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Sevoflurane reduces dysrhythmias during reperfusion in the working rat heart

Takeshi Oguchi, Satoshi Kashimoto, Toshiaki Yamaguchi, Kenichi Masui, and Teruo Kumazawa

Department of Anesthesiology, Yamanashi Medical University, 1110 Shimokato, Tamaho-cho, Nakakoma-gun, Yamanashi 409-3898, Japan

Abstract

Purpose. The effects of sevoflurane on myocardial reperfusion injury have not been well studied. The purpose of this study was to determine the effects of sevoflurane on myocardial function, arrhythmia, and metabolism during reperfusion in an isolated working rat heart model.

Methods. Thirty-two hearts were divided into four groups according to the timing of 2.5% sevoflurane administration: group I, control, no sevoflurane; group II, sevoflurane administered only before ischemia; group III, sevoflurane only during reperfusion; group IV, sevoflurane during the whole study period. Myocardial contractility, myocardial ATP, lactate, and glycogen levels were assessed in the reperfusion period following global heart ischemia of 15 min duration. The incidence and duration of ventricular fibrillation were also observed in the reperfusion period.

Results. There was no difference in cardiac output and left ventricular dP/dt max among the four groups at 10, 15, and 20 min after reperfusion. There was no difference in myocardial ATP, lactate and glycogen contents between the groups. The incidences of ventricular fibrillation during reperfusion were 100%, 63%, 100%, and 25% ($P < 0.05$ *vs* control), and the durations of ventricular fibrillation during reperfusion were 375 ± 269, 104 ± 98 ($P < 0.05$ *vs* control), 303 ± 189, and 93 ± 245 ($P < 0.05$ *vs* control) in groups I, II, III, and IV, respectively (mean ± SD).

Conclusion. The administration of sevoflurane prior to reperfusion appears to provide myocardial protection, as assessed by reduced dysrhythmias during reperfusion.

Key words Sevoflurane · Dysrhythmia · Myocardial metabolism · Postischemic reperfusion

Address correspondence to: T. Oguchi

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Introduction

Cardiac dysfunction and arrhythmias arise as a consequence of myocardial ischemia and reperfusion. Reperfusion injury has been reported to be associated with oxygen free radicals and calcium paradox [1,2]. The pathogenesis of this injury appears to be multifactorial. Halothane, enflurane, and isoflurane have been shown to decrease the incidence of dysrhythmias during reperfusion [3–5]. Furthermore, the administration of enflurane and isoflurane enhanced metabolic recovery after postischemic reperfusion [4,6,7]. Warltier et al. [8] studied the effects of halothane and isoflurane on recovery of contractile function in stunned myocardium and showed that both of these volatile agents enhanced recovery. In these experiments, volatile anesthetics were delivered before ischemia, during ischemia, and during the reperfusion period. In contrast to the above studies, Mattheussen et al. [9] showed that exposure to halothane or isoflurane only prior to ischemia had no effect on functional and metabolic indices of recovery after myocardial stunning. In addition, Belo et al. [10] demonstrated that subsequent administration of halothane or isoflurane after reperfusion did not improve contractile function in stunned myocardium. Therefore, the timing of drug administration is supposed to be an important factor for the beneficial effects of inhalation anesthetics against reperfusion injury.

Sevoflurane was reported to depress cardiac function and increase coronary flow in similar degrees as isoflurane in the chronically instrumented intact dog [11]. Graf et al. [12] also reported that sevoflurane and isoflurane produced equivalent negative inotropic, chronotropic, metabolic, and vasodilatory effects in a concentration-dependent manner in isolated hearts. In the canine model of multivessel coronary artery obstruction, sevoflurane did not decrease myocardial blood flow derived from coronary collateral vessels [13].

However, the direct effects of sevoflurane on reperfusion injury have not been well studied.

The present study was designed to examine whether the timing of sevoflurane administration affects its protective property against reperfusion injury in the ischemic working heart preparation. We recently showed that the administration of sevoflurane before, during, and after ischemia reduced the incidence of reperfusion-induced ventricular fibrillation in the same model [4]. However, 3.3% and 5.0% sevoflurane did not have any effect on myocardial metabolism during reperfusion. Therefore, we employed a lower concentration of sevoflurane than we used before to evaluate the effects on myocardial metabolism.

Materials and methods

These experiments were approved by the Animal Ethical Committee of the Yamanashi Medical University. Isolation and preparation of hearts as used for this report have been detailed in the earlier study [4]. Thirty-two 3-month-old male Wistar rats weighing 280–320 g were used. The animals were anesthetized with sevoflurane. The hearts were rapidly excised and perfused according to the Langendorff procedure. Nonrecirculating modified Krebs-Henseleit bicarbonate buffer (KHB) was used as a perfusate. The perfusate was maintained at 37.0 ± 0.3°C and contained (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.0, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, di-NaEDTA 0.5, and glucose 11. The solution was equilibrated with a gas mixture of 95% O_2 and 5% CO_2. During retrograde perfusion, the left atrium was connected to an angled steel cannula via a pulmonary vein. The remaining pulmonary veins were ligated to avoid leakage. Thereafter, the heart was converted to a Neely's working model [14] by perfusing the left atrium and by releasing the aortic outflow for 10 min. The KHB was used also during the working heart system. In this preparation, the preload was adjusted at a constant level by placing an atrial bubble trap reservoir above the left atrium at 10 cm H_2O. The afterload was determined at a constant level by setting the height of the aortic bubble trap over the heart level (60 mmHg).

Left ventricular pressure was measured with a transducer (P10EZ, Gould, Oxnard, CA, USA) connected to a thin catheter (18G, Argyle Intramedicut Catheter, Sherwood, Tokyo, Japan) inserted into the left ventricle through the mitral valve from the angled steel cannula in the left atrium. The rate of development of tension (dP/dt) was electronically measured as the derivative of left ventricular pressure. Aortic outflow was recorded with an electromagnetic blood flow meter (MFV-3200, Nihon Kohden, Tokyo, Japan). Coronary flow was measured by timed collection of the pulmonary artery outflow and surface runoff of the heart resulting from the coronary sinus and Thebesian vessel drainage. Cardiac output was estimated as the sum of the aortic and coronary outflows. The coronary effluent was not recirculated at any time.

For measurement of oxygen tension of the coronary effluent, a catheter was placed in the pulmonary artery. The oxygen tension was measured in an intermittently self-calibrating blood gas analyzer system (Instrumentation Laboratory Model 1306, Lexington, MA, USA). Myocardial oxygen consumption, MVO_2 ($\mu mol \cdot min^{-1} \cdot g^{-1}$), was calculated as O_2 solubility multiplied by coronary flow per gram of heart tissue multiplied by the difference between the inflow and outflow O_2 tensions. Oxygen delivery (DO_2) was calculated from the inflow O_2 tension multiplied by O_2 solubility multiplied by coronary flow per gram of heart tissue.

After an initial stabilization period, the experimental protocol had three phases. In the first phase, the hearts were perfused under aerobic conditions for 10 min. In the second phase, global heart ischemia was induced for 15 min. In this model, whole heart ischemia was induced by the one-way aortic valve. Since the largest fraction of coronary flow occurs during diastole, this one-way valve severely restricted coronary perfusion [14]. During the ischemic period only, the hearts were paced at 333 beats·min^{-1}. In the last phase, reperfusion of the heart after this ischemic period was performed for 30 min.

The hearts were randomly assigned to one of four groups according to the timing of sevoflurane administration ($n = 8$ in each group): group I, control, no sevoflurane; group II, sevoflurane was administered only before ischemia; group III, sevoflurane was administered during reperfusion; group IV, sevoflurane was administered during the whole period. The minimum alveolar concentration (MAC) of sevoflurane in rats was given as 2.5% in unpublished data of Cook et al. [15]; this value was utilized in the present study. The hearts were initially exposed for 10 min to the perfusate without (groups I and III) or with (groups II and IV) 2.5% sevoflurane. In group II, sevoflurane was discontinued just before ischemia. Afterwards, global ischemia was induced by a one-way aortic valve for 15 min, followed by reperfusion for 30 min. The concentration of sevoflurane was measured continuously in the gas phase of the oxygenating chamber by an anesthetic agent monitor (Acoma, Tokyo, Japan).

At the end of reperfusion, the heart was quickly frozen in liquid nitrogen and was freeze-dried for 6 days. An aliquot was extracted with perchloric acid and centrifuged at 3000 rpm. The concentration of ATP was measured by high-performance liquid chromatography according to the modified methods of Wynants and Van Bele [16]. The concentration of lactate was measured

spectrophotometrically by an enzymatic technique [17]. Another piece of freeze-dried sample was placed in 30% potassium hydroxide and digested at 100°C. Tissue glycogen was extracted, hydrolyzed, and assayed as glucose equivalents in neutralized, potassium-hydrated extracts [18]. The values were expressed as micromoles per gram of dry heart weight.

The data were expressed as means ± SD. Testing for significant differences between group I and the other groups was performed by one-way analysis of variance (ANOVA), followed by Dunnett's test. The incidence of ventricular fibrillation was analyzed by the chi-square test. The duration of ventricular fibrillation during reperfusion was analyzed by one-way ANOVA, followed by nonpaired *t*-tests with the Bonferroni correction. $P < 0.05$ was regarded as statistically significant.

Results

Before ischemia, the administration of sevoflurane was associated with significant decreases in cardiac output and left ventricular *dP/dt* maximum (LV_{max}). At 5 min after reperfusion, cardiac output and LV_{max} in group IV were higher than in group I. However, there were no differences in myocardial contractility among the four groups 10, 15, 20 min after reperfusion (Figs. 1 and 2).

Irrespective of the time at which sevoflurane was administered, sevoflurane did not affect heart rate and coronary flow (Figs. 3 and 4). Figure 5 shows the relationship between DO_2 and MVO_2. Sevoflurane did not affect myocardial oxygen balance at any time.

The incidences of ventricular fibrillation were 100%, 63%, 100%, and 25% in groups I, II, III, and IV, respectively. A significant reduction was observed in the incidence of ventricular fibrillation only when sevoflurane was administered before, during, and after ischemia. When sevoflurane was given only during reperfusion, sevoflurane did not have any effect on reperfusion-induced arrhythmia. When it was given before ischemia, we recorded a 37% reduction in the incidence of dysrhythmia (statistically nonsignificant). The duration of ventricular fibrillation during reperfusion was 375 ± 269, 104 ± 98, 303 ± 189, and 93 ± 245 in groups I, II, III, and IV, respectively. The duration of fibrillation in groups II and IV was significantly shorter than that in group I.

There were no significant differences in myocardial ATP, lactate, and glycogen contents between the control and sevoflurane groups (Fig. 6).

Discussion

The present study was designed to examine whether the presence of sevoflurane during reperfusion is crucially important in the protective mechanism. Our results showed that 2.5% sevoflurane provided myocardial protection, as assessed by the reduced incidence of ventricular fibrillation after reperfusion in the group receiving sevoflurane during the whole period. However, the protective property was not detected when sevoflurane was given only during reperfusion. These findings suggest that administration of sevoflurane

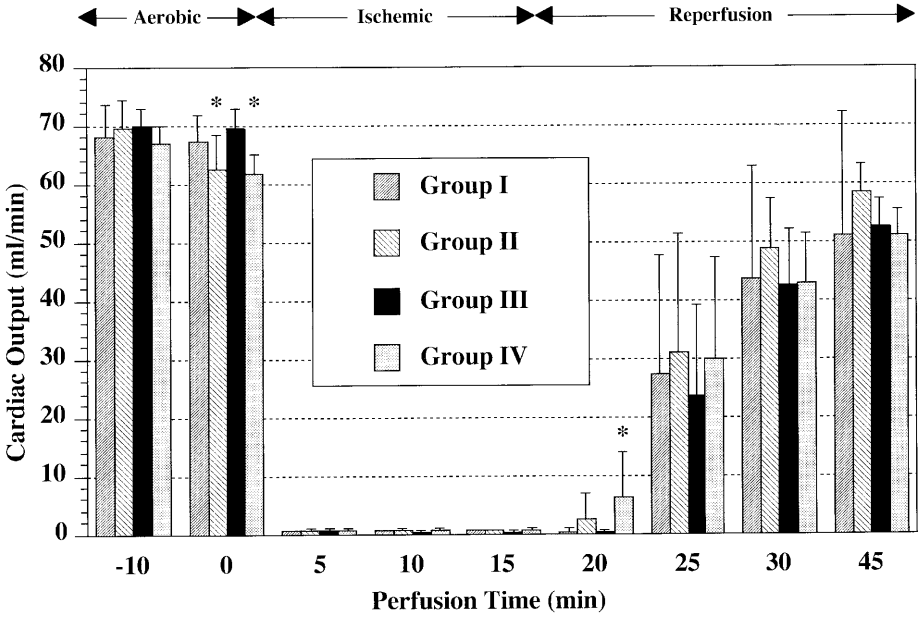

Fig. 1. Changes in cardiac output in the control and sevoflurane groups. The X-axis represents perfusion time in minutes. At zero time, ischemia was induced by one-way aortic valve procedure. Perfusion of the ischemic heart was continued for 15 min, followed by reperfusion for 30 min. Each point represents mean ± SD for eight hearts. *$P < 0.05$ as compared with the control group

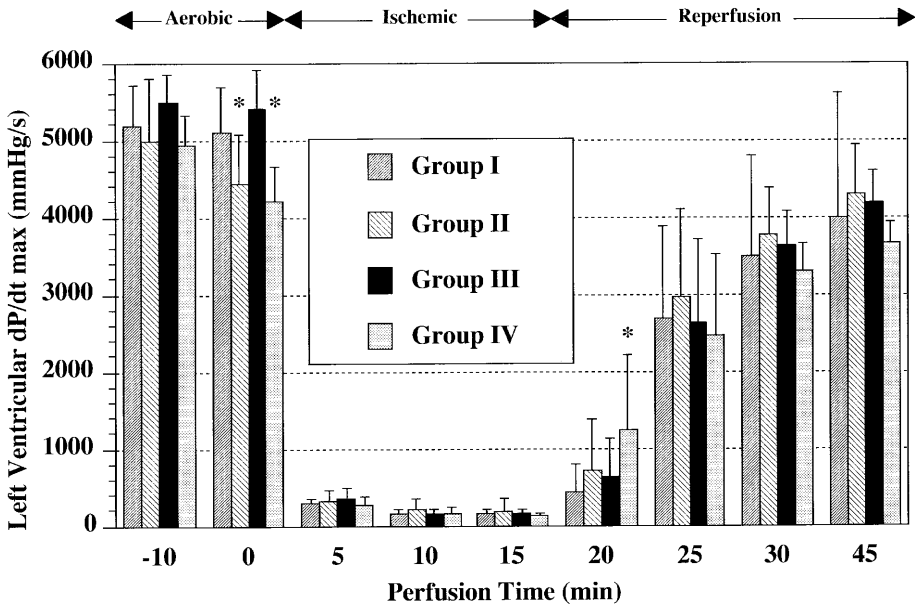

Fig. 2. Changes in left ventricular *dP/dt* maximum in the control and sevoflurane groups. Each point represents mean ± SD for eight hearts. See Fig. 1 for explanation

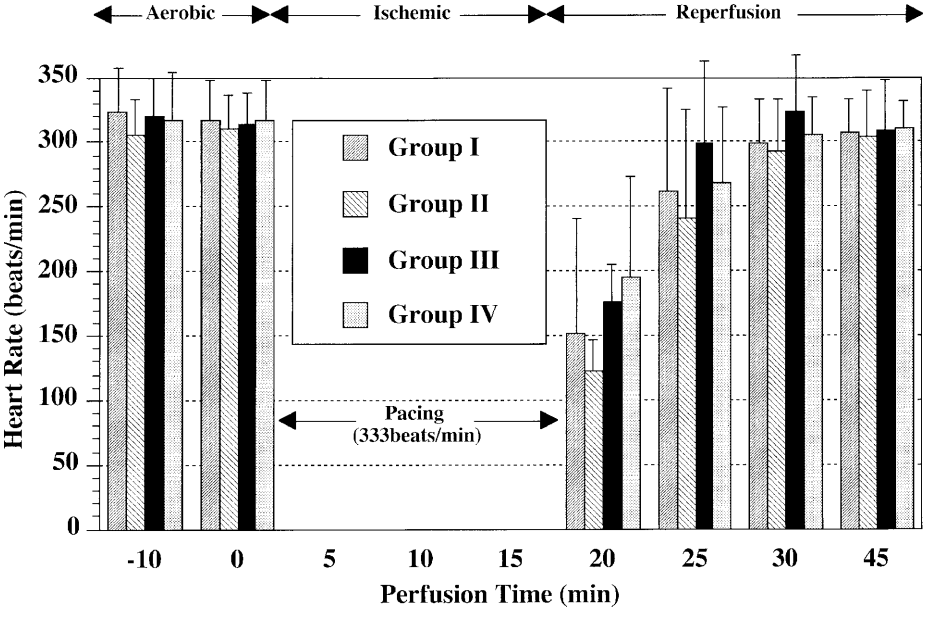

Fig. 3. Changes in heart rate in the control and sevoflurane groups. Each point represents mean ± SD for eight hearts. See Fig. 1 for explanation

preceding reperfusion may be important for protective effect. When given during the preischemic phase, sevoflurane decreased the duration of ventricular fibrillation. In this group, a small amount of sevoflurane probably still remained in the myocardium in the first minutes of ischemia. Therefore, the presence of sevoflurane in the early stage of ischemia may play a consequential role in sevoflurane's protective mechanism. Enflurane and isoflurane were reported to have beneficial effects on myocardial recovery after reperfusion [6,7]. However, 2.5% sevoflurane did not have any effect on metabolism.

Halothane, enflurane, and isoflurane have been shown to reduce the severity of dysrhythmias during reperfusion in the isolated heart [3,4,19] and in the intact animal model [5,20]. It was predicted that these myocardial protective effects may be due to a decrease in oxygen demand relative to oxygen supply [6,19,21–23]. However, in our study, sevoflurane did not affect myocardial oxygen balance during the whole period. These results suggest that the effect of sevoflurane on myocardial oxygen balance cannot contribute to improved recovery during reperfusion. Coetzee et al. [24,25] suggested that the beneficial effects of inhalation

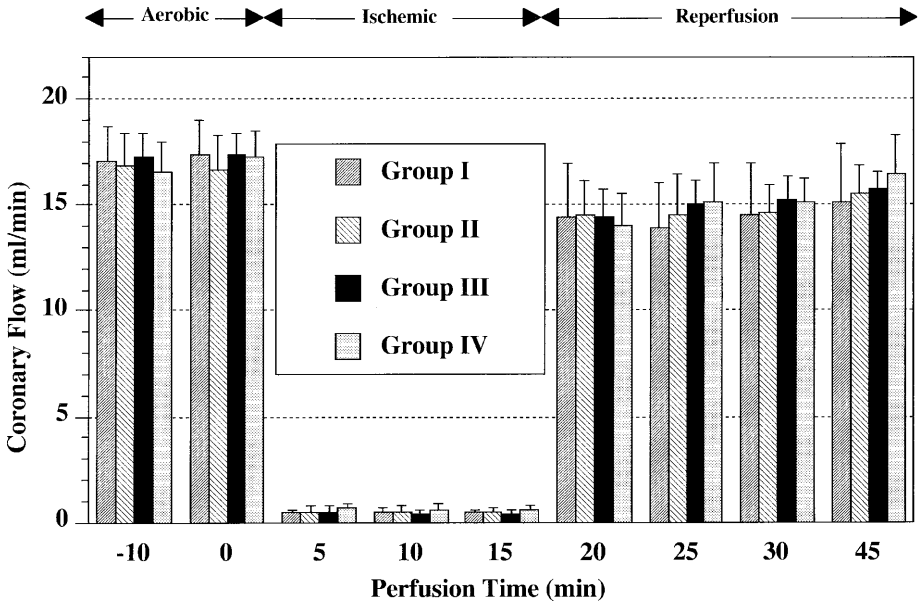

Fig. 4. Changes in coronary flow in the control and sevoflurane groups. Each point represents mean ± SD for eight hearts. See Fig. 1 for explanation

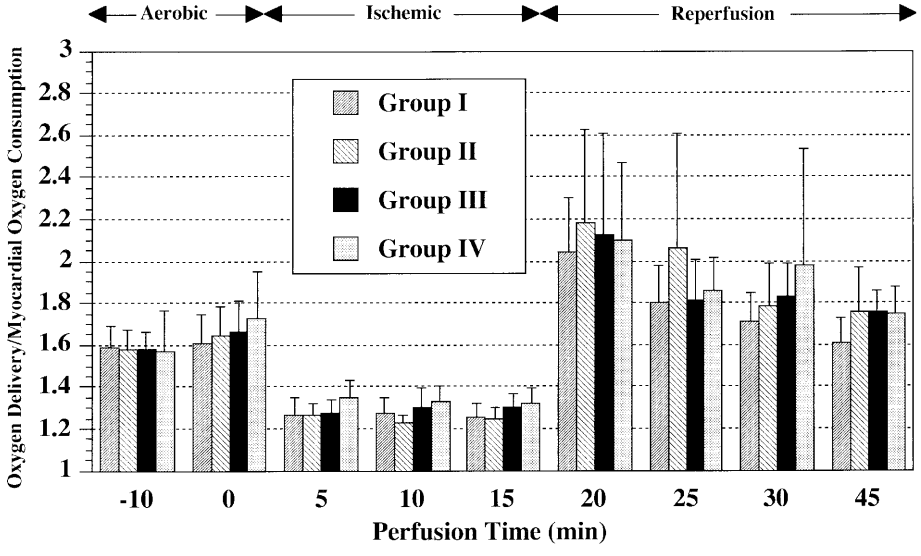

Fig. 5. Changes in the oxygen delivery (DO_2) to myocardial oxygen consumption (MVO_2) ratio in the control and sevoflurane groups as a function of time. Each point represents mean ± SD for eight hearts. See Fig. 1 for explanation

anesthetics after cardioplegic arrest could not be attributed to depression of myocardial contractile function.

The abrupt occlusion and reperfusion of a coronary artery is well known to be arrhythmogenic and may result in ventricular fibrillation [26]. A variety of mechanisms have been proposed to explain the genesis of reperfusion arrhythmias. These include the stimulation of alpha and beta adrenergic receptors [27], the formation of lysophosphatides [28] and toxic free radicals [29], the products of the arachidonic acid pathway [30], disturbances of ionic homeostasis [31], and the heterogeneity of injury and recovery [26]. Considerable controversy exists as to the relative importance of each of these potential mechanisms. Lynch has suggested that the antiarrhythmic actions of inhalation anesthetics might be associated with their effects upon intracellular handling of calcium [32]. Therefore, the cellular mechanisms of sevoflurane remain to be examined to elucidate the antidysrhythmic effect.

There are some problems in our study. First, in the present investigation, sevoflurane did not change myocardial oxygen balance before, during, and after ischemia. However, we could not measure this ratio for the first 2 min after the beginning of ischemia. In our preparation, there was still a small amount of stroke volume until 2 min after the beginning of myocardial ischemia. Therefore, myocardial oxygen consumption in the early stage of ischemia might be reduced by sevoflurane. Second, regarding recovery of myocardial

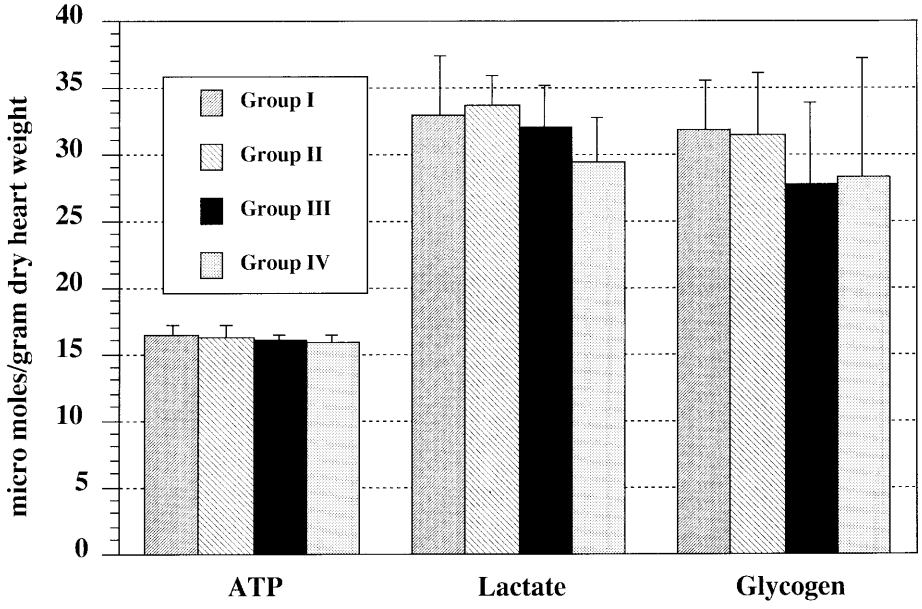

Fig. 6. ATP, lactate, and glycogen in the control and sevoflurane groups ($n = 8$ in each group). See Fig. 1 for explanation

contractility, small differences were observed at 5 min after reperfusion in cardiac output and LV_{max} between the control and sevoflurane groups. However, this may be due not to the difference in myocardial contractile function itself, but to the difference in the incidence of ventricular fibrillation. Reduced dysrhythmias during reperfusion may contribute to the recovery of cardiac function. Finally, there is also the question of extrapolation from global to regional ischemia.

In conclusion, sevoflurane had myocardial protective effects, as assessed by the reduced incidence of ventricular fibrillation after reperfusion when it was administered during the whole period. When sevoflurane was given only during the preischemic phase, the duration of ventricular fibrillation was decreased significantly. However, these protective effects could not be detected when it was given only during reperfusion. With regard to metabolic recovery after reperfusion, enflurane and isoflurane were reported to have beneficial effects. However, sevoflurane did not appear to have similar beneficial properties.

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