The role of mitochondria in cardioprotection
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Hyperglycaemia blocks Sevoflurane-induced postconditioning in the rat heart \textit{in vivo}: Cardioprotection can be restored by blocking the mitochondrial permeability transition pore

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Sevoflurane-induced postconditioning and hyperglycemia

ABSTRACT

Recent studies showed that hyperglycaemia (HG) blocks anaesthetic-induced preconditioning. The influence of HG on anaesthetic-induced postconditioning (post) has not yet been determined. We investigated whether sevoflurane (Sevo)-induced postconditioning is blocked by HG and whether the blockade could be reversed by inhibiting the mitochondrial permeability transition pore (mPTP) with cyclosporine A (CsA). Chloralose-anaesthetized rats (n=7–11 per group) were subjected to 25 min coronary artery occlusion followed by 120 min reperfusion. Postconditioning was achieved by administration of 1 or 2 MAC sevoflurane for the first 5 min of early reperfusion. HG was induced by infusion of glucose 50% (G 50) for 35 min, starting 5 min before ischaemia up to 5 min of reperfusion. CsA (5 or 10 mg kg⁻¹) was administered i.v. 5 min before the onset of reperfusion. At the end of the experiments, hearts were excised for infarct size measurements. Infarct size (% of area at risk) was reduced from 51.4 (5.0)% [mean (SD)] in controls to 32.7 (12.8)% after sevoflurane postconditioning (Sevo-post) (P<0.05). This infarct size reduction was completely abolished by HG [51.1 (13.2)%, P<0.05 vs Sevo-post], but was restored by administration of sevoflurane with CsA [35.2 (5.2)%, P<0.05 vs HG+Sevo-post]. Increased concentrations of sevoflurane or CsA alone could not restore cardioprotection in a state of HG [Sevo-post2, 54.1 (12.6)%, P>0.05 vs HG+Sevo-post; CsA10, 58.8 (11.3)%, P>0.05 vs HG+CsA]. Sevoflurane-induced postconditioning is blocked by HG. Inhibition of the mPTP with CsA is able to reverse this loss of cardioprotection.
INTRODUCTION

Hyperglycaemia correlates with increased mortality after acute myocardial infarction in diabetic patients as well as in patients without diabetes mellitus. (2; 13) Hyperglycaemia was shown to abolish cardioprotection induced by ischaemic and anaesthetic preconditioning. (14; 15)

Besides preconditioning, also postconditioning (i.e. cardioprotection by administration of the substance after ischaemia during early reperfusion) can be induced by volatile anaesthetics. (3; 23) Recent studies demonstrated, that the volatile anaesthetic sevoflurane offers cardioprotection by postconditioning. (5; 21) In both studies, postconditioning induced a cardioprotective effect that was comparable to the extent of cardioprotection induced by sevoflurane preconditioning. Furthermore, Obal et al. showed that sevoflurane induces maximal cardioprotection by postconditioning at a concentration of only 1 MAC. (22) It is not known whether anaesthetic-induced postconditioning can be induced in hyperglycaemic subjects. This question was tested in the first part of the study using the in vivo rat model.

Recent studies showed that the mPTP is involved in isoflurane-induced postconditioning via phosphorylation and inhibition of GSK3β. (8) Krolikowski et al. demonstrated that keeping the mPTP closed with CsA enhanced cardioprotection of isoflurane-induced postconditioning. (17) Therefore, in the second part of the study, we tested if administration of CsA shortly before the reperfusion period could restore the assumed abolished cardioprotection.

We hypothesized that (1) sevoflurane postconditioning is abolished by hyperglycaemia and (2) that the cardioprotection can be restored by inhibiting the mPTP in hyperglycaemic animals.

MATERIALS AND METHODS

The study was performed in accordance with the requirements of the Animal Ethics Committee of the University of Amsterdam and was in line with European Union directives on the care and use of experimental animals.
Sevoflurane-induced postconditioning and hyperglycemia

Materials

Sevoflurane was purchased from Abbott (SEVOrane®, Abbott B.V., Hoofddorp, the Netherlands). Cyclosporine A was purchased from Fluka Biochemika (Sigma Aldrich, Steinheim, Germany). The Glucose 50% was purchased from B. Braun (B. Braun Melsungen AG, Melsungen, Germany). For measurement of the blood glucose levels, we used the FreeStyle Freedom blood glucose meter from Abbott. Rat insulin samples were measured with a Rat Insulin ELISA from Orange Medical (Orange Medical, Tilburg, the Netherlands).

Experimental protocol for infarct size determination:

Animals had free access to food and water at all times before the start of the experiments. Male Wistar rats (250-350 g, 7-11 per group) were anaesthetized by intraperitoneal S-ketamine injection (150 mg kg⁻¹). S-ketamine does not interfere with cardioprotection in animals in vivo. (19)

Rats were divided into ten groups (Fig. 1A):

Control group (Con) (n = 9): After surgical preparation, rats received 30% oxygen plus 70% nitrogen. Normal saline was given intravenously over 35 minutes starting 5 min prior ischaemia up to 5 minutes of reperfusion.

Sevoflurane postconditioned group (Sevo-post) (n = 11): Rats received sevoflurane with an endtidal concentration of 1 MAC (± 2.4 Vol%) for 5 minutes starting 1 minute prior to the onset of reperfusion; saline 0.9% was infused intravenously over 35 minutes starting 5 min prior ischaemia up to 5 minutes of reperfusion.

Glucose 50% group (HG) (n = 9): Glucose 50% was administered intravenously over 35 minutes starting 5 minutes prior to ischaemia and was continued until 5 minutes of reperfusion. Target blood glucose level before ischaemia was 22 mmol l⁻¹ or higher and was maintained at this level.

Glucose 50% + Sevoflurane postconditioned group (HG+Sevo-post) (n = 9): glucose 50% and sevoflurane were both given as described above.

CsA group (CsA) (n = 9): CsA (5 mg kg⁻¹ in DMSO 1% aqueous solution) (17) was administered intravenously 5 minutes before reperfusion; saline 0.9% was infused intravenously over 35 minutes starting 5 min prior ischaemia up to 5 minutes of reperfusion.
CsA + Sevoflurane postconditioned group (CsA+Sevo-post) (n = 8): rats received CsA and sevoflurane as described above.

Glucose 50% + CsA group (HG+CsA) (n = 8): Rats received Glucose 50% and CsA (5 mg kg⁻¹) intravenously as described above.

Glucose 50% + CsA + Sevoflurane postconditioned group (HG+CsA+Sevo-post) (n = 8): rats received glucose 50%, CsA (5 mg kg⁻¹) intravenously, and inhaled sevoflurane as described above.

To investigate whether a higher concentration of sevoflurane or CsA alone could restore cardioprotection in state of hyperglycaemic condition we added two more groups with 2 MAC sevoflurane and 10 mg kg⁻¹ CsA:

Glucose 50% + Sevoflurane postconditioned group (HG+Sevo-post2) (n = 9): glucose 50% and 2 MAC sevoflurane were both given as described above.

Glucose 50% + CsA group (HG+CsA10) (n = 7): Rats received Glucose 50% and CsA (10 mg kg⁻¹) intravenously as described above.

Surgical preparation and infarct size measurement:
Surgical preparation was performed as described previously. (22; 24) In brief, male Wistar rats (250-350 g) were anaesthetized by intraperitoneal S-ketamine injection (150 mg kg⁻¹). Respiratory rate was adjusted to maintain $P_{CO_2}$ within physiological limits. Body temperature was maintained at 38°C by the use of a heating pad. Anaesthesia was maintained by continuous α-chloralose infusion. A lateral left sided thoracotomy followed by pericardiotomy was performed and a ligature (5-0 Prolene) was passed below a major branch of the left coronary artery. All animals were left untreated for 25 minutes before the start of the respective experimental protocol. Arterial blood gases were analyzed at baseline and $P_{CO_2}$ and $P_{O_2}$ were kept within physiological ranges by adjusting ventilation. Sevoflurane concentration was measured in the expiratory gas (Datex Capnomac Ultima, Division of Instrumentarium Corp., Helsinki, Finland). Aortic pressure and electrocardiographic signals were digitized using an analogue to digital converter (PowerLab/8SP, ADInstruments Pty Ltd, Castle Hill, Australia) at a sampling rate of 500 Hz and were continuously recorded on a personal computer using Chart for Windows v5.0 (ADInstruments).

After 120 minutes of reperfusion, the heart was excised and infarct size was determined as previously described. (22) The area of risk and the infarcted area were determined by planimetry using SigmaScan Pro 5® computer software (SPSS Science Software, Chicago, IL) and corrected for dry weight of each slice.
Sevoflurane-induced postconditioning and hyperglycemia

Figure 1:

(A) Experimental protocol. Sevo, sevoflurane; post, postconditioning; HG, hyperglycaemia; CsA, cyclosporine A. (B) Infarct size measurement. Histogram shows the infarct size (per cent of area at risk, AAR) of controls (Con), sevoflurane postconditioning (Sevo), hyperglycaemia (HG) alone, hyperglycaemia and sevoflurane postconditioning (HG+Sevo), cyclosporine A (CsA) alone, cyclosporine A and sevoflurane postconditioning (CsA+Sevo), hyperglycaemia and cyclosporine A (HG+CsA), hyperglycaemia and cyclosporine A and sevoflurane postconditioning (HG+CsA+Sevo), hyperglycaemia and sevoflurane postconditioning with 2 MAC (HG+Sevo2), hyperglycaemia and cyclosporine A with 10 mg kg⁻¹ (HG+Csa10). Data shown are mean (SD). *P<0.05 vs control group; #P<0.05 vs HG+Sevo; §P<0.05 vs HG+CsA (n = 7–11 per group).
Blood glucose and insulin measurement

Blood samples were collected at different times to measure blood glucose in each group (see Tab. 1). Insulin levels were measured in order to determine a physiological endocrinial reaction to hyperglycaemia. Samples were taken before ischaemia, during ischaemia and after 30 min of reperfusion. During ischaemia, insulin level was 4 fold increased in the hyperglycaemic groups compared to non hyperglycaemic groups. After 30 min of reperfusion, insulin level was still 7 fold higher in the hyperglycaemic groups compared with the non hyperglycaemic groups.

Statistical Analysis

Data are expressed as mean (SD). Heart rate (HR, in bpm) and mean aortic pressure (AOPmean, in mmHg) were measured during baseline, coronary artery occlusion, and reperfusion period. Inter-group differences of haemodynamic data were analyzed (SPSS Science Software, version 12.0.1) by performing a One-way ANOVA followed by Tukey’s post-hoc test. Time effects (changes from baseline value) during the experiments were analyzed by using a One-way ANOVA followed by Dunnett’s post-hoc test. Infarct sizes were analyzed by a One-way ANOVA followed by Tukey’s post-hoc test. Changes within and between groups were considered statistically significant if p<0.05.

RESULTS

Blood glucose measurement

Glucose levels during the experimental protocol are shown in table 1. Mean baseline blood glucose levels were 7.1 (1.2) mmol l\(^{-1}\) and did not differ between the groups. Before ischaemia, mean blood glucose levels were 25.5 (1.5) mmol l\(^{-1}\) in the hyperglycaemic groups and 7.1 (0.7) mmol l\(^{-1}\) in the non-hyperglycaemic groups. During ischaemia, blood glucose levels remained high at 26.6 (1.2) mmol l\(^{-1}\) in hyperglycaemic groups, while blood glucose was 7.0 (0.6) mmol l\(^{-1}\) in the non-hyperglycaemic groups. After 5 minutes of reperfusion, blood glucose levels were 26.0 (1.3) mmol l\(^{-1}\) in the hyperglycaemic groups and then declined to 5.9 (0.9) mmol l\(^{-1}\) at the end of the reperfusion period. In the non-hyperglycaemic groups, blood glucose levels were 6.8 (0.5) mmol l\(^{-1}\) after 5 minutes of reperfusion and declined to 4.9 (0.6) mmol l\(^{-1}\) at the end of the reperfusion period. Blood glucose levels at the end of the reperfusion period of the non-hyperglycaemic groups were significantly decreased compared to baseline. In contrast, no
Sevoflurane-induced postconditioning and hyperglycemia

significant differences were found in hyperglycaemic groups when comparing baseline values to values at the end of reperfusion.

Table 1:

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Ischaemia</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Con</td>
<td>7.7 (2.1)</td>
<td>7.5 (2.4)</td>
<td>7.1 (1.5)</td>
</tr>
<tr>
<td>Sevo-post</td>
<td>7.8 (1.2)</td>
<td>7.3 (1.3)</td>
<td>7.3 (1.5)</td>
</tr>
<tr>
<td>HG</td>
<td>7.6 (1.8)</td>
<td>26.5 (0.9)</td>
<td>25.1 (1.4)</td>
</tr>
<tr>
<td>HG+Sevo-post</td>
<td>8.4 (1.4)</td>
<td>27.0 (1.3)</td>
<td>25.8 (1.1)</td>
</tr>
<tr>
<td>CsA</td>
<td>6.8 (1.0)</td>
<td>6.3 (0.8)</td>
<td>6.4 (1.0)</td>
</tr>
<tr>
<td>CsA+Sevo-post</td>
<td>6.7 (1.3)</td>
<td>6.3 (0.9)</td>
<td>6.4 (1.3)</td>
</tr>
<tr>
<td>HG+CsA</td>
<td>7.1 (0.8)</td>
<td>25.9 (1.4)</td>
<td>25.6 (1.2)</td>
</tr>
<tr>
<td>HG+CsA+Sevo-post</td>
<td>6.2 (0.4)</td>
<td>27.2 (0.6)</td>
<td>26.3 (1.7)</td>
</tr>
<tr>
<td>HG+Sevo-post2</td>
<td>6.5 (0.7)</td>
<td>26.5 (0.9)</td>
<td>26.8 (0.9)</td>
</tr>
<tr>
<td>HG+CsA10</td>
<td>6.7 (0.7)</td>
<td>27.4 (1.1)</td>
<td>26.2 (1.8)</td>
</tr>
</tbody>
</table>

Blood glucose values (mmol litre\(^{-1}\)). Data are mean (SD). Con, control; Sevo-post, 1 MAC sevoflurane postconditioning; Sevo-post2, 2 MAC sevoflurane postconditioning; HG, hyperglycaemia; CsA, cyclosporine A 5 mg kg\(^{-1}\); CsA10, cyclosporine A 10 mg kg\(^{-1}\). *P<0.05 vs baseline

**Haemodynamic variables**

Haemodynamic variables are summarized in table 2. No significant differences in heart rate and aortic pressure were observed between the experimental groups during baseline. In sevoflurane treated groups, mean aortic pressure was transiently reduced during the postconditioning period with exception of the CsA+Sevo-post group. At the end of the experiments, mean aortic pressure was significantly decreased compared with baseline in all groups with the exception of the hyperglycaemic group.

**Infarct size measurement**

Infarct size was reduced from 51.4 (5.0)% in controls to 32.7 (12.8)% after sevoflurane postconditioning (p<0.05, Fig. 1B). Hyperglycaemia alone had no effect on infarct size (56.0 (10.7)%) but abolished the postconditioning effect of sevoflurane (51.1 (13.2)%, p<0.05 vs. Sevo-post). In normoglycaemic rats, CsA had a similar infarct reducing effect as sevoflurane (31.8 (7.7)%), but combination of both drugs did not further reduce infarct size (31.3 (6.3)%, p<0.05 vs. controls). The cardioprotective effect of CsA alone was also
Chapter 8

blocked by hyperglycaemia (55.0 (8.7)%, p>0.05 vs. controls). However, combination of CsA and Sevo provided the infarct sparing effect against hyperglycaemia (35.2 (5.2)%, p<0.05 vs. HG+Sevo-post respectively HG+CsA). Increasing the sevoflurane concentration to 2 MAC with hyperglycaemia (54.1 (12.6)%, p>0.05 vs. HG+Sevo-post) or CsA to 10 mg kg\(^{-1}\) CsA (58.8 (11.3)%, p>0.05 vs. HG+CsA) had no effect on infarct size.

Table 2:

<table>
<thead>
<tr>
<th>HR (BPM)</th>
<th>Baseline</th>
<th>Ischaemia</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 25</td>
<td>15 30 120</td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>431(21)</td>
<td>432(21)</td>
<td>430(31)</td>
</tr>
<tr>
<td>Sevo-post</td>
<td>421(61)</td>
<td>411(88)</td>
<td>444(27)</td>
</tr>
<tr>
<td>HG</td>
<td>423(45)</td>
<td>432(15)</td>
<td>420(27)</td>
</tr>
<tr>
<td>HG+Sevo-post</td>
<td>426(55)</td>
<td>423(32)</td>
<td>415(53)</td>
</tr>
<tr>
<td>CsA</td>
<td>416(40)</td>
<td>418(44)</td>
<td>409(54)</td>
</tr>
<tr>
<td>CsA+Sevo-post</td>
<td>402(19)</td>
<td>406(30)</td>
<td>397(33)</td>
</tr>
<tr>
<td>HG+CsA</td>
<td>418(42)</td>
<td>399(32)</td>
<td>414(42)</td>
</tr>
<tr>
<td>HG+CsA+Sevo-post</td>
<td>428(46)</td>
<td>435(24)</td>
<td>413(61)</td>
</tr>
<tr>
<td>HG+Sevo-post2</td>
<td>412(29)</td>
<td>400(29)</td>
<td>398(31)</td>
</tr>
<tr>
<td>HG+CsA10</td>
<td>405(25)</td>
<td>389(24)</td>
<td>392(16)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AOPmean (mmHg)</th>
<th>Baseline</th>
<th>Ischaemia</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 25</td>
<td>15 30 120</td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>127(21)</td>
<td>124(22)</td>
<td>111(20)</td>
</tr>
<tr>
<td>Sevo-post</td>
<td>140(17)</td>
<td>139(18)</td>
<td>122(34)</td>
</tr>
<tr>
<td>HG</td>
<td>135(19)</td>
<td>144(17)</td>
<td>126(34)</td>
</tr>
<tr>
<td>HG+Sevo-post</td>
<td>141(16)</td>
<td>148(28)</td>
<td>129(35)</td>
</tr>
<tr>
<td>CsA</td>
<td>136(15)</td>
<td>123(18)</td>
<td>118(15)</td>
</tr>
<tr>
<td>CsA+Sevo-post</td>
<td>126(13)</td>
<td>125(19)</td>
<td>114(22)</td>
</tr>
<tr>
<td>HG+CsA</td>
<td>135(20)</td>
<td>145(19)</td>
<td>134(15)</td>
</tr>
<tr>
<td>HG+CsA+Sevo-post</td>
<td>136(14)</td>
<td>148(16)</td>
<td>119(22)</td>
</tr>
<tr>
<td>HG+Sevo-post2</td>
<td>136(16)</td>
<td>144(16)</td>
<td>133(15)</td>
</tr>
<tr>
<td>HG+CsA10</td>
<td>143(10)</td>
<td>149(12)</td>
<td>131(10)</td>
</tr>
</tbody>
</table>

Haemodynamic variables. Data are mean (SD). Con, control; Sevo-post, 1 MAC sevoflurane postconditioning; Sevo-post2, 2 MAC sevoflurane postconditioning; HG, hyperglycaemia; CsA, cyclosporine A 5 mg kg\(^{-1}\); CsA10, cyclosporine A 10 mg kg\(^{-1}\). *P<0.05 vs baseline; **P<0.05 vs control group
Sevoflurane-induced postconditioning and hyperglycemia

DISCUSSION

In the present study we investigated the effects of sevoflurane-induced postconditioning during hyperglycaemia. The main results show that: 1) hyperglycaemia abolishes cardioprotection by sevoflurane postconditioning and that 2) inhibition of mPTP with CsA reverses this loss of cardioprotection.

Diabetic and also hyperglycaemic non-diabetic patients with myocardial ischaemia-reperfusion like infarction or cardiac surgery, have a poorer prognosis than non diabetic or normoglycaemic controls. (2; 18) It is hypothetized that hyperglycaemia might cause a loss of (endogenous) cardioprotective mechanisms.

Beside cardioprotection by preconditioning, also cardioprotection by postconditioning, can be induced by ischaemic and pharmacological stimulus. (26) The protective effects of early as well as late preconditioning can be blocked by hyperglycaemia or diabetes mellitus. (7; 15) For ischaemic preconditioning it was shown that diabetes and hyperglycaemia of 17 and 34 mmol l⁻¹ blocked cardioprotection in vivo. (15) The blockade was independent of plasma insulin concentrations and plasma osmolality. (16) Another study showed that, isoflurane-induced preconditioning was blocked by hyperglycaemia. (14) So far, there is no study available investigating the influence of hyperglycaemia on postconditioning. Postconditioning describes a cardioprotective intervention at the onset of myocardial reperfusion. In our study, postconditioning by sevoflurane reduced infarct size by nearly 40%, but sevoflurane postconditioning was abolished in state of hyperglycaemia.

For the hyperglycaemic groups we chose a blood glucose target level from 22 mmol l⁻¹. From a former study we know that this blood glucose concentration blocks desflurane-induced preconditioning. (6) Blood glucose levels used in the literature investigating the effect of hyperglycaemia on ischaemic- and isoflurane-induced preconditioning are quite in the same range. (14; 15) In our study blood glucose levels declined significantly at the end of the reperfusion period compared to baseline in the control group. Furthermore, this is the fact in all non-hyperglycaemic groups. There are two possible explanations for this blood glucose decrease: first, after preparation the animals were in a slight hyperglycaemic condition because of surgical stress and, second, the animals did not receive any substrates (e.g. glucose, free fatty acids) over the whole experimental protocol and reached normoglycaemic levels at the end of the experiments.

The opening of the mPTP occurs in the early minutes of reperfusion and is associated with the pathogenesis of necrosis and apoptosis. mPTP-opening might thus be regarded as a crucial step from reversible to irreversible cell death. (4) Inhibition of mPTP with CsA at the
onset of reperfusion was shown to protect the myocardium. (1; 11) In addition, it was demonstrated that 0.5 MAC isoflurane combined with CsA 5 mg kg\(^{-1}\) induced postconditioning, while 0.5 MAC isoflurane or CsA 5 mg kg\(^{-1}\) alone could not induce cardioprotection. In contrast, application of 1 MAC isoflurane or CsA at a dosage of 10 mg kg\(^{-1}\) were able to induce postconditioning. (17) In the current investigation, we used CsA in a concentration of 5 mg kg\(^{-1}\). In our study, this low dosage led to a strong cardioprotection in rats in vivo. In two additional groups, 2 MAC sevoflurane alone or 10 mg kg\(^{-1}\) CsA alone in the hyperglycaemic condition were studied in order to investigate if higher doses of these agents given alone could restore cardioprotection. The data show that a single substance even at higher concentrations had no protective effect, in contrast to combination of the two substances (see figure 1).

A study by Chiari et al. showed that a non protective intervention with three times 10 seconds of ischaemic postconditioning, was enhanced by additional administration of 0.5 MAC isoflurane, a dosage which itself was also not protective. (3) These studies indicate that a triggered cardioprotective intervention with a non protective stimulus, a stimulus which does not confer cardioprotection by its own, could be enhanced by a second stimulus, assuming that there exist different and/or parallel cardioprotective pathways which could alter myocardial infarct size by various activation. The cited studies did not combine the two single protective interventions and the studies were not performed in hyperglycaemic animals. In our study, the combination of the two protective stimuli, 1 MAC Sevo and CsA did not result in enhanced cardioprotection in normoglycaemic animals. To our knowledge there is no study available showing that two protective stimuli by the same cardioprotective intervention, in this case postconditioning, could enhance the cardioprotective effect significantly in comparison to the single intervention. With regard to the combination of two different cardioprotective interventions, i.e. combination of ischaemic late preconditioning and early ischaemic preconditioning or early preconditioning and postconditioning, the literature is ambiguous. (5; 20) Our present results show that during hyperglycaemia, 1 MAC Sevo or 5 mg kg\(^{-1}\) CsA alone were not protective, but combination of both stimuli resulted in the full cardioprotective effect as observed in non-hyperglycaemic animals. Enhancement of the doses of sevoflurane to 2 MAC or CsA to a concentration of 10 mg kg\(^{-1}\) had no effect on infarct size in state of hyperglycaemia when the substances were given alone.

Elucidation of the molecular mechanisms involved in this cardioprotective interaction during hyperglycaemia is beyond the scope of the present study. The signal transduction pathways described for pharmacological postconditioning so far include i.e. PI3K/Akt,
Sevoflurane-induced postconditioning and hyperglycemia

MEK1/2, ERK1/2 and eNOS. (10; 12; 26) The signal transduction cascade consists of two parallel ways. Activation of PI3K/Akt leads to inhibition of the mPTP, whereas MEK1/2 via ERK1/2 activation finally leads to protein translation. (25) Both pathways interact with each other. The inhibition of the mPTP with CsA occurs downstream in the cascade of pharmacological postconditioning. (9) We speculate that sevoflurane amplifies the inhibition of the mPTP by CsA and additionally activates protein translation via a parallel pathway of the postconditioning cascade. Another explanation could be that the sole cardioprotective intervention with CsA or sevoflurane is not strong enough to protect the hyperglycaemic myocardium, but possibly the threshold for cardioprotection is lowered after combination of both protective pathways. Further research is needed to elucidate the molecular mechanisms contributing to this cardioprotective effect.

In summary, we demonstrated that hyperglycaemia blocks cardioprotection by sevoflurane-induced postconditioning, and that this loss of cardioprotection can be restored by CsA administration briefly before the onset of reperfusion.

REFERENCES
