesthesia.

MATERIALS AND METHODS

Twenty-two mongrel dogs of both sexes weighing 9-21 kg (mean 13.5 kg), fasted overnight, were used. They were divided into two groups of 11 animals each: halothane group and control group.

Anesthesia was induced with thiopental 25 mg/kg given intravenously (i.v.), and maintained with 50% N_2O in oxygen. After induction of anesthesia, 2 mg pancuronium bromide was injected i.v. and the trachea was intubated. The animals were ventilated mechanically with a Harvard Animal Ventilator Model 625, the minute volume being adjusted to maintain the $Paco_2$ in the range of 35-45 mmHg. Pancuronium bromide was given to prevent spontaneous respiratory activity. The femoral artery and a foreleg cutaneous vein were cannulated for arterial pressure display, blood gas analyses and i.v. fluid infusion.

Laparotomy was carried out under local infiltration anesthesia with 1% bupivacaine. The hepatic artery and the portal vein were exposed for the purpose of occlusion to halt the hepatic blood flow.

In the halothane group, 2% halothane from a precalibrated Fluotec III® Vaporizor was added to the anesthetic gases at the end of the preparatory operation described above. Thirty min were allowed to pass for each group. The hepatic artery and the portal vein were then occluded for 15 min to halt the hepatic blood flow completely.

Samples of liver tissue and arterial blood were collected sequentially five times: before the occlusion; at the end of the occlusion; and 10 (RI0), 30 (R30), and 60 (R60) min after the resumption of perfusion. Sam-

ples of liver tissue were obtained, in principle, from different liver lobes; if obtained from the same lobe, care was taken to place enough distance between the sampling sites.

Rectal temperature was kept at 37±0.5℃ with a thermal blanket. Ringer solution was given i.v. at a rate of 10 ml/kg/hr for both groups. Metabolic acidosis, greater than -5 meq/l, was corrected by i.v. infusion of sodium bicarbonate, but after the vascular occlusion was initiated, no correction was made for metabolic or respiratory acid-base imbalance.

Lactate and pyruvate contents of liver tissue and blood lactate were measured enzymatically (Lactate UV Test, Pyruvate UV Test, Boehringer-Mannheim).

Analysis of tissue contents for adenine nucleotides

Samples weighing 0.5-1.0 g were frozen immediately on a pair of metal blocks (Wollenberger's tongs),[8] precooled in liquid nitrogen, and then crushed to powder in a stainless steel cylinder fitted with a stainless steel piston, both precooled in liquid nitrogen. The tissue powder was weighed and 0.6 N perchloric acid solution —nearly three times the weight— was added to the powder in a homogenizer tube. The mixture was then homogenized in an ice bath for about 10 min with a teflon pestle. The precipitated protein was centrifuged for 10 min at 4,000 RPM at 0℃. The clear supernatant fluid was decanted into another tube and the residue was rehomogenized with a 0.2 N perchloric acid solution —nearly twice the weight of the residue— for 3 min and recentrifuged. The combined supernatant fluids were neutralized to pH 7 by the addition of 5 N KOH. This last solution was used for the enzymatic determination of ATP, ADP and AMP.[9,10]

Data were reported as mean±SEM, and compared using the unpaired *t* test. Observed differences were considered significant when $p<0.05$.

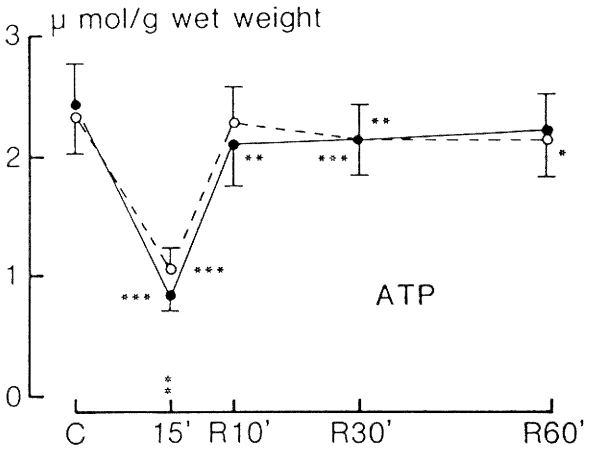

Fig. 1. Changes in ATP by interception and resumption of hepatic circulation. Mean±SD, (●) control group and (○) halothane group *Significant difference from control value. Significant difference between groups is shown by asterisks on the abscissa.
*P<0.05 **P<0.01 ***P<0.001

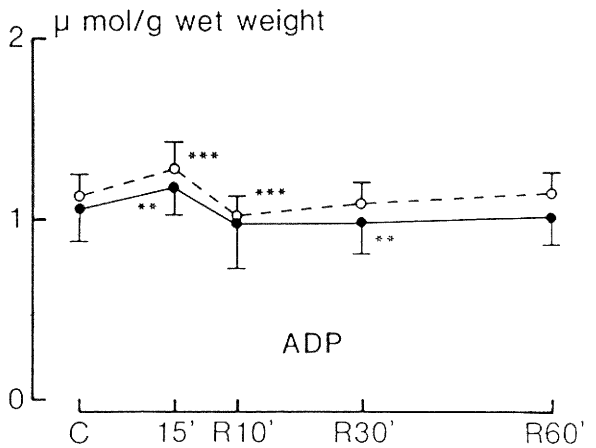

Fig. 2. Changes in ADP (Refer to Fig. 1 for legend).

RESULTS

Changes in ATP, ADP, AMP, total adenine nucleotides and adenylate energy charge potential (AECP)[11] in the liver tissue are shown in Figures 1-5. Concentrations of ATP and AECP decreased markedly in both groups after 15 min interception of the hepatic blood flow, and the decrease in the control (N_2O) group was significantly greater than that in the halothane (N_2O and halothane) group.

ATP and AECP suppressed by ischemic hypoxia recovered nearly to the control levels within 10 min of resumption of the hepatic circulation and no significant differences were found at R10, R30 and R60 between the two groups.

The contents of total adenine nucleotides decreased in both groups by vascular occlusion, and recovered slowly after the resumption of the hepatic circulation. However, the values in both groups at R10, R30 and R60 were significantly lower than their control values (Fig. 4). There were no inter-group differences.

The lactate content (Fig. 6) and the lactate-pyruvate ratio (Fig. 7) of the liver tissue changed in a

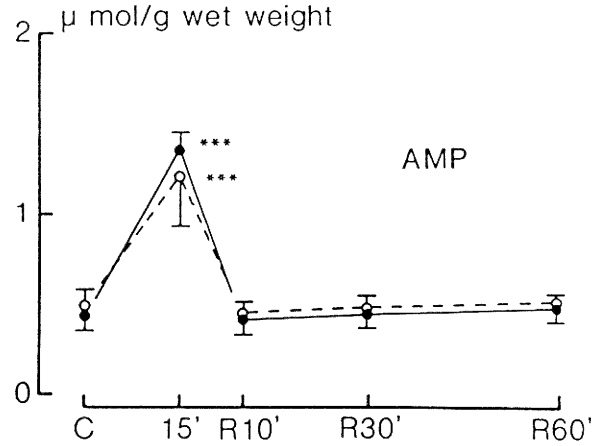

Fig. 3. Changes in AMP (Refer to Fig. 1 for legend).

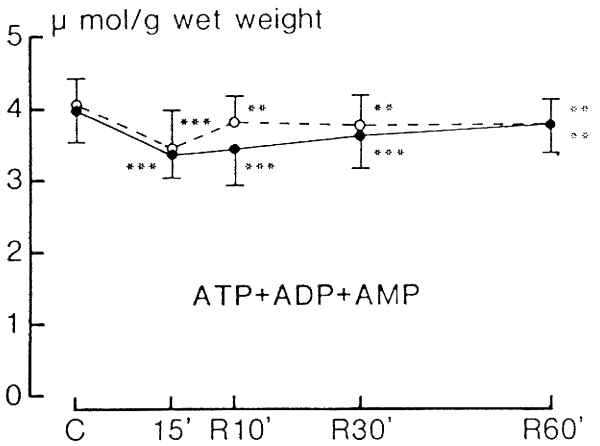

Fig. 4. Changes in total adenine nucleotides (Refer to Fig. 1 for legend).

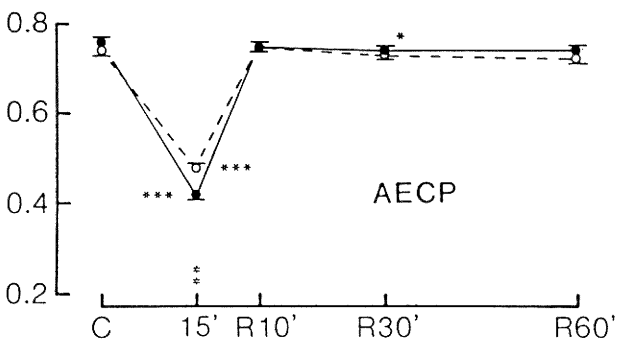

Fig. 5. Changes in AECP (Refer to Fig. 1 for legend).

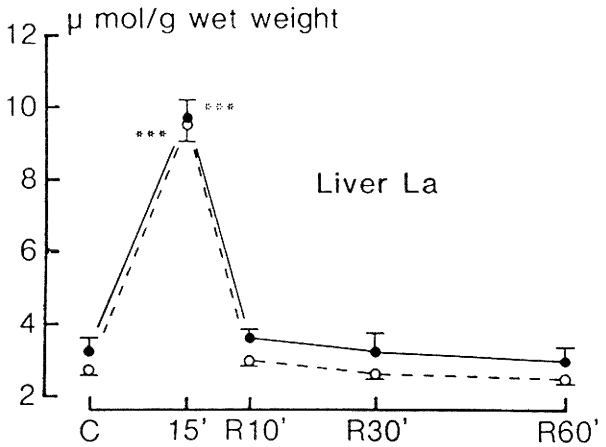

Fig. 6. Changes in liver lactate (Refer to Fig. 1 for legend).

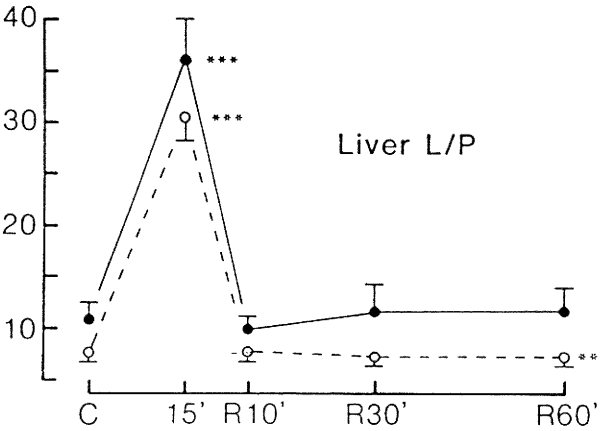

Fig. 7. Changes in liver lactate-pyruvate ratio (Refer to Fig. 1 for legend).

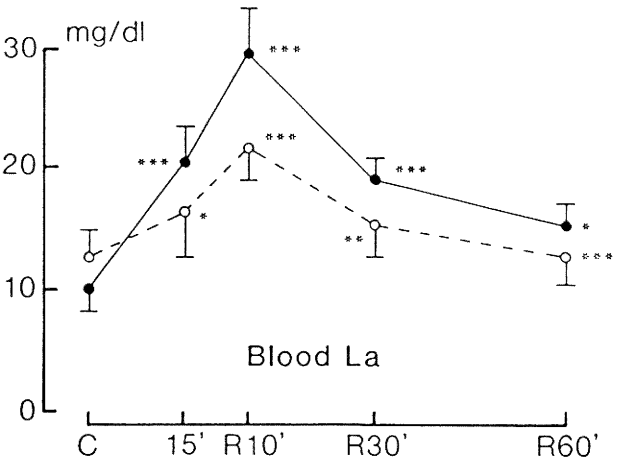

Fig. 8. Changes in blood lactate (Refer to Fig. 1 for legend).

similar fashion for both groups. These increased to about three times the control values when the hepatic circulation was halted, but returned to near control levels within 10 min of the resumption of circulation. No significant difference was found between the two groups.

The concentration of blood lactate increased 10 min after the resumption of the circulation and decreased gradually thereafter (Fig. 8). No significant difference was found between the two groups.

Pa_{O_2} was higher than 200 mmHg throughout the experiment. Although a temporary drop of Pa_{CO_2} was seen at the interception of the hepatic circulation, it recovered rapidly to near normal range after the hepatic circulation was reestablished. Base excess values of the control group were significantly lower than those of the halothane group at all measuring points except the control values.

DISCUSSION

Halothane has been known to inhibit markedly the state 3 mitochondrial respiration *in vitro*. The state 3 mitochondrial respiration is defined as strongly activated oxydative phosphorylation with sufficient supply of oxygen and substrate (glutamate) at a low ATP-ADP ratio.

In the present study, we first rendered the dog liver ischemic to decrease the ATP-ADP ratio, and sufficient amounts of oxygen and substrate were then supplied by reperfusion of the liver. In the resting cells, mitochondrial respiration has been generally thought to be suited to the state 4.[12] We therefore inflicted the above-mentioned insult onto the dog liver to render the hepatic cells as close to state 3 *in vitro* as possible.

The tissue ATP content is reduced and the damage to mitochondrial function increased in proportion to the duration of ischemia. Farkouh et al.,[13] Ishikawa[14] and Marubayashi et al.[15] reported that the reduction of ATP concentration resulting from ischemia was drastic in the initial 30 min and became slower thereafter. They demonstrated that the liver ATP content was reduced to 58% of the control value after 30 min ischemia in the dog (Farkouh et al.), to 67.5% after 15 min ischemia in the dog (Ishikawa) and to 85% after 15 min ischemia in the rat (Marubayashi et al.).

Marubayashi et al.[15] reported that the recovery of reduced ATP content after temporary interruption of the hepatic circulation became poorer in proportion to the duration of ischemia. It was demonstrated that the reduced liver ATP content induced by hepatic ischemia returned promptly to near control levels when the duration of ischemia was less than 15 min. Changes in the liver lactate and lactate-pyruvate ratio, observed in our preliminary experiments in the dog, indicated that once the hepatic perfusion was reestablished after 15 min ischemia, aerobic metabolism resumes almost completely. Taking the above fact into consideration, we chose 15 min as a proper length of time for the purpose of the study.

In the present experiment, a portocaval shunt was not made during the period of portal occlusion. According to Marubayashi et al.,[15] the survival rate of rats after portal occlusion was influenced by the presence of a portocaval shunt. If the duration of portal occlusion was less than 30 min, however, all the rats survived in the absence of a portocaval shunt. In our experiment, we observed venous stagnation in the peripheral portion of the portal vein during portal occlusion, and many petechiae were seen all over the serosa of the bowel after the portal occlusion was released. The surface of the bowel, however, seemed almost normal except for the petechiae.

Hepatic ATP content and AECP decreased markedly in both groups during 15 min interruption of the hepatic circulation. Depletion of hepatic ATP content and AECP in the control group was significantly greater than that in the halothane group. We inferred that this difference between the two groups was due partly to the inhibition of cellular activity and ATP consumption caused by halothane. Therefore, the possibility that inhibition of mitochondrial respiration (reduced ATP production) was offset by decreased cellular activity (reduced ATP consumption) under halothane anesthesia could not be completly denied.

Several authors reported that halothane anesthesia decreased the hepatic blood flow.[16–18] Andreen et al.[19] and Hughes et al.[20] also reported reduction of hepatic blood flow under halothane anesthesia, but demonstrated that the oxygen consumption did not change significantly. In our experiment, the hepatic blood flow was probably reduced by halothane, but this reduced hepatic blood flow may have been still sufficient to allow mitochondria to produce ATP and to maintain the liver tissue ATP level.

Other factors that may be responsible for the lack of significant difference between the two groups concerning the course of the recovery of the nucleotide contents and AECP are: 1) though mitochondrial respiration was inhibited by halothane, the capacity of the mitochondria to produce ATP was still well maintained; 2) inhibition of mitochondrial

respiration by halothane, seen *in vitro*, does not occur *in vivo*, or this inhibition is too mild to make a significant difference in the recovery time of ATP between the two groups; 3) in view of the very rapid recovery seen for ATP and AECP, a period of 10 min from the resumption of the hepatic inflow to the first sampling (R10) may not have been appropriate to demonstrate a difference. If we had taken the first sample earlier than 10 min, there may have been a significant difference in ATP content and AECP between the two groups.

In conclusion, 2% halothane did not inhibit the recovery of ATP content and AECP of the hepatic tissue in the dog as induced by 15 min occlusion of the hepatic circulation.

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