Comparative effects of halothane, enflurane, isoflurane and sevoflurane on function and metabolism in the ischaemic rat heart

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Summary
This study was designed to examine the effects of inhalation anaesthetics on function and metabolism in isolated ischaemic rat hearts. Four volatile anaesthetics in two different concentrations (1.0 to 1.5 MAC) were used before whole heart ischaemia was induced for 15 min followed by reperfusion for 30 min. The data were compared with a control group in which inhalation anaesthetics were not used. Before ischaemia, volatile anaesthetics depressed ventricular function. During reperfusion, ventricular function and coronary flow in both halothane groups were significantly lower than those in the control group. Myocardial ATP concentrations in the 1.0 MAC of enflurane and isoflurane groups were significantly higher than those in the control group. We conclude that halothane had more depressant effects than the other anaesthetics and that enflurane and isoflurane may enhance metabolic recovery in the ischaemic working rat heart. (Br. J. Anaesth. 1995; 74: 569-575)

Key words

It has been reported that reperfusion injury is associated with oxygen free radicals and calcium paradox [1, 2]. The cause of this injury is apparently multifactorial and inhalation anaesthetics have been reported to exert both protective [3, 4] and deleterious [5] effects against ischaemic myocardium. It is of interest therefore to investigate the effects of inhalation anaesthetics on reperfusion injury in ischaemic hearts.

The inhalation anaesthetics, halothane, enflurane and isoflurane have been shown to depress myocardial contractility in a dose-dependent manner. Previous studies have suggested that isoflurane may depress myocardial contractility less than halothane or enflurane under aerobic conditions [6, 7]. However, the relative functional and metabolic effects of equipotent concentrations of these anaesthetics have not been compared well in in vitro ischaemic heart preparations. In addition to these three anaesthetics, sevoflurane has recently been used clinically in Japan and is now under investigation in the United States and Europe. However, the direct effects of sevo-flurane on myocardial function have not been compared satisfactorily with the other inhalation anaesthetics in a well-controlled and randomized fashion. Moreover, the metabolic effect of this anaesthetic on the ischaemic heart has not been well studied.

In the present study, we have used the ischaemic rat heart preparation to compare the direct effects of four inhalation anaesthetics on cardiac performance, coronary flow, oxygen delivery and consumption, reperfusion-induced arrhythmia and myocardial energy metabolites.

Materials and methods
These experiments were approved by the Animal Ethics Committee of the Yamanashi Medical University. We used 73 3-month-old male Wistar rats weighing 280–320 g; they were allocated randomly to nine groups as follows (n = 8 each group): control (no inhalation anaesthetic), 1.0 or 1.5 MAC [8, 9] of halothane, enflurane, isoflurane and sevoflurane for rats. The animals were anaesthetized with each inhalation anaesthetic. In the control group, rats were anaesthetized with isoflurane. The hearts were then excised rapidly and perfused according to the Langendorff procedure. Non-recirculating modified Krebs–Henseleit bicarbonate buffer was used as perfusate. The perfusate was maintained at 37.0±0.3 °C and contained (mmol litre−1): NaCl 118, KCl 4.7, CaCl2 3.0, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25, di-NaEDTA 0.5 and glucose 11. The solution was equilibrated with a gas mixture of 95% oxygen and 5% carbon dioxide. During retrograde perfusion, the left atrium was connected via a pulmonary vein to an angled steel cannula. After this preliminary perfusion, the heart was converted to a working preparation for a stabilization period of 10 min. A non-recirculating Krebs–Henseleit bicarbonate buffer was used also during the working heart systems. In this preparation, the perfusate was ejected from the heart into an aortic bubble trap, placed above the heart. The afterload could be...
determined at a constant level by setting the height of the aortic bubble trap over the heart level (60 mm Hg).

Left ventricular pressure was measured with a transducer (PI10EZ, Gould, Oxnard, CA, USA) connected to a thin catheter (18-gauge, Argyle Intramedicut Catheter, Sherwood, Tokyo, Japan) inserted into the left ventricle through the mitral valve from the angled steel cannula in the left atrium. Rates of development of tension (dP/dt) were measured from the derivatives of left ventricular pressure obtained electronically. Aortic outflow was recorded with an electromagnetic blood flow meter (MFV-3200, Nihonkohden, Tokyo, Japan). Coronary flow was measured by timed collection of the pulmonary artery outflow and surface run-off of the heart resulting from coronary sinus and thebesian vessel drainage. Cardiac output was considered as the sum of aortic and coronary outflows. The coronary effluent was not recirculated.

For measurement of oxygen tension of coronary effluent, a catheter was placed in the pulmonary artery. Oxygen tension was measured in an intermittently self-calibrating blood-gas analyser system (Instrumentation Laboratory Model 1306, Lexington, MA, USA). Myocardial oxygen consumption (MV02; μmol min⁻¹ g⁻¹) was calculated as oxygen solubility multiplied by coronary flow per gram of heart tissue multiplied by the difference between inflow oxygen and outflow oxygen tensions. Oxygen delivery (DO2) was calculated from the inflow oxygen tension multiplied by oxygen solubility multiplied by coronary flow per gram of heart tissue.

After an initial stabilization period (10 min), the heart was exposed for 15 min to the perfusates equilibrated without (control group) or with 1.0 or 1.5 MAC of halothane, enflurane, isoflurane, or sevoflurane in the oxygenating chamber. MAC of halothane, enflurane and isoflurane used were 1.0%, 2.2% and 1.5%, respectively, which are considered the MAC values in male rats [8]. We decided that the sevoflurane MAC for rats was 3.3% according to the modified data of Tamada and colleagues [9], which, to our knowledge, is the only published report examining the MAC value of sevoflurane in rats. In their paper, MAC of sevoflurane was 2.2% whereas that of enflurane was 1.45%. This MAC value of enflurane is too low for rats in general. Mazze, Rice and Baden have reported the value for MAC of enflurane as 2.2% [8]. Therefore, MAC of sevo-
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Inhalation anaesthetics

- Aerobic
- Ischaemic
- Reperfusion

Control
1.5 MAC halothane
1.5 MAC enflurane
1.5 MAC isoflurane
1.5 MAC sevoflurane

Reperfusion

Control
1.0 MAC halothane
1.0 MAC enflurane
1.0 MAC isoflurane
1.0 MAC sevoflurane

Figure 2 Changes (mean, SD) in coronary flow in the control and anaesthetic groups (n = 8 hearts). (See fig. 1 for explanation.)

flurane was calculated from the sevoflurane MAC value of Tamada and colleagues (2.2 %) multiplied by enflurane MAC value of Mazze, Rice and Baden (2.2 %) and divided by the enflurane MAC value of Tamada and colleagues (1.45 %). The concentration of the anaesthetic was measured continuously in the gas phase of the oxygenating chamber by an Acoma anaesthetic agent monitor (Acoma, Tokyo, Japan).

Subsequently, whole heart ischaemia was induced by clamping the one-way aortic valve bypass for 15 min [10]. During the ischaemic period only, the heart was paced at 333 beat min⁻¹. Reperfusion of the heart after this ischaemic period of 15 min was performed by declamping the one-way aortic valve bypass tube for 30 min. Inhalation anaesthetics were administered until the end of reperfusion.

At the end of the perfusion, the heart was quickly frozen in liquid nitrogen and freeze-dried for 6 days. An aliquot was extracted with perchloric acid and centrifuged at 3000 rpm. Concentrations of adenosine triphosphate (ATP) and lactate were measured spectrophotometrically by enzymatic techniques [11]. We determined the decrease in absorbance at 340 nm that resulted when NADH was oxidized to NAD, lactate and lactate dehydrogenase. Another piece of freeze-dried sample was placed in 30 % potassium hydroxide and digested at 100 °C. Tissue glycogen was extracted, hydrolysed and assayed as glucose equivalents in neutralized, potassium-hydrated extracts [12]. In each assay technique of myocardial metabolites, the coefficient of variation was less than 5 % and reproducibility was regarded as high. The values were expressed as µmol per gram of dry heart weight.

Data are expressed as mean (SD). Significance of differences among the different groups was tested by one-way ANOVA, followed by Duncan's multiple range test. Intra-group comparisons were performed by two-way ANOVA for repeated measures, followed by paired t tests with Bonferroni correction. The incidence of ventricular fibrillation was analysed by a chi-square test. P < 0.05 was regarded as statistically significant.

Results

Cardiac output in all anaesthetic groups with 1.0 MAC was significantly lower than that in the control group both before and after ischaemia. Moreover, cardiac output in the halothane group was signifi-
Inhalation anaesthetics

Aerobic — Ischaemic — Reperfusion

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Figure 3 Changes (mean, SD) in oxygen delivery ($D_{O_2}$) to myocardial oxygen consumption ($M\dot{V}_O_2$) ratio in the control and anaesthetic groups ($n = 8$ hearts). (See fig. 1 for explanation.)

Figure 3 shows the relationship between $D_{O_2}$ and $M\dot{V}_O_2$. Both before and after the ischaemic periods, the $D_{O_2}:M\dot{V}_O_2$ ratio in the 1.0 and 1.5 MAC of halothane groups was significantly higher than that in the control group. Five and 10 min after ischaemia, that in the 1.5 MAC of halothane group was also significantly greater than the values in the other anaesthetic groups. There were also differences in the $D_{O_2}:M\dot{V}_O_2$ ratio between the control and 1.5 MAC of enflurane groups before ischaemia and at the end of reperfusion.

All control hearts had ventricular fibrillation during reperfusion (100%). However, the incidences of ventricular fibrillation were 25%, 25%, 25% and 38% in the 1.0 MAC of halothane, enflurane, isoflurane and sevoflurane groups, respectively. These values were significantly lower than that in the control group. The incidences of ventricular fibrillation were 50%, 50%, 63%, and 63% in the 1.5 MAC of halothane, enflurane, isoflurane and sevoflurane groups, respectively.

There were no significant differences in lactate and glycogen concentrations among the control and 1.0 MAC groups. ATP content in the 1.0 MAC of enflurane and isoflurane groups was significantly higher than that in the control group. However, there were no significant differences in ATP and lactate concentrations among the control and 1.5 MAC groups. Glycogen concentrations in the 1.5 MAC anaesthetic groups, except sevoflurane, were significantly higher than those in the control group (fig. 4).
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Results during aerobic perfusion are similar to those could not be demonstrated before ischaemia. These enflurane and isoflurane at lower concentrations reduced coronary flow significantly compared with a constant afterload in the working heart model. In our study, atropine and propranolol were administered until the end of reperfusion to compare the effects of the inhalation agents on coronary autoregulation. Although there is controversy in the literature on whether or not enflurane and isoflurane affect coronary autoregulation, halothane disturbed autoregulation most in all three experimental designs. In the present study, even during reperfusion, halothane produced the most pronounced attenuation of coronary autoregulation. However, it is well known that after a period of ischaemia there is a massive hyperaemic response in the early phase of reperfusion and it could have influenced the data on oxygen supply-demand ratio. Indeed, we observed elevation of this ratio during reperfusion even in the absence of an anaesthetic agent. Therefore, changes in autoregulation during reperfusion may have resulted from ischaemia and not exclusively from the inhalation agents.

Smith and colleagues demonstrated that in dogs with an area of acute myocardial ischaemia induced by ligation of the anterior descending branch of the left main coronary artery, halothane and enflurane caused a significant improvement in the oxygen supply-demand relationship in the ischaemic area in comparison with the non-ischaemic area. They suggested that these beneficial effects were probably secondary to changes in heart rate and not a result of specific mechanisms.

Inhalation anaesthetics can increase the incidence of ventricular ectopic beats, but paradoxically they can also decrease the occurrence of ectopy under certain situations. Halothane has been demonstrated to reduce the severity of arrhythmias during reperfusion in the isolated Langendorff preparation and in the intact animal model. In our working rat model, all four inhalation anaesthetics at lower concentrations had an anti-arrhythmic effect against ventricular fibrillation induced by ischaemia. Lynch [23] has suggested that...
the antiarrhythmic effects of inhalation anaesthetics might be related to their actions on intracellular handling of calcium. However, this cannot explain the fact that higher concentrations of inhalation anaesthetics had less antiarrhythmic effects against ventricular fibrillation during and after ischaemia. Several mechanisms have been proposed to explain the genesis of reperfusion arrhythmias [24, 25]. Therefore, studies on the cellular mechanisms of inhalation anaesthetics are necessary to explain the phenomena.

We also performed this study to see if exposure to inhalation anaesthetics during ischaemia improved post-ischaemic recovery of myocardial metabolism. The results of our study showed that 1.0 MAC of enflurane and 1.0 MAC of isoflurane increased myocardial ATP content at the end of reperfusion compared with the control. With regard to isoflurane, our finding was consistent with data from our previous study [4] showing that the administration of isoflurane before and after ischaemia enhanced metabolic recovery in the heart–lung preparation. With regard to enflurane, there are comparable data from Freedman and co-workers [3] showing that administration of 2% enflurane to the Langendorff rat heart before a severe ischaemic insult enhanced metabolic recovery in the post-ischaemic state. Buljubasic and colleagues suggested that the myocardial protective effects of halothane may be caused, at least in part, to a decrease in oxygen demand relative to oxygen supply [26]. However, in our study there were no significant differences in the oxygen supply–demand ratio among the control, 1.0 MAC of enflurane and 1.0 MAC of isoflurane groups. Moreover, the high dose of anaesthetics did not produce any ATP recovery in spite of a significant increase in the oxygen supply–demand ratio. These results suggest that the effects of volatile agents on coronary blood flow autoregulation cannot always contribute to recovery of myocardial metabolism in the ischaemic heart. The potent depressant effects of the high dose of anaesthetic might inhibit post-ischaemic metabolic recovery.

Mattheussen and colleagues [27] have assessed the effect of inhalation anaesthetics on myocardial metabolism using the ischaemic working heart preparation. Although they used the same experimental model as us, they found that inhalation anaesthetics did not affect myocardial high energy phosphates at the end of reperfusion. In contrast with our study, they administered volatile agents only during the pre-ischaemic period. The conflicting data suggest that the presence of volatile anaesthetics in the period of reperfusion may play an important role in protective mechanisms. Coetzee and co-workers [28] also suggested that the beneficial effects of inhalation anaesthetics after cardioplegic arrest might be related to the prevention of the reperfusion injury and could not be ascribed to depression of global myocardial contractile function.

In the present experiment, inhalation anaesthetics did not influence myocardial lactate concentration at the end of reperfusion. These data are not consistent with the results of our previous study, indicating that halothane and enflurane, but not isoflurane, increased myocardial lactate concentrations at the end of reperfusion [4, 29]. This may result from a difference in methods. Myocardial glycogen concentrations in the halothane, enflurane and isoflurane 1.5 MAC groups were increased after reperfusion. These high concentrations of inhalation agents severely depressed mechanical activity and may decrease glycogen utilization. Therefore, an increase in glycogen concentration does not always indicate a myocardial protective effect induced by anaesthetics.

There are several problems with this study. Many of the differences which were statistically significant were not necessarily physiologically significant between the enflurane, isoflurane and sevoflurane groups. Furthermore, extrapolation of our data to clinical anaesthesia or other animal studies is questionable as it may not be possible to extrapolate from global to regional ischaemia. Furthermore, the anatomy—including that of the coronary circulation and physiology, especially with respect to the sources of activator calcium—of different species differ markedly.

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References

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