Hyperglycaemia blocks sevoflurane-induced postconditioning in the rat heart in vivo: cardioprotection can be restored by blocking the mitochondrial permeability transition pore


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Background. 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).

Methods. 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.

Results. 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].

Conclusions. Sevoflurane-induced postconditioning is blocked by HG. Inhibition of the mPTP with CsA is able to reverse this loss of cardioprotection.

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Hyperglycaemia (HG) correlates with increased mortality after acute myocardial infarction in diabetic patients and in patients without diabetes mellitus.\(^1\)\(^2\) HG was shown to abolish cardioprotection induced by ischaemic and anaesthetic preconditioning.\(^3\)\(^4\)

Besides preconditioning, postconditioning (i.e. cardioprotection by administration of the substance after ischaemia during early reperfusion) can also be induced by volatile anaesthetics.\(^5\)\(^6\) Recent studies demonstrated that the volatile anaesthetic sevoflurane offers cardioprotection by postconditioning.\(^7\)\(^8\) In both studies, postconditioning induced a cardioprotective effect that was comparable with the extent of cardioprotection induced by sevoflurane preconditioning. Furthermore, Obal and colleagues\(^9\) showed that sevoflurane induces maximal cardioprotection by postconditioning at a concentration of only 1 MAC. It is not known whether anaesthetic-induced postconditioning can be induced in hyperglycaemic subjects. This question was tested in the initial phase of the study using an in vivo rat model.

Recent studies have shown that the mitochondrial permeability transition pore (mPTP) is involved in
isoflurane-induced postconditioning via phosphorylation and inhibition of GSK3β.10 Krolikowski and colleagues11 demonstrated that keeping the mPTP closed with cyclosporine A (CsA) enhanced cardioprotection produced by isoflurane-induced postconditioning. Therefore, in the second phase of the study, we tested if administration of CsA shortly before the reperfusion period could restore the cardioprotection.

We hypothesized that (i) sevoflurane postconditioning is abolished by HG and (ii) cardioprotection can be restored by inhibiting the mPTP in hyperglycaemic animals.

**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.

**Materials**

Sevoflurane was purchased from Abbott (SEVOrrane®, Abbott B.V., Hoofddorp, The Netherlands). Cyclosporine A was purchased from Fluka Biochemika (Sigma Aldrich, Steinheim, Germany). Glucose 50% was purchased from B. Braun (B. Braun Melsungen AG, Melsungen, Germany).

**Surgical preparation and infarct size measurement**

Animals had free access to food and water at all times before the start of the experiments. Surgical preparation was performed as described previously.9 12 In brief, male Wistar rats (250–350 g, 7–11 per group) were anaesthetized by intraperitoneal S-ketamine injection (150 mg kg⁻¹); this does not interfere with in vivo experimental cardioprotection.13 Ventilatory frequency was adjusted to maintain PCO₂ 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 pericardiectomy 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 min before the start of the respective experimental protocol. Arterial blood gases were analysed at baseline and PCO₂ and PO₂ were maintained within physiological ranges by adjusting ventilation. Sevoflurane concentration was measured in the expired 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 min of reperfusion, the heart was excised and infarct size was determined as previously described.7 The area of risk and the infarcted area were determined by planimetry using SigmaScan Pro 5® computer software (SPSS Science Software, Chicago, IL, USA) and corrected for dry weight of each slice.

**Experimental protocol**

Rats were divided into 10 groups (Fig. 1A): all animals underwent 25 min of coronary artery occlusion and 2 h of reperfusion (I/R).

- **Control group (Con) (n=9):** After surgical preparation, rats received oxygen 30% plus nitrogen 70%. Normal saline was given i.v. over 35 min starting 5 min before ischaemia up to 5 min of reperfusion.
- **Sevoflurane postconditioned group (Sevo-post) (n=11):** Rats received sevoflurane with an end-tidal concentration of 1 MAC (Δ2.4 vol%) for 5 min starting 1 min before the onset of reperfusion; saline 0.9% was infused i.v. over 35 min starting 5 min before ischaemia up to 5 min of reperfusion.
- **Glucose 50% group (HG) (n=9):** Glucose 50% was administered i.v. over 35 min starting 5 min before ischaemia and was maintained until 5 min of reperfusion. Target blood glucose level before ischaemia was 22 mmol litre⁻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 dimethyl sulphoxide 1% aqueous solution)11 was administered i.v. 5 min before reperfusion; saline 0.9% was infused i.v. over 35 min starting 5 min before ischaemia up to 5 min 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) i.v. as described above.
- **Glucose 50%+CsA+sevoflurane postconditioned group (HG+CsA+Sevo-post) (n=8):** Rats received glucose 50%, CsA (5 mg kg⁻¹) i.v., and inhaled sevoflurane as described above.

To investigate whether a higher concentration of sevoflurane or CsA alone could restore cardioprotection during HG, 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) i.v. as described above.
Fig 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 samples were collected to measure blood glucose in each group using the FreeStyle Freedom blood glucose meter from Abbott. Plasma insulin levels were measured using a Rat Insulin ELISA from Orange Medical (Orange Medical, Tilburg, The Netherlands) in order to determine a physiological endocrine reaction to HG. Samples were taken before ischaemia, during ischaemia, and after 30 min of reperfusion. During ischaemia, insulin was four-fold increased in the hyperglycaemic groups compared with non-hyperglycaemic groups. After 30 min of reperfusion, insulin was still seven-fold higher in the hyperglycaemic groups compared with the non-hyperglycaemic groups.

Statistical analysis
Data are expressed as mean (SD). Heart rate (HR, in beats min\(^{-1}\)) and mean aortic pressure (AOPmean, in mm Hg) were measured during baseline, coronary artery occlusion, and reperfusion. Inter-group differences in haemodynamic data were analysed (SPSS Science Software, version 12.0.1) using a one-way ANOVA followed by Tukey’s post hoc test. Time effects (changes from baseline value) during the experiments were analysed using a one-way ANOVA followed by Dunnett’s post hoc test. Infarct sizes were analysed by a one-way ANOVA followed by Tukey’s post hoc test. Changes within and between the 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 litre\(^{-1}\) and did not differ between the groups. Before ischaemia, mean blood glucose levels were 25.5 (1.5) mmol litre\(^{-1}\) in the hyperglycaemic groups and 7.1 (0.7) mmol litre\(^{-1}\) in the non-hyperglycaemic groups. During ischaemia, blood glucose levels remained high at 26.6 (1.2) mmol litre\(^{-1}\) in hyperglycaemic groups, whereas blood glucose was 7.0 (0.6) mmol litre\(^{-1}\) in the non-hyperglycaemic groups. After 5 min of reperfusion, blood glucose levels were 26.0 (1.3) mmol litre\(^{-1}\) in the hyperglycaemic groups and then declined to 5.9 (0.9) mmol litre\(^{-1}\) at the end of the reperfusion period. In the non-hyperglycaemic groups, blood glucose levels were 6.8 (0.5) mmol litre\(^{-1}\) after 5 min of reperfusion and declined to 4.9 (0.6) mmol litre\(^{-1}\) 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 with baseline. In contrast, no significant differences were found in the hyperglycaemic groups when comparing baseline values with values at the end of reperfusion.

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). HG 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 a 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 blocked by HG [55.0 (8.7)%, \(P>0.05\) vs controls]. However, the combination of CsA and Sevo provided an infarct sparing effect against HG [35.2 (5.2)%, \(P<0.05\) vs HG+Sevo-post, respectively, HG+CsA]. Increasing the sevoflurane concentration to 2 MAC with HG [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.

Discussion
In the present study, we investigated the effects of sevoflurane-induced postconditioning during HG. The main results show that: (i) HG abolishes cardioprotection by sevoflurane postconditioning and (ii) 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.\(^1\)\(^{14}\) It is hypothesized that HG might cause a loss of (endogenous) cardioprotective mechanisms.

In addition to cardioprotection produced by preconditioning, cardioprotection produced by postconditioning can be induced by ischaemic and pharmacological stimuli.\(^15\) The protective effects of early and late preconditioning can be blocked by HG or diabetes mellitus.\(^3\)\(^{16}\) For ischaemic preconditioning, it was shown that diabetes and HG of 17 and 34 mmol litre\(^{-1}\) blocked cardioprotection in vivo.\(^3\) The blockade was independent of plasma insulin.
concentrations and osmolality. Another study showed that isoflurane-induced preconditioning was blocked by HG. So far, there is no study available investigating the influence of HG 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 during HG.

For the hyperglycaemic groups, we chose a blood glucose target level from 22 mmol litre$^{-1}$. From a former study, we know that this blood glucose concentration blocks desflurane-induced preconditioning. Blood glucose levels used in the literature investigating the effect of HG on ischaemic- and isoflurane-induced preconditioning are in the same range. In our study, blood glucose levels declined significantly at the end of the reperfusion period compared with baseline in the control group. Furthermore, this is true in all non-hyperglycaemic groups. There are two possible explanations for this decrease in blood glucose: first, after preparation the animals were in a mild hyperglycaemic state due to surgical stress and, secondly, 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. Inhibition of mPTP with CsA at the onset of reperfusion was shown to protect the myocardium.

### Table 1 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

<table>
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<th>Baseline</th>
<th>Ischaemia</th>
<th>Reperfusion</th>
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<tr>
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<tr>
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<td>26.2 (1.8)</td>
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### Table 2 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

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<th>HR (beats min$^{-1}$)</th>
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<th>Reperfusion</th>
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addition, it was demonstrated that 0.5 MAC isoflurane combined with CsA 5 mg kg\(^{-1}\) induced postconditioning, whereas 0.5 MAC isoflurane or CsA 5 mg kg\(^{-1}\) alone could not induce cardioprotection. In contrast, administration of 1 MAC isoflurane or CsA at a dosage of 10 mg kg\(^{-1}\) was able to induce postconditioning\(^{11}\). 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 with HG were studied in order to investigate if higher doses of these agents given alone could restore cardioprotection. The data show that a single agent even at higher dose had no protective effect, in contrast to combination of the two agents (Fig. 1).

A study by Chiari and colleagues\(^{6}\) showed that a non-protective intervention with three 10 s periods of ischaemic postconditioning was enhanced by additional administration of 0.5 MAC isoflurane, a dose which was itself not protective. These studies indicate that a triggered cardioprotective intervention with a non-protective stimulus could be enhanced by a second stimulus, assuming that there are different and/or parallel cardioprotective pathways which could alter myocardial infarct size by various pathways. The cited studies did not combine the two 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 with the single intervention. With regard to the combination of two different cardioprotective interventions, that is, combination of ischaemic late preconditioning and early ischaemic preconditioning or early preconditioning and postconditioning, the literature is ambiguous.\(^{7,22}\) Our present results show that during HG, 1 MAC Sevo or 5 mg kg\(^{-1}\) CsA alone were not protective, but the 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 during HG when the agents were given alone.

Elucidation of the molecular mechanisms involved in this cardioprotective interaction during HG is beyond the scope of the present study. The signal transduction pathways described for pharmacological postconditioning so far include: PI3K/Akt, MEK1/2, ERK1/2, and eNOS.\(^{15,23,24}\) 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.\(^{26}\) We speculate that sevoflurane amplifies the inhibition of the mPTP by CsA and additionally activates protein translation via a parallel pathway in 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 HG blocks cardioprotection by sevoflurane-induced postconditioning and that this loss of cardioprotection can be restored by CsA administration briefly before the onset of reperfusion.

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