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Chapter 5

Helium-induced preconditioning in young and old rat heart - Impact of mK_{Ca} channel activation

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ABSTRACT

The noble gas helium induces cardiac preconditioning. It is unknown whether helium-preconditioning is mediated by activation of mitochondrial K⁺ channels. We investigated if helium preconditioning is 1) mediated by activation of K_{Ca} channels, 2) results in mitochondrial uncoupling, and 3) whether helium-preconditioning is age-dependent. Anesthetized Wistar rats were assigned to one of six groups (each n = 10). Young (2-3 months) and aged (22-24 months) control animals were not further treated (Con and Age-Con). Preconditioning groups (He-PC and Age-He-PC) inhaled 70% helium for 3x5 min. The K_{Ca} blocker Iberitoxin (Ibtx, 6 µg kg⁻¹ min⁻¹) was administered in young animals, with and without helium (Ibtx+He-PC and Ibtx). Animals were exposed to 25 min regional myocardial ischemia followed by 120 min reperfusion, and infarct size was determined. In additional experiments, cardiac mitochondria were isolated and the respiratory control index (RCI) was determined as state 3 respiration / state 4 respiration. Helium reduced infarct size in young rats from 61±7 % to 36±14 % (P<0.05 vs. Con). Infarct size reduction was abolished by Iberitoxin (60±11 %; P<0.05 vs. He-PC), whereas Ibtx alone had no effect (59±8 %; n.s. vs. Con). In aged animals Helium had no effect on infarct size (Age-Con: 59±7 % vs. Age-He-PC: 58±8 %, n.s.). Helium reduced the RCI in young rats (2.76±0.05 to 2.43±0.15, P<0.05) but not in aged animals (Age-Con: 2.87±0.17 vs. Age-He-PC: 2.87±0.07, n.s.). Ibtx abrogated the effect of helium on RCI (2.73±0.15, P<0.05 vs. He-PC), but had no effect on mitochondrial respiration alone (2.75±0.05; n.s. vs. Con). Helium causes mitochondrial uncoupling, and induces preconditioning in young rats via K_{Ca} channel activation. However, these effects are lost in aged rats.

INTRODUCTION

Ischemic heart disease, with its clinical consequences of acute myocardial infarction, sudden cardiac death, arrhythmias and heart failure is the leading cause of morbidity and mortality in industrialized nations. Several studies demonstrated tissue protective effects of PC during ischemia-reperfusion interventions, both in animals (15; 16; 23) and humans. (4; 5)

However, most of these studies were conducted in young and healthy animals. The morbidity and mortality of myocardial infarction is increased with increasing age, (6; 7; 19) possibly partly due to an aging related loss of the protective potency of cardioprotective strategies, e.g. preconditioning (PC). (1; 11; 12; 32) The underlying reason for this loss of cardioprotection in the senescent heart is unknown. Lee et al. (12) demonstrated a loss of protection in elderly patients (older than 65 years) undergoing coronary angioplasty compared to patients younger than 55 years. Since a prolonged period of ischemia and the mitochondrial ATP-sensitive potassium channel activator nicorandil were able to (re)initiate a preconditioning state in the older patients, the authors concluded that the impaired preconditioning response is caused by some defects in signal transduction of activation of ATP-sensitive potassium (K_{ATP}) channels with aging. There is evidence that regulation of mitochondrial function by activation of potassium (K^+) channels in the inner mitochondrial membrane with the consequence of K^+ influx into the mitochondrial matrix is a key step in the signal transduction cascade of PC. (22; 25) Recently, we discovered that the effect of activation of calcium sensitive potassium (K_{Ca}) channels on mitochondrial function is age dependent. (10) It was shown that activation of this channel is critically involved in the signal transduction pathway of PC. (30)

A recent study demonstrated that the noble gas helium is able to mimic the cardioprotective effect of PC. (28) Helium confers cardioprotection via modulation of the mitochondrial permeability transition pore (mPTP). (28) It is suggested that opening of the mPTP can be prevented by alterations in mitochondrial function. (8) However, it is unknown whether helium-induced preconditioning is mediated by K_{Ca} channels with the consequence of altered mitochondrial respiration, and whether helium initiates preconditioning in the senescent heart. Here, we hypothesize that helium-induced preconditioning 1) is mediated by activation of K_{Ca} channels, 2) results in mitochondrial uncoupling, and 3) is abolished in the aged myocardium.

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MATERIALS AND METHODS

The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and was performed in accordance with the requirements of the Animal Ethics Committee of the University of Amsterdam.

Materials

Helium was purchased from Linde Gas (Linde Gas Benelux BV, Dieren, the Netherlands). KCl was purchased from EMD Chemicals (Gibbstown, NJ); all other chemicals were purchased from Sigma Chemical Co. (Taufkirchen, Germany). The polyclonal K_{Ca} channel beta 1 subunit antibody and the immunizing peptide were purchased from Abcam (Cambridge, UK).

Surgical preparation and experimental protocol for infarct size determination

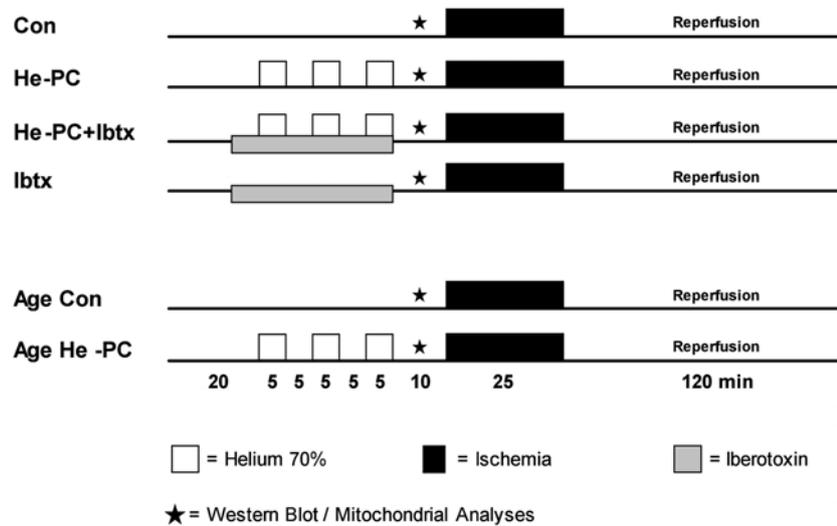
Animals had free access to food and water at all times before the start of the experiments. Young (3-4 months) male Hannover Wistar rats (352 ± 15 g) and old (22-24 months) male Hannover Wistar rats (621 ± 34 g) were anesthetized by intraperitoneal S-ketamine injection (150 mg/kg) and diazepam (1.5 mg/kg).

Surgical preparation was performed as described previously.(26; 33) In brief, after tracheal intubation, the lungs were ventilated, and respiratory rate was adjusted to maintain *PCO*₂ within physiological limits. 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 α -chloralose infusion. A lateral left sided thoracotomy 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 minutes before the start of the respective experimental protocol. Aortic pressure was digitized using an analogue to digital converter (PowerLab/8SP, ADInstruments Pty Ltd, Castle Hill, Australia) at a sampling rate of 500 Hz and was continuously recorded on a personal computer using Chart for Windows v5.0 (ADInstruments).

Rats were divided into six groups (Fig. 1):

All animals underwent 25 min of coronary artery occlusion and 2 hours of reperfusion (I/R).

Figure 1: Experimental protocol



Control group (Con) (n = 10): After surgical preparation, rats received 30% oxygen plus 70% nitrogen.

Helium preconditioned group (He-PC) (n = 10): Rats received Helium 70% for three 5-min periods, interspersed with two 5-min wash-out periods 10 min before I/R. The other 30% gas consisted of 30% oxygen.

Helium preconditioned group with Iberiotoxin (He-PC+Ibtx) (n = 10): Rats received Helium 70% for three 5-min periods, interspersed with two 5-min wash-out periods 10 min before I/R. The other 30% gas consisted of 30% oxygen. Ibtx was administered continuously over a time period of 30 minutes starting 5 min prior to the first preconditioning stimulus.

Iberiotoxin group (Ibtx) (n = 10): Rats received Ibtx continuously over a time period of 30 minutes starting 5 min prior to the first preconditioning stimulus.

Aged control group (Age Con) (n = 10): After surgical preparation, rats received 30% oxygen plus 70% nitrogen.

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Aged Helium preconditioned group (Age He-PC) (n = 10): Rats received Helium 70% for three 5-min periods, interspersed with two 5-min wash-out periods 10 min before I/R. The other 30% gas consisted of 30% oxygen.

Infarct size measurement

After 120 minutes 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 H₂O in order to wash out intravascular blood. After 5 minutes of perfusion, the coronary artery was re-occluded and the remainder of the myocardium was perfused through the aortic root with 0.2% Evans blue in normal saline for 10 minutes. Intravascular Evans blue was then washed out by perfusion for 10 minutes 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 minutes at 37°C, and fixed in 4% formalin solution for 24 hours at room temperature. The area of risk and the infarcted area were determined by planimetry using SigmaScan Pro 5[®] computer software (SPSS Science Software, Chicago, IL).

For mitochondrial respiration and Western Blot analysis additional experiments (each n = 8) were performed. Hearts were excised 5 min before the onset of ischemia (total baseline 50 min).

Mitochondrial isolation

Heart mitochondria were isolated by differential centrifugation as described previously.⁽¹⁰⁾ Briefly, atria were removed and ventricles were placed in isolation buffer [200 mmol/L mannitol, 50 mmol/L sucrose, 5 mmol/L KH₂PO₄, 5 mmol/L 3-(n-morpholino) propanesulfonic acid (MOPS), 1 mmol/L Ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), 0.1% bovine serum albumin (BSA), pH 7.15 adjusted with KOH], and minced into 1 mm³ pieces. The suspension was homogenized for 15 sec in 2.5 ml isolation buffer containing 5 U/ml protease (from *Bacillus licheniformis*, Enzyme Commission Number 3.4.21.14), and for another 15 sec after addition of 17 ml isolation buffer. The suspension was centrifuged at 3220g for 10 min, the supernatant was removed, and the pellet was resuspended in 25 ml 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 isolation buffer and kept on ice. Protein content was determined by the Bradford method. All isolation procedures were conducted at 4°C.

Mitochondrial respiration

Oxygen consumption was measured polarographically at 37°C using a respirometric system (System S 200A, Strathkelvin Instruments, Glasgow, Scotland). Mitochondria (0.3 mg protein/ml) were suspended in respiration buffer containing 130 mmol/L KCl, 5 mmol/L K₂HPO₄, 20 mmol/L MOPS, 2.5 mmol/L EGTA, 1 μmol/L Na₄P₂O₇, 0.1% BSA, pH 7.15 adjusted with KOH. Mitochondrial respiration was initiated by administration of 10 mmol/L complex II substrate succinate (+10 μmol/L complex I blocker rotenone) after 60 sec. State 3 respiration was initiated after 120 sec by addition of 200 μmol/L adenosine-diphosphate (ADP). Respiration rates were recorded under state 3 conditions and after complete phosphorylation of ADP to adenosine-triphosphate (ATP) (State 4). The respiratory control index (RCI, state 3/state 4) and the P/O ratio (phosphate incorporated into ATP to oxygen consumed) were calculated as parameter of mitochondrial coupling between respiration and oxidative phosphorylation, and mitochondrial efficiency, respectively. From each heart, respiration measurements were repeated in 3 mitochondrial samples and the average was taken (and counted as n=1). Respiration rates are expressed as absolute rates in nmol O₂/mg/min.

Western blot analysis

The content of K_{Ca} channels in the mitochondria was determined by Western blot analysis. 100 μl of mitochondrial suspension was treated with 5 μl Triton X 100 (10%), 20 μl KCL (4.5 M), and protease inhibitor mix (aprotinin, leupeptin and pepstatin), stirred, and incubated at room temperature for 5 min. After centrifugation (10000 g, 5 min), the protein concentration was determined by the Lowry method. (18) Subsequently, equal amounts of mitochondrial protein (30 μg) were mixed with loading buffer (1:1) containing Tris-HCl, glycerol and bromphenol blue. Samples were loaded on a 12% SDS-PAGE gel, separated by electrophoresis and transferred to a PVDF membrane by tank blotting (100V, 2h). Unspecific binding of the antibody was blocked by incubation with 5% skimmed milk solution in Tris buffered saline containing Tween (TBS-T) for 2 hours. Subsequently, the membrane was incubated over night at 4°C with the K_{Ca} channel beta 1 subunit antibody (1:1000). After washing in fresh, cold TBS-T, the blot was subjected to the appropriate horseradish peroxidase conjugated secondary antibody for 2 hours at room temperature. Immunoreactive bands were visualized by chemiluminescence and detected on X-ray film (Hyperfilm ECL, Amersham) using the enhanced chemiluminescence system Santa Cruz. The blots were quantified using a Kodak Image Station[®] (Eastman Kodak Comp., Rochester, NY) and the results are presented as ratio of K_{Ca} beta1 subunit (arbitrary units)

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to citrate synthase activity (mU/mg). Equal loading of protein on the gel was additionally proved by Coomassie blue staining of the gels. For identification of the specific K_{Ca} beta 1 subunit band, additional blocking experiments were conducted using the immunizing peptide in a large molar excess (~70 fold) for competitive inhibition of antibody-protein binding.

Determination of enzyme activities

Citrate synthase (CS) activity, a mitochondrial marker, was measured according to standard spectrophotometric procedures (2) and served as a control for Western blot results of mitochondrial K_{Ca} channels. It was shown that CS activity does not change with increasing age. (21; 29)

Statistical Analysis

Data are expressed as mean \pm SD. Heart rate (HR, in bpm) and mean AOP (AOP_{mean}, in mmHg) were measured during baseline, coronary artery occlusion, and reperfusion period. Inter-group differences of hemodynamic data were analyzed (SPSS Science Software, version 12.0.1) by performing a One-way ANOVA followed by Tukey's post-hoc test. Time effects (changes from baseline value) during the experiments were analyzed by using a One-way ANOVA followed by Dunnett's post-hoc test. Infarct sizes were analyzed by a One-way ANOVA followed by Tukey's post-hoc test. Changes within and between groups were considered statistically significant if $p < 0.05$. Mitochondrial respiration results and Western blot data were analyzed by a One-way ANOVA followed by Tukey's post-hoc test.

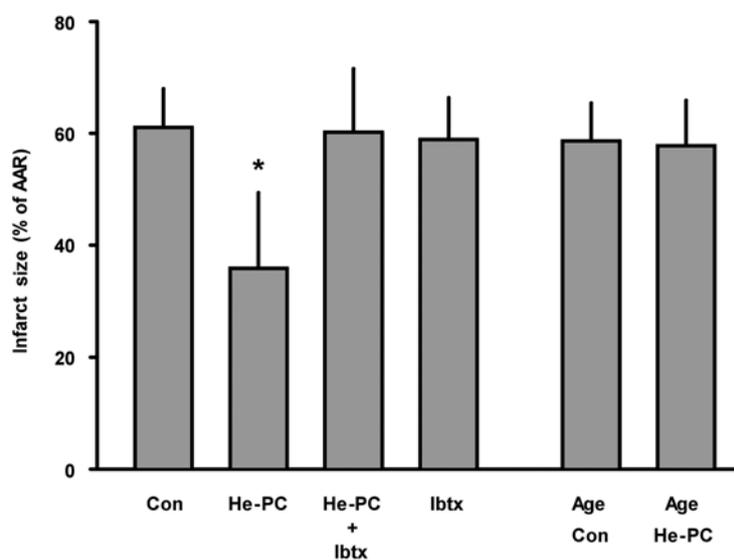
RESULTS

Infarct size measurement

Helium-induced preconditioning reduced infarct size in young animals from 61 ± 7 % in controls (n = 10) to 36 ± 14 % (n = 10, $P < 0.05$, Fig. 2). Administration of Iberiotoxin during the preconditioning period (n = 10) completely abolished cardioprotection (60 ± 11 %; ns vs. Con). Iberiotoxin alone (n = 10) had no effect on infarct size (59 ± 8 %; ns vs. Con). Infarct size in aged controls (n = 10) was comparable to young controls (59 ± 7 %). In contrast to young rats, Helium did not reduce infarct size in aged rats (58 ± 8 %, n = 10, ns vs. Age Con, 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 or reperfusion. At the end of the experiments, mean aortic pressure and heart rate were significantly decreased compared with baseline in all groups.

Figure 2: Infarct size measurement

Histogram shows the infarct size (percent of area at risk, AAR) of controls (Con), preconditioning with 70% Helium (He-PC), preconditioning with 70% Helium combined with Iberitoxin (He-PC+Ibtx), Iberitoxin alone (Ibtx), controls in aged rats (Age Con) and preconditioning in aged rats with 70% Helium (Age He-PC). Data are presented as mean \pm SD, * $p < 0.05$ vs. control group.

Mitochondrial function

The respiratory control indices are shown in figure 3. There was no significant difference in the RCI between young ($n = 8$) and aged ($n = 8$) control rats (2.76 ± 0.05 vs. 2.87 ± 0.10 , ns). Helium preconditioning reduced the RCI in young rats ($n = 8$; 2.43 ± 0.12 , $p < 0.05$ vs. Con), but had no effect on the RCI in aged rats ($n = 8$; 2.87 ± 0.09 , ns vs. Age Con). RCI reduction was completely abolished by administration of the mK_{Ca} channel blocker Ibtx (2.73 ± 0.15 , ns vs. Con), while Ibtx itself had no effect on RCI (2.75 ± 0.05 , ns vs. Con).

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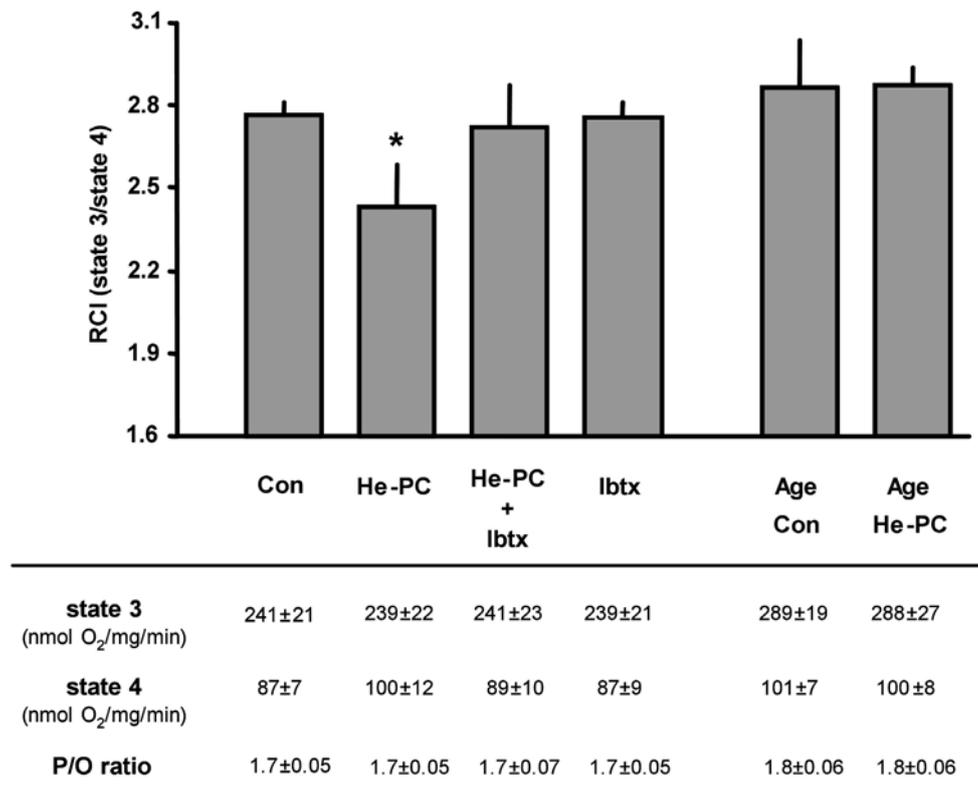
There was no difference between all groups in the efficiency of oxidative phosphorylation as demonstrated by no changes in the P/O ratio.

Table 1: Hemodynamic variables

	Baseline	Washout 3	Ischemia	Reperfusion	
			15	30	120
<i>Heart rate (bpm)</i>					
Con	445 ± 27	428 ± 35	424 ± 35	397 ± 36	369 ± 33*
He-PC	448 ± 12	443 ± 21	449 ± 27	397 ± 36*	374 ± 32*
He-PC+Ibtx	435 ± 29	417 ± 31	426 ± 28	390 ± 23*	361 ± 25*
Ibtx	461 ± 31	439 ± 27	459 ± 20	429 ± 27	385 ± 40*
Age Con	421 ± 28	405 ± 24	410 ± 28	374 ± 37*	323 ± 44*
Age He-PC	409 ± 27	407 ± 28	402 ± 29	367 ± 33*	334 ± 34*
<i>Mean aortic pressure (mmHg)</i>					
Con	127 ± 18	112 ± 20	101 ± 22	91 ± 23*	68 ± 11*
He-PC	133 ± 25	125 ± 23	108 ± 31	95 ± 23*	76 ± 19*
He-PC+Ibtx	117 ± 21	119 ± 17	101 ± 25	88 ± 21*	72 ± 9*
Ibtx	127 ± 24	129 ± 16	124 ± 16	94 ± 18*	65 ± 17*
Age Con	114 ± 26	114 ± 18	115 ± 24	98 ± 21	78 ± 21*
Age He-PC	119 ± 21	120 ± 20	111 ± 25	92 ± 16*	77 ± 19*

Data are Mean ± SD. Con = control group; Age = aged rats; He-PC = Helium preconditioning; Ibtx = Iberiotoxin. *P<0.05 vs. baseline.

Figure 3: Mitochondrial respiration



Summarized data for the effects of Helium-induced preconditioning on mitochondrial respiration. RCI = respiratory control index, a parameter for the coupling between mitochondrial respiration and oxidative phosphorylation. P/O ratio = ratio between phosphate incorporated into adenosine-triphosphate and oxygen consumed; a parameter for the efficiency of oxidative phosphorylation. Data are presented as mean \pm SD, *p < 0.05 vs. control group.

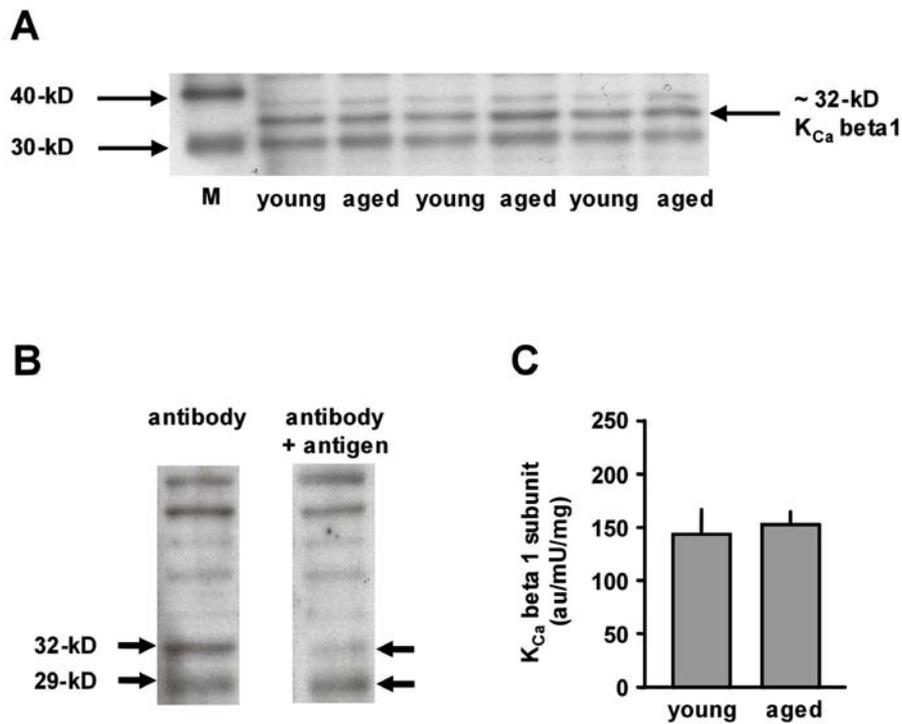
Western blot analysis

Figure 4 (panel C) shows that there was no difference of K_{Ca} beta1 subunit expression in mitochondrial lysates from young and aged rat heart mitochondria (normalized to citrate synthase activity; young: 143 \pm 23 au, old: 153 \pm 12 au, n.s.).

The analysis of citrate synthase activity showed no difference between young and old mitochondria (young: 1012 \pm 109 mU/mg, aged: 1065 \pm 61 mU/mg, n.s.).

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Figure 4: Western blot analysis



A) Representative Western blot (K_{Ca} channel beta 1 subunit) showing two major bands at ~32-kD and ~29-kD, respectively in mitochondrial lysate from both young and old aged heart mitochondria. B) Identification of specific band by immunizing peptide blocking experiment. The arrows denote positions of the 32-kD and the 29-kD bands. Blocking the antibody with the antigen demasks the specific band (32-kD) by strongly reducing the intensity of the band (right), while the intensity of the 29-kD band (and other non-specific bands) remains unchanged. C) Summarized data of the Western blot analysis of K_{Ca} channel beta 1 subunit normalized to citrate synthase activity.

DISCUSSION

The main findings of our study are that helium-induced preconditioning 1) is mediated by activation of K_{Ca} channels, 2) is accompanied by alterations in mitochondrial respiration, and 3) is abolished in the senescent heart.

In a recent study, the noble gas helium, a gas without anesthetic properties, was found to mimic the cardioprotective effect of preconditioning. (28) The results of the present study

are in line with these previous findings that helium confers cardioprotection *in vivo* as seen by a strong infarct size reduction in the helium preconditioning group compared with control hearts. (28) It was beyond the scope of the present study to unravel the complete mechanism of helium-induced preconditioning. However, our results demonstrate that activation of K_{Ca} channels is critically involved in the signal transduction pathway because the infarct size reducing effect of helium was completely abrogated by the K_{Ca} channel antagonist iberiotoxin. A central role of K_{Ca} channels in preconditioning has been shown by several studies demonstrating that either pharmacological activation of these channels initiates cardioprotection, or that pharmacological preconditioning can be blocked by K_{Ca} channel antagonists. (3; 27; 30; 31; 34) In 2002, Xu et al. reported not only evidence for the existence of K_{Ca} channels in the inner mitochondrial membrane of ventricular myocytes, the authors also demonstrated a cardioprotective potency of mitochondrial K_{Ca} (mK_{Ca}) channel activation. (34) Recently, we showed that activation of mK_{Ca} channels increases mitochondrial state 4 respiration and reduces the respiratory control index in isolated guinea pig heart mitochondria. (9) In the present study, helium-induced preconditioning did not only reduce infarct size, it also caused a significant reduction in the mitochondrial respiratory control index. Furthermore, helium-induced reduction in the respiratory control index was completely abolished by co-administration of iberiotoxin. We conclude from these data that helium confers cardioprotection by activation of mK_{Ca} channels with the consequence of mild mitochondrial uncoupling. A mild mitochondrial uncoupling during the trigger phase of preconditioning may represent a common characteristic of mitochondria in a “preconditioned” state. (13; 17; 20; 34)

The mechanism by which mK_{Ca} channel activation mediates cardioprotection is still incompletely understood. Opening of mK_{Ca} channels is capable to cause a slight increase in mitochondrial reactive oxygen species generation. (9) Stowe et al. (31) demonstrated that the cardioprotective effect of K_{Ca} channel agonist 1,3-Dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one (NS1619) requires superoxide radical generation during the preconditioning stimulus. Furthermore, the authors demonstrated that preconditioning by NS1619 reduces mitochondrial calcium overload and mitochondrial reactive oxygen species production during the subsequent period of ischemia and early reperfusion. (31) Such a reduction in mitochondrial calcium overload and reactive oxygen species generation has been suggested to prevent mPTP opening. (8; 14) Pagel et al. demonstrated that the infarct size reducing effect of helium was abolished by co-administration of the mPTP opener atractyloside, thereby showing that modulation of the mPTP is involved in helium-induced preconditioning. (28)

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In the present study, helium-induced preconditioning did not reduce infarct size in the aged rat heart. The underlying reason for the age-related loss of the cardioprotective potency of helium-induced preconditioning is yet unknown. Our results show that not only the infarct size reducing effect of helium is lost in the senescent rat heart, but that also the helium-induced effect on mitochondrial respiration before the onset of the lethal ischemia is abolished. Based on these data we suggest that the aging related blockade of helium-induced preconditioning is related to some defects at the level of the mK_{Ca} channel or its upstream signaling cascade. Previously, we demonstrated that the effects of mK_{Ca} channel activation by NS1619 on mitochondrial respiration were reduced in isolated cardiac mitochondria from aged rats. (10) Furthermore, there is evidence that aging is associated with decrease of K_{Ca} channel beta 1 subunit expression in the plasma membrane of coronary myocytes, (24) but it is completely unknown whether also mK_{Ca} channel expression is changed with increasing age. In the present study, we found that aging was without effect on mK_{Ca} channel beta 1 subunit expression, a finding that suggests that the aging-related loss of helium-induced cardioprotection is not caused by a decrease in mK_{Ca} channel density.

In summary, our results demonstrate that helium initiates preconditioning via activation of mK_{Ca} channels in the rat heart *in vivo*, that but that helium's protective potency is abolished in the senescent heart.

REFERENCES

1. **Abete P, Ferrara N, Cioppa A, Ferrara P, Bianco S, Calabrese C, Cacciatore F, Longobardi G and Rengo F.** Preconditioning does not prevent postischemic dysfunction in aging heart. *J Am Coll Cardiol* 27: 1777-1786, 1996.
2. **Bergmeyer HU.** *Methoden der enzymatischen Analyse.* Weinheim: Verlag Chemie, 1970.
3. **Cao CM, Xia Q, Gao Q, Chen M and Wong TM.** Calcium-activated potassium channel triggers cardioprotection of ischemic preconditioning. *J Pharmacol Exp Ther* 312: 644-650, 2005.
4. **Cribier A, Korsatz L, Koning R, Rath P, Gamra H, Stix G, Merchant S, Chan C and Letac B.** Improved myocardial ischemic response and enhanced collateral circulation with long repetitive coronary occlusion during angioplasty: a prospective study. *J Am Coll Cardiol* 20: 578-586, 1992.
5. **Deutsch E, Berger M, Kussmaul WG, Hirshfeld JW, Jr., Herrmann HC and Laskey WK.** Adaptation to ischemia during percutaneous transluminal coronary angioplasty. Clinical, hemodynamic, and metabolic features. *Circulation* 82: 2044-2051, 1990.
6. **Devlin W, Cragg D, Jacks M, Friedman H, O'Neill W and Grines C.** Comparison of outcome in patients with acute myocardial infarction aged > 75 years with that in younger patients. *Am J Cardiol* 75: 573-576, 1995.
7. **Haase KK, Schiele R, Wagner S, Fischer F, Burczyk U, Zahn R, Schuster S and Senges J.** In-hospital mortality of elderly patients with acute myocardial infarction: data from the

- MITRA (Maximal Individual Therapy in Acute Myocardial Infarction) registry. *Clin Cardiol* 23: 831-836, 2000.
8. **Halestrap AP, Clarke SJ and Khaliulin I.** The role of mitochondria in protection of the heart by preconditioning. *Biochim Biophys Acta* 1767: 1007-1031, 2007.
 9. **Heinen A, Camara AK, Aldakkak M, Rhodes SS, Riess ML and Stowe DF.** Mitochondrial Ca^{2+} -induced K^+ influx increases respiration and enhances ROS production while maintaining membrane potential. *Am J Physiol Cell Physiol* 292: C148-C156, 2007.
 10. **Heinen A, Winning A, Schlack W, Hollmann MW, Preckel B, Frassdorf J and Weber NC.** The regulation of mitochondrial respiration by opening of mK_{Ca} channels is age-dependent. *Eur J Pharmacol* 578: 108-113, 2008.
 11. **Juhaszova M, Rabuel C, Zorov DB, Lakatta EG and Sollott SJ.** Protection in the aged heart: preventing the heart-break of old age? *Cardiovasc Res* 66: 233-244, 2005.
 12. **Lee TM, Su SF, Chou TF, Lee YT and Tsai CH.** Loss of preconditioning by attenuated activation of myocardial ATP-sensitive potassium channels in elderly patients undergoing coronary angioplasty. *Circulation* 105: 334-340, 2002.
 13. **Liem DA, Manintveld OC, Schoonderwoerd K, McFalls EO, Heinen A, Verdouw PD, Sluiter W and Duncker DJ.** Ischemic preconditioning modulates mitochondrial respiration, irrespective of the employed signal transduction pathway. *Transl Res* 151: 17-26, 2008.
 14. **Lim SY, Davidson SM, Hausenloy DJ and Yellon DM.** Preconditioning and postconditioning: the essential role of the mitochondrial permeability transition pore. *Cardiovasc Res* 75: 530-535, 2007.
 15. **Liu GS, Thornton J, Van Winkle DM, Stanley AW, Olsson RA and Downey JM.** Protection against infarction afforded by preconditioning is mediated by A1 adenosine receptors in rabbit heart. *Circulation* 84: 350-356, 1991.
 16. **Liu Y and Downey JM.** Ischemic preconditioning protects against infarction in rat heart. *Am J Physiol* 263: H1107-H1112, 1992.
 17. **Ljubkovic M, Mio Y, Marinovic J, Stadnicka A, Warltier DC, Bosnjak ZJ and Bienengraeber M.** Isoflurane preconditioning uncouples mitochondria and protects against hypoxia-reoxygenation. *Am J Physiol Cell Physiol* 292: C1583-C1590, 2007.
 18. **Lowry OH, Rosebrough NJ, Farr AL and Randall RJ.** Protein measurement with the Folin phenol reagent. *J Biol Chem* 193: 265-275, 1951.
 19. **Maggioni AP, Maseri A, Fresco C, Franzosi MG, Mauri F, Santoro E and Tognoni G.** Age-related increase in mortality among patients with first myocardial infarctions treated with thrombolysis. The Investigators of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI-2). *N Engl J Med* 329: 1442-1448, 1993.
 20. **Minners J, Lacerda L, McCarthy J, Meiring JJ, Yellon DM and Sack MN.** Ischemic and pharmacological preconditioning in Girardi cells and C2C12 myotubes induce mitochondrial uncoupling. *Circ Res* 89: 787-792, 2001.
 21. **Moreau R, Heath SH, Doneanu CE, Harris RA and Hagen TM.** Age-related compensatory activation of pyruvate dehydrogenase complex in rat heart. *Biochem Biophys Res Commun* 325: 48-58, 2004.
 22. **Murphy E and Steenbergen C.** Preconditioning: The mitochondrial connection. *Annu Rev Physiol* 69: 51-67, 2007.
 23. **Murry CE, Jennings RB and Reimer KA.** Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 74: 1124-1136, 1986.
 24. **Nishimaru K, Eghbali M, Lu R, Marjic J, Stefani E and Toro L.** Functional and molecular evidence of MaxiK channel beta1 subunit decrease with coronary artery ageing in the rat. *J Physiol* 559: 849-862, 2004.
 25. **O'Rourke B.** Evidence for mitochondrial K^+ channels and their role in cardioprotection. *Circ Res* 94: 420-432, 2004.

mK_{Ca} channels, aging and helium preconditioning

26. **Obal D, Weber NC, Zacharowski K, Toma O, Dettwiler S, Wolter JI, Kratz M, Mullenheim J, Preckel B and Schlack W.** Role of protein kinase C-epsilon (PKCepsilon) in isoflurane-induced cardioprotection. *Br J Anaesth* 94: 166-173, 2005.
27. **Ohya S, Kuwata Y, Sakamoto K, Muraki K and Imaizumi Y.** Cardioprotective effects of estradiol include the activation of large-conductance Ca²⁺-activated K⁺ channels in cardiac mitochondria. *Am J Physiol Heart Circ Physiol* 289: H1635-H1642, 2005.
28. **Pagel PS, Krolikowski JG, Shim YH, Venkatapuram S, Kersten JR, Weihrauch D, Warltier DC and Pratt PF, Jr.** Noble gases without anesthetic properties protect myocardium against infarction by activating prosurvival signaling kinases and inhibiting mitochondrial permeability transition in vivo. *Anesth Analg* 105: 562-569, 2007.
29. **Sample J, Cleland JG and Seymour AM.** Metabolic remodeling in the aging heart. *J Mol Cell Cardiol* 40: 56-63, 2006.
30. **Shintani Y, Node K, Asanuma H, Sanada S, Takashima S, Asano Y, Liao Y, Fujita M, Hirata A, Shinozaki Y, Fukushima T, Nagamachi Y, Okuda H, Kim J, Tomoike H, Hori M and Kitakaze M.** Opening of Ca²⁺-activated K⁺ channels is involved in ischemic preconditioning in canine hearts. *J Mol Cell Cardiol* 37: 1213-1218, 2004.
31. **Stowe DF, Aldakkak M, Camara AK, Riess ML, Heinen A, Varadarajan SG and Jiang MT.** Cardiac mitochondrial preconditioning by Big Ca²⁺-sensitive K⁺ channel opening requires superoxide radical generation. *Am J Physiol Heart Circ Physiol* 290: H434-H440, 2006.
32. **Tani M, Suganuma Y, Hasegawa H, Shinmura K, Ebihara Y, Hayashi Y, Guo X and Takayama M.** Decrease in ischemic tolerance with aging in isolated perfused Fischer 344 rat hearts: relation to increases in intracellular Na⁺ after ischemia. *J Mol Cell Cardiol* 29: 3081-3089, 1997.
33. **Toma O, Weber NC, Wolter JI, Obal D, Preckel B and Schlack W.** Desflurane preconditioning induces time-dependent activation of protein kinase C epsilon and extracellular signal-regulated kinase 1 and 2 in the rat heart in vivo. *Anesthesiology* 101: 1372-1380, 2004.
34. **Xu W, Liu Y, Wang S, McDonald T, Van Eyk JE, Sidor A and O'Rourke B.** Cytoprotective role of Ca²⁺-activated K⁺ channels in the cardiac inner mitochondrial membrane. *Science* 298: 1029-1033, 2002.