One MAC of sevoflurane provides protection against reperfusion injury in the rat heart *in vivo*

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Volatile anaesthetics protect the heart against reperfusion injury. We investigated whether the cardioprotection induced by sevoflurane against myocardial reperfusion injury was concentration-dependent. Fifty-eight α-chloralose anaesthetized rats were subjected to 25 min of coronary artery occlusion followed by 90 min of reperfusion. Sevoflurane was administered for the first 15 min of reperfusion at concentrations corresponding to 0.75 (n=11), 1.0 (n=11), 1.5 (n=13), or 2.0 MAC (n=12). Eleven rats served as untreated controls. Left ventricular peak systolic pressure (LVPSP, tipmanometer) and cardiac output (CO, flowprobe) was measured. Infarct size (IS, triphenyltetrazolium staining) was determined as percentage of the area at risk. Baseline LVPSP was 131 (126±135) mm Hg (mean (95% confidence interval)) and CO 33 (31±36) ml min⁻¹, similar in all groups. During early reperfusion, sevoflurane reduced LVPSP in a concentration-dependent manner to 78 (67±89)% of baseline at 0.75 MAC (not significant vs controls 99 (86±112)%), 71 (62±80)% at 1 MAC (P<0.05), 66 (49±83)% at 1.5 MAC (P<0.05) and 56 (47±65)% at 2 MAC (P<0.05). CO remained constant. While 0.75 MAC of sevoflurane had no effect on IS (34 (27–41)% of the area at risk) compared with controls (38 (31–45)%), 1.0 MAC reduced IS markedly to 23 (17–30)% (P<0.05). Increasing the concentration to 1.5 MAC (23 (17–30)%) and 2 MAC (23 (13–32)%, both P<0.05 vs controls) had no additional protective effect. One MAC sevoflurane protected against myocardial reperfusion injury. Increasing the sevoflurane concentration above 1 MAC resulted in no further protection.

Keywords: anaesthetics volatile, sevoflurane; heart, reperfusion injury

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Sevoflurane protects against reperfusion injury after myocardial ischaemia *in vitro*¹² and *in vivo*.³ Sevoflurane like isoflurane has only minimal cardiovascular effects.⁴ Compared with other anaesthetics,⁵ depression of myocardial contractility lowers with sevoflurane. However, all previous studies investigating the effects of volatile anaesthetics against reperfusion injury used only one anaesthetic concentration. In isolated rat hearts, 1.5 MAC (minimum alveolar concentration) of sevoflurane reduced creatine kinase release and improved myocardial function;² in rabbits, 1.0 MAC sevoflurane reduced infarct size after regional myocardial ischaemia and reperfusion.³ It remains unknown, whether the cardioprotective effect of sevoflurane is concentration-dependent.

Therefore, we investigated the effects of four different concentrations of sevoflurane on myocardial reperfusion injury after occlusion of a major branch of the left coronary artery in rats *in vivo*.

Materials and methods

The study was performed in accordance with the regulations of the German Animal Protection Law and was approved by the Bioethics Committee of the District of Düsseldorf.

Animal preparation

Fifty-eight Wistar rats (body weight mean 486 (95% confidence interval, 479–493) g) were anaesthetized by intraperitoneal pentobarbital 60 mg kg⁻¹. After intubation of the trachea, the lungs were ventilated (Rhema-Labortechnik Beatmungsgerät, Typ 10ml, Cass 34931, Germany) with a tidal volume of 5 ml at 60 breaths min⁻¹ to maintain P⁰₂ within physiological limits. Surface electrocardiogram (Siemens Elema AB EKG-Gerät, Germany) was recorded continuously. After cannulation of the femoral vein, the rats received a single dose of α-chloralose (50 mg kg⁻¹). For compensation of fluid losses and maintenance of anaesthesia.
sia, saline 0.9% (5 ml h\(^{-1}\)), and α-chloralose (25 mg kg\(^{-1}\) h\(^{-1}\)) were infused continuously. For measurement of aortic pressure, a polyethylene catheter was inserted into the descending aorta via the femoral artery and connected to a pressure transducer (Statham, PD23, Gould, Cleveland, OH, USA).

After median sternotomy and pericardiotomy were performed, an ultrasonic flowprobe was placed around the pulmonary artery in order to measure cardiac output (CO, 6S ultrasonic flowprobe, T 208, Transonic Systems Inc., Ithaca, NY, USA). A ligature snare was passed around a major coronary artery for later occlusion. Left ventricular (LV) pressure (LVP) was monitored using a catheter tip manometer (Millar Microtip-Catheter Model SPR-407, size 2F, Houston, TX, USA) advanced from the right carotid artery via the aortic arch into the left ventricle. A temperature probe was placed sub-diaphragmatically (GTH 1160, Digital Thermometer, Geisinger Electronic, Germany) and body temperature was maintained constant at 38.8 (0.3)°C by adjusting a heating pad and a warming lamp.

**Experimental programme**

After 15 min of recovery from surgical preparation, baseline measurements were performed. Tightening the snare around the prepared coronary artery induced myocardial ischaemia. The effectiveness of this manoeuvre was verified by the appearance of epicardial cyanosis and changes in surface ECG. After 25 min of occlusion, the snare occluder was released and successful reperfusion was verified by the disappearance of epicardial cyanosis. During the initial reperfusion period (first 15 min), the rats inhaled sevoflurane in different concentrations. After 90 min of reperfusion, the hearts were quickly excised and mounted on a modified Langendorff perfusion system for determination of infarct size.

Eleven rats underwent the ischaemia-reperfusion programme without further intervention and served as controls. In 47 rats, sevoflurane was added to the inspired gas starting 1 min before reperfusion to achieve a stable concentration at the beginning of the reperfusion period. The anaesthetic was then continued for the first 15 min of reperfusion. The volatile anaesthetic was titrated to an end-tidal concentration (Datex Capnomac Ultima, Division of Instrumentarium Corp., Helsinki, Finland) of 1.8 (0.75 MAC; n=11), 2.4 (1 MAC, n=11), 3.6 (1.5 MAC, n=13), and 4.8 vol.% (2 MAC, n=12), respectively. An end-tidal concentration of 2.4% corresponds to 1.0 MAC of sevoflurane in rats.6

**Measurement of infarct size**

Hearts were perfused on a modified Langendorff apparatus with normal saline at 80 mm Hg perfusion pressure to wash out any remaining blood. The coronary artery was then re-occluded and 5–10 ml of 0.2% Evans Blue dye in 1% dextrane was infused via the aortic root into the coronary system. This manoeuvre identifies the area at risk as unstained. The heart was then frozen and cut into 8–15 transverse slices of equal thickness (1 mm). The slices were incubated (37°C) for 15 min in buffered 1% triphenyltetrazoliumchloride adjusted to pH 7.4 and then incubated for 2 days in 4% formaldehyde. Viable myocardium was then identified as red stained by triphenyltetrazoliumchloride, whereas necrotic myocardium appears pale grey. The area at risk and the infarcted area were determined by planimetry using Sigma Scan Pro 5 computer software (SPSS Science Software) and corrected for dry weight.

**Data analysis**

LVP, its first derivative dP/dt, aortic pressure and CO were recorded continuously on a polygraph (Hellige 120 710 94, Freiburg, Germany) and were digitized using an analogue to digital converter (Data Translation, Marlboro, MA, USA) at a sampling rate of 500 Hz and processed later on a personal computer.

**Haemodynamic variables**

Global systolic function was measured as LV peak systolic pressure (LVSP) and the maximum rate of pressure increase (dP/dt\(_{max}\)). Global LV end-systole was defined as the point of minimum dP/dt and LV end-diastole as the beginning of the sharp upslope of the LV dP/dt tracing. Systemic vascular resistance (SVR) was calculated from mean aortic pressure and CO, assuming a right atrial pressure of 0 mm Hg in the open-chest preparation.

**Statistical analysis**

Data are presented as means and 95% confidence interval. Statistical analysis was performed by a two-way analysis of variance (ANOVA) for time and treatment (sevoflurane concentration) effects. Time effects (changes from baseline value) were analysed by using Dunnett’s post-hoc test. If an overall significance between groups was found, comparison was made for each time point using one-way ANOVA followed by the Dunnett’s post-hoc test where appropriate.

**Results**

Sixty-four rats were used. Six rats died from ventricular fibrillation during coronary artery occlusion. In the remaining 58 animals, complete data sets were obtained.

**Haemodynamic function**

Figure 1 shows a recording of the haemodynamic variables during a single experiment. During baseline conditions, no between group differences in haemodynamics were observed (data are summarized in Table 1 and Fig. 2). Coronary artery occlusion resulted in a reduction of LVSP, dP/dt\(_{max}\) and SVR to 91 (85–96)% of baseline values, 94...
(87–102)% and 83 (79–88)% respectively. They were similar in all groups (data at 23 min ischaemia). CO remained constant (99 (95–104)%), and LV end-diastolic pressure increased to 182 (155–208)%.

During reperfusion, no further changes from ischaemic values were observed in controls, except for a further reduction in SVR to 83 (71–96)% of baseline. Sevo-urane slightly reduced heart rate during the early reperfusion.

Table 1  
**Haemodynamic variables during ischaemia-reperfusion experiments (% changes from baseline) in the control group and the groups receiving 0.75 (0.75 MAC), 1 (1 MAC), 1.5 (1.5 MAC), and 2 MAC (2 MAC) of sevo-urane. Data are mean (95% confidence interval). *P<0.05 vs controls; tP<0.05 vs baseline**

<table>
<thead>
<tr>
<th>Coronary artery occlusion</th>
<th>15 min</th>
<th>24 min</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 min</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>Control</td>
<td>10 (7–12)</td>
<td>163 (143–183)</td>
<td>171 (141–203)</td>
</tr>
<tr>
<td>0.75 MAC</td>
<td>10 (5–15)</td>
<td>162 (110–214)</td>
<td>167 (110–214)</td>
</tr>
<tr>
<td>1 MAC</td>
<td>9 (7–11)</td>
<td>165 (130–200)</td>
<td>176 (129–223)</td>
</tr>
<tr>
<td>1.5 MAC</td>
<td>9 (7–11)</td>
<td>205 (147–264)</td>
<td>237 (167–307)</td>
</tr>
<tr>
<td>dP/dt max (mm Hg s⁻¹)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>Control</td>
<td>6845 (5794–7897)</td>
<td>105 (87–123)</td>
<td>108 (89–126)</td>
</tr>
<tr>
<td>0.75 MAC</td>
<td>6222 (5444–7001)</td>
<td>92 (81–103)</td>
<td>105 (86–125)</td>
</tr>
<tr>
<td>1 MAC</td>
<td>6972 (5643–8301)</td>
<td>95 (84–107)</td>
<td>91 (79–104)</td>
</tr>
<tr>
<td>1.5 MAC</td>
<td>7584 (6552–8636)</td>
<td>101 (74–128)</td>
<td>102 (65–138)</td>
</tr>
<tr>
<td>CO (ml min⁻¹)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>0.75 MAC</td>
<td>30 (26–34)</td>
<td>103 (90–116)</td>
<td>104 (88–119)</td>
</tr>
<tr>
<td>1 MAC</td>
<td>35 (27–42)</td>
<td>95 (88–101)</td>
<td>97 (89–105)</td>
</tr>
<tr>
<td>1.5 MAC</td>
<td>37 (32–42)</td>
<td>104 (92–116)</td>
<td>99 (83–114)</td>
</tr>
<tr>
<td>2 MAC</td>
<td>34 (26–41)</td>
<td>92 (84–100)</td>
<td>93 (82–103)</td>
</tr>
</tbody>
</table>

**Fig 1** Registration showing selected times from one experiment where 1 MAC sevo-urane was given during initial reperfusion. LVP=left ventricular pressure, dP/dt=rate of change of LVP, AOP=aortic pressure, Flow A.pulm.=pulmonary artery flow, ECG=electrocardiogram.
Fig 2 Line plot showing the time course of heart rate (HR), left ventricular peak systolic pressure (LVPSP) and systemic vascular resistance (SVR) during the experiments (data are mean (95% confidence interval), *P<0.05 vs baseline). LVPSP and SVR decreased in a concentration-dependent manner during initial reperfusion, but no differences between the treatment groups were observed at the end of the experiments. Baseline values were:

- HR (beats min⁻¹): controls, 441 (413–467); 0.75 MAC, 431 (407–456); 1 MAC, 443 (423–462); 1.5 MAC, 444 (426–462); 2 MAC, 441 (412–470).
- LVPSP (mm Hg): controls, 125 (112–138); 0.75 MAC, 130 (122–137); 1 MAC, 132 (120–144); 1.5 MAC, 135 (126–144); 2 MAC, 129 (116–142).
- SVR (mm Hg ml⁻¹ min⁻¹): controls, 3.2 (2.6–3.8); 0.75 MAC, 3.7 (3.2–4.2); 1 MAC, 3.5 (3.0–4.1); 1.5 MAC, 3.3 (2.8–3.7); 2 MAC, 3.5 (2.7–4.3).
A concentration-dependent depression of LVPSP to 56 (47–65)% (P<0.05 vs controls) with 2.0 MAC was observed after 15 min of sevoflurane administration (Fig. 2). A similar reduction was seen for dP/dt max (to 43 (34–52)%; P<0.05 vs controls, Table 1) and SVR (to 48 (38–57)%; P<0.05 vs controls, Fig. 2). Administration of sevoflurane had no effect on CO (99 (87–102)% of baseline) after 15 min of reperfusion.

Infarct size
Mean heart dry weight was 0.19 (0.18–0.21) g with no differences between groups (data for the individual groups are given in Table 2). Ischaemic-reperfused area (area at risk) constituted 28 (24–31)% of the total heart size. In controls, infarct size was 38 (31–45)% of the area at risk (Fig. 3). Infarct size was reduced by 1 MAC sevoflurane to 23 (17–30)% of the area at risk (P<0.05). Increasing the sevoflurane concentration had no further effect on infarct size reduction: 1.5 MAC, 23 (17–30)%; 2 MAC, 23 (13–33)% of the area at risk (both P<0.05 vs controls). However, a lower concentration of sevoflurane (0.75 MAC) had no effect on infarct size (0.75 MAC, 33 (27–41)% of the area at risk, P=0.83 vs controls).

Table 2 Heart weight and area at risk size in the control group and the groups receiving 0.75 (0.75 MAC), 1 (1 MAC), 1.5 (1.5 MAC), and 2 MAC (2 MAC) of sevoflurane. Data are mean (95% confidence interval)

<table>
<thead>
<tr>
<th>Group</th>
<th>Heart weight (g)</th>
<th>Area at risk (% of heart weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.19 (0.17–0.21)</td>
<td>29 (21–38)</td>
</tr>
<tr>
<td>0.75 MAC</td>
<td>0.18 (0.17–0.20)</td>
<td>32 (21–43)</td>
</tr>
<tr>
<td>1 MAC</td>
<td>0.19 (0.17–0.22)</td>
<td>26 (20–31)</td>
</tr>
<tr>
<td>1.5 MAC</td>
<td>0.19 (0.18–0.20)</td>
<td>23 (16–30)</td>
</tr>
<tr>
<td>2 MAC</td>
<td>0.21 (0.15–0.27)</td>
<td>28 (20–37)</td>
</tr>
</tbody>
</table>

Discussion
Reperfusion injury characterizes a situation in which the myocardium loses viability by pathological mechanisms that are triggered by restoration of oxygen and substrate supply after myocardial ischaemia. This type of myocardial cell damage is caused by mechanisms which take place during reperfusion and not during the preceding ischaemia.

Inhalation anaesthetics offer specific protection against myocardial reperfusion injury in a variety of experimental settings. For sevoflurane, it was shown that a concentration corresponding to 1.5 MAC reduced creatine kinase release and improved myocardial function after global ischaemia in isolated rat hearts. In addition, 1.5 MAC sevoflurane provided further protection against reperfusion injury even if the heart was already protected against the ischaemic damage using a cardioplegic solution. Sevoflurane at a concentration corresponding to 1 MAC reduced infarct size after regional ischaemia in rabbits in vivo. No data concerning concentration-dependent effects of sevoflurane or other anaesthetics against myocardial reperfusion injury are available.

The present study demonstrates that 1 MAC sevoflurane reduces infarct size after regional ischaemia in the rat heart in vivo. A lower concentration (0.75 MAC) has no effect on infarct size. Increasing the concentration of sevoflurane to 2 MAC produced a similar reduction of infarct size as 1 MAC sevoflurane. However, during administration of these higher concentrations, more pronounced haemodynamic side effects were observed.

One mechanism responsible for the phenomenon of early reperfusion injury was first described in isolated cardiomyocytes as the ‘oxygen paradox’. The hypoxic-reoxygenated cells developed cellular hypercontracture during reoxygenation, resulting in cytolysis. This hypercontracture is caused by simultaneous resupply of ATP after reactivation of oxidative phosphorylation and uncontrolled Ca2+-release from the sarcoplasmic reticulum, leading to uncontrolled cellular contraction. For halothane, a protection against hypercontracture was demonstrated by an action on the Ca2+-dependent Ca2+-release channel of the sarcoplasmic reticulum. One might speculate that the interaction between this channel and volatile anaesthetics follows a ligand-binding kinetic. This would result in a dose-dependent effect of the volatile anaesthetics, with no further increase in protection after blocking all channels. However, volatile anaesthetics might have different effects on sarcoplasmic Ca2+-handling and actions of sevoflurane on these channels are not known. Therefore, it remains speculative whether the observed maximal protection with 1 MAC of sevoflurane results from a specific effect at this channel.
In the intact animal, haemodynamic effects during reperfusion might also influence the amount of infarcted tissue. For example, a reduction of myocardial afterload or a staged reperfusion (in contrast to a sudden reperfusion) might reduce reperfusion injury. Despite significant necrosis, control hearts showed no changes in LVSP or CO (Fig. 2, Table 1). This finding can be explained by the mass of the infarcted tissue, which constituted 38 (31–34)% of the area at risk, but only 7.5 (6.2–8.8)% of the total ventricular mass. Taking into account that the large potential increase in CO during exercise, it is not surprising that a 7.5% decrease of the contractile mass can be compensated for without major changes in haemodynamics.

Haemodynamic effects of volatile anaesthetics are known to be concentration-dependent. Sevoflurane caused a significant reduction of SVR and only a small reduction in heart rate. A reduction of SVR does not necessarily reduce CO, and a reduced heart rate can be compensated for by an increase in stroke volume, resulting in maintained CO (Table 1, Fig. 1). There was a more pronounced reduction in LVSP during reperfusion with the higher concentrations of sevoflurane in our experiments (Fig. 2). In contrast, infarct size was no further reduced by higher concentrations of sevoflurane. Therefore, it is likely that specific actions of sevoflurane on reperfused myocardium rather than haemodynamic changes are responsible for the observed reduction in infarct size. These results are in line with previous findings that the action of volatile anaesthetics on reperfusion injury is independent of haemodynamic changes.

In addition to direct effects at the myocardium and haemodynamic alterations, interactions with leukocyte activation and accumulation may also reduce reperfusion injury. By plugging capillaries, leukocytes may play an important role in the ‘delayed no-reflow phenomenon’, resulting in persistent ischaemia. Sevoflurane (0.5–1.0 MAC) has been shown to attenuate adhesion of neutrophils and platelets in the coronary system after ischaemia and reperfusion. As a result, changes in regional myocardial blood flow could occur. Myocardial blood flow was not measured in the present study and it cannot be excluded that alterations of endo- or epicardial blood flow contributed to the protection against reperfusion injury by sevoflurane. Activation of leukocytes results in release of oxygen-derived free radicals, which can further increase cellular damage. Sevoflurane also reduced the production of radicals as well as the damage caused by free radicals.

Reperfusion of ischaemic myocardium may occur in a variety of clinical situations, for example after coronary artery bypass surgery. In cardio-compromised patients, optimal protection against myocardial reperfusion injury in the absence of haemodynamic side effects might be beneficial. We demonstrated that 1 MAC of sevoflurane protects against ischaemia-reperfusion injury in the rat heart in vivo and that increasing the concentration further did not improve cardioprotection, but increased the cardiodepressant side effects.

References
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