Effects of Thiopental on Cardiac Energy Metabolisms in Postischemic Reperfusion in Rat

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In experiments on isolated rat heart lung preparation, the effects of thiopental on myocardial metabolisms in postischemic reperfusion were evaluated with intramyocardial high energy phosphates, lactate, pyruvate and glycogen. The release of CPK in the perfusate blood was also measured at the end of reperfusion. After 10 min perfusion, hearts were made globally ischemic for 8 min and reperfused for 12 min. Large dose of thiopental (100 μg/ml) reduced the energy charge and glycogen content. Reperfusion with an anesthetic dose of thiopental (10 μg/ml) resulted in an exacerbation of the CPK release. Protection by thiopental during ischemia was not observed and its high dose may be harmful. (Key words: cardiac metabolism, CPK, ischemia, thiopental)

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Barbiturates have been shown to have a protective effect[1-2] or an adverse effect in the heart[3]. Variable results may be attributed to differences between species or in techniques used for the induction of hypoxia or ischemia. In a previous study we found that thiopental depressed the cardiac function and metabolisms in the isolated rat heart[4].

The purpose of the present study was to investigate the effects of thiopental on cardiac energy metabolisms during the postischemic reperfusion in the rat heart-lung preparation. We have compared the effects of thiopental in an anesthetic dose (10 μg/ml) and large dose (100 μg/ml).

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Methods

Male Wistar rats (300–330g) were 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 80 breaths/min with the ambient air which produced a Po_2 range of 85–110 mmHg, a Pco_2 range of 12–16 mmHg. 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. The cannula of the superior vena cava was used for the monitor of central venous pressure. 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. The concentrations (mM) of the buffer constituents were :NaCl 127, KCl 5.1, $CaCl_2$ 2.2, KH_2PO_4 1.3, $MgSO_4$ 2.6, $NaHCO_3$ 15 and

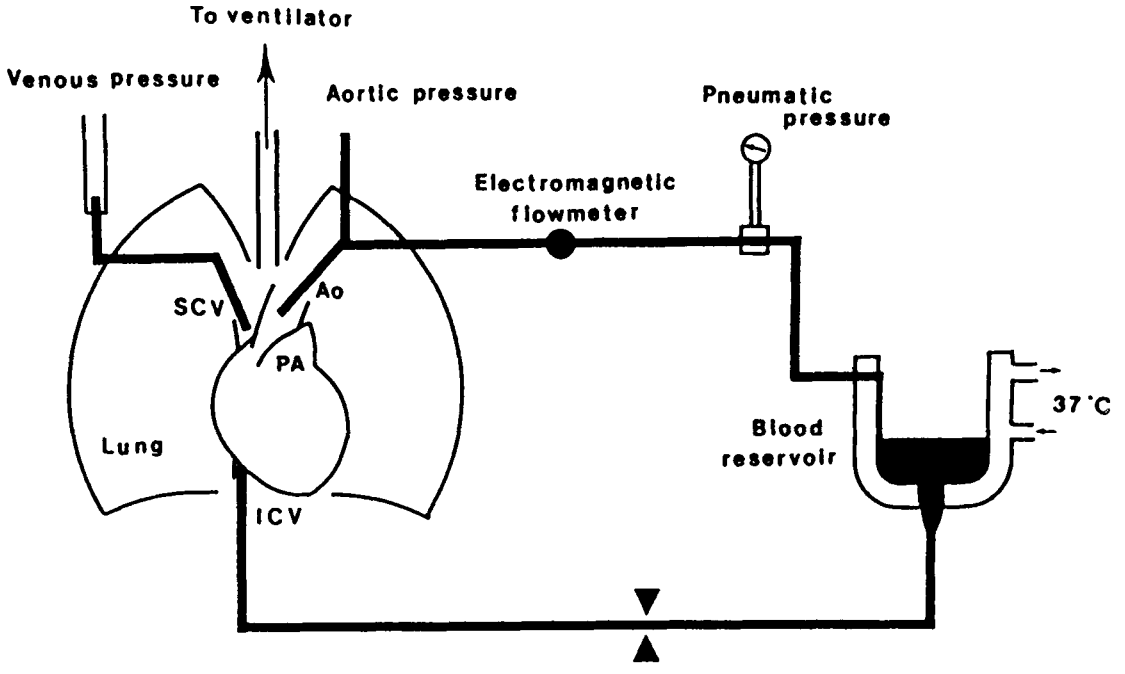

Fig. 1. The schema of heart lung preparation
Perfusate blood pumped from the aorta was collected in the reservoir, warmed at 37°C and then returned to the inferior vena cava.
Abbreviations: Ao; Aorta, SCV; Superior vena cava, PA; Pulmonary artery, ICV; Inferior vena cava

heparin. 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 (fig. 1).

All hearts were perfused initially at a cardiac output of 30 ml/min and a mean arterial pressure of 80 mmHg. The animals were divided into three groups of control (C:n=7), T10 (n=7) and T100 (n=7). Five minutes after the start of perfusion, thiopental 10 μg/ml and 100 μg/ml were added to the perfusate blood in groups T10 and T100 respectively. Ten minutes after the start of perfusion, all hearts were made globally ischemic for 8 minutes by clamping the venous return and making the pneumatic resistance zero. Afterwards, they were reperfused for 12 min by regulating the venous return and the pneumatic resistance. At the end of reperfusion, hearts were freeze-clamped by liquid nitrogen and the perfusate blood was collected and analyzed for creatine phosphokinase (CPK) activity with the tetrazolium method. The heart was estimated as "failure" when the cardiac output was less than 20 ml/min.

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 rpm. High energy phosphates (ATP: adenosine triphosphate, ADP: adenosine diphosphate, AMP: adenosine monophosphate and CP: creatine phosphate), lactate and pyruvate were determined spectrophotometrically by standard techniques according to Bergmeyer[5]. 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[6]. The values were expressed as micromoles per gram of dry weight.

Significant differences were determined by the analysis of variance followed by the least significant difference method for multiple comparison. A probability of $P<0.05$ was

Table 1. Myocardial high energy phosphates and energy charge

	ATP	ADP	AMP	CP	EC
C (n=7)	16.21±1.52	3.075±0.128	0.207±0.067	13.01±1.53	0.909±0.011
T10 (n=7)	16.11±1.23	3.249±0.203	0.384±0.145	13.40±1.31	0.896±0.013
T100 (n=7)	13.54±1.27	4.260±0.426★	2.020±0.495★★	10.19±1.04	0.786±0.037★★

ATP: adenosine triphosphate, ADP: adenosine diphosphate, AMP: adenosine monophosphate, CP: creatine phosphate (μ mole/g.dry.tissue)
EC: energy charge
★P<0.05, ★★P<0.01

Table 2. Myocardial lactate, pyruvate, glycogen contents and L/P ratio

	lactate	pyruvate	glycogen	L/P
C (n=7)	34.97± 5.75	0.966±0.105	41.49±6.21	40.5± 8.9
T10 (n=7)	35.78± 7.68	0.926±0.125	29.05±5.36	37.9± 7.0
T100 (n=7)	60.60±11.65	1.017±0.124	16.86±3.94★	74.7±21.4

μ mole/g.dry.tissue
★P<0.05: when compared with group C.
L/P: lactate/pyruvate

regarded as statistically significant. The data were given as means ± SEM.

Results

One heart in group C, two hearts in group T10 and three hearts in group T100 failed (cardiac output<20 ml/min) at the end of reperfusion.

In cardiac energy metabolisms, there were no significant differences in ATP and CP contents between the three groups. However, ADP and AMP contents in group T100 were higher than those in groups C and T10. Therefore, energy charge (EC: [ATP + 0.5 × ADP]/[ATP + ADP + AMP]) in group T100 was lower than that in group C (table 1). Although there were no significant differences in lactate, pyruvate contents and lactate/pyruvate (L/P) ratio between the three groups, glycogen contents was lower in group T100 significantly (table 2).

There was no significant difference in the release of CPK between the groups T100 and C. But the CPK release from the hearts in group T10 increased significantly (table 3).

Table 3. The CPK release from the reperfused hearts

	CPK (mU/ml)
C (n=7)	27.1 ± 5.1
T10 (n=7)	44.4 ± 8.2★
T100 (n=7)	17.2 ± 3.4

★ $P < 0.05$

Discussion

The results of the present study show that thiopental did not protect heart muscle against the total ischemia in the cardiac energy metabolisms. There were no significant differences between thiopental treated and untreated hearts in the myocardial CP, ATP, lactate and pyruvate contents. However, large dose of thiopental (100 μg/ml) reduced the EC and glycogen content significantly.

These results may be explained as follows. The first, thiopental reduced contraction and worsened recovery from the total ischemia. This seems to have been due to its cardiodepressant effect. Frankl et al.[7] have shown that 60 μg/ml thiopental reduced developed tension to less than 20% in the rabbit ventricular myocardium. Although there was no significant difference between the three groups, three hearts in group T100 failed at the end of reperfusion. A cardiodepressant effect might have caused the heart failure and resulted in the deterioration of myocardial metabolisms. The second, thiopental entered the cell, inhibited mitochondrial function directly and limited the production of high energy phosphates[8]. Ruigrok et al.[9] also have reported that thiopental 100 μg/ml reduced the CP content of hearts after control perfusion in the rat.

In the present study, thiopental was used in concentrations of 10 and 100 μg/ml, the latter is grossly excess of the dose used for induction of anesthesia. In a previous study, we showed that even thiopental 6 μg/ml decreased cardiac output, coronary blood flow and contents of CP and ATP in the reperfused rat heart[4]. This is not compatible with the present study.

Reperfusion with the anesthetic dose of thiopental resulted in an exacerbation of the CPK release. However, the CPK release from the hearts with high dose of thiopental did not increase. Ruigrok et al[9], comparing the effects of thiopental during severe hypoxia, total ischemia, and low-flow ischemia in rat heart muscle, have shown that the creatine kinase release from the thiopental treated hearts was less than from the untreated hearts in both severe hypoxia and low-flow ischemia, but not in total ischemia. Their results are partly compatible with our observations in that the release of CPK on reperfusion after total ischemia was not reduced by high dose of thiopental. We don't know why the CPK release from the hearts with the anesthetic dose of thiopental increased and that from the hearts with high dose was low.

Variable results in the cardiac energy metabolisms as well as in the enzyme release may be attributed to differences between preparations of model or in techniques used for the induction of ischemia. The overall result in any experiment will depend on the factors, such as the cardiodepressant effect, the reduction of the slow calcium current, the block of the uptake of calcium by the sarcoplasmic reticulum, and the inhibition of mitochondrial function[4,7-9].

Anyway, protection by thiopental during ischemia was not observed in the present study. And its high dose seems to be detrimental to the cardiac metabolisms. An attention should be paid when barbiturates are used during cardiac arrest or cardiopulmonary bypass.

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