Early and late post-ischaemic recovery of contractile function is affected to different degrees by isoflurane and halothane in the anaesthetized rabbit model

S. A. ROSS, R. KATO AND P. FOËX

Summary
The protective efficacy of halogenated anaesthetics on myocardial injury has never been compared during early reperfusion and late reperfusion in an in vivo animal model. We compared recovery of left ventricular function under isoflurane (0.5 MAC) and halothane (0.5 MAC) anaesthesia after a brief period of regional ischaemia (15 min) in acutely instrumented rabbits. Rabbits were instrumented for the measurement of regional segment length and left ventricular pressure. Rabbits receiving isoflurane showed greater recovery of systolic shortening fraction (%SS) both during early and late reperfusion compared with halothane anaesthesia. Isoflurane protected the post-ischaemic myocardium to a greater extent than halothane anaesthesia. Early recovery of contractile function may be a predictor of contractile recovery during the later stages of reperfusion. (Br. J. Anaesth. 1998; 81: 224–229).

Keywords: ischaemia; anaesthetics volatile, isoflurane; anaesthetics volatile, halothane; reperfusion; model, rabbit

“Stunning” is a phenomenon which describes the left ventricular dysfunction observed after a brief period of ischaemia.1 This is a reversible ischaemia-reperfusion induced injury in which the time course of recovery of left ventricular function depends on the duration of the preceding ischaemia.2 Recovery of left ventricular contractile function is biphasic in nature such that during the early stages, 2–5 min into reperfusion, there is a period where contractile function recovers to normal or supranormal levels.3,4 This is very short-lived and, thereafter, contractile function gradually deteriorates to a minimum level from which full recovery to normal levels takes place over a longer period of time, possibly hours, days, or weeks. There has been some suggestion that this brief period represents the myocardium returning to normal function. However, the many different processes involved in ischaemia-reperfusion injury, such as free radical production, calcium overload, and impairment of myocardial perfusion, intervene and cause the subsequent deterioration of function.5

There have been a number of studies examining the protective effects of halogenated anaesthetics on reversible ischaemia-reperfusion injury.6–8 The protective effects of these agents have been attributed to a number of factors; alterations in calcium fluxes,9 free radical scavenging10,11 and the opening of ATP sensitive K+ channels.12,13 Studies have shown that different anaesthetics affect these factors to differing degrees.10 If this is the case, then it is possible that recovery during early reperfusion may differ depending on the protective potency of the anaesthetic and may be a predictor of recovery during the later stages of reperfusion.

We decided to compare the effects of two commonly used anaesthetics, halothane and isoflurane on both early recovery and later recovery to investigate if they were affected in different ways.

Materials and methods

EXPERIMENTAL PREPARATION

The study conformed to United Kingdom Animals Acts (Scientific Procedures, 1986). Nineteen New Zealand White rabbits of either sex (2.5–3.5 kg) were given xylazine (1 mg kg–1 i.m.) pre-medication and then anaesthetized with ketamine HCl (75 mg kg–1 i.m.). Adequate depth of anaesthesia was ensured before any surgical procedures by the absence of pedal and palpebral reflexes. The marginal ear vein was cannulated (20-gauge cannula) for the administration of fluids (hetastarch, 3 ml kg–1 h–1). The central ear artery was also cannulated (18-gauge cannula) and connected to a pressure transducer (Druck Ltd, Groby, Leicester, UK) for measurement of arterial pressure during surgery. All catheters were flushed with heparinized saline (10 i.u ml–1) to prevent clotting during the experiment. A tracheotomy was performed (tracheal tube size 4 mm outer diameter) and the rabbits mechanically ventilated (Servo ventilator 900B, Siemens-Elema, Sweden) with 100% oxygen. The tidal volume was set at 15 ml kg–1 and the respiratory rate at 35 breaths min–1. Ventilation was adjusted to keep the end-tidal carbon dioxide in the physiological range (4–5%). End-tidal gas concentrations were measured continuously (gas analyser M1025A, Hewlett Packard, Bracknell, UK). Anaesthesia was maintained with the appropriate anaesthetic (isoflurane 1 MAC or halothane 1 MAC14) depending on which group the subject was assigned to. Body temperature was recorded by the thermistor of a 7F pulmonary artery catheter (Swan-Ganz, American Edwards Laboratories, Anasco,


Correspondence to S. R.
Puerto Rico) inserted orally into the oesophagus and maintained between 39.0–40.5°C through a heating element incorporated into the table. Limb lead II of the electrocardiogram was continually monitored by means of s.c. needle electrodes. The heart was exposed via a left thoracotomy and suspended in a pericardial cradle. A 5–0 Dexon suture was passed around the major marginal branch of the left coronary artery approximately half way between apex and base and the suture ends threaded through a small vinyl tube to make a coronary snare. Where possible we tried to avoid snaring coronary veins but in some experiments this was not possible. The pressure in the left ventricle was measured by inserting a micro-manometer-tipped 5F catheter (Millar, Houston, Texas, USA) into the apex of the heart. Two pairs of piezoelectric crystals (0.8 mm diameter, Triton, San Diego, USA) for ultrasonic measurement of segmental lengths were implanted in the subendocardium in the apical region (supplied by the snared marginal coronary artery) and the basal region (supplied by the unrestricted left circumflex coronary artery). These were implanted approximately 8 mm apart through epicardial stab wounds.

**EXPERIMENTAL PROCEDURES**

After surgery the anaesthetic concentration was reduced to 0.5 MAC and there was a stabilization period of 30 min after which control measurements were obtained. Regional ischaemia was then imposed for 15 min. This was followed by 120-min reperfusion. Measurements were taken after 15 min of ischaemia and then every 5 min after reperfusion for a period of 15 min with measurements being taken every 30 s during the first 5 min. Measurements were then taken at 30, 60, 90 and 120 min. To produce ischaemia the coronary snare was tightened by pulling the silk through the tubing. Ischaemia was confirmed by changes in the shape of the pressure length loop (reduction in systolic shortening, increase in post-systolic shortening and/or systolic bulging). Visually myocardial ischaemia was confirmed by the appearance of regional myocardial surface cyanosis distal to the snare, and akinesia or systolic bulging in this area. After 15 min, the snare was released and reperfusion begun for a period of 120 min. Reperfusion was confirmed by changes in the pressure length loop (increase in systolic shortening, reductions in post-systolic shortening and systolic bulging). Visually reperfusion was confirmed by the appearance of hyperaemia.

Steady state parameters were recorded for 5 s. All measurements were taken during expiratory apnoea to obtain an identical respiratory phase.

Two groups were studied; one group anaesthetized with isoflurane (group I) \((n = 10)\), and the other group anaesthetized with halothane (group H) \((n = 9)\).

**DATA COLLECTION**

ECG, pressures (central ear artery, left ventricular) and regional dimension signals were converted using an analogue-digital converter (AT-MIO-16, National Instruments Corporation, Austin Texas, USA), and continuously displayed on the screen of an IBM AT personal computer by means of the real-time mode of software designed in this department. Data were sampled at a frequency of 500 Hz and stored on the hard disk. Pressure-length loops were continuously displayed on an oscilloscope.

**DATA ANALYSIS**

Data analysis was performed on a IBM AT personal computer using the play back mode of the above mentioned software. Peak-positive and peak-negative left ventricular dP/dt were obtained by electronic differentiation of the left ventricular pressure signal. End-diastole was defined as the first upward (positive) deflection of the left ventricular dP/dt signal. End-systole was defined as occurring at peak-negative dP/dt.

Length measurements were normalized to an initial value of end-diastolic length of 10 mm at control. The following definitions were used to quantify regional wall motion: EDL=end-diastolic length; MSL=minimum length during systole; ESL=end-systolic length; L\text{max}=maximum length during systole; L\text{min}=minimum length during diastole. Two sets of definitions were used to describe regional wall motion.

Set 1: Systolic shortening (SS), systolic bulging (SB), and post-systolic shortening (PSS) were defined as follows:

\[
\text{SS} = (\text{EDL} - \text{ESL})/\left(\text{L}_{\text{max}} - \text{L}_{\text{min}}\right) \times 100
\]

\[
\text{SB} = (\text{L}_{\text{max}} - \text{EDL})/\left(\text{L}_{\text{max}} - \text{L}_{\text{min}}\right) \times 100
\]

\[
\text{PSS} = (\text{ESL} - \text{L}_{\text{min}})/\left(\text{L}_{\text{max}} - \text{L}_{\text{min}}\right) \times 100
\]

SS, PSS and SB were expressed as a percentage of total shortening \((\text{L}_{\text{max}} - \text{L}_{\text{min}})\) to show the individual contribution of each of these phases; note SS + SB + PSS = total shortening.

Set 2: Systolic shortening fraction (%SS) was defined as follows:

\[
\%\text{SS} = (\text{EDL} - \text{MSL})/\text{EDL} \times 100
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**STATISTICS**

All values were expressed as mean (SD). Statistical analyses were performed using analysis of variance (ANOVA) with repeated measures. If ANOVA indicated significant differences with time during the procedure further comparisons were performed using a paired \(t\) test with Bonferroni’s correction. Differences between groups were compared using an unpaired \(t\) test with Bonferroni’s correction. Statistical significance was assumed at the \(P<0.05\) level.

**Results**

There was no difference in general haemodynamics compared with control values or between the groups during early reperfusion therefore these data have not been included.

Table 1 shows the changes in general haemodynamics before and during ischaemia and during late reperfusion only. The only index to show any difference during control measurements was +dP/dt\text{max} which was significantly higher in group I. Heart rate and rate-pressure product (RPP) were significantly lower in group I compared with group H at various time points during late reperfusion.
Table 1 shows the changes in regional mechanical function before and during ischaemia and during early reperfusion. EDL showed a biphasic response during early reperfusion with a decrease from ischaemia to 150 s into reperfusion. EDL increased thereafter. %SS decreased to similar levels in groups I and H during ischaemia and increased to peak values at 90 s into reperfusion. This increase was significantly greater in group I (−1.6 (3.2)% to 20.1 (7.0)% compared with group H (−2.7 (1.5)% to 14.8 (6.6)%). Thereafter %SS decreased in both groups with group H reaching a significantly lower value (0.7 (3.0)% compared with group I (5.1 (5.1%). SB and PSS showed biphasic responses with decreases during the initial stages of early reperfusion and then returning towards ischaemic values in the later stages.

Table 3 shows the changes in regional mechanical function before and during ischaemia and during late reperfusion. %SS was greater at all time points in group I except at 10 min into reperfusion. At the end of reperfusion %SS in group I had recovered to 11.8 (4.4)% whereas in group H %SS had only recovered to 7.5 (3.3)%.

Figure 1 shows the time course of changes in %SS over the entire reperfusion period, including early and late reperfusion.

Discussion

The results of our study show that in the acutely instrumented rabbit model with similar MAC concentrations of isoflurane and halothane, recovery of contractile function is greater under isoflurane anaesthesia than halothane anaesthesia. Recovery during early as well as late reperfusion was greater under isoflurane anaesthesia.

"Stunning" was first described by Braunwald and Kloner more than a decade ago. The hallmark of this condition is the presence of a flow-function “mismatch” of the myocardium, with normal flow but abnormal function after a brief period of ischaemia. Since then there has been an explosion of studies examining the mechanisms involved and possible approaches to attenuate the deleterious consequences of this phenomenon. The majority of studies that examine recovery of post-ischaemic function show a simple progression from a minimum level during ischaemia to the recovery at the end of the reperfusion period. However, the recovery profile is more complex and is biphasic in nature. There is in fact a brief period where contractile function recovers to normal or supranormal levels (fig. 1). Manche, Edmondson and Hearse performed a particularly detailed study in isolated blood perfused rat hearts examining both early and late recovery after different durations of ischaemia. Their results showed that the initial recovery of function reached its maximum during the early reperfusion period. However, the recovery profile is more complex and is biphasic in nature. The majority of studies that examine recovery of post-ischaemic function show a simple progression from a minimum level during ischaemia to the recovery at the end of the reperfusion period. However, the recovery profile is more complex and is biphasic in nature.

It has been known for a number of years that halogenated anaesthetics cause depression of cardiac
Table 2  Regional mechanical function before and during ischaemia and during early reperfusion. EDL = end-diastolic length; %SS = systolic shortening fraction; SS = systolic shortening; SB = systolic bulging; PSS = post-systolic shortening. Results are mean (± SD). † = P<0.05 compared with group H and * = P<0.05 compared with control values

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Table 3  Regional mechanical function before and during ischaemia and during early reperfusion. EDL = end-diastolic length; %SS = systolic shortening fraction; SS = systolic shortening; SB = systolic bulging; PSS = post-systolic shortening. Results are mean (± SD). † = P<0.05 compared with group H and * = P<0.05 compared with control values

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function and therefore a reduction in oxygen demand of the myocardium. This property of halogenated anaesthetics led researchers to consider the potential protective effects that these anaesthetics may have on the myocardium subjected to ischaemia and reperfusion. Warltier and colleagues were the first group to conduct a detailed study of the recovery of myocardial function under anaesthesia with halogenated agents. They showed that recovery was greatly enhanced compared with conscious controls. Subsequent studies in both in vivo and in vitro models have shown that the protective effects of these agents cannot be solely attributed to a reduction in oxygen demand but to properties such as inhibition of calcium fluxes, free radical scavenging and KATP channel opening activities.

In our study we found that early and late recovery of contractile function were greater under isoflurane than halothane anaesthesia. Unfortunately we could not compare these results with a conscious control. Therefore we cannot be sure that the concentration of halothane was high enough to afford protection. However, the data demonstrate that isoflurane has a greater protective effect than halothane. Studies examining recovery under isoflurane (1 MAC) and halothane (1 MAC) have shown even greater recovery at higher concentrations with these agents with isoflurane still providing greater protection. In the present study the rate-pressure product was higher in the halothane group and this may have accounted for the reduced recovery with this agent. However, systolic shortening during ischaemia was similar under isoflurane or halothane suggesting that the severity of the ischaemia was comparable in both groups.

Examining early recovery of contractile function we found that under isoflurane anaesthesia systolic shortening reached a higher value. One possible explanation is that isoflurane and halothane exhibit different free radical scavenging powers. At low concentrations (0.5% halothane, 0.7% isoflurane) Tanquay and colleagues found in isolated hearts exposed to free radicals that isoflurane inhibited the decrease in systolic pressure to a greater extent than halothane. If isoflurane was acting as a more potent free radical scavenger at the concentrations used in the present study this may explain why contractile recovery during both early and late reperfusion was greater. A burst in the production of free radicals is known to take place within the first few minutes of reperfusion. This may account for the decline in contractile function observed after the early return to normal contractile function. Early recovery under halothane did not reach the same levels as isoflurane possibly because free radicals were scavenged less and therefore affected the recovery to a greater extent.

Another explanation behind the return to normal function observed during early reperfusion is stimulation by catecholamines or calcium influx. However, Manche, Edmondson and Hearse found that epinephrine and norepinephrine concentrations were constant as long as adequate anaesthesia was maintained. A transient increase in calcium influx would also stimulate hyperactivity. If this was the case one may expect contractility to reach supranormal levels. We did not find this; recovery under isoflurane reached 98% of control values. The myocardial cell also expresses transporters (Na+/Ca2+ exchange) which can rapidly normalize calcium levels.

We believe that this is the first study to show that early recovery as well as late recovery can be affected to different degrees by different anaesthetics. As contractile recovery during this early phase followed the same pattern as during the later stages (%SS in group I> group H) it would seem reasonable to assume that the greater recovery during early reperfusion implies a greater degree of protection and therefore greater recovery during the later stages of reperfusion.

An effect of an anaesthetic on this hypercontractile phase has been reported previously. Wouters and colleagues observed that during reperfusion of the myocardium the hypercontractile phase was greater in isoflurane anaesthetized dogs than conscious dogs. The authors could not provide an explanation for this effect but eliminated drug-specific effects on

**Figure 1** Change in systolic shortening (SS) fraction before and during ischaemia and during early and late reperfusion. † = P < 0.05 compared with group H and * = P < 0.05 compared with control values.
coronary and systemic vessels. Again this would suggest that isoflurane protects the myocardium during the early stages of reperfusion.

We have only studied the effects of two anaesthetics at one concentration in this study. Further studies are in progress using other anaesthetics at different concentrations to determine if our theory that early recovery may be a predictor of late recovery after reversible ischaemia-reperfusion injury holds true.

Acknowledgements
Dr Sean Ross was in receipt of an MRC studentship.

References