Helium-Induced Early Preconditioning and Postconditioning Are Abolished in Obese Zucker Rats in Vivo


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ABSTRACT

Preconditioning is abolished in the prediabetic Zucker obese rat. It has been shown that prevention of mitochondrial permeability transition pore (mPTP) opening is involved in preconditioning by the noble gas helium. Here, we investigated: 1) whether helium induces pre- and postconditioning in Zucker rats and 2) whether possible regulators of the mPTP [i.e., mitochondrial respiration or the extracellular signal-regulated kinase (Erk) 1/2, Akt/glycogen synthase kinase (GSK)-3β signaling pathway] are influenced. Anesthetized Zucker lean (ZL) and Zucker obese (ZO) rats were randomized to seven groups. Control animals were not treated (ZL-/ZO-Con). Preconditioning groups (ZL-/ZO-He-PC) inhaled 70% helium for 3 × 5 or 6 × 5 min, and postconditioning groups (ZL-/ZO-He-PostC) inhaled 70% helium for 15 min at the onset of reperfusion. Animals underwent 25 min of ischemia and 120 min of reperfusion. In additional experiments, hearts were excised after the third helium exposure for analysis of mitochondrial respiration and for Western blot analysis of Erk1/2, Akt, and GSK-3β phosphorylation. Helium reduced infarct size from 52 ± 3% (mean ± S.E.) to 32 ± 2% and 37 ± 2% in ZL rats (ZL-HE-PC, ZL-He-PostC), respectively, but not in ZO rats (ZO-He-PC, 56 ± 3%; ZO-He-PC (6×), 57 ± 4%; and ZO-He-PostC, 51 ± 3% versus ZO-Con, 54 ± 3%). Mitochondrial respiration analysis showed that helium causes mild uncoupling in ZL rats (2.27 ± 0.03 versus 2.51 ± 0.03) but not in ZO rats (2.52 ± 0.04 versus 2.52 ± 0.03). Helium had no effect on Erk1/2 and Akt phosphorylation. GSK-3β phosphorylation during ischemia was reduced after helium application in ZL but not in ZO rats. Helium-induced preconditioning is abolished in obese Zucker rats in vivo, probably caused by a diminished effect of helium on mitochondrial respiration.

Diabetes mellitus is a known risk factor for the development of ischemic heart disease and myocardial infarction (Haffner et al., 1998). It was shown that acute myocardial infarction is consistently associated with an increased mortality in patients with type 2 diabetes (Donnan et al., 2002). Furthermore, diabetes mellitus is associated with a loss of the protective potency of cardioprotective strategies, e.g., preconditioning, both in humans and animals (Donnan et al., 2002; del Valle et al., 2003; Katakam et al., 2007). Katakam et al. (2007) demonstrated that both ischemic and pharmacological preconditioning by the mitochondrial ATP-activated potassium (mKATP) channel agonist diazoxide is abolished in Zucker obese rats, a widely used animal model of insulin resistance and type 2 diabetes.

Recently, it was shown that exposure to the noble gas helium initiates a pronounced protection of the myocardium against ischemia reperfusion injury (Pagel et al., 2007). Helium is easy and safe to administer, and compared with volatile anesthetics or xenon, the absence of anesthetic effects and the lack of hemodynamic side effects would make helium an optimal agent for cardioprotection (Koblin et al., 1998; Gannier and Forel, 2006). These properties might offer the possibility to use helium in various groups of patients, e.g., during the perioperative period in patients at risk for cardiac events and in nonsurgical patients, e.g., in patients with unstable angina or myocardial infarction. Helium is already safely used in the therapy of asthma and chronic obstructive pulmonary disease and in young children with ventilation disorders (Gannier and Forel, 2006; Myers, 2006).

ABBREVIATIONS: mKATP, mitochondrial ATP-activated potassium; mPTP, mitochondrial permeability transition pore; GSK, glycogen synthase kinase; ZL, Zucker lean; ZO, Zucker obese; Con, control; He-PC, helium preconditioning; Erk, extracellular signal-regulated kinase; RCI, respiratory control index; mKCa, mitochondrial calcium-sensitive potassium channel; NS1619, 1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H-benimidazol-2-one.
Cardioprotective effects of helium are mediated by activation of prosurvival signaling kinases and prevention of mitochondrial permeability transition pore (mPTP) opening (Pagle et al., 2007). Opening of the mPTP can be regulated by different mechanisms including alterations in mitochondrial bioenergetics or regulation of glycogen synthase kinase (GSK)-3β activity (Juhászova et al., 2004; Halestrap et al., 2007). The underlying mechanism, however, by which helium confers cardioprotection via mPTP is unknown. We aimed to investigate: 1) whether the noble gas helium initiates cardiac preconditioning in the Zucker obese rat in vivo and 2) the underlying subcellular mechanism by which helium prevents mPTP opening, i.e., regulation of mitochondrial bioenergetics and/or inhibition of prosurvival kinase-dependent pathways.

Materials and Methods

All experiments were performed in accordance with the Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996) and were approved by the Institutional Committee for Animal Care and Use (Academic Medical Center Amsterdam, The Netherlands).

Materials

Helium was purchased from Linde Gas Benelux B.V. (Dieren, The Netherlands). KCl was purchased from EMD Biosciences (San Diego, CA); all antibodies were purchased from Cell Signaling Technology Inc. (Danvers, MA), except the anti-α-tubulin and the anti-actin antibodies (Sigma-Aldrich, Saint Louis, MO). All other chemicals were purchased from Sigma-Aldrich Chemie B.V. (Zwijndrecht, The Netherlands). Rat insulin samples were measured with a Rat Insulin ELISA from Orange Medical (Orange Medical, Tilburg, The Netherlands).

Surgical Preparation

Animals had free access to food and water at all times before the start of the experiments. Male Zucker lean rats (248 ± 5 g) and male Zucker obese rats (334 ± 5 g) were anesthetized by intraperitoneal injection of S-ketamine (150 mg/kg) and diazepam (1.5 mg/kg).

Surgical preparation was performed as described previously (Toma et al., 2004). In brief, after tracheal intubation, the lungs were ventilated with 30% oxygen and 70% nitrogen and a positive end-expiratory pressure of 2 to 3 cm of H2O. During the experiments, the end-tidal CO2 concentration was measured in the expiratory gas (Instrumentarium Corp., Helsinki, Finland). Respiratory rate was adjusted to maintain end-tidal CO2 between 35 and 45 mm Hg. Body temperature was maintained at 38°C by the use of a heating pad.

The right jugular vein was cannulated for saline and drug infusion, and the left carotid artery was cannulated for measurement of aortic pressure. Anesthesia was maintained by continuous 0-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 20 min before the start of the respective experimental protocol. Aortic pressure was digitized using an analog 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 version 5.0 (AD- Instruments Pty Ltd.).

Experimental Protocol

Rats were assigned to seven groups (Fig. 1). Animals for infarct size measurements underwent 25 min of coronary artery occlusion and 2 h of reperfusion (ischemia/reperfusion).

**Zucker Lean Control Group (ZL Con; n = 8).** After surgical preparation, rats received 30% oxygen/70% nitrogen.

**Zucker Lean Helium-Preconditioned Group (ZL He-PC; ZL He-PC; n = 8).** Rats received 70% helium/30% oxygen for three 5-min periods, interspersed with two 5-min wash-out periods 10 min before ischemia and reperfusion.

**Zucker Obese Control Group (ZO Con; n = 8).** After surgical preparation, rats received 30% oxygen/70% nitrogen.

**Zucker Obese Helium-Preconditioned Group (ZO He-PC; ZO He-PC; n = 8).** Rats received 70% helium/30% oxygen for three 5-min periods, interspersed with two 5-min wash-out periods 10 min before ischemia and reperfusion.

**Zucker Obese Helium-Preconditioned Group (ZO He-PC (6X); n = 8).** Rats received 70% helium/30% oxygen for six 5-min periods, interspersed with five 5-min wash-out periods 10 min before ischemia and reperfusion. In two additional groups, we investigated whether we could induce cardioprotection in ZO rats by helium postconditioning. In these groups, ZL and ZO rats (each n = 8) received 70% helium/30% oxygen for 15 min at the onset of reperfusion (Preckel et al., 2000).

Infarct Size Measurement

After 120 min of reperfusion, the heart was excised and mounted on a modified Langendorff apparatus for perfusion with ice-cold normal saline via the aortic root at a perfusion pressure of 80 cm of H2O to wash out intravascular blood. After 2 min of perfusion, the coronary artery was reoccluded, and the remainder of the myocardium was perfused through the aortic root with 0.2% Evans blue in normal saline for 10 min. Intravascular Evans blue was then washed out by perfusion for 10 min with normal saline. This treatment identified the area at risk as unstained. The heart was then cut into transverse slices, 2 mm thick. The slices were stained with 0.75% triphenyltetrazolium chloride solution for 10 min at 37°C, fixed in 4% formalin solution for 24 h at room temperature. The area of risk and the infarcted area were determined by planimetry using SigmaScan Pro 5 computer software (SPSS Inc., Chicago, IL) and corrected for dry weight of each slide.

To investigate the effects of helium preconditioning on mitochondrial respiration, additional experiments (n = 8 for each group) were conducted using the same preconditioning protocol, except that the hearts were excised 5 min after the third helium administration (see Fig. 1). The effect of helium preconditioning on phosphorylation of the enzymes GSK-3β (Ser9), Akt (Thr308 and Ser473), and extracellular-regulated kinase (Erk) 1/2 (p42/p44) were determined in additional experiments in groups 1 to 4 at four different time points (n = 4 for each time point in duplicate): time point 1 after the first helium administration, time point 2 after the third helium administration, time point 3 after 15 min of ischemia, and time point 4 after 15 min of reperfusion (see Fig. 1).
Mitochondrial Isolation

Heart mitochondria were isolated by differential centrifugation as described previously (Heinen et al., 2008b). In brief, atria were removed, and ventricles were placed in isolation buffer (200 mM mannitol, 50 mM sucrose, 5 mM KH2PO4, 5 mM 3-(n-morpholino)propanesulfonic acid, 1 mM ethylene glycol-bis(2-aminoethyl)ether)-N,N,N',N'-tetraacetic acid, 0.1% bovine serum albumin, pH 7.15 adjusted with KOH) and minced into 1-mm3 pieces. The suspension was homogenized for 15 s in 2.5 ml of isolation buffer containing 5 U/ml protease and for another 15 s after addition of 17 ml of isolation buffer. The suspension was centrifuged at 3220g for 10 min, the supernatant was removed, and the pellet was resuspended in 25 ml of isolation buffer and centrifuged at 800g for 10 min. The supernatant was centrifuged at 3220g for 10 min, and the final pellet was suspended in 0.5 ml of isolation buffer and kept on ice. Protein content was determined by the Bradford method (Bradford, 1976). All isolation procedures were conducted at 4°C.

Mitochondrial Respiration

Oxygen consumption was measured polarographically at 37°C using a respirometer system (System S 200A; Strathkelvin Instruments Limited, North Lanarkshire, UK). Mitochondria (0.3 mg/ml protein) were suspended in respiration buffer containing 130 mM KCl, 5 mM KH2PO4, 20 mM 5 mM 3-(n-morpholino)propanesulfonic acid, 2.5 mM ethylene glycol-bis(2-aminoethyl)ether)-N,N,N',N'-tetraacetic acid, 1 μM Na3P2O7, and 0.1% bovine serum albumin, pH 7.15, adjusted with KOH. Mitochondrial respiration was initiated by administration of 10 mM complex II substrate succinate (+10 μM complex I blocker rotenone) after 60 s. State 3 respiration was initiated after 120 s by the addition of 200 μM ADP. Respiration rates were recorded under state 3 conditions and after complete phosphorylation of ADP to ATP (state 4). The respiratory control index (RCI; state 3/state 4) and the P/O ratio (phosphate incorpo- rated into ATP/oxygen consumed) were calculated as a parameter of mitochondrial coupling between respiration and oxidative phosphorylation, and mitochondrial efficiency, respectively. From each heart, respiration measurements were repeated in three mitochondrial samples, and the average was taken (and counted as n = 1). Respiration rates are expressed as absolute rates in nanomoles of O2 per milligram per minute.

Separation of Cytosolic Fraction

For cellular fractionation and subsequent Western blot assay, tissue specimens were prepared for protein analysis of GSK-3β (Ser9), Akt (Thr308 and Ser473), and Erk1/2 (p42/p44), respectively. The excised hearts were frozen in liquid nitrogen. A cellular fractionation was performed subsequently, as described previously (Weber et al., 2006). The frozen tissue was pulverized and dissolved in lysis buffer containing Tris base, EGTA, NaF, and Na3VO4 (as phosphatase inhibitors), a freshly added protease inhibitor mix (aproti- nin, leupeptin, and pepstatin), and dithiothreitol. The solution was vigorously homogenized on ice (Homogenisator; IK-A-Werke GmbH & Co. KG, Staufen, Germany) and then centrifuged at 1000g, 4°C, for 10 min. The supernatant, containing the cytosolic fraction, was cen- trifuged again at 16,000g, 4°C, for 15 min to clean up this fraction for further Western blot assay.

Western Blot Analysis

After protein concentration was determined by the Lowry method (Lowry et al., 1951), equal amounts of protein were prepared and loaded on a 10% SDS-polyacrylamide gel electrophoresis gel. The proteins were separated by electrophoresis (100 V, 85 min) and then transferred to a polyvinylidene difluoride membrane by tank blotting (100 V, 1 h). To prevent unspecific antibody binding, the membrane was subsequently blocked with 5% skimmed milk solution in Tris-buffered saline containing Tween for 2 h. Then, the membrane was incubated overnight at 4°C with the respective primary antibody GSK-3β (1:10,000), Akt (Thr308) (1:10,000), or Akt (Ser473) (1:10,000), Erk1/2 (p42/p44) (1:10,000). After washing in fresh, cold Tris-buffered saline containing Tween, the blot was subjected to the appropriate horseradish peroxidase-conjugated secondary antibody for 2 h at room temperature. Immunoreactive bands were visualized by chemiluminescence detected on X-ray film (Amersham Hyperfilm ECL; GE Healthcare, Chalfont St. Giles, UK) using the enhanced chemiluminescence system Santa Cruz (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). The blots were quantified using a Kodak Image station (Eastman Kodak, Rochester, NY), and the results are presented as ratio of phosphorylated protein to total protein. Equal loading of protein on the gel was additionally proved by the detection of α-tubulin or actin, respectively, and Coomassie Blue staining of the gels.

Statistical Analysis

Data are expressed as mean ± S.E.M. Heart rate (in beats per minute) and mean aortic pressure (in millimeters of mercury) were measured during baseline, coronary artery occlusion, and reperfu- sion period. Comparisons of hemodynamics between groups or be- tween time points in a group were performed (SPSS Science Software, version 12.0.1) using two-way analysis of variance followed by Tukey’s post hoc test. Infarct sizes were analyzed by one-way analysis of variance followed by Tukey’s post hoc test. Data from mito- chondrial and Western blot experiments were analyzed by Student’s t test with Bonferroni’s correction for multiple comparisons. Changes within and between groups were considered statistically significant if p < 0.05.

Results

Infarct Size Measurement. Helium preconditioning re-duced infarct size in ZL rats from 52 ± 3% in controls to 32 ± 2% (p < 0.05; Fig. 2). In ZO control rats, infarct size was similar to ZL controls (54 ± 3%, N.S. versus ZL Con). In contrast to the protection seen in ZL rats, in ZO rats helium did not reduce infarct size (56 ± 3%, N.S.; Fig. 2). Further- more, an increased preconditioning stimulus by six cycles of helium could not protect the ZO rat heart (57 ± 4%, N.S.; Fig. 2). Helium postconditioning reduced infarct size in ZL rats (37 ± 2%, p < 0.05; Fig. 2). This effect was also completely abolished in ZO rats (51 ± 3%, N.S.; Fig. 2).

Hemodynamic Variables. Hemodynamic variables are summarized in Table 1. No significant differences in heart rate and aortic pressure were observed between the experimental groups during baseline, ischemia, and reperfusion. At the end of the experiments, mean aortic pressure was signif- icantly decreased compared with baseline in all groups, with the exception of the ZO control group.

Weights and Blood Glucose Levels. The body weights (grams) of ZO rats were significantly higher than in ZL rats (Table 2). Blood glucose levels were not different between groups (Table 2). Insulin levels were significantly higher in ZO compared with ZL rats (Table 2).

Mitochondrial Respiration. Mitochondrial respiration results are summarized in Fig. 3. There was no significant difference in the RCI between ZL (n = 8) and ZO (n = 7) control rats (2.51 ± 0.03 versus 2.52 ± 0.03, N.S.). Helium preconditioning reduced the RCI in ZL rats (2.27 ± 0.03; n = 8; p < 0.05 versus ZL Con) but had no effect on the RCI in ZO rats (2.52 ± 0.04; n = 8; N.S. versus ZO Con). The reduction in the RCI in ZL He-PC was caused by an increase in state 4 respiration (155 ± 4 versus 139 ± 3 nmol O2/mg/min, p < 0.05); state 3 respiration was not affected by helium precon-
Data are mean ± S.E. *p < 0.05 versus ZL Con.

**Discussion**

The main findings of our study are that the cardioprotective effect of helium-induced preconditioning: 1) is abolished in the prediabetic rat heart and 2) is mediated in the nondiabetic heart rather than regulation of mitochondrial respiration, i.e., mild mitochondrial uncoupling, than by activation of pro-survival signaling kinases. We also demonstrate that there is a similar effect of helium-induced postconditioning.

**Regulation of GSK-3β, Akt, and Erk1/2 Phosphorylation during Helium Preconditioning.** Figure 4 shows that there was no difference at any time point in Akt and Erk1/2 phosphorylation during the experiments in ZL and ZO rats. Helium reduced GSK-3β phosphorylation during ischemia in ZL rats compared with respective controls (0.49 ± 0.07 versus 0.72 ± 0.07, p < 0.05; Fig. 4A3).

**TABLE 1**

<table>
<thead>
<tr>
<th>Hemodynamic variables</th>
<th>Baseline</th>
<th>Washout 3</th>
<th>Ischemia: 15 Min</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZL Con</td>
<td>419 ± 9</td>
<td>428 ± 9</td>
<td>435 ± 8</td>
<td>378 ± 17</td>
</tr>
<tr>
<td>ZL He-PC</td>
<td>417 ± 12</td>
<td>420 ± 11</td>
<td>423 ± 8</td>
<td>387 ± 11</td>
</tr>
<tr>
<td>ZL He-PostC</td>
<td>407 ± 12</td>
<td>389 ± 5</td>
<td>391 ± 6</td>
<td>360 ± 10</td>
</tr>
<tr>
<td>ZO Con</td>
<td>414 ± 12</td>
<td>397 ± 11</td>
<td>406 ± 18</td>
<td>381 ± 18</td>
</tr>
<tr>
<td>ZO He-PC</td>
<td>416 ± 14</td>
<td>410 ± 8</td>
<td>415 ± 7</td>
<td>398 ± 11</td>
</tr>
<tr>
<td>ZO He-PC (6x)</td>
<td>407 ± 7</td>
<td>389 ± 13</td>
<td>404 ± 5</td>
<td>375 ± 9</td>
</tr>
<tr>
<td>ZO He-PostC</td>
<td>400 ± 7</td>
<td>392 ± 3</td>
<td>398 ± 10</td>
<td>361 ± 9</td>
</tr>
<tr>
<td>Mean AOP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZL Con</td>
<td>116 ± 6</td>
<td>105 ± 9</td>
<td>96 ± 7</td>
<td>80 ± 6*</td>
</tr>
<tr>
<td>ZL He-PC</td>
<td>120 ± 7</td>
<td>103 ± 5</td>
<td>90 ± 8</td>
<td>74 ± 9*</td>
</tr>
<tr>
<td>ZL He-PostC</td>
<td>110 ± 5</td>
<td>95 ± 4</td>
<td>87 ± 6*</td>
<td>70 ± 5*</td>
</tr>
<tr>
<td>ZO Con</td>
<td>111 ± 14</td>
<td>106 ± 15</td>
<td>100 ± 17</td>
<td>81 ± 11</td>
</tr>
<tr>
<td>ZO He-PC</td>
<td>120 ± 4</td>
<td>113 ± 4</td>
<td>107 ± 7</td>
<td>91 ± 10</td>
</tr>
<tr>
<td>ZO He-PC (6x)</td>
<td>117 ± 3</td>
<td>101 ± 6</td>
<td>103 ± 4</td>
<td>86 ± 4*</td>
</tr>
<tr>
<td>ZO He-PostC</td>
<td>121 ± 2</td>
<td>117 ± 3</td>
<td>106 ± 6</td>
<td>83 ± 5*</td>
</tr>
</tbody>
</table>

He-PostC, helium postconditioning.

*p < 0.05 vs. baseline.
The mechanism by which helium-induced preconditioning is blocked in the prediabetic heart is unknown. Ischemic and pharmacological preconditioning failed to protect the diabetic myocardium, possibly caused by dysfunctional potassium channels in the inner mitochondrial membrane (Hassouna et al., 2006). Alterations in mitochondrial function caused by potassium channel activation have been proposed to protect the myocardium by reducing mPTP opening (Halestrap et al., 2007). Helium-induced preconditioning is abrogated by administration of the mPTP opener atractyloside (Pagel et al., 2007). It also has been suggested that preconditioning prevents mPTP opening by regulation of prosurvival signaling kinases, including Erk1/2, Akt, and GSK-3β, and/or by regulation of mitochondrial bioenergetics, i.e., mild uncoupling of mitochondrial respiration (Halestrap et al., 2007; Nishihara et al., 2007). Pagel et al. (2008a) demonstrated that helium preconditioning is abolished by 5-hydroxydecanoate, an mKATP channel blocker. It is interesting that activation of the K_{ATP} channel with diazoxide could not induce preconditioning in the Zucker obese rat heart (Katakam et al., 2007), suggesting that the blockade of diazoxide-induced preconditioning is related to defects at the level of the K_{ATP} channel or its downstream signaling cascade. We demonstrated very recently that opening of another mitochondrial potassium channel, namely the mitochondrial calcium-sensitive potassium channel (mK_{Ca}), is involved in helium-induced preconditioning (Heinen et al., 2008a). This study, together with the study from Pagel et al. (2008a), suggests a crucial role of mitochondrial potassium channels in helium preconditioning. mK_{Ca} channel opening causes a slight increase in mitochondrial reactive oxygen species generation (Heinen et al., 2007). Stowe et al. (2006) demonstrated that the cardioprotective effect of the mK_{Ca} channel agonist NS1619 requires superoxide radical generation during the preconditioning stimulus. Furthermore, preconditioning by NS1619 reduces mitochondrial calcium overload and mitochondrial reactive oxygen species production during the subsequent period of ischemia and early reperfusion (Stowe et al., 2006). Such a reduction in mitochondrial calcium overload and reactive oxygen species generation has been suggested to prevent mPTP opening (Halestrap et al., 2007; Lim et al., 2007). Both mK_{ATP} and mK_{Ca} channel activation trigger preconditioning independent from each other and by involvement of the mPTP (Cao et al., 2005).

Our results suggest that regulation of mitochondrial respiration is involved in helium-induced preconditioning; helium induced a reduction of the respiratory control index in

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood Sugar</th>
<th>Insulin Levels</th>
<th>Body Weight</th>
<th>Heart Dry Weight</th>
<th>Area at Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mM</td>
<td>mM</td>
<td>g</td>
<td></td>
<td>%</td>
</tr>
<tr>
<td>ZL Con</td>
<td>6.7 ± 0.1</td>
<td>0.24 ± 0.05</td>
<td>244 ± 10</td>
<td>0.176 ± 0.007</td>
<td>24 ± 5</td>
</tr>
<tr>
<td>ZL He-PC</td>
<td>6.3 ± 0.6</td>
<td>0.26 ± 0.06</td>
<td>262 ± 10</td>
<td>0.172 ± 0.008</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>ZL He-PostC</td>
<td>6.0 ± 0.1</td>
<td>0.20 ± 0.05</td>
<td>238 ± 4</td>
<td>0.174 ± 0.006</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>ZO Con</td>
<td>6.9 ± 0.4</td>
<td>2.20 ± 0.48</td>
<td>315 ± 12</td>
<td>0.182 ± 0.007</td>
<td>23 ± 3</td>
</tr>
<tr>
<td>ZO He-PC</td>
<td>7.8 ± 0.8</td>
<td>2.18 ± 0.42</td>
<td>330 ± 10</td>
<td>0.186 ± 0.008</td>
<td>23 ± 5</td>
</tr>
<tr>
<td>ZO He-PC (6×)</td>
<td>6.4 ± 0.2</td>
<td>2.65 ± 0.38</td>
<td>343 ± 4*</td>
<td>0.194 ± 0.002</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>ZO He-PostC</td>
<td>6.2 ± 0.2</td>
<td>2.64 ± 0.68</td>
<td>348 ± 4*#†</td>
<td>0.186 ± 0.005</td>
<td>18 ± 3</td>
</tr>
</tbody>
</table>

* He-PostC, helium postconditioning
# p < 0.05 vs. ZL Con.
† p < 0.05 vs. ZL He-PC.
Fig. 4. Effects of helium-induced preconditioning on GSK-3β (Ser9) (A), Akt (Thr308 and Ser473) (B and C), and Erk1/2 (D and E) phosphorylation. Summarized data presenting ratio of phosphorylated enzyme to total enzyme are shown. Time point 1 after the first helium administration (A1–E1, respectively), time point 2 after the third helium administration (A2–E2, respectively), time point 3 after 15 min of ischemia (A3–E3, respectively), time point 4 after 15 min of reperfusion (A4–E4, respectively). LC, Zucker lean control; OC, Zucker obese control; LH, Zucker lean helium preconditioning; OH, Zucker obese helium preconditioning. Data are presented as mean ± S.E. *p < 0.05 versus LC.
enzymes reported to be involved in helium preconditioning is mediated by endothelial but not by potassium channels in the present study. It was shown that mitochondrial uncoupling is capable of inducing cardioprotection; pharmacological uncoupling by 2,4-dinitrophenol or FCCP reduced infarct size in rat heart in vitro (Minners et al., 2000; Brennan et al., 2006a,b).

The role of prosurvival signaling kinases in helium-induced preconditioning remains unclear. In the rabbit heart, the protective effect of helium was blocked by pharmacological inhibition of phosphatidylinositol-3-kinase, extracellular signal-regulated kinase, and 70-kDa ribosomal protein s6 kinase. Hausenloy et al. (2005) demonstrated for ischemic preconditioning that Akt and Erk1/2 were phosphorylated before and after the ischemic period compared with control group. In the current study, we tested time-dependent phosphorylation of GSK-3β, Akt, and Erk1/2. We did not detect an effect of helium on Erk1/2 and Akt phosphorylation. GSK-3β shows a decreased activity in Zucker lean heart-treat rats after 15 min of ischemia compared with respective controls. The importance of Akt phosphorylation in the signal transduction of ischemic preconditioning was demonstrated by Tsang et al. (2005) in their study, preconditioning caused an increased Akt phosphorylation 5 min after the last preconditioning cycle, i.e., the same timing of tissue sampling as we used in the present study. However, to our knowledge, there is no evidence that “prosurvival kinases” activate mitochondrial K<sub>m</sub> channels to regulate mitochondrial function. It was shown recently that adenomedullin treatment before ischemia reduces infarct size via protein kinase A-mediated activation of m<sub>K<sub>m</sub></sub> channels (Nishida et al., 2008). This effect was independent of phosphatidylinositol-3-kinase. In the present study, we did not test whether protein kinase A is involved in helium preconditioning.

The results of the present study have to be interpreted within the scope of some limitations. First, our experiments were conducted in Zucker obese and Zucker lean rats. Zucker obese rats have a lephti receptor mutation and develop obesity at an early age (Zucker and Antoniades, 1972; Bray, 1977). The Zucker obese rat is described to be hyperphagic compared with lean littersmates from an early age on, and the obese condition is evident at 5 weeks. In the present study, feeding of the Zucker obese rat was not different from Zucker lean rats. However, we did not measure differences in caloric intake. Second, in the present study, we did not investigate the effect of helium on the m<sub>PTP</sub> directly. However, it has already been demonstrated that helium confers cardioprotection by prevention of m<sub>PTP</sub> opening (Pagel et al., 2007). Therefore, the present study was designed to investigate the effect of helium on possible regulators of the m<sub>PTP</sub> (i.e., mitochondrial respiration, GSK-3β phosphorylation). Third, we did not investigate other possible avenues of preconditioning like endothelial or inducible nitric oxide synthase and/or potassium channels in the present study. It was shown that mitochondrial preconditioning is mediated by endothelial but not by inducible nitric oxide synthase (Pagel et al., 2008b). All these enzymes reported to be involved in helium preconditioning are located upstream of mitochondrial potassium channels and the mitochondria. Abolished mitochondrial uncoupling in the Zucker obese rat heart before lethal ischemia, together with no effects on the expression of enzymes of the prosurvival cascade, suggest a blockade of cardioprotection in the preischemic heart caused by yet unknown mechanisms.

Taken together, the present study demonstrates that the noble gas helium can induce pre- and postconditioning in the rat heart in vivo. The protective effect of preconditioning could be explained by mild mitochondrial uncoupling, an alteration that is capable to prevent m<sub>PTP</sub> opening. Furthermore, the protective potency of helium-induced preconditioning is completely abrogated in the Zucker obese rat, a widely used animal model for metabolic preconditioning of diabetics and for the study of other diabetics. Whether this cardioprotection can be re-established in the preischemic heart by further pharmacological intervention needs further investigation.

References


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