Desflurane-induced Postconditioning Is Mediated by β-Adrenergic Signaling

Role of β₁ and β₂-Adrenergic Receptors, Protein Kinase A, and Calcium/Calmodulin-dependent Protein Kinase II

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Background: Anesthetic preconditioning is mediated by β-adrenergic signaling. This study was designed to elucidate the role of β-adrenergic signaling in desflurane-induced postconditioning.

Methods: Pentobarbital-anesthetized New Zealand White rabbits were subjected to 30 min of coronary artery occlusion followed by 3 h of reperfusion and were randomly assigned to receive vehicle (control), 1.0 minimum alveolar concentration of desflurane, esmolol (30 mg·kg⁻¹·h⁻¹) for the initial 30 min of reperfusion or throughout reperfusion, the β₂-adrenergic receptor blocker ICI 118,551 (0.2 mg/kg), the protein kinase A (PKA) inhibitor H-89 (250 μg/kg), or the calcium/calmodulin-dependent protein kinase II (CaMK II) inhibitor KN-93 (300 μg/kg) in the absence or absence of desflurane. Protein expression of protein kinase B, calcium/calmodulin-dependent protein kinase II, and phospholamban was measured by Western immunoblotting. Myocardial infarct size was assessed by triphenyltetrazolium staining.

Results: Infarct size was 57 ± 5% in control. Desflurane postconditioning reduced infarct size to 36 ± 5%. Esmolol given during the initial 30 min of reperfusion had no effect on infarct size (54 ± 4%) but blocked desflurane-induced postconditioning (58 ± 5%), whereas esmolol administered throughout reperfusion reduced infarct size in the absence or presence of desflurane to 42 ± 6% and 41 ± 7%, respectively. ICI 118,551 and KN-93 did not affect infarct size (62 ± 4% and 62 ± 6%, respectively) but abolished desflurane-induced postconditioning (57 ± 5% and 64 ± 3%, respectively). H-89 decreased infarct size in the absence (36 ± 5%) or presence (33 ± 5%) of desflurane.

Conclusions: Desflurane-induced postconditioning is mediated by β-adrenergic signaling. However, β-adrenergic signaling displays a differential role in cardioprotection during reperfusion.

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Materials and Methods

All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal Care and Use Committee of the local authorities (Government of Unterfranken, Würzburg, Germany) and REPERFUSION of the myocardium after sustained ischemia induces myocardial cell necrosis and apoptosis, resulting in increased infarct size despite the restoration of coronary blood flow. Intracellular mechanisms underlying this so-called reperfusion injury presumably include increased levels of reactive oxygen species, Ca²⁺ overload, rapid restoration of physiologic pH, and inflammatory processes resulting in opening of the mitochondrial permeability transition pore (mPTP).¹ Volatile anesthetics confer marked protection against myocardial reperfusion injury when administered during early reperfusion.²³ This phenomenon was termed anesthetic-induced postconditioning and might broaden the perspective for the treatment of patients with perioperative myocardial ischemia and infarction. The mechanisms underlying the cardioprotective effects of anesthetic-induced postconditioning have not completely been clarified. However, there is substantial evidence that anesthetic-induced preconditioning and postconditioning share similar signal transduction pathways and that prevention of intracellular Ca²⁺ accumulation might be a primary target of volatile anesthetics in the prevention of the sequelae of myocardial reperfusion injury.⁴⁵ Among other G protein–coupled receptors, β-adrenergic receptors play a pivotal role in anesthetic-induced preconditioning.⁶ However, the role of the β-adrenergic pathway in anesthetic-induced postconditioning is unclear. The current study was designed to elucidate the role of β-adrenergic signaling in desflurane-induced postconditioning. Therefore, the hypothesis was tested that selective blockade of β₁ and β₂-adrenergic receptors and of the intracellular β-adrenergic pathway components protein kinase A (PKA) and Ca²⁺/calmodulin-dependent protein kinase II (CaMK II) abolishes desflurane-induced postconditioning. Furthermore, we tested the hypothesis that the prolonged blockade of β₁-adrenergic receptors throughout the complete reperfusion period after experimental ischemia reduces myocardial infarct size.
conformed to the regulations of the German animal protection law. Furthermore, all conformed to the Guiding Principles in the Care and Use of Animals of the American Physiologic Society and were in accordance with the Guide for the Care and Use of Laboratory Animals.7

General Preparation

General preparation was performed as previously described.6 Briefly, male New Zealand White rabbits were anesthetized with sodium pentobarbital (30-mg/kg intravenous bolus, followed by an infusion of 20–30 mg kg⁻¹ h⁻¹) via the left marginal auricular vein. Sodium pentobarbital was chosen because of its negligible effects on preconditioning.8 No opioids or neuromuscular blocking agents were used throughout the investigation. Depth of anesthesia was verified by recurrent testing of palpebral reflexes and hind paw withdrawal throughout the experiment. After tracheotomy and tracheal cannulation, animals were artificially ventilated (Cicero®; Dräger, Lübeck, Germany) using positive pressure with an air-and-oxygen mixture (70%/30%). Arterial blood gases drawn from the auricular artery was analyzed using an ABL 505 blood gas analyzer (Radiometer, Copenhagen, Denmark), and blood gases were maintained within a normal physiologic range by adjusting respiratory rate or tidal volume. End-tidal concentration of desflurane was measured at the tip of the endotracheal tube by an infrared anesthetic gas analyzer that was calibrated with known standards before and during experimentation. The rabbit minimum alveolar concentration (MAC) of desflurane used in the current investigation was 8.9%.9

Left ventricular (LV) pressure and the maximum increase in left ventricular pressure (+dP/dt max) were measured with a saline-filled PE-50 polyethylene catheter inserted into the left ventricle via the right carotid artery. Mean arterial pressure was monitored by insertion of a 2.5-French microcannulated catheter (Millar Instruments Inc., Houston, TX) via the right femoral artery into the descending aorta. Rectal body temperature was maintained at 38.5 ± 0.5°C by a servo-controlled heating pad (Föhr Instruments, Seeheim, Germany). After a left fourth thoracotomy and pericardiotomy, the left heart was exposed and suspended in a pericardial cradle. A silk ligature (2–0) was placed halfway between the base and the apex of the heart around a prominent branch of the left anterior descending coronary artery to form a snare. By tightening the snare, a coronary artery occlusion was produced, and reperfusion was instituted by loosening the snare. Each rabbit received 300 U/kg heparin 5 min before coronary artery occlusion for anticoagulation. Coronary artery occlusion was verified by epicardial cyanosis, regional dyskinesia in the ischemic zone, and electrocardiographic changes. Adequate reperfusion was confirmed by epicardial hyperemic response and reversion of electrocardiographic changes. Hemodynamic parameters, body temperature, and electrocardiogram were continuously recorded and analyzed using a personal computer (Hewlett Packard, Palo Alto, CA) and hemodynamic data acquisition and analysis software (Notocord® hem 3.5; Croissy sur Seine, France). Data were digitized at a sampling rate of 1,000 Hz.

Experimental Protocol

The experimental protocol used in this investigation is illustrated in figure 1. Baseline systemic hemodynamics were recorded after a 30-min equilibration period after completion of instrumentation and calibration. All rabbits were subjected to 30 min of coronary artery occlusion followed by 3 h of reperfusion. Rabbits were randomly assigned to one of the study groups by opening a sealed envelope containing information about the study group after completion of the preparation of each animal. With the initiation of coronary reperfusion, rabbits received either vehicle (0.9% saline [control]), 1.0 MAC of desflurane, or the selective β₁-adrenergic receptor blocker esmolol (30 mg kg⁻¹ h⁻¹) continuously for the first 30 min of reperfusion (esmolol 30 min) or throughout the complete 3 h of reperfusion (esmolol 180 min). The selective β₂-adrenergic receptor blocker ICI 118,551 (0.2 mg/kg), the specific PKA inhibitor H-89 (250 μg/kg; Sigma-Aldrich, Munich, Germany), and the specific CaMK II inhibitor KN-93 (300 μg/kg; Sigma-Aldrich) were administered as boluses directly into the left ventricle with the initiation of coronary reperfusion. In five separate groups, esmolol 30 min and 180 min, ICI 118,551, H-89, and KN-93 were coadministered with desflurane.

Measurement of Myocardial Infarct Size

Infarct size and area at risk (AAR) were determined gravimetrically according to standard procedures.11 Briefly, at the end of each experiment, the coronary artery was reoccluded and the AAR was determined by infusion of 2 ml patent blue (0.1 g/ml; Sigma-Aldrich, Taufkirchen, Germany). The rabbits were then killed with a lethal dose of pentobarbital, and the heart was rapidly excised. The heart was cut into five slices from apex to base, and the nonstained red myocardium (AAR) was carefully separated and weighed. Infarct size was expressed as a percentage of the AAR. Rabbits with an AAR less than 15% of LV mass and those with intractable ventricular fibrillation or LV pump failure were excluded from the study. Infarct size was determined by an investigator blinded to the study protocol.
Western Immunoblotting

In a separate set of experiments, protein expression of CaMK II, phospholamban, phosphorylated phospholamban, protein kinase B (PKB)/Akt, and phosphorylated PKB/Akt was determined by Western blot analysis at two different time points: during early reperfusion (35 min of reperfusion) in all groups and in additional experiments during late reperfusion at the end of the protocol (180 min of reperfusion) only in the control group and the groups receiving desflurane, esmolol for 180 min, and desflurane in combination with esmolol given for 180 min (n = 6 per group) (fig. 1). At the respective time point, the hearts were rapidly excised, and the left ventricle was shock frozen in liquid nitrogen and stored at −80°C until further treatment. The samples were homogenized in ice cold RIPA buffer (1× phosphate-buffered saline, 1% Igepal CA-630, 0.5% sodium deoxycholic acid, 0.1% sodium dodecylsulfate polyacrylamide, 20 mM sodium fluoride, and 1 mM sodium orthovanadate, containing a protease-inhibitor-cocktail [Roche, Grenzach-Wyhlen, Germany]) and centrifuged at 12,000g. Proteins were separated on 15% polyacrylamide sodium dodecylsulfate polyacrylamide gels and subsequently transferred electrophoretically on nitrocellulose membranes (Whatman, Maidstone, United Kingdom). After transfer, nonspecific background was blocked using 5% nonfat milk powder in phosphate-buffered saline–Tween 20 (1 h at room temperature). Membranes were then incubated for
1 h at room temperature with the following specific antibodies: mouse anti-phospholamban 1:3,000 (Affinity BioReagents, Golden, CO), goat anti-Akt 1 (C-20) 1:200 (Santa Cruz Biotechnology, Santa Cruz, CA), mouse anti-glyceraldehyde-3-phosphate dehydrogenase 1:3,000 (Santa Cruz Biotechnology), and rabbit anti-αB-crystallin (Assay Designs, Ann Arbor, MI) in 5% nonfat milk in phosphate-buffered saline–Tween 20 or incubated overnight (rabbit anti-p-phospholamban 1:1,000 [Badrilla, Leeds, United Kingdom], mouse anti-p-AKT1 [Ser473] 1:100 [Abcam, Cambridge, United Kingdom], and goat anti-CaMK II 1:200 [Santa Cruz Biotechnology]) at 4°C, respectively. Horseradish peroxidase–linked antibodies against mouse, rabbit, or goat at 1:5,000 (Santa Cruz Biotechnology) were used as secondary antibodies. The protein bands were detected by ECL® detection reagent (GE Healthcare, Buckinghamshire, United Kingdom) and were visualized using an x-ray film. The films were scanned and the band optical density was analyzed using Scan pack Software 3.0 (Bio-Rad Laboratories, Munich, Germany). Gel loading was normalized to αB-crystallin or glyceraldehyde-3-phosphate dehydrogenase expression, respectively.

**Statistical Analysis**

Power analysis revealed a group size of n = 8 to detect a difference in infarct size in means of 15% with a power of 0.8 at an α level of 0.05. Statistical analysis of hemodynamic data was performed using an overall 12 (control vs. desflurane vs. esmolol 30 min vs. esmolol 180 min vs. desflurane + esmolol 30 min vs. desflurane + esmolol 180 min vs. ICI 118,551 vs. desflurane + ICI 118,551 vs. H-89 vs. desflurane + H-89 vs. KN-93 vs. desflurane + KN-93) × 6 (base vs. coronary artery occlusion vs. reperfusion 0.5 vs. reperfusion 1 vs. reperfusion 2 vs. reperfusion 3) two-way analysis of variance with repeated measures. In case of significant main effects of interaction, post hoc one-way analyses of variance with Duncan post hoc tests were conducted for each group and for each time. Statistical analysis for body weight, LV, AAR, AAR/LV, infarct size/AAR, and densitometry was performed using one-way analysis of variance (control vs. desflurane vs. esmolol 30 min vs. esmolol 180 min vs. desflurane + esmolol 30 min vs. desflurane + esmolol 180 min vs. ICI 118,551 vs. desflurane + ICI 118,551 vs. H-89 vs. desflurane + H-89 vs. KN-93 vs. desflurane + KN-93) and post hoc Duncan test where appropriate. Statistical analysis of data was performed on a personal computer using SPSS 15.0 software (The Apache Software Foundation, Forest Hill, MD). All data are expressed as mean ± SEM.

**Results**

One hundred four rabbits were instrumented to obtain 88 successful experiments in the groups determined for infarct size measurement. Six rabbits were excluded because of intractable ventricular fibrillation during the experimental protocol (1 control, 1 desflurane, 1 esmolol 180 min, 1 ICI 118,551, 1 H-89, and 1 desflurane + KN-93), 6 because of LV pump failure (2 desflurane, 1 desflurane + esmolol 30 min, 2 desflurane + esmolol 180 min, and 1 KN-93), and 4 because LV AAR was less than 15% of the LV mass (1 control, 1 desflurane + ICI 118,551, 1 H-89, and 1 desflurane + H-89). Seventy-five rabbits were studied in the Western blot groups.

**Hemodynamics**

There were no differences in hemodynamic parameters between experimental groups at baseline (table 1). Esmolol given for 180 min, H-89, and KN-93 reduced heart rate during the complete reperfusion period, while the heart rate–reducing effect of esmolol administered only during the initial 30 min of reperfusion was suspended after cessation of esmolol. Mean arterial pressure was reduced by desflurane and esmolol. However, mean arterial pressure returned to baseline values after the cessation of desflurane and esmolol administration after 30 min of reperfusion. Mean arterial pressure reduction by esmolol given for 180 min persisted throughout the complete reperfusion period. LV end-diastolic pressure elevation during coronary artery occlusion did not reach statistical significance in any of the study groups.

**Myocardial Infarct Size**

There were no differences between groups with respect to body weight, LV weight, or AAR (table 2). Myocardial infarct size (infarct size/AAR) was 57 ± 5% in control experiments. Desflurane at 1.0 MAC administered during the first 30 min of reperfusion reduced infarct size to 36 ± 5%. The selective β₂-adrenergic receptor blocker esmolol given only during the initial 30 min of reperfusion had no effect on infarct size (54 ± 4%) but blocked desflurane-induced postconditioning (58 ± 5%). In contrast, esmolol administered throughout the complete 180 min of reperfusion reduced infarct size to 42 ± 6%. Coadministration of desflurane for the first 30 min of reperfusion and esmolol throughout the complete reperfusion period did not confer additional infarct size reduction and resulted in an infarct size of 41 ± 7%. The selective β₂-adrenergic receptor blocker ICI 118,551 did not affect infarct size (62 ± 4%) but completely abolished desflurane-induced postconditioning to an infarct size of 57 ± 5% (n = 8 in all groups). (fig. 2) Specific inhibition of PKA during early reperfusion by H-89 decreased infarct size in the absence (36 ± 5%) or presence (33 ± 5%) of desflurane. The specific inhibitor of CaMK II, KN-93, had no effect on infarct size that was 62 ± 6%. However, desflurane-induced postconditioning was blocked by KN-93 (64 ± 3%) (n = 6 in all groups) (fig. 5).
## Table 1. Systemic Hemodynamic Parameters

| HR, beats/min | CON 272 ± 11 | DES 258 ± 7 | ESMO 30 min 263 ± 7 | DES + ESMO 30 min 265 ± 10 | ESMO 180 min 250 ± 8 | DES + ESMO 180 min 251 ± 7 | DES + ESMO 180 min 257 ± 8 | DES + ESMO 180 min 257 ± 8 | ICI 118,551 257 ± 8 | DES + ICI 118,551 259 ± 9 | DES + ICI 118,551 278 ± 9 | DES + ICI 118,551 265 ± 10 | DES + ICI 118,551 282 ± 8 | DES + ICI 118,551 277 ± 13 | MAP, mmHg CON 84 ± 5 | DES 86 ± 5 | ESMO 30 min 84 ± 5 | DES + ESMO 30 min 75 ± 3 | ESMO 180 min 81 ± 3 | DES + ESMO 180 min 76 ± 4 | DES + ESMO 180 min 78 ± 5 | DES + ICI 118,551 77 ± 3 | H-89 81 ± 4 | DES + H-89 80 ± 2 | KN-93 70 ± 8 | DES + KN-93 79 ± 4 | LVEDP, mmHg CON 3 ± 1 | DES 2 ± 2 | ESMO 30 min 4 ± 1 | DES + ESMO 30 min 2 ± 2 | ESMO 180 min 4 ± 1 | DES + ESMO 180 min 3 ± 1 | ICI 118,551 3 ± 1 | DES + ICI 118,551 4 ± 1 | H-89 4 ± 2 | DES + H-89 3 ± 1 | KN-93 4 ± 2 | DES + KN-93 3 ± 2 | +dP/dtmax, mmHg/s CON 4,013 ± 560 | DES 3,435 ± 460 | ESMO 30 min 3,779 ± 490 | DES + ESMO 30 min 3,315 ± 570 | ESMO 180 min 3,502 ± 470 | DES + ESMO 180 min 3,849 ± 730 | ICI 118,551 3,355 ± 570 | DES + ICI 118,551 3,527 ± 750 | H-89 3,050 ± 890 | DES + H-89 3,790 ± 590 | KN-93 3,517 ± 660 | DES + KN-93 3,627 ± 490 |

Data are mean ± SEM.

* Significantly (P < 0.05) different from baseline. † Significantly (P < 0.05) different from the respective value in control experiments.

Groups: CON = control; DES = 1.0 minimum alveolar concentration of desflurane; ESMO 30 min = 30 mg · kg⁻¹ · h⁻¹ esmolol for 30 min; DES + ESMO 30 min = desflurane + 30 mg · kg⁻¹ · h⁻¹ esmolol for 30 min; ESMO 180 min = 30 mg · kg⁻¹ · h⁻¹ esmolol for 180 min; DES + ESMO 180 min = desflurane + 30 mg · kg⁻¹ · h⁻¹ esmolol for 180 min (n = 8 per group); ICI 118,551 = 0.2 mg/kg ICI 118,551; DES + ICI 118,551 = desflurane + ICI 118,551; H-89 = 250 μg/kg H-89; DES + H-89 = desflurane + H-89; KN-93 = 300 μg/kg KN-93; DES + KN-93 = desflurane + KN-93 (n = 6 per group).

CAO = coronary artery occlusion; +dP/dtmax = maximal rate of increase of left ventricular pressure; HR = heart rate; LVEDP = left ventricular end-diastolic pressure; MAP = mean aortic blood pressure.
**Table 2. Area at Risk**

<table>
<thead>
<tr>
<th>Body Weight, kg</th>
<th>LV, g</th>
<th>AAR, g</th>
<th>AAR/LV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>2.43 ± 0.15</td>
<td>3.12 ± 0.09</td>
<td>1.33 ± 0.15</td>
</tr>
<tr>
<td>DES</td>
<td>2.34 ± 0.12</td>
<td>3.29 ± 0.09</td>
<td>1.26 ± 0.19</td>
</tr>
<tr>
<td>ESMO 30 min</td>
<td>2.60 ± 0.07</td>
<td>3.28 ± 0.25</td>
<td>1.23 ± 0.14</td>
</tr>
<tr>
<td>DES + ESMO 30 min</td>
<td>2.53 ± 0.06</td>
<td>3.46 ± 0.17</td>
<td>1.07 ± 0.12</td>
</tr>
<tr>
<td>ESMO 180 min</td>
<td>2.41 ± 0.05</td>
<td>3.52 ± 0.18</td>
<td>1.07 ± 0.13</td>
</tr>
<tr>
<td>DES + ESMO 180 min</td>
<td>2.39 ± 0.05</td>
<td>3.50 ± 0.07</td>
<td>1.04 ± 0.08</td>
</tr>
<tr>
<td>ICI 118,551</td>
<td>2.26 ± 0.15</td>
<td>2.93 ± 0.16</td>
<td>1.05 ± 0.12</td>
</tr>
<tr>
<td>DES + ICI 118,551</td>
<td>2.39 ± 0.06</td>
<td>2.93 ± 0.16</td>
<td>1.07 ± 0.11</td>
</tr>
<tr>
<td>H-89</td>
<td>2.25 ± 0.12</td>
<td>2.99 ± 0.17</td>
<td>1.24 ± 0.21</td>
</tr>
<tr>
<td>DES + H-89</td>
<td>2.51 ± 0.06</td>
<td>3.22 ± 0.25</td>
<td>1.04 ± 0.16</td>
</tr>
<tr>
<td>KN-93</td>
<td>2.62 ± 0.05</td>
<td>3.39 ± 0.26</td>
<td>1.18 ± 0.14</td>
</tr>
<tr>
<td>DES + KN-93</td>
<td>2.73 ± 0.05</td>
<td>3.60 ± 0.24</td>
<td>1.43 ± 0.18</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

Groups: CON = control; DES = 1.0 minimum alveolar concentration of desflurane (30 min); ESMO 30 min = desflurane + 30 mg·kg⁻¹·h⁻¹ esmolol for 30 min; DES + ESMO 30 min = desflurane + 30 mg·kg⁻¹·h⁻¹ esmolol for 30 min; ESMO 180 min = desflurane + 30 mg·kg⁻¹·h⁻¹ esmolol for 180 min; DES + ESMO 180 min = desflurane + DES 118,551; H-89 = 300 μg/kg H-89; DES + H-89 = desflurane + H-89; KN-93 = 300 μg/kg KN-93; DES + KN-93 = desflurane + KN-93

AAR = area at risk; LV = left ventricle.

**Western Immunoblotting**

**CAMK II, Phospholamban Phosphorylation in Early Reperfusion.** Ca²⁺/calmodulin-dependent protein kinase II (PKA) and phospholamban (PLB) protein expression were not affected by any of the study drugs at early reperfusion. Desflurane given alone had no impact on the phosphorylation of phospholamban at serine 16 or threonine 17 (fig. 6). PKA-dependent phosphorylation of phospholamban at serine 16 was inhibited by esmolol, ICI 118,551, and H-89 (fig. 6). However, in the case of H-89, this effect was abolished in the presence of desflurane. KN-93 did not affect phospholamban phosphorylation at serine 16. CaMK II-dependent phospholamban phosphorylation at threonine 17 was blocked by esmolol, ICI 118,551, and H-89, and the specific CaMK II inhibitor KN-93 (fig. 6). However, in the presence of desflurane, the inhibitory activity of esmolol and H-89 on phospholamban phosphorylation at threonine 17 was abolished (fig. 6).

**CAMK II, Phospholamban Phosphorylation in Late Reperfusion.** In late reperfusion, desflurane and esmolol given alone did not affect protein expression of CaMK II. However, CaMK II protein expression was increased by the combined administration of desflurane and esmolol given throughout reperfusion (fig. 4). Phospholamban protein expression was not affected by desflurane or esmolol (fig. 5). Phosphorylation of phospholamban at serine 16 and at threonine 17 was inhibited by esmolol given alone or combined with desflurane (fig. 6).

**PKB/Akt.** At early reperfusion, protein expression of total PKB/Akt was reduced by ICI 118,551 and H-89 in the presence or absence of desflurane. However, phosphorylation of PKB/Akt was inhibited only by ICI 118,551 in the presence or absence of desflurane, whereas all other study drugs did not affect PKB/Akt phosphorylation. In late reperfusion, desflurane and esmolol had no impact on PKB/Akt protein expression or phosphorylation (fig. 7).

**Discussion**

Ischemic injury to the heart results in a stress response with increased norepinephrine and epinephrine release and accumulation in ischemic myocardium, subsequent β-adrenergic receptor stimulation, and activation of the classic Gs protein-adenyl cyclase-cyclic adenosine monophosphate-PKA signaling pathway. β-Adrenergic receptors have been shown to play a complex and differential role in ischemia and reperfusion (I/R) injury.

![Fig. 2. Myocardial infarct size expressed as a percentage of the area at risk in rabbits, receiving vehicle (CON), 1.0 minimum alveolar concentration of desflurane (DES), or the β₁-adrenergic blocker esmolol for the first 30 min of reperfusion (ESMO 30 min) or throughout the reperfusion period (ESMO 180 min) or the β₂-adrenergic blocker ICI 118,551 (ICI). The combination of desflurane and the β-adrenergic receptor blockers esmolol (DES + ESMO 30 min, DES + ESMO 180 min) and ICI 118,551 (DES + ICI) was administered in separate groups (n = 8 per group). * Significantly (P < 0.05) different from CON.]

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and in cardioprotection. Increased $\beta_1$-adrenergic receptor and PKA activity during ischemia have been shown to contribute to I/R injury. Consecutively, pharmacologic $\beta$ blockade in acute myocardial infarction is cardioprotective and reduces infarct size in patients and in animals. However, short-term direct activation of $\beta_1$-adrenergic receptors by isoproterenol before an ischemic event induces preconditioning. Moreover, ischemic and anesthetic-induced preconditioning have been shown to be mediated by $\beta_1$-adrenergic signaling. Considering that volatile anesthetic preconditioning and postconditioning share similar signal transduction pathways, it is likely that desflurane-induced postconditioning also depends on $\beta$-adrenergic signaling. The results of the current study now confirm for the first time that desflurane-induced postconditioning is mediated by $\beta$-adrenergic signaling, because selective blockade of $\beta_1$- and $\beta_2$-adrenergic receptors abolishes cardioprotection conferred by desflurane administered during early reperfusion. Moreover, although a direct effect of desflurane on $\beta$-adrenergic receptors or PKA activity can not be proven by the results of our study, Western blot analysis demonstrates that the inhibitory effects of $\beta$-adrenergic receptor and PKA antagonists on PKA-dependent phosphorylation of phospholamban are decreased by the presence of desflurane. This finding provides indirect evidence for the sympathomimetic effects of desflurane. However, if blockade of $\beta_1$-adrenergic receptors by esmolol is maintained throughout the complete reperfusion period, myocardial infarct size is reduced to the same extent as by desflurane-induced postconditioning. Although alternative protective mechanisms of $\beta$ blockers have been proposed, the energy sparing effects of $\beta$ blockade, mainly heart rate reduction, probably account primarily for infarct size reduction induced by esmolol. It is obvious that the reduction of myocardial energy demand limited to the initial 30 min of reperfusion is not sufficient to confer cardio-

Fig. 3. Myocardial infarct size expressed as a percentage of the area at risk in rabbits receiving vehicle (CON), 1.0 minimum alveolar concentration of desflurane (DES), or the protein kinase A blocker H-89 or the Ca$^{2+}$/calmodulin-dependent protein kinase II inhibitor KN-93 alone (H-89, KN-93) or in combination with desflurane (DES + H-89, DES + KN-93) (n = 6 per group). Note that data of CON and DES are the same as in figure 2. * Significantly ($P < 0.05$) different from CON.

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In early reperfusion, inhibition of the downstream mediator of β-adrenergic receptor stimulation, PKA, with H-89 is cardioprotective and reduces infarct size, independent of the absence or presence of desflurane. This observation is in line with previous findings that H-89 reduces infarct size when given before ischemia in isolated rat hearts. However, PKA has been shown to mediate ischemic and anesthetic-induced preconditioning, likely by activation of mitochondrial large-conductance calcium-activated K⁺ channels. It is unlikely that this discrepancy is caused by differences in the signal transduction pathways underlying preconditioning and postconditioning. Rather, PKA blockade by H-89 parallels the effect of prolonged β₁-adrenergic receptor blockade throughout reperfusion by esmolol. The duration of action of H-89 in the rabbit heart in vivo is unknown. However, the fact that H-89 given at the onset of reperfusion reduces heart rate throughout the complete reperfusion period suggests that PKA blockade persists for the complete reperfusion period. Prolonged PKA inhibition would consequently have the same cardioprotective effect as persistent β₁-adrenergic receptor blockade, probably by conferring similar reduction of myocardial oxygen demand as β-adrenergic receptor blockade.

Persistent stimulation of β₁-adrenergic receptors shifts the intracellular signal transduction pathway from the classic cyclic adenosine monophosphate–PKA to predominant CaMK II activation. There is emerging evidence that the adverse effects of sustained β-adrenergic stimulation, including induction of myocyte apoptotic cell death and maladaptive remodeling processes, refer to CaMK II rather than to PKA activation. CaMK II activity and expression are increased in myocardial infarction. Therefore, inhibition of CaMK II is thought to be a novel approach in the prevention of long-term sequelae of myocardial infarction, namely heart failure. However, in the acute phase of myocardial infarction, CaMK II activation is involved in the restoration of myocardial contractility. Moreover, experimental evidence suggests that CaMK II mediates ischemic and
desflurane-induced preconditioning,40 because blockade of CaMK II by KN-93 abrogated the beneficial effects of ischemic preconditioning on LV contractile function after simulated ischemia in isolated rat hearts and abolished infarct size reduction conferred by desflurane preconditioning in the rabbit heart in vivo. Therefore, short-term activation of CaMK II might be involved in the signaling pathway of ischemic preconditioning. The results of the current investigation demonstrate for the first time that blockade of CaMK II abolishes desflurane-induced postconditioning. Western blot analysis confirms effective CaMK II blockade by the specific CaMK II inhibitor KN-93, because CaMK II–dependent phosphorylation of phospholamban at threonine 17 is blocked, whereas PKA-dependent phosphorylation of phospholamban at serine 16 is not. The mechanism by which CaMK II is involved in anesthetic-induced cardioprotection is unclear. However, I/R injury results in critical cytosolic Ca2+ overload,1 and CaMK II plays a pivotal role in the regulation of intracellular Ca2+ levels. By phosphorylating ryanodine receptors and regulating sarcoplasmatic reticulum Ca2+-pump adenosine triphosphatase activity via phospholamban phosphorylation, CaMK II regulates Ca2+ release from and uptake to the sarcoplasmatic reticulum.41 Anesthetic-induced preconditioning has been shown to reduce Ca2+ overload after I/R injury by the preservation of the calcium cycling proteins sarcoplasmatic reticulum Ca2+-pump adenosine triphosphatase and ryanodine receptors and to improve ventricular function.4,42 It is likely that the impact of desflurane-induced postconditioning on CaMK II activity contributes to improved Ca2+ handling after I/R injury.

Several potential limitations must be considered in the interpretation of the study results. Six rabbits had to be excluded from the study because of LV pump failure. Five of these animals received desflurane. This finding

Fig. 5. Western blot analysis of myocardial phospholamban (PLB) content at early reperfusion after blockade of β-adrenergic receptors (A) and downstream kinases protein kinase A (PKA) and Ca2+/calmodulin-dependent protein kinase (CaMK) II (B) and at late reperfusion after continuous blockade of β-adrenergic receptors (C). Rabbits received vehicle (CON), desflurane (DES), esmolol for 30 min (ESMO 30) or 180 min of reperfusion (ESMO 180), and ICI 118,551 (ICI), H-89, or KN-93 alone or in combination with desflurane. Results are presented as representative original immunoblottings and average densitometric results of immunoblotting as percentage of control (n = 5 per group). Note that data from Sham, CON, and DES in B are the same as in A. Values are mean ± SEM.
might suggest that 1 MAC of desflurane administered at the onset of reperfusion after experimental ischemia increases the risk for LV pump failure. However, desflurane is administrated in conjunction with the background anesthetic sodium pentobarbital. The negative inotropic effects of both anesthetics during the vulnerable phase of early reperfusion might in combination be responsible for this adverse effect. Although esmolol is a selective inhibitor with a high affinity to β1-adrenergic receptors, it might also block β2-adrenergic receptors. In addition to its action on β adrenoceptors, esmolol has non-receptor-mediated effects that reduce action potential duration and plateau voltage.43 It cannot be excluded that these β2-adrenergic and non-receptor-mediated effects influenced infarct size. The PKA inhibitor H-89, although considered selective to PKA, also blocks sev-

**Figure 6.** Phosphorylation of phospholamban (p-PLB) at serine 16 and threonine 17 at early reperfusion after blockade of β1- and β2-adrenergic receptors (A and C) and downstream kinases protein kinase A (PKA) and Ca2+/calmodulin-dependent protein kinase (CaMK) II (B and D) and at late reperfusion after continuous blockade of β-adrenergic receptors (E and F). Rabbits received vehicle (CON), desflurane (DES), esmolol for 30 min (ESMO 30) or 180 min of reperfusion (ESMO 180), and ICI 118,551 (ICI), H-89, or KN-93 alone or in combination with desflurane. Results are presented as representative original immunoblottings and average densitometric results of immunoblotting as percentage of control (n = 5 per group). Note that data from Sham, CON, and DES in B and D are the same as in A and C. * Significantly (P < 0.05) different from CON. Values are mean ± SEM.
eral other protein kinases, including MSK1, S6K1, ROCKII, and protein kinase G, and directly inhibits adenosine triphosphate–sensitive potassium channels in rabbit coronary artery smooth muscle cells. It has been demonstrated that I/R injury induces compartmentalization of CaMK II during transient ischemia with subsequent redistribution during reperfusion. CaMK II translocation was not tested in our study. However, it is possible that CaMK II translocation might play a role in desflurane-induced postconditioning. KN-93 is a selective competitive inhibitor of calmodulin binding to CaMK II and CaMK II autophosphorylation and has no inhibitory effect on other protein kinases. However, KN-93 is known to directly inhibit L-type Ca$^{2+}$/H1 channels and K$^{+}$ currents. These additional effects of H-89 and KN-93 should be borne in mind when interpreting the current findings.

In conclusion, the results of the current study demonstrate a differential role for β-adrenergic signaling in cardioprotection during reperfusion. Blockade of β-ad-
renergic receptor subtypes or the downstream kinase CaMK II abolishes desflurane-induced postconditioning, whereas prolonged β1-renergic receptor or PKA blockade confers marked cardioprotection that is independent of the presence or absence of desflurane. Optimization of Ca\(^{2+}\) homeostasis by the preservation of calcium handling proteins might play a major role in volatile anesthetic-induced cardioprotection and might explain the role of β-adrenergic pathways in this phenomenon. Further studies are needed to elucidate the complex interactions of anesthetic-induced postconditioning and adrenergic signaling.

References


From Resuscitation to Extermination: Chloroforming Rodents

In November of 1974, San Francisco’s Daniel B. Meyers and his grandson, Gary C. Yerby, filed for two U.S. patents, the first of which was granted in 1976 for their “Portable Rodent Exterminator” (pictured above), “for transporting and/or exterminating rodents collected in container-type traps designed to couple to the exterminator during transfer . . . .” To preclude rodent escape during collection, the device employed an “absorbent material . . . [that could] be partially soaked with a liquid, such as . . . chloroform [CHCl₃], to . . . anesthetize collected rodents . . . .” The Meyers-Yerby device consisted “of a container having a removable top, a retractable divider plate, and a viewing window in the side of the container for viewing the transfer and extermination process.” About 95 yr before this patent, rather than extermination, chloroforming rodents had yielded valuable clues to resuscitation. Back then, a French surgeon had observed that most mice chloroformed to unconsciousness had tried to bite him when lifted up by their tails and then resumed their “insensibility” when placed flat again on a table. Inverting patients “tail in the air” or at least raising their legs soon became standard in treating anesthetic overdose. (Copyright © the American Society of Anesthesiologists, Inc. This image appears in the Anesthesiology Reflections online collection available at www.anesthesiology.org.)

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