Desflurane-induced postconditioning of diabetic human right atrial myocardium in vitro

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Abstract

Aim. – We tested the hypothesis that brief exposure to desflurane at the time of reoxygenation might be able to protect against hypoxia–reoxygenation injury in human myocardium from diabetic (insulin-dependent, ID; and non-insulin-dependent, NID) patients and non-diabetic (ND) subjects.

Methods. – The force of contraction (34°C, stimulation frequency 1 Hz) in the right atrial trabeculae was recorded during 30 min of hypoxia followed by 60 min of reoxygenation. Desflurane (at 3, 6 and 9%) was administered during the first 5 min of reoxygenation. The force of contraction at the end of the 60-min reoxygenation period (FoC 60) was compared in the study groups (means ± SD).

Results. – In the ND group, desflurane at 3, 6 and 9% (FoC 60: respectively 78 ± 10%, 84 ± 4% and 85 ± 12% of baseline) enhanced the recovery of FoC 60 compared with the ND-controls (53 ± 7%; P < 0.05). In the ID group, desflurane at 3% (61 ± 4%) did not modify the recovery of FoC 60 compared with the ID-controls (54 ± 6%), whereas desflurane at 6 and 9% (75 ± 11% and 81 ± 8%, respectively) enhanced the recovery of FoC 60 vs the controls (P < 0.05). In the NID group, desflurane at 3% (57 ± 5%) also failed to modify the recovery of FoC 60 compared with the NID-controls (52 ± 10%), while desflurane at 6 and 9% (80 ± 10% and 79 ± 7%, respectively) enhanced the recovery of FoC 60 vs the controls (P < 0.05).

Conclusion. – Desflurane in vitro was able to postcondition diabetic (both ID and NID) human myocardium at 6 and 9%, but not at 3%.

Keywords: Diabetes; Human myocardium; Desflurane; Postconditioning

Résumé

Postconditionnement in vitro induit par le desflurane du myocarde humain diabétique.

But. – Observer si la brève exposition au desflurane en début de réoxygénation pouvait protéger contre les dommages liés à l’hypoxie-réoxygénation le myocarde humain prélevé sur des patients diabétiques (insulinodépendant, ID ; et non insulinodépendant, NID).

Méthodes. – La force de la contraction (34 °C, fréquence de stimulation 1 Hz) des trabécules isolés d’oreillettes droites humaines a été enregistrée durant une hypoxie de 30 minutes suivie d’une réoxygénation de 60 minutes. Le desflurane 3, 6 et 9 % a été administré durant les cinq premières minutes de réoxygénation. La force de la contraction à 60 minutes de réoxygénation (FoC 60) a été comparée entre les groupes (moyenne ± écart type).

Résultats. – Dans le groupe non diabétique, le desflurane 3, 6 et 9 % (FoC 60 : 78 ± 10 %, 84 ± 4 %, 85 ± 12 % de la valeur de base) augmente la FoC 60 par comparaison au groupe témoin (53 ± 7 % ; P < 0.05). Dans le groupe ID, le desflurane 3 % (61 ± 4 %) ne modifie pas la FoC 60 par comparaison au groupe témoin (54 ± 6 %) ; le desflurane 6 et 9 % (75 ± 11 % et 81 ± 8 %) augmente la FoC 60 par comparaison au groupe témoin (P < 0.05). Dans le groupe NID, le desflurane 3 % (57 ± 5 %) ne modifie pas la FoC 60 par comparaison au groupe témoin (52 ± 10 %) ; le desflurane 6 et 9 % (80 ± 10 % et 79 ± 7 %) augmentent la FoC 60 par comparaison au groupe témoin (P < 0.05).

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1. Introduction

Diabetes and its related complications and co-morbidities are well-recognized major risk factors of ischaemic heart disease that are also able to influence the risk profile, postoperative outcome and long-term survival of patients undergoing coronary artery bypass graft (CABG) surgery [1]. During CABG surgery using cardiopulmonary bypass and cardioplegic arrest, global myocardial ischemia injury can occur. Although the off-pump CABG procedure has been suggested to decrease morbidity and mortality in high-risk patients [2], it also has resulted in myocardial ischemic injury, as measured by postoperative troponin I levels [3]. For this reason, it is important to study perioperative cardioprotective strategies using myocardium from diabetic patients.

Of the interventions that may contribute to perioperative cardioprotection, ischaemic- and volatile-anaesthetic-induced preconditioning (PreC) and postconditioning (PostC) have both received significant interest [4,5]. Recent studies of CABG surgery have indirectly shown that the choice of a volatile anaesthetic regimen may result in perioperative cardioprotection, as suggested by the lower postoperative release of troponin I, improved early postoperative recovery and decreased requirement for postoperative positive inotropic support [6,7]. In addition, the probability of cardiac-event-free survival appears to be favourably influenced by the perioperative use of a volatile anaesthetic [8].

Nevertheless, these studies excluded diabetic patients, as experimental studies have shown that both ischaemic- and volatile–anaesthetic-induced cardioprotection was abolished in diabetic animals, and in the presence of high glucose concentrations [9–12]. However, it needs to be emphasized that the glucose concentrations reported in those experimental studies (270–600 mg/dL) were far greater than those observed in diabetic patients scheduled for surgery. This suggests that the clinical relevance of such experimental studies is limited and that, in turn, led us to hypothesize that these results could not be extended to human diabetic myocardium. As studies in vitro of isolated human right atrial trabeculae exposed to hypoxia–reoxygenation have proved useful in the study of hypoxic- and anaesthetic-induced PreC [5], similar studies on isolated human myocardium obtained from diabetic patients scheduled for cardiac surgery appeared to be mandatory.

The goals of the present study were:

- to examine the sensitivity of isolated human diabetic myocardium to hypoxia–reoxygenation;
- to study myocardial PostC following desflurane administration and during the first few minutes of reoxygenation.

2. Materials and methods

After receiving the approval of the local medical ethics committee and written informed consent from the study participants, right atrial appendages were obtained during cannulation for cardiopulmonary bypass from ND subjects, and from patients with either insulin-dependent (ID) or non-insulin-dependent (NID) diabetes, who were scheduled for routine CABG surgery or aortic valve replacement. In patients with ID diabetes, diabetic treatment remained unchanged until the evening of the day before surgery. In patients with NID diabetes, oral diabetic treatment was discontinued 24 h before surgery except for metformin, which was discontinued one week before surgery. All treated diabetic patients undergoing cardiac surgery received the preoperative standardized local-protocol service to maintain blood glucose concentrations at 150–200 mg/dL. Also, in all diabetic patients, an infusion of dextrose solution was started (5 g/h) on the evening of the day before surgery, together with 0.15 U/kg of subcutaneous intermediary insulin (Umut line NPH; Lilly France, Suresnes, France). Blood glucose was measured every 8 h using a glucose analyzer (Accu-Chek Performa; Roche Diagnostics, Meylan, France). During the operative period, all participants (with and without diabetes) received continuous-infusion fast-acting insulin (Actrapid HM; Novo Nordisk Pharmaceutique, Puteaux, France) as soon as arterial blood glucose levels exceeded 180 mg/dL. Thereafter, the infusion rate was titrated according to the local protocol (< 180 mg/dL, 0 U/h; 180–220 mg/dL, 1 U/h; 221–249 mg/dL, 2 U/h; > 249 mg/dL, 3 U/h). Arterial blood glucose concentration was measured every 30 and 60 min after changes in the rate of infusion, which was titrated according to the following protocol: lesser than 140 mg/dL, the infusion was stopped until 180 mg/dL; 140–179 mg/dL, the rate of infusion was decreased by 0.5 U/h; 180–220 mg/dL, no change in the rate of infusion; 221–249 mg/dL, the rate of infusion was increased or decreased by 0.5 U/h, according to the previous blood glucose concentration; and greater than 249 mg/dL, the rate of infusion was increased by 1.0 U/h.

All study participants also received total intravenous anaesthesia with propofol, sufentanil and pancuronium. Patients with chronic atrial arrhythmias were excluded from the study. The participants’ clinical characteristics and age, preoperative drug treatments, preoperative left ventricular ejection fractions and HbA1c values are presented in Table 1, and their blood glucose concentrations at the time of atrial appendage dissection are shown in Table 2.

2.1. Experimental conditions

Right atrial trabeculae (one to two per appendage) were dissected, and then suspended vertically between an isometric...
Table 1
Study participants’ clinical data, including age, left ventricular ejection fraction (LVEF) and HbA1c.

<table>
<thead>
<tr>
<th>Group and type of heart disease</th>
<th>Age (years)</th>
<th>Preoperative drug treatments</th>
<th>LVEF (%)</th>
<th>HbA1c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>63 ± 13</td>
<td>ACE (4), b-bl (5), BZD (0), CA (1), COR (1), FUR (1), K’ (0), K’A (0), K’AT (0), INS (0), STA (5), NG (0)</td>
<td>58 ± 10</td>
<td>5.6 ± 0.5</td>
</tr>
<tr>
<td>AVR (n = 5); CABB (n = 4)</td>
<td>64 ± 11</td>
<td>ACE (5), b-bl (5), BZD (3), CA (1), COR (0), FUR (2), K’ (0), K’A (0), K’AT (3) INS (7), STA (8), NG (0)</td>
<td>58 ± 16</td>
<td>7.2 ± 0.7</td>
</tr>
<tr>
<td>ID-Control</td>
<td>65 ± 14</td>
<td>ACE (5), b-bl (4), BZD (0), CA (1), COR (0), FUR (2), K’ (0), K’A (0), K’AT (0), INS (0), MET (2), STA (8), NG (0)</td>
<td>63 ± 16</td>
<td>7.0 ± 1.2</td>
</tr>
<tr>
<td>NID-Control</td>
<td>69 ± 18</td>
<td>ACE (1), b-bl (3), BZD (1), CA (0), COR (1), FUR (2), K’ (0), K’A (0), K’AT (0), INS (0), MET (2), STA (8), NG (0)</td>
<td>67 ± 9</td>
<td>5.7 ± 0.3</td>
</tr>
<tr>
<td>AVR (n = 4); CABB (n = 5)</td>
<td>78 ± 5</td>
<td>ACE (2), b-bl (2), BZD (0), CA (2), COR (0), FUR (2), K’ (0), K’A (0), K’AT (0), INS (0), MET (2), STA (8), NG (0)</td>
<td>76 ± 3</td>
<td>6.8 ± 0.1</td>
</tr>
<tr>
<td>Des3</td>
<td>66 ± 6</td>
<td>ACE (4), b-bl (4), BZD (1), CA (1), COR (1), FUR (1), K’ (0), K’A (0), K’AT (0), INS (0), MET (2), STA (8), NG (0)</td>
<td>47 ± 17</td>
<td>6.5 ± 0.7</td>
</tr>
<tr>
<td>NID-Des3</td>
<td>65 ± 14</td>
<td>ACE (2), b-bl (2), BZD (1), CA (1), COR (1), FUR (1), K’ (0), K’A (0), K’AT (0), INS (0), MET (2), STA (8), NG (0)</td>
<td>69 ± 4</td>
<td>6.7 ± 1.0</td>
</tr>
<tr>
<td>AVR (n = 5); CABB (n = 2)</td>
<td>63 ± 6</td>
<td>ACE (2), b-bl (5), BZD (0), CA (0), COR (0), FUR (0), K’ (0), K’A (0), K’AT (0), INS (0), MET (2), STA (8), NG (0)</td>
<td>63 ± 19</td>
<td>7.4 ± 0.6</td>
</tr>
<tr>
<td>NID-Des6</td>
<td>70 ± 4</td>
<td>ACE (1), b-bl (2), BZD (1), CA (1), COR (1), FUR (0), K’ (0), K’A (0), K’AT (0), INS (0), MET (2), STA (8), NG (0)</td>
<td>76 ± 11</td>
<td>6.9 ± 0.9</td>
</tr>
<tr>
<td>AVR (n = 2); CABB (n = 4)</td>
<td>67 ± 9</td>
<td>ACE (5), b-bl (5), BZD (2), CA (2), COR (0), FUR (2), K’ (0), K’A (0), K’AT (0), INS (0), MET (2), STA (8), NG (0)</td>
<td>65 ± 5</td>
<td>5.9 ± 0.4</td>
</tr>
<tr>
<td>Des3</td>
<td>67 ± 11</td>
<td>ACE (2), b-bl (4), BZD (1), CA (0), COR (0), FUR (0), K’ (0), K’A (0), K’AT (0), INS (0), MET (2), STA (8), NG (0)</td>
<td>64 ± 10</td>
<td>7.2 ± 1.0</td>
</tr>
<tr>
<td>NID-Des3</td>
<td>60 ± 3</td>
<td>ACE (4), b-bl (4), BZD (1), CA (0), COR (1), FUR (1), K’ (0), K’A (0), K’AT (0), INS (0), MET (2), STA (8), NG (0)</td>
<td>51 ± 16</td>
<td>6.8 ± 1.0</td>
</tr>
<tr>
<td>AVR (n = 3); CABB (n = 3)</td>
<td>70 ± 6</td>
<td>ACE (4), b-bl (5), BZD (0), CA (0), COR (1), FUR (5), K’ (0), K’A (0), K’AT (0), INS (0), MET (2), STA (8), NG (0)</td>
<td>53 ± 15</td>
<td>6.8 ± 1.0</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD; the numbers in parentheses after drug abbreviations are the number of patients; AVR: aortic valve replacement; CABB: coronary artery bypass graft; ACE: angiotensin-converting enzyme inhibitors; b-bl: beta-adrenergic blockers; BZD: benzodiazepine; CA: calcium-channel antagonists; COR: amiodarone; FUR: furosemide; INS: insulin; K’ A: potassium-channel agonists; K’ AT: potassium-channel antagonists; NG: nitroglycerin; STA: statin; ID: insulin-dependent diabetes; NID: non-insulin-dependent diabetes; Des3: desflurane 3%; Des6: desflurane 6%; Des9: desflurane 9%.

force transducer (MLT0202; ADInstruments, Sydney, Australia) and a stationary stainless clip in a 200-mL jacketed reservoir filled with daily-prepared modified Tyrode’s solution, containing (mM) 120 NaCl, 3.5 KCl, 1.1 MgCl2, 1.8 Na2HPO4, 25.7 NaHCO3, 2.0 CaCl2 and 5.5 glucose. The jacketed reservoir was maintained at 34°C by a thermostatic water circulator (Polystat Micropros; Fisher Scientific Bioblock, Illkirch, France), and the bath solution was oxygenated with 95% O2 and 5% CO2, resulting in a pH of 7.40 and a pO2 of 600 mmHg. The isolated muscles were field-stimulated by two platinum electrodes at 1 Hz, with rectangular wave pulses of 5-ms duration at 20% above threshold (CMS 95107; Bionics Instrument, Paris, France).

Table 2
Control values of mechanical parameters of human right atrial trabeculae and blood glucose concentrations at the time of atrial appendage dissection.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Lmax (mm)</th>
<th>CSA (mm²)</th>
<th>FoC (mN.mm⁻²)</th>
<th>RF/TF</th>
<th>BGC (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 9)</td>
<td>6.0 ± 1.8</td>
<td>0.50 ± 0.17</td>
<td>23 ± 12</td>
<td>0.39 ± 0.17</td>
<td>99 ± 15</td>
</tr>
<tr>
<td>ID-Control (n = 9)</td>
<td>7.0 ± 1.1</td>
<td>0.49 ± 0.22</td>
<td>28 ± 14</td>
<td>0.31 ± 0.10</td>
<td>138 ± 45</td>
</tr>
<tr>
<td>NID-Control (n = 9)</td>
<td>6.6 ± 2.6</td>
<td>0.51 ± 0.25</td>
<td>22 ± 10</td>
<td>0.37 ± 0.17</td>
<td>115 ± 12</td>
</tr>
<tr>
<td>Des3 (n = 6)</td>
<td>7.1 ± 1.0</td>
<td>0.48 ± 0.17</td>
<td>24 ± 8</td>
<td>0.29 ± 0.04</td>
<td>111 ± 10</td>
</tr>
<tr>
<td>ID-Des3 (n = 6)</td>
<td>6.0 ± 1.1</td>
<td>0.44 ± 0.23</td>
<td>30 ± 11</td>
<td>0.33 ± 0.07</td>
<td>125 ± 32</td>
</tr>
<tr>
<td>NID-Des3 (n = 6)</td>
<td>7.0 ± 1.8</td>
<td>0.50 ± 0.20</td>
<td>24 ± 11</td>
<td>0.35 ± 0.13</td>
<td>94 ± 10</td>
</tr>
<tr>
<td>Des6 (n = 6)</td>
<td>7.5 ± 0.6</td>
<td>0.60 ± 0.26</td>
<td>28 ± 17</td>
<td>0.30 ± 0.1</td>
<td>118 ± 11</td>
</tr>
<tr>
<td>ID-Des6 (n = 6)</td>
<td>7.2 ± 2.1</td>
<td>0.52 ± 0.17</td>
<td>19 ± 5</td>
<td>0.35 ± 0.06</td>
<td>126 ± 38</td>
</tr>
<tr>
<td>NID-Des6 (n = 6)</td>
<td>7.4 ± 1.7</td>
<td>0.53 ± 0.22</td>
<td>26 ± 6</td>
<td>0.29 ± 0.13</td>
<td>128 ± 43</td>
</tr>
<tr>
<td>Des9 (n = 6)</td>
<td>6.4 ± 1.6</td>
<td>0.49 ± 0.28</td>
<td>22 ± 7</td>
<td>0.34 ± 0.12</td>
<td>119 ± 14</td>
</tr>
<tr>
<td>ID-Des9 (n = 6)</td>
<td>7.0 ± 0.7</td>
<td>0.46 ± 0.20</td>
<td>23 ± 8</td>
<td>0.37 ± 0.10</td>
<td>121 ± 17</td>
</tr>
<tr>
<td>NID-Des9 (n = 6)</td>
<td>5.8 ± 1.5</td>
<td>0.48 ± 0.20</td>
<td>26 ± 12</td>
<td>0.33 ± 0.10</td>
<td>112 ± 20</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD; Lmax: maximum length at apex of length-active force curve; CSA: cross-sectional area; FoC: force of contraction normalized per cross-sectional area; RF/TF: resting force/total force ratio; BGC: blood glucose concentration at time of atrial appendage dissection; ID: insulin-dependent diabetes; NID: non-insulin-dependent diabetes; Des3: desflurane 3%; Des6: desflurane 6%; Des9: desflurane 9%.
The trabeculae were equilibrated for 60–90 min to allow optimal mechanical recovery and performance at the apex of the length-active isometric tension curve. At the end of the stabilization period, the trabeculae were randomized to one of the experimental groups detailed below, but making sure that trabeculae obtained from the same appendage were distributed to different experimental groups. Force of contraction (FoC) was measured continuously, and digitized at a sampling frequency of 400 Hz and stored on a writable compact disc for analysis (MacLab; ADInstruments, Sydney, Australia). At the end of each experiment, trabecular length and weight were measured, and its cross-sectional area calculated, assuming a cylindrical shape and density of 1. Trabeculae with a cross-sectional area greater than 1.0 mm² were excluded, while the included preparations had FoC normalized per cross-sectional area to greater than 0.45. The endpoint of the study was the recovery of FoC during 60 min of reoxygenation (FoC₆₀, expressed as percent of baseline).

2.2. Experimental protocol

At the end of the stabilization period, the trabeculae were randomized (in sealed envelopes) to one of the experimental groups. In all groups, hypoxia–reoxygenation was performed by replacing the 95% O₂–5% CO₂ bath solution with 95% N₂–5% CO₂ in buffer for 30 min, followed by 60 min of reoxygenation (95% O₂–5% CO₂).

The tolerance of the isolated trabeculae to hypoxia–reoxygenation obtained from the controls of each study group (ND-control, n = 9; ID-control, n = 9; and NID-control, n = 9) was studied separately.

Desflurane-induced PostC was triggered by administration of desflurane at 3, 6 and 9% during the first 5 min of reoxygenation. Desflurane was delivered to the organ bath in a gas flow passing through a specific calibrated vaporizer, while desflurane concentration in the carrier-gas phase was measured continuously by an infrared analyzer (Datex Capnomac Ultima; Datex-Ohmeda, Helsinki, Finland). Desflurane-induced PostC was studied in isolated trabeculae obtained from ND subjects (Des3, Des6, Des9; n = 6 in each group), ID patients (ID-Des3, ID-Des6, ID-Des9; n = 6 in each group) and NID patients (NID-Des3, NID-Des6, NID-Des9; n = 6 in each group). Isolated trabeculae underwent 30 min of hypoxia and 60 min of reoxygenation.

2.3. HbA₁c measurement

Arterial blood samples were measured for HbA₁c (n = 68) using high-performance liquid chromatography (ADAMS HA-8160 HbA₁c Analyzer; Menarini Diagnostics, Florence, Italy) before cardiopulmonary bypass.

2.4. Statistical analysis

Taking into account the number of comparisons, the power analysis calculated a group size of five to detect a difference of 40% in FoC₆₀ with a power of 0.8 at an alpha-level of 0.05. Data are expressed as means ± standard deviation (SD). Baseline values of the main mechanical parameters, age, preoperative left ventricular ejection fraction and FoC₆₀ were compared by univariate analysis of variance (ANOVA) with the group factor as the independent variable. If the P value was < 0.05, Bonferroni post-hoc analysis was performed using Student’s t-test.

Within-group data were analyzed over time using ANOVA for repeated measures and Bonferroni post-hoc analysis, with group factor and time (baseline, hypoxia at 5, 10 and 30 min, and reoxygenation at 5, 10, 20, 30, 40, 50 and 60 min) as independent variables.

All P values were two-tailed and P < 0.05 was required to reject the null hypothesis. Statistical analyses were performed using Statview 5.0 software (DeltaSoft, Meylan, France).

3. Results

The patients’ characteristics, preoperative treatments, left ventricular ejection fraction and HbA₁c are shown in Table 1. A total of 80 human right atrial trabeculae were studied. There were no significant differences between groups in trabecular length at the apex of the length-active isometric tension curve, cross-sectional area, ratio of resting-to-total force, FoC and blood glucose concentration at the time of atrial appendage dissection (Table 2).

3.1. Effect of hypoxia–reoxygenation on human atrial trabeculae

As shown in Fig. 1, FoC₆₀ did not differ between the ND-control (FoC₆₀: 53 ± 7% of baseline value), ID-control (FoC₆₀: 54 ± 6% of baseline value, P = 0.29 vs controls) and NID-control (FoC₆₀: 52 ± 10% of baseline value; P = 0.78 vs ND-control; P = 0.18 vs ID-control) groups.

3.2. Effects of desflurane on hypoxia–reoxygenation

In trabeculae obtained from ND subjects, desflurane 3% (Des3; FoC₆₀: 78 ± 10% of baseline), desflurane 6% (Des6; FoC₆₀: 84 ± 4% of baseline) and desflurane 9% (Des9; FoC₆₀: 85 ± 12% of baseline), all administered during the first 5 min of reoxygenation, increased FoC₆₀ compared with the ND-control group (FoC₆₀: 53 ± 7% of baseline; P < 0.05). The FoC₆₀ did not differ across the desflurane groups (Fig. 1).

In trabeculae from ID patients, the recovery of FoC₆₀ measured in the presence of desflurane 3% did not differ from that in ID-control group (ID-Des3: 61 ± 4% vs ID-control: 54 ± 6%; P = 0.21). In contrast, desflurane 6% and 9% both increased FoC₆₀ (75 ± 11% and 81 ± 8% of baseline, respectively) compared with control groups (P < 0.05). The recovery of FoC₆₀ measured in the desflurane 6 and 9% groups was significantly different from that measured in the ID-Des3 group (Fig. 1).

In NID patients, the recovery of FoC₆₀ measured in the desflurane 3% group did not differ from that measured in NID-control group (NID-Des3: 57 ± 5% vs NID-control: 52 ± 10%; P = 0.06). Desflurane 6% (80 ± 10% of baseline) and 9%
(79 ± 7% of baseline) both increased FoC₆₀ vs control groups (P<0.05), and the recovery of FoC₆₀ measured in these two groups was significantly different from that measured in the NID-Des3 group (Fig. 1).

Finally, FoC₆₀ measured in the desflurane 3% ND group was significantly different from that measured in the ID and NID desflurane 3% groups (P<0.05). In contrast, the recovery of FoC₆₀ measured in the desflurane 6 and 9% groups did not significantly differ from those measured in the ND, ID and NID groups (Fig. 1).

4. Discussion

In the present study, we found:

- that human myocardium in vitro, whether obtained from diabetic or non-diabetic patients, has the same sensitivity to hypoxia–reoxygenation injury;
- that brief exposure to desflurane was able to postcondition human myocardium against hypoxia–reoxygenation injury, regardless of whether it was obtained from non-diabetic or diabetic patients;
- that in human myocardium isolated from ID and NID patients, a higher concentration of desflurane was required to trigger PostC.

Although the clinical data demonstrated that diabetic patients had an increased susceptibility to ischaemia injury [1], experimental studies are inconsistent, showing both an increased [13] and decreased [14] sensitivity of the diabetic heart to ischaemia–reperfusion injury. In diabetic db/db mice, the sensitivity to ischaemia was similar to that in non-diabetic mice at six weeks, but increased at 12 weeks [15]. These mixed findings may be explained by important differences in the experimental models of diabetes mellitus, and the species and experimental protocols used in the various studies. This suggests that, despite several limitations, studies on isolated human myocardium obtained from diabetic patients may have clinical relevance. Ghosh et al. [16] showed that, in isolated quiescent human myocardium, the diabetic heart had the same sensitivity to hypoxia–reoxygenation as the non-diabetic heart. In addition, Mudalagiri et al. [17] demonstrated that isolated human trabeculae from both diabetic and non-diabetic subjects had comparable recovery of FoC following hypoxia–reoxygenation despite a 10-mM glucose concentration in the organ bath.

The present study also found that human atrial myocardium obtained from diabetic and non-diabetic subjects had comparable recovery of FoC₆₀ following hypoxia–reoxygenation (Fig. 1). However, it needs to be emphasized that HbA₁c levels were comparable between groups (close to 6%), suggesting that glycaemic control was good in the diabetic patients included in our study. Furthermore, arterial blood glucose concentrations were controlled during surgery (Table 2), and the glucose concentration in the organ bath was 5.5 mM. Nevertheless, when considered altogether, the present study findings and those of previous studies strongly suggest that, in vitro, human diabetic myocardium has the same sensitivity to hypoxia–reoxygenation injury as that of non-diabetic myocardium.

Volatile-anaesthetic-induced myocardial PreC and PostC are now well established [5,12]. However, experimental results have shown that both hyperglycaemia and diabetes can abolish volatile-anaesthetic-induced PreC and PostC. Thus,
in alloxan- and streptozotocin-induced diabetes in dogs in vivo, isoflurane-induced myocardial PreC was attenuated [10]. However, in the diabetic dogs during this experiment, the blood glucose concentration decreased from 400 mg/dL to 150 mg/dL. On the other hand, in non-diabetic dogs, raising the blood glucose concentration up to 600 mg/dL through dextrose infusion abolished isoflurane-induced PreC, whereas a blood glucose concentration of 300 mg/dL only attenuated it [11]. Similarly, in rats in vivo, Huhn et al. [12] reported that acute hyperglycaemia (500 mg/dL), obtained by glucose infusion during the ischaemic period, and early reperfusion abolished sevoflurane-induced PostC, and blood glucose concentrations decreased from 500 mg/dL to normal concentrations until the end of the reperfusion period.

Nevertheless, these experiments had important limitations. First, the blood glucose concentrations were two- to threefold higher than those observed in diabetic patients [18]. This is important, as isoflurane-induced PreC is inhibited in the presence of a blood glucose concentration of 600 mg/dL, but only attenuated in the presence of 300 mg/dL [11]. Also, diabetic animals were studied after three weeks of pharmacologically induced diabetes. Yet, 6–12 weeks have been shown to be required for the development of diabetic cardiomyopathy [15]. Furthermore, the experimental protocols for increasing blood glucose concentrations using glucose infusion are not representative of diabetic myocardium and, moreover, in these experiments, blood glucose concentrations were increased only during the PreC stimulus period [11], or during the ischaemia and PostC stimulus [12].

In the present study, we found that, in isolated human myocardium obtained from diabetic patients, desflurane-induced PostC was attenuated, but not abolished. Also, desflurane 3% during early reoxygenation triggered PostC in the atrial trabeculae from non-diabetic patients, but not in those from diabetic patients. On the other hand, we have shown that desflurane 6 and 9% both triggered myocardial PostC in both diabetic and non-diabetic myocardium in vitro. This is in agreement with the findings reported by Tanaka et al. [10], showing that the efficiency of isoflurane-induced PreC was attenuated not only in pharmacologically induced diabetes in dogs, but also by acute hyperglycaemia [11]. Similarly, it has been shown that, in rat type 2 diabetic (NID) myocardium, three cycles of ischaemia reperfusion were needed to trigger myocardial protection of the same magnitude as that following one cycle of ischaemia reperfusion in non-diabetic rat heart tissue [19]. The present study extends these results to human myocardium obtained from diabetic patients who were scheduled for cardiac surgery, and to desflurane-induced PostC. Taken altogether, the present and previous results suggest that diabetic myocardium might be more resistant to the cardioprotection afforded by PreC and PostC. This suggests that despite having well-controlled diabetes, as indicated by preoperative HbA1C (Table 1), and having controlled blood glucose concentrations both pre- and perioperatively, diabetic cardiomyopathy may nevertheless result in an altered response to PostC stimuli. In addition, in the present study, six patients received intravenous insulin before their right atrial appendage was removed. Of the trabeculae thus obtained, two were in the control, two were in the NID-Des3, one was in the NID-Des6 and one was in the ID-Des6 groups. Finally, arterial blood glucose concentrations immediately prior to atrial appendage dissection were comparable between groups (Table 2). It may be hypothesized that these uncontrolled factors had an influence, at least in part, on our results.

Although the precise mechanisms underlying desflurane-induced PostC of diabetic myocardium remain incompletely studied, the present study was not designed to address this issue [20]. Recently, it was shown in rats in vivo that sevoflurane- and isoflurane-induced PostC was mediated by activation of mitochondrial adenosine triphosphate (ATP)-sensitive potassium channels [21,22]. It has also been shown that diabetes and hyperglycaemia can impair activation of mitochondrial ATP-sensitive potassium channels in dogs, thereby abolishing the cardioprotective effect of ischaemic PreC. In addition, Ghosh et al. [16] have suggested that failure to precondition the diabetic heart was due to dysfunction of the mitochondrial ATP-sensitive potassium channels. Although several signalling pathways have been identified in ischaemic and pharmacological PostC [17,20–22], they need to be studied in diabetic myocardium.

Desflurane administration has also been shown to result in β-adrenergic pathway stimulation in isolated human myocardium [5], and both desflurane-induced PreC and PostC have been found to be mediated, at least in part, through β-adrenoceptor stimulation [5,23]. In addition, the response to β-adrenoceptor stimulation is altered in rat diabetic myocardium related to β3-adrenoceptor subtype overexpression [24]. Although the precise role of β-adrenoceptor subtypes in myocardial PreC remains unknown, the decreased sensitivity of diabetic myocardium to desflurane-induced postC reported in the present study may be related to changes in the relative β-adrenoceptor subtype expression in diabetic myocardium. Indeed, further experimental studies are required to determine the precise role of β-adrenoceptor subtypes in myocardial PostC and in diabetic myocardium. The present study has several limitations that should be borne in mind when interpreting the results. First, the effects of anaesthetic drugs, diseases and medical treatments in the patients before the atrial specimens were removed cannot be completely ignored, although there were no differences in chronic medical treatments between groups (Table 1). More important, however, is that the patients included in the present study were representative of those in whom desflurane may be used and cardioprotective strategies applied. Most of our patients had been chronically treated with statin therapy, which has been shown to restore ischaemic PreC in the presence of hyperglycaemia [25]. Second, the effect of ageing has to be taken into account. Although no data are available on the effect of age on pharmacological PostC, it has been suggested that age may decrease anaesthetic-induced PreC in isolated human myocytes exposed to oxidative stress [26]. In the present study, only 17 trabeculae were obtained from donors ≤ 75 years old. However, the effect of ageing may predominate before age 60, but not after, as shown by contraction findings in isolated human myocardium [27]. Nevertheless, there was no significant difference in age between our groups (Table 1). Third, in several patients, intravenous insulin was used to control blood glucose
concentration before atrial appendage dissection (two patients in the controls, two in the NID-Des3, one in the NID-Des6 and one in the ID-Des6 group). Insulin has been shown to activate cell survival pathways, including the PI3-kinase/Akt-dependent pathway [28]. In addition, we studied right atrial myocardium, which is structurally and functionally different from ventricular myocardium [29]. However, for ethical reasons, it is not possible to obtain human ventricular myocardium by biopsy. Furthermore, our experiments were performed under conditions of moderate hypothermia (34°C) to ensure stability of the trabeculae over time. However, at present, there are no data on the PostC effects of hypothermia. This means that hypothermia may have decreased mitochondrial ATP-sensitive potassium channel sensitivity; however, during surgical procedures, moderate hypothermia may also occur. Fourth, rather than inducing myocardial ischaemia by coronary occlusion, we used a 30-min period of hypoxia to simulate ischaemia, as hypoxia has proved effective in inducing myocardial PostC. Finally, and most important of all, our present investigation included a control group that could have been equally affected by any of these potentially modifying factors.

In conclusion, the present study shows that the brief administration of desflurane in early reoxygenation was able to induce PostC in diabetic human right atrial myocardium in vitro. Also, it found that, to trigger desflurane-induced PostC, the concentration of desflurane administered had to be higher in diabetic myocardium compared with non-diabetic myocardium.

5. Conflicts of interest

The authors have not declared any conflicts of interest.

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References


