Effect of halothane on myocardial reoxygenation injury in the isolated rat heart

W. SCHLACK, M. HOLLMANN, J. STUNNECK AND V. THÄMER

Summary

Several studies have reported a protective effect of halothane on myocardial injury in an ischaemia–reperfusion situation. It is unclear if the protection is a result of the haemodynamic effects of halothane or if halothane has a specific action on ischaemia or reperfusion pathomechanisms. To examine this question, we have used an isolated rat heart model where heart rate (300 beat min⁻¹), ventricular volume and coronary flow are constant. Left ventricular developed pressure (LVDP) and release of creatine kinase (CK) were measured as variables of myocardial performance and cellular injury, respectively. Five control hearts were subjected to 35 min of cardiogenic performance and cellular injury, respectively. Mean basal CK release was 149.1 (9.1) iu g⁻¹, and LVDP was 52.0 (2.6) mm Hg. Under normoxic conditions, halothane reduced LVDP to 52.0 (2.6) mm Hg. In the treatment groups, halothane 0.4 mmol litre⁻¹ was added during the first 30 min of anoxic perfusion (n = 5) or during the first 30 min of reoxygenation (n = 5). In five additional hearts, the effect of halothane 0.4 mmol litre⁻¹ was tested under normoxic conditions. Mean basal CK release was 0.29 (SEM 0.13) iu g⁻¹ min⁻¹ and LVDP was 105.5 (4.0) mm Hg. Under normoxic conditions, halothane reduced LVDP to 52.0 (2.6) mm Hg. In control hearts, the major cell injury occurred at the onset of reoxygenation (CK release increased to 149.1 (9.1) iu g⁻¹ min⁻¹) and functional recovery after 1 h of reoxygenation was poor (control LVDP, 14.2(2.1)% of baseline). Halothane during anoxia attenuated myocardial injury only moderately (CK release 50.2(6.7) iu g⁻¹ min⁻¹ and LVDP recovered to 30.8(3.9)% (each P < 0.05 vs control). When halothane was administered at reoxygenation, CK release was reduced to 10.1(0.9) iu g⁻¹ min⁻¹ and LVDP recovered to 69.4(4.9)% (each P <.05 vs control). We conclude that halothane not only attenuated ischaemic injury but had a specific protective action against reoxygenation injury. (Br. J. Anaesth. 1996; 76: 860–867).

Key words


Reperfusion of temporarily ischaemic myocardium can initiate cellular and biochemical changes which reduce the amount of potentially salvageable myocardium (“reperfusion injury”). The term “lethal reperfusion injury” is used for the irreversible deterioration of myocardium that can be reduced by modifications of the conditions of reperfusion [1]. Some experimental evidence suggests that halothane may have myocardial protective properties in an ischaemia–reperfusion situation. Halothane anaesthesia reduced infarct size in a rabbit model with coronary occlusion and subsequent reperfusion, compared with i.v. anaesthesia with propofol, pentobarbitone or ketamine [2]. The results in non-reperfusion infarct models are contradictory; in a dog model, a reduction in infarct size was reported compared with awake controls [3], while an increase in infarct size was found in rats [4]; both studies explained their findings by the haemodynamic effects of halothane. In a pig model (with collateral circulation after a stenosis operation at a young age), halothane had no effect on infarct size [5].

Administration of halothane during hypoperfusion (low coronary perfusion pressure) to isolated guinea pig hearts, reduced the loss of adenosine and inosine during early reperfusion [6] and improved functional recovery [7]. When supplied to isolated working rat hearts before or during cardioplegic arrest, halothane did not cause preservation of ATP during the ischaemic period, but led to an improved recovery of ATP content and myocardial function during the following reperfusion period [8]. In the same model, lower calcium accumulation during cardioplegic arrest and reperfusion, and better recovery of microscopic signs of ischaemic cell damage were found when halothane was added to the perfusion medium (before and after ischaemia). Similarly, administration of halothane during hypoxic perfusion, and for the first 10 min of reoxygenation, resulted in better recovery of contractile function in isolated guinea pig hearts [9].

While most of the above studies found evidence for the cardioprotective effects of halothane, it is unclear if this protection is caused by the haemodynamic effects of halothane (i.e. a reduction in sympathetic tone, myocardial inotropy and myocardial work) or if halothane has specific protective...
effects against ischaemia or reperfusion pathomechanisms. In addition, it is not possible to distinguish from these studies if the protective effects occur before, during or after the ischaemic period.

To examine these questions, we have used an isolated rat heart model so that extrinsic humoral and autonomic nervous system influences could be excluded and ventricular volume, coronary flow and heart rate could be kept constant. In order to differentiate between anti-ischaemic and protective effects against reperfusion injury, halothane was supplied to the myocardium only during a state of oxygen and substrate deprivation (simulated ischaemia) or only during the initial period, when oxygen and substrates were re-supplied (simulated reperfusion).

Materials and methods
The study was performed in accordance with the regulations of the German Animal Protection Law and local institutional regulations.

EXPERIMENTAL PREPARATION

We excised hearts from 200–250-g male Wistar rats, anaesthetized with halothane, and mounted them on a Langendorff perfusion system. Retrograde perfusion was initiated with an oxygenated modified Krebs-Henseleit buffer containing (mmol litre\(^{-1}\)): NaCl 116, KCl 4.7, MgSO\(_4\) 1.1, KH\(_2\)PO\(_4\) 1.17, NaHCO\(_3\) 24.9, CaCl\(_2\) 2.52, glucose 8.3 and pyruvate 2.0, and was gassed with 95% oxygen–5% carbon dioxide. Flow in the system was controlled by two calibrated roller pumps (Model 7518, Cole-Parmer Instruments, IL, USA). After ligating both venae cavae, the right ventricle was vented via the pulmonary artery with a Teflon catheter (1.2-mm outer diameter). Heart rate was maintained at 300 beat mean\(^{-1}\) by atrial pacing. The stimulation voltage was maintained at 20% above threshold (control 2–4 V) and was adjusted continuously throughout the experiment (up to 12 V in reperfused hearts, if necessary). After completion of the experimental preparation, the heart was placed in a water-jacketed chamber at 37°C, filled with humidified, warmed air. During surgical preparation, hearts were perfused at a flow rate of 15 ml min\(^{-1}\). Coronary flow rate was adjusted in order to achieve a coronary perfusion pressure \((P_{\text{LVDP}})\) of 100 mm Hg and CF\(_{\text{max}}\) and \(P_{\text{LVDP}}\)\(_{\text{max}}\) and \(P_{\text{LVDP}}\)\(_{\text{min}}\) and \(P_{\text{LVDP}}\)\(_{\text{min}}\) were measured continuously on an ink recorder (Gould, Mark 260, Cleveland, OH, USA). At the beginning of each experiment, the latex balloon was filled, air-bubble free, with normal saline to achieve an end-diastolic left ventricular pressure of 5 mm Hg. This volume was then held constant for the remainder of the experiment. Left ventricular pressure and coronary perfusion pressure signals were digitized at a sampling rate of 2000 Hz using an analogue-to-digital converter (Data Translation 2801, Marlboro, MA, USA) and then processed further on a personal computer system.

Left ventricular end-diastole was determined as the point when left ventricular \(dP/dt\) started its rapid upstroke after crossing the zero line. Left ventricular end-systole was defined as the point of minimum \(dP/dt\) [10]. Left ventricular peak systolic pressure (LVSP), end-diastolic pressure (LVEDP), developed pressure (LVDP), and maximum and minimum \(dP/dt\) (\(dP/dt_{\text{max}}\) and \(dP/dt_{\text{min}}\)) were obtained from the digitized signals. Only hearts with an LVSP greater than 95 mm Hg during the initial control period were used for the study.

METABOLIC MEASUREMENTS

Aliquots from the perfusion medium and the coronary venous effluent perfusate were sampled anaerobically at the times indicated. Samples were processed immediately for PO\(_2\) measurements (ABL 30, Radiometer, Copenhagen, Denmark). Oxygen consumption \((\dot{V}_{\text{O}})\) was calculated according to Fick’s principle with the use of Bunsen’s absorption coefficient \((\alpha = 0.036 \mu l \times mm \ Hg^{-1} \times ml^{-1})\) at 37°C as follows:

\[
\dot{V}_{\text{O}} = (P_{\text{aO}} - P_{\text{V}}) \alpha CF
\]

where \(P_{\text{aO}}\) = arterial PO\(_2\), \(P_{\text{V}}\) = venous PO\(_2\) (both in mm Hg) and CF = coronary flow (ml min\(^{-1}\)).

For determination of creatine kinase (CK) release, 1-min samples of the effluent were collected at the times indicated (fig. 3). CK activity was measured with an “optimized standard method” according to the recommendations of the Deutsche Gesellschaft für Klinische Chemie (CK test, Boehringer, Mannheim, Germany). After each experiment, the dry weight of the hearts was measured. Myocardial oxygen consumption and creatine kinase release were expressed per gram of dry tissue.
After mounting the heart on the Langendorff apparatus, a preparation and stabilization period of 30 min was allowed. The experimental programme consisted of three experimental phases: baseline, anoxia and reoxygenation (fig. 1). After a 15-min baseline period with two control measurements of all experimental variables, the hearts underwent 35 min of anoxic and substrate-free perfusion at a coronary flow of 2 ml min⁻¹. After the anoxic period, the hearts were re-supplied with oxygen, glucose and pyruvate by changing back to the initial normoxic perfusion conditions for 1 h (reoxygenation). Halothane was added to the perfusion medium to achieve a concentration of 0.4 mmol litre⁻¹ during the first 30 min of anoxic perfusion (halothane–anoxia, n = 5) or during the first 30 min of reoxygenation starting with a 1-min wash-in of the substance (halothane–reoxygenation, n = 5). Five hearts served as controls and underwent the anoxia–reoxygenation programme without receiving halothane. Five hearts received halothane 0.4 mmol litre⁻¹ for 15 min during normoxic perfusion conditions for assessment of the haemodynamic effects of halothane on normal myocardium in this experimental model.

**DATA ANALYSIS**

Data are presented as mean (SEM). The effects of halothane on normal myocardium were assessed by Student’s t test for paired observations. In the anoxia–reoxygenation experiments, statistical analysis was performed by two-way analysis of variance (ANOVA) for time and treatment (experimental group) effects. If an overall significant difference between groups was found, comparison was made for each time using one-way ANOVA followed by the Tukey–Kramer post-test when appropriate.

**Results**

A total of 28 hearts were used. Eight did not fulfil the predefined quality criteria (LVDP > 95 mm Hg at a coronary perfusion pressure of 100 mm Hg) and were excluded from the study. Mean dry heart weight was 0.168 g (range 0.15–0.18 g).

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**EXPERIMENTAL PROGRAMME**

Figure 1 Experimental programme.

**Figure 2** Effect of halothane 0.4 mmol litre⁻¹, given during the first 30 min of anoxic perfusion (●), or during the first 30 min of reoxygenation (○), on left ventricular end-diastolic pressure (LVEDP) as a variable of myocardial contracture (mean, SEM). †P < 0.05 vs controls hearts; *P < 0.05 halothane–anoxia vs halothane–reoxygenation. Note that in control hearts (●) contracture developed with the onset of reoxygenation. This phenomenon was absent when halothane was given during reoxygenation, but persisted when halothane was given during anoxia.

**HAEMODYNAMIC FUNCTION**

**Basal values and effect of halothane on normal myocardium**

In five additional hearts, we investigated the effects of halothane 0.4 mmol litre⁻¹ on normal myocardium (measurements were made after 15 min under steady state conditions). Halothane reduced LVDP from 99.2 (1.8) to 52.0 (2.6) mm Hg (P < 0.001). LVEDP was 4.0 (0.3) mm Hg before and 2.9 (0.4) mm Hg after administration of halothane (P < 0.05). VO₂ decreased from 869.6 (29.5) to 681.0 (21.0) μl min⁻¹ g⁻¹ (P < 0.001). After discontinuation of halothane, recovery to baseline values occurred within 5 min.

**Anoxic contracture development**

During anoxic perfusion, a progressive increase in LVEDP was noted in all groups (fig. 2). The time of onset of anoxic contracture was defined as the time at which the end-diastolic pressure had increased by 2 mm Hg; it was similar in all three groups (control 140 (17) s; halothane–anoxia 133 (18) s; halothane–reoxygenation 150 (10) s). The maximum level of contracture was reached within 10 min (ns between groups) and LVEDP decreased gradually during the anoxic period. The level of contracture was reduced in the halothane–anoxia group; at the end of the anoxic period, LVEDP was 55.2 (0.3) mm Hg compared with 71.2 (5.1) mm Hg in control hearts (P < 0.05) and 69.2 (4.3) mm Hg in hearts treated during reperfusion (P < 0.05).

**End-diastolic pressure after reoxygenation**

The main effect on functional recovery during reoxygenation was a difference in the behaviour of end-diastolic pressure of the experimental groups
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In the control group, there was an increase in LVEDP by a mean of 40.0 mm Hg with the onset of reoxygenation (reoxygenation-induced contracture), followed by a slow decrease in LVEDP during the rest of the reoxygenation period to 85.3 mm Hg at 60 min of reoxygenation. The hearts treated with halothane during anoxia had a lower LVEDP before the onset of reoxygenation (51.8 mm Hg) than the two other groups, but then also showed reoxygenation-induced contracture (by 30.0 mm Hg). LVEDP was, however, lower compared with untreated hearts and showed a faster recovery to 43.9 mm Hg at 60 min of reoxygenation. In the group treated with halothane at reoxygenation, reoxygenation-induced contracture was absent and LVEDP decreased rapidly, reaching 24.2 mm Hg at 60 min of reoxygenation.

Recovery of contractile function during reoxygenation

LVDP was similar in all groups (about 105 mm Hg) during baseline measurements before anoxia and then declined rapidly to 0 mm Hg during anoxic perfusion (fig. 3, top). In control hearts, recovery of LVDP started at 30–40 min of reoxygenation and reached 15.4 % of baseline values after 60 min. In hearts treated with halothane during anoxia, recovery was better, starting at 20–30 min of reoxygenation and reaching 32.4 % of baseline values. The best functional recovery was seen in hearts treated with halothane only during reoxygenation; recovery started within the first 5 min and reached 70.4 % of baseline at 60 min of reoxygenation. After discontinuation of halothane at 30 min of reoxygenation, LVDP showed a steep increase by a mean of 32 mm Hg within the next 5 min.

LVSP (table 1) in this experimental model can be regarded as the sum of LVEDP and LVDP. Consequently, it showed a different behaviour between the three groups. In control hearts, LVSP remained almost unchanged during the course of the experiment. In hearts treated with halothane during anoxia, LVSP was reduced compared with baseline values at the onset of reoxygenation (to 81.8 (2.6) mm Hg) and remained at a lower level throughout the reoxygenation period. In hearts that received halothane during the first 30 min of reoxygenation, LVSP was reduced during the first 30 min and then increased after discontinuation of halothane to values not different from baseline.

METABOLIC MEASUREMENTS

Creatine kinase

In untreated control hearts, CK release, as an indicator of cell damage, did not increase significantly during anoxic perfusion but increased markedly during the early reoxygenation phase with a peak value (149.1 iu min⁻¹ g⁻¹) at 1 min of reoxygenation (fig. 3, bottom). CK release was increased in control hearts during the first 40 min of reoxygenation. In hearts treated with halothane during anoxia, maximum CK release was reduced to 33.7 % compared with control hearts and in hearts treated with halothane only during early reoxygenation to 6.7 %. As a consequence, total cumulative CK release (fig. 4) was reduced significantly when halothane was given during reoxygenation (60 (12) iu g⁻¹) compared with untreated controls (712 (52) iu g⁻¹) or hearts treated with halothane during anoxia (188 (8) iu g⁻¹).

Oxygen consumption

\( V_O^2 \) was approximately 1000 μl min⁻¹ g⁻¹ dry tissue under baseline conditions in all three groups (table 1). In control hearts, \( V_O^2 \) increased to 100 % of baseline with the onset of reoxygenation and then decreased to 57.3 % at the end of reoxygenation. In hearts treated with halothane during the anoxic period, \( V_O^2 \) was 108 % of baseline at the onset of
Effects of halothane in anoxia–reoxygenation experiments. Data are mean (SEM), n = 5. LVSP = Left ventricular systolic pressure, CPP = coronary perfusion pressure; \( \dot{V}_02 \) = myocardial oxygen consumption. *P < 0.05 compared with untreated hearts (control); †P < 0.05 compared with hearts treated with halothane during anoxia (halothane anoxia).

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<th>Control</th>
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Discussion

We have found, in an isolated rat heart model, that halothane only moderately attenuated myocardial injury during a state of oxygen and substrate deprivation, but had a pronounced protective action on myocardial injury at reoxygenation after an anoxic period.

Critique of Methods

In this study, we used a period of anoxic and substrate-free perfusion to simulate myocardial ischaemia, and subsequent reoxygenation and resupply of metabolic substrates to simulate myocardial reperfusion. Although the terms “reperfusion” and “reoxygenation” and the terms “anoxic perfusion” and “ischaemia” are often used synonymously, some important differences should be taken into account in the interpretation of our results. First, anoxic perfusion was used to allow the possibility of halothane administration during simulated ischaemia. Anoxic perfusion may have attenuated the development of intracellular acidosis and accumulation of intracellular adenosine; in vitro, both mechanisms may have some endogenous myocardial protective effects. Second, during buffer perfusion there are no neutrophils and thrombocytes; both may interact with the development of in vivo ischaemia–reperfusion injury (for review see [11]). Thus this experimental model excludes some of the (patho)mechanisms that play a role in an in vivo ischaemia–reperfusion situation.

In most previous in vitro and in vivo studies that have found a reduction in myocardial injury in an ischaemia–reperfusion situation in the presence of volatile anaesthetics, the beneficial effects were explained primarily by the action of anaesthesia on sympathoadrenal activity and on myocardial oxygen demand [6, 7, 9], rather than by any intrinsic property of the volatile anaesthetics. Some studies also discussed a protective effect on myocardial reperfusion injury [8, 12], but the interpretation of these data is difficult because halothane was given also before reperfusion (during a period of ischaemia [13], hypoxia [6, 7, 9] or cardioplegic arrest [8]) and often also before the onset of oxygen deprivation (hypoxia [6, 7, 9], ischaemia [13] or cardioplegic arrest [8, 12]). However, it is known that other negative inotropic drugs (e.g. calcium antagonists or β blockers) exert potent anti-ischaemic effects when given before or during ischaemia. As the extent of reperfusion-induced injury is proportional to the severity of the preceding ischaemia, it is impossible from these studies to ascertain if a protective effect is caused by a reduction in reperfusion injury or if it is secondary to an anti-ischaemic action.

In order to reduce haemodynamic side effects in the experimental model used in this study, we controlled heart rate, ventricular volume and coronary flow. Nevertheless, loading conditions between the experimental groups were not identical during reoxygenation; halothane-treated hearts had...
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a higher LVDP and consequently a greater wall stress during reoxygenation compared with untreated controls. However, in the treatment group, myocardial injury was reduced markedly and increased loading conditions should have had the opposite effect. Therefore, differences in loading conditions can be regarded as a "conservative" effect that cannot explain the observed protection.

To differentiate between anti-ischaemic effects and protection against reperfusion injury, halothane was administered only during the first 30 min of anoxia or for the first 30 min of reoxygenation. Because the danger of impending contracture in the reoxygenated myocardial cell is evoked by the reactivation of mitochondrial energy production [14], administration of halothane was started with a 1-min anoxic wash-in of the substance into the perfusion system before reoxygenation in the halothane–reoxygenation group. In the hearts treated with halothane during the anoxic perfusion, halothane was discontinued 5 min before the onset of reoxygenation. Although we found in preliminary experiments in normoxic hearts that a 5-min wash-out of halothane was sufficient for complete reversal of the haemodynamic effects of halothane, it should be taken into consideration that an unknown amount of halothane may have been present at the onset of reoxygenation in this group. Therefore, some protection against reoxygenation-induced injury may have been present in addition to the anti-ischaemic effect in this experimental group.

INTERPRETATION OF RESULTS

In the ischaemia–reperfusion model used in this study, the manifestation of lethal cell injury occurs at the onset of reoxygenation, as evident from the massive release of creatine kinase, an intracellular marker enzyme. Because the extent of this injury can be reduced through an intervention at the time when oxygen is re-supplied, cell injury represents not only the manifestation of ischaemic injury, but in part true "reperfusion injury" in the sense defined by Rosenkranz and Buckberg [1] as "metabolic, functional and structural consequences of restoring coronary arterial flow ... that can be avoided or reversed by modifications of the conditions of reperfusion". The mechanisms underlying the type of "reperfusion injury" that occurs in the present model have been studied in the past years; one important mechanism is cellular contracture that occurs at reoxygenation and most likely results from the combination of a high cytosolic calcium concentration, accumulated during the anoxic period, and the re-supply of metabolic energy with the onset of reoxygenation (for review, see [15]). Contracture may result in mechanical disruption of the cells [15] that have an increased mechanical fragility after the period of energy depletion [16]. Consequently, in the isolated heart model used in this study, reoxygenation induced a sudden increase in LVEDP (contracture) and a high myocardial oxygen consumption. Simultaneously, there was a massive release of creatine kinase. Oxygen consumption decreased to approximately 57 % of baseline values, probably an effect of the reduced amount of viable myocardium. These findings were identical to others in similar models [17–19].

When present at the critical moment of reoxygenation, halothane had a pronounced protective effect against this type of "reperfusion injury"; it completely prevented the development of contracture and reduced the peak release of creatine kinase to 6.7 % of that seen in untreated hearts. In addition, myocardial oxygen consumption was reduced compared with untreated controls despite an increased mechanical work index (LVDP). From these results it seems likely that the protective effect of halothane against "reperfusion injury" consists of attenuation of cellular contracture.

It was clearly beyond the scope of the study to identify the cellular mechanisms of the protective effect of halothane against reperfusion-induced contracture. However, in parallel with the recent understanding of the underlying pathophysiology, some protective mechanisms have been identified, and from the mechanisms of action that have been described for halothane in normal myocardium, several protective mechanisms against contracture development are possible. First, contractile inhibition at the level of the myofibrils. It has been shown previously that temporary block of the contractile apparatus (using the substance BDM) can prevent or reduce "reperfusion injury" in isolated cardiomyocytes [20], isolated hearts [17] and intact hearts [21]. Even partial contractile inhibition (by SIN-1C) had a protective effect in isolated hearts [18] and in vitro [22], probably by reducing developed force in the re-energized cardiomyocyte below a critical threshold [22]. A reduction in the calcium sensitivity of myofibrils by halothane has been described [23, 24] and the resulting contractile inhibition may have contributed to the observed protective effect.

Second, effects on intracellular calcium. The high intracellular calcium concentration accumulated during the ischaemic period represents the trigger for cellular contracture during reperfusion. With the onset of reperfusion, intracellular calcium may increase even further by calcium influx via the Na+/Ca" exchange [25], and phasic oscillations of intracellular calcium occur as a result of pathological calcium cycling the the sarcoplasmic reticulum [26]; both mechanisms can increase further contractile activation. In normal myocardium, inhibition of the Na+/Ca" exchanger by halothane has been shown [27]. In addition, halothane interferes with the calcium handling of the sarcoplasmic reticulum (of normal myocardium) by direct inhibition of sarcoplasmic function [28] and an action on the calcium-dependent calcium-release channel [29]. At low intracellular pH and ATP concentrations (conditions similar to early reperfusion), halothane may have an opposite effect on sarcoplasmic reticulum calcium handling, enhancing calcium uptake [30]. Halothane may also interact with the Na+/H" exchanger at reperfusion preserves intracellular acidosis, prevents pathological calcium cycling [32]
Lochner and co-workers [12], who found reduced ischaemic period seems to attenuate ischaemic injury during reperfusion. As the latter reperfusion injury, although the extent of reoxygenation was high despite a reduced myocardial astolic tension was reduced in the present model. Halothane was also protective when given during anoxic perfusion (reduced oxidative stress and improved functional recovery), but the protective effect was smaller. A beneficial role of volatile anaesthetics in myocardial ischemia was observed in 1969 by Spieckermann and colleges [36], who found a prolonged tolerance to global ischemia and enhanced preservation of high energy compounds in dog hearts. It was shown recently that halothane slow calcium accumulation during "ischaemia" [12]. Consequently, the level of diastolic tension was reduced in the present model. However, reoxygenation-induced contracture still occurred in these hearts and initial $V_{O_2}$ during reoxygenation was high despite a reduced myocardial work index. Thus the hearts showed typical signs of reperfusion injury. Nevertheless, although the extent of reperfusion injury was reduced. As the latter depends mainly on the severity of the preceding ischaemia, the presence of halothane during an ischaemic period seems to attenuate ischaemic injury. This is also in accordance with findings of Lochner and co-workers [12], who found reduced cellular damage when halothane was given during cardioplegic arrest to isolated rat hearts.

Acknowledgement

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