Anesthetic induced cardioprotection: from bench to bedside and retour

Frässdorf, J.

Publication date
2012

Chapter 1: The regulation of mitochondrial respiration by opening of mK(Ca) channels is age-dependent

André Heinen, Adrian Winning, Wolfgang Schlack, Markus W. Hollmann, Benedikt Preckel, Jan Fräßdorf, Nina C. Weber

European Journal of Pharmacology 578: 108–113 (2008)

Abstract

The protective potency of ischemic preconditioning decreases with increasing age. A key step in ischemic preconditioning is the opening of mitochondrial Ca2+ sensitive K+ (mK_{Ca}) channels, which causes mild uncoupling of mitochondrial respiration. We hypothesized that aging reduces the effects of mK_{Ca} channel opening on mitochondrial respiration.

We measured the effects ofmKCa channel openerNS1619 (30 µM) on mitochondrial respiration in isolated heart mitochondria from young (2–3months) and old (22–26 months) Wistar rats. Oxygen consumption was monitored online after addition of 250 µM ADP (state 3 respiration), and after complete phosphorylation of ADP to ATP (state 4 respiration) in the presence or absence of the mK_{Ca} channel blocker paxilline (5 µM). The respiratory control index (RCI) was calculated as state 3/state 4.

In mitochondria from young rats, NS1619 increased state 4 respiration by 11.9±4.1% (mean±S.E.M.), decreased state 3 respiration by 7.6±2.5%, and reduced the RCI from 2.6±0.03 (control) to 2.1±0.06 (all $P<0.05$, n=12 for all groups). Paxilline blocked the effect of NS1619 on state 4 respiration (0.7±2.8%), but did not affect the decrease in state 3 respiration; paxilline blunted the decrease of RCI. In mitochondria from old rats, NS1619 had neither effect on state 4 (0.4±1.6%), and state 3 respiration (−7.4±1.5%), nor on RCI (3.0±0.13 vs. 3.2±0.11, n=12).

Increasing age reduced the effects of mKCa opening on mitochondrial respiration. This might be one underlying reason of the decreased protective potency of ischemic preconditioning in the aged myocardium.

1. Introduction

The worldwide population of persons aged ≥65 years will increase from 420 million in 2000 to about 973 million in 2030. An increasing lifespan, expected to extend 10 years by 2050, is associated with an increase in the incidence and prevalence of chronic diseases such as coronary artery disease, which is a major cause for myocardial infarction (Lakatta and Levy, 2003). The morbidity and mortality ofmyocardial infarction is enhanced with increasing age (Devlin et al., 1995); possibly due to an aging related loss of the protective potency of cardioprotective strategies, e.g. ischemic preconditioning (Juhaszova et al., 2005).

Ischemic preconditioning is a cardioprotective phenomenon by which short periods of ischemia reduce the deleterious consequences of a subsequent prolonged period of ischemia/ reperfusion of the heart (Murry et al., 1986). So far, most studies that investigated the protective effects and the underlying mechanism of ischemic preconditioning were conducted in young animals. There is strong evidence from the literature that the cardioprotective effect of ischemic preconditioning decreases with increasing age both in animals (Abete et al., 1996; Tani et al., 1997) and in humans (Lee et al., 2002). Lee et al. demonstrated a loss of protection by ischemic preconditioning in elderly patients undergoing coronary angioplasty (Lee et al., 2002). A prolonged period of ischemia and the mitochondrial ATP-sensitive potassium (mK_{ATP}) channel activator nicorandil were able to (re)initiate a preconditioning state in these patients. The authors concluded that the impaired preconditioning response must result from some defects in the signal transduction of K+ channel activation of the aged myocardium.

It is proposed that signalling pathways in preconditioning converge on the mitochondria (Murphy, 2004). Many reports strongly support the hypothesis that regulation of mitochondrial function by activation of K+ channels in the inner mitochondrial membrane resulting in an increased K+ influx into the mitochondrial matrix is a key step in the signal transduction cascade of ischemic preconditioning (Murphy and Steenbergen, 2007; O'Rourke, 2004).

In addition to the importance of mK_{ATP} channels, there is increasing evidence for a role of Ca2+ sensitive potassium (K_{Ca}) channel opening in ischemic preconditioning. Recently, Cao et al. (Cao et al., 2005) demonstrated in isolated perfused rat hearts that blockade of K_{Ca} channels by paxilline abolished the re-

duction of infarct size caused by ischemic preconditioning. Furthermore, pharmacological preconditioning was initiated by the K_{Ca} channel activator 1,3-Dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one (NS1619). Xu et al. (Xu et al., 2002) detected mitochondrial (m) K_{Ca} channels in the inner mitochondrial membrane of guinea pig ventricular cells and suggested a role for mK_{Ca} channels in protection against ischemic injury.

We aimed to investigate the effect of age-dependent changes in mK_{Ca} channel activation on mitochondrial respiration, and analyzed the effects of the mK_{Ca} channel agonist NS1619 and the antagonist paxilline on mitochondrial respiration and oxidative phosphorylation in mitochondria isolated from young and old rat hearts.

2. Materials and methods

All experiments were performed in accordance 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 approved by the Institutional Committee for Animal Care and Use (Heinrich-Heine-University Düsseldorf, Germany).

2.1. Chemicals and reagents

KCl was purchased from EMD Chemicals (Gibbstown, NJ); all other chemicals were purchased from Sigma Chemical Co. (Taufkirchen, Germany). NS1619 and paxilline were dissolved in DMSO before they were added to the experimental buffer.

2.2. Mitochondrial isolation

Heart mitochondria were isolated from young (2–3months) and old (22–26 months) Wistar rats. Animals were anesthetized by an intraperitoneal injection of S(+)-ketamine (150 mg/kg). After decapitation, hearts were excised and heart mitochondria were isolated by differential centrifugation as described previously (Heinen et al., 2007a,b; Riess et al., 2004). Briefly, atria were removed and ventricles were placed in isolation buffer [200 mmol/L mannitol, 50 mmol/L sucrose, 5 mmol/L KH_2PO_4, 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 mm^3 pieces. The suspension was homogenized for 15 s 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 s after addition of 17 mL isolation buffer. The suspension was centrifuged at 3220 g for 10 min, the supernatant was removed, and the pellet was resuspended in 25 mL isolation buffer and centrifuged at 800 g for 10 min. The supernatant was centrifuged at 3220 g 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 Bradfordmethod (Bradford, 1976). All isolation procedures were conducted at 4 °C.

2.3. Mitochondrial O_2 consumption

Oxygen consumption was measured polarographically at 27 °C using a respirometric system (System S 200A, Strathkelvin Instruments, Glasgow, Scotland). Mitochondria (0.25 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 s (Fig. 1) in the presence or absence of 5 µmol/L mKCa channel blocker paxilline (Pax). The mKCa channel activator NS1619 (20, 30, or 50 µmol/L) or its vehicle DMSO (0.3%) were injected into the respiration chamber after 120 s. State 3 respiration was initiated after 180 s by addition of 250 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 2 to 3 mitochondrial samples and the average was taken (and counted as n=1). Respiration rates are expressed as absolute rates in nmol O_2/mg/min or as percent of control.

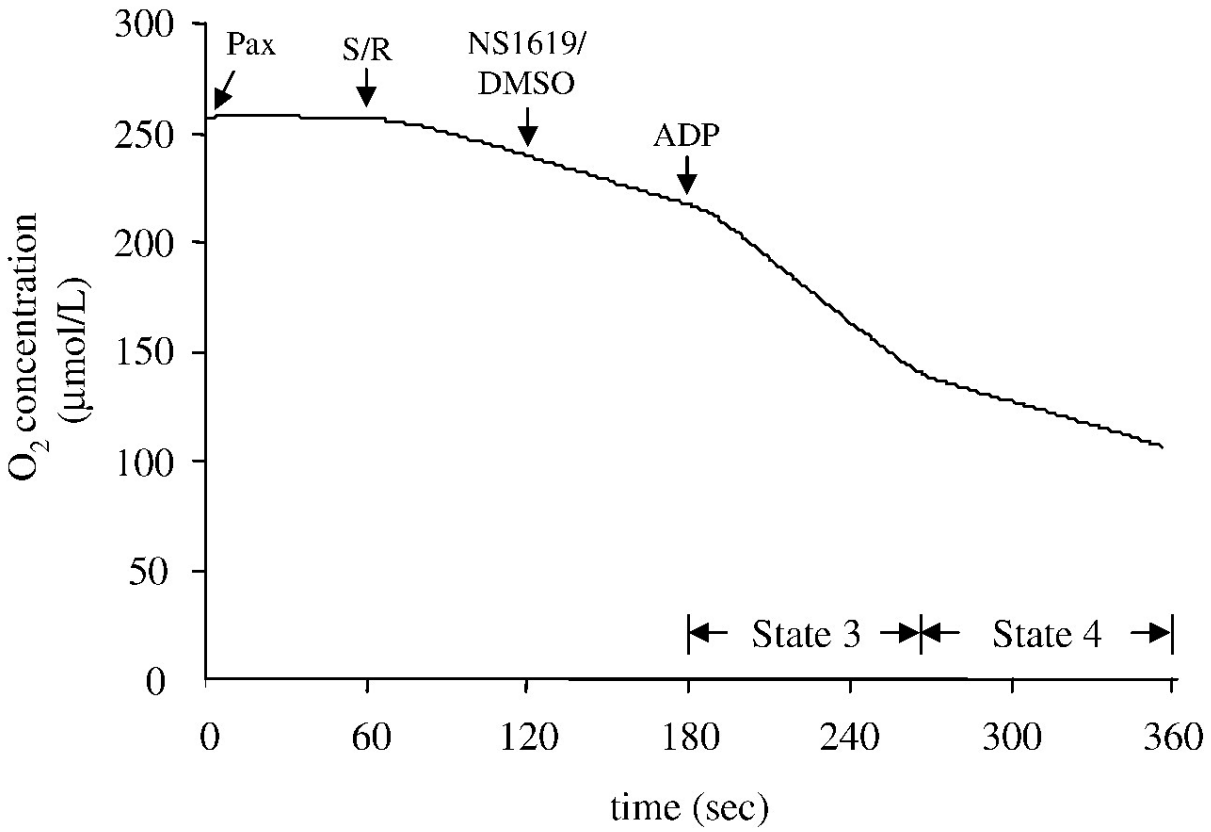

Figure. 1: *Representative traces of mitochondrial respiration experiments. Mitochondrial respiration was initiated by addition of 10 mmol/L succinate + 10µmol/L rotenone (S/R); State 3 respiration was initiated by addition of 250 µmol/L ADP. NS1619 or its vehicle DMSO (0.3%) was administered at 120 s. The respiration rates were analyzed both under state 3 conditions and after complete phosphorylation of ADP to ATP (state 4). When the effect of mKCa channel blockade by paxilline (Pax) was investigated, 5 µmol/L Pax were present during the whole experimental protocol.*

To investigate concentration-dependent effects of mK_{Ca} channel opening on mitochondrial bioenergetics, we measured in a first series of experiments mitochondrial respiration in the absence (control) or presence of 20, 30, or 50 µmol/L NS1619 (NS20, NS30, or NS50, respectively).

To test if the effects of NS1619 were caused by mK_{Ca} channel opening, we added in a second series of experiments 5 µmol/L mK_{Ca} channel blocker Pax in the absence or presence of 30 µmol/L NS1619.

2.4. Statistical analysis

To analyse concentration-dependent effects of NS1619 (experimental series 1), group data were compared by analysis of variance, followed by Dunett's post hoc test (all vs. control).

To compare if the effects of NS30 were caused by mK_{Ca} channel opening (experimental series 2), group data were compared by analysis of variance, followed by Tukey's post hoc test. Data were considered statistically significant when $P<0.05$ and are presented as means±S.E.M.

3. Results

3.1. Effect of aging on mitochondrial respiration

The respiration rates of isolated mitochondria from old rat hearts are reduced compared to mitochondria from young rat hearts both under "resting" state 4 conditions (72.5±6.3 nmol O_2/mg/min vs. 100.5±5.4 nmol O_2/mg/min) and "stimulated" state 3 conditions (218.4±13.9 nmol O_2/mg/min vs. 260.8± 13.8 nmol O_2/mg/min) (Table 1). These age-dependent changes in respiration rates resulted in an increased RCI in old rats compared to young rats (3.1±0.08 vs. 2.6±0.04). Aging did not affect the efficiency of oxidative phosphorylation as demonstrated by no change in P/O ratio.

Table 1 Respiration rates under control conditions

	Young	Old
State 4 (nmol O_2/mg/min)	100.5 ± 5.4	72.5 ± 6.3[a]
State 3 (nmol O_2/mg/min)	260.8 ± 13.8	218.4 ± 13.9[a]
RCI (state 3/state 4)	2.6 ± 0.04	3.1 ± 0.08[a]
P/O ratio	1.40 ± 0.03	1.45 ± 0.04

Data are mean ± S.E.M.; [a]$P < 0.05$ vs. young. RCI = respiratory control index (state 3/state 4). P/O ratio = ratio between phosphate incorporated into ATP to atoms O_2 consumed.

3.2. Concentration effects

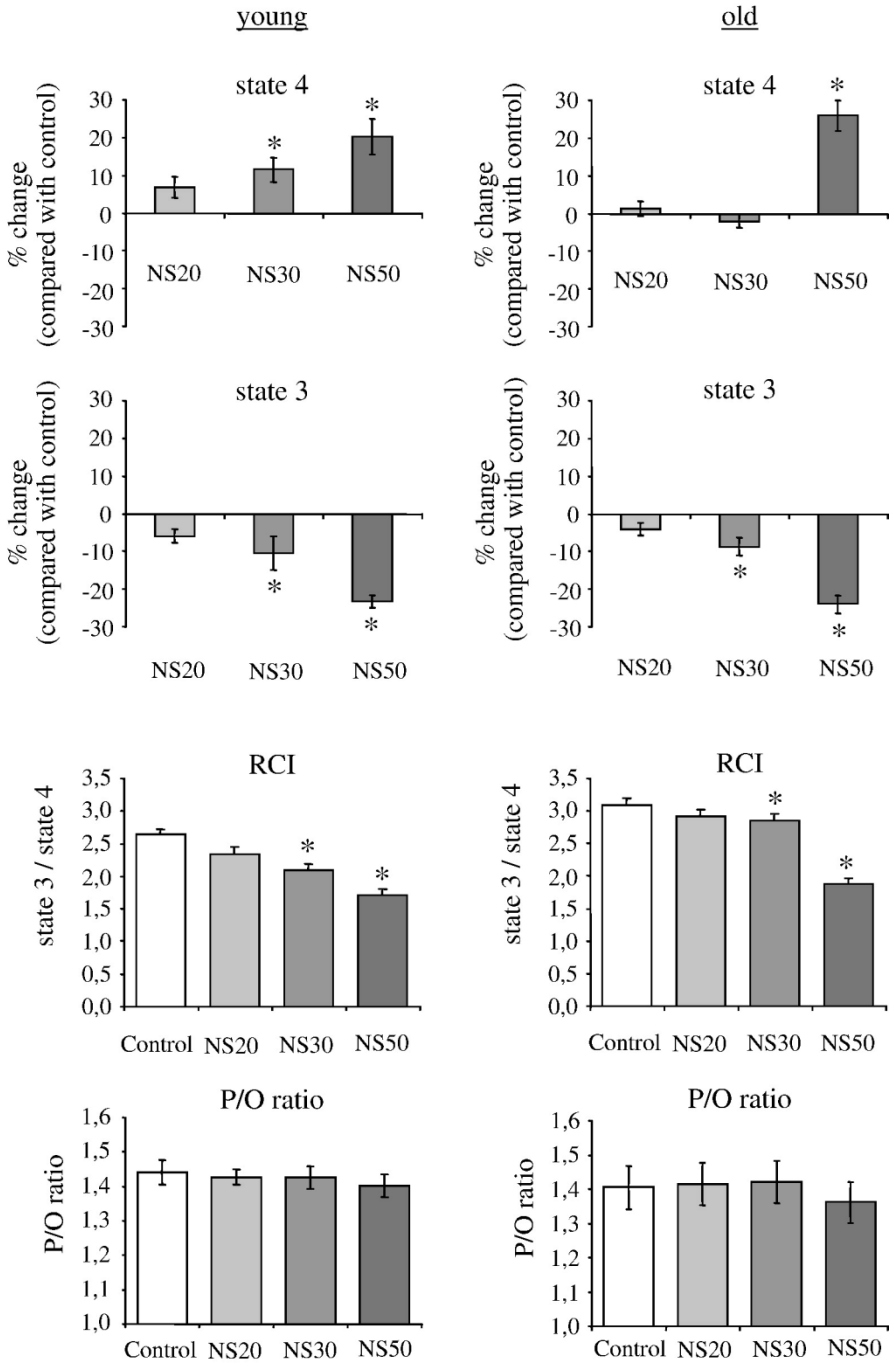

Figure 2.*Summarized data for the concentration effects of 20, 30, or 50 µmol/L NS1619 (NS) on mitochondrial respiration in young (left panel, n = 12 for all groups) or old (right panel, n = 15 for all groups) rat heart mitochondria. Data are mean ± S.E.M.; ANOVA followed by Dunnett's post hoc test; *P < 0.05 vs. control. RCI = respiratory control index, a parameter for the coupling between mitochondrial respiration and oxidative phosphorylation. P/O ratio = ratio between phosphate incorporated into ATP and oxygen consumed; a parameter for the efficiency of oxidative phosphorylation.*

The concentration-dependent effects of mK_{Ca} channel opening by NS1619 on mitochondrial bioenergetics were measured in our first series of experiments.

Mitochondrial respiration was measured in the absence (control) or presence of 20, 30, or 50 µmol/L NS1619 (NS20, NS30, or NS50, respectively) (Fig. 2).

We detected an age-dependent difference in respiration rates under state 4 conditions. In young rats, NS1619 increased oxygen consumption state 4 in a dosedependent manner (NS20: 6.9±2.8%, P=ns; NS30: 11.5±3.2%, Pb0.05; NS50: 20.3±4.8%, Pb0.05). In old rats, only the highest concentration of 50 µmol/L NS1619 increased state 4 respiration (NS50: 26.0±4.0%, Pb0.05). Furthermore, NS1619 decreased state 3 respiration (NS20: −5.9±1.8%, P=ns; NS30: −10.5±4.4%, P<0.05; NS50: −23.3±1.8%, P<0.05) in young rats. In old rats, state 3 was decreased by NS30 (−8.7 ± 2.4%, P<0.05) and NS50 (−24.0 ± 2.3%, P<0.05). The respiratory control index, a parameter of the coupling between mitochondrial respiration and oxidative phosphorylation, was decreased by NS30, and NS50 both in young rats (−0.5±0.1, and −0.9±0.1, both P<0.05, respectively) and old rats, respectively (−0.3±0.1, and −1.0±0.1, both P<0.05). Opening of mK_{Ca} channel activation did not affect the efficiency of oxidative phosphorylation as shown by no changes in the P/O ratio neither in young nor in old rats.

3.3. Blockade of mK_{Ca} channel activation

In the second series of experiments we tested by using mK_{Ca} channel blocker Pax, if the effects of NS1619 were caused by mK_{Ca} channel opening (Fig. 3). Pax alone had no effect on state 4 respiration, indicating mK_{Ca} channels were closed under the experimental conditions in both young (−0.7±2.5%, P=ns) and old rats (1.7±1.5%, P=ns). Pre-administration of Pax blocked the NS30-induced increase in state 4 respiration in young rats (11.9±4.1% vs. 0.7±2.8%, P<0.05), but had no effect on state 4 respiration in the presence of NS30 in old rats (0.4±1.6%, P=ns). Pax did not affect state 3 respiration neither in young (−1.0±2.0%, P=ns) nor in old rats (−2.4±1.4%, P=ns), and did not reduce the NS30 induced decrease in state 3 respiration in both young (−9.9±2.5% vs. −7.6±2.5%, P=ns) and old rats (−10.9±1.5% vs. −7.4±1.5%, P=ns). The NS30 induced decrease in RCI in young (−0.4±0.1 vs. −0.3±0.1, P=ns) and old rats (−0.3±0.2 vs. −0.1±0.2, P=ns) was not affected by Pax. Furthermore, blockade of mK_{Ca} channels did not affect the efficiency of oxidative phosphorylation as demonstrated by no changes in the P/O ratio neither in young nor in old rats.

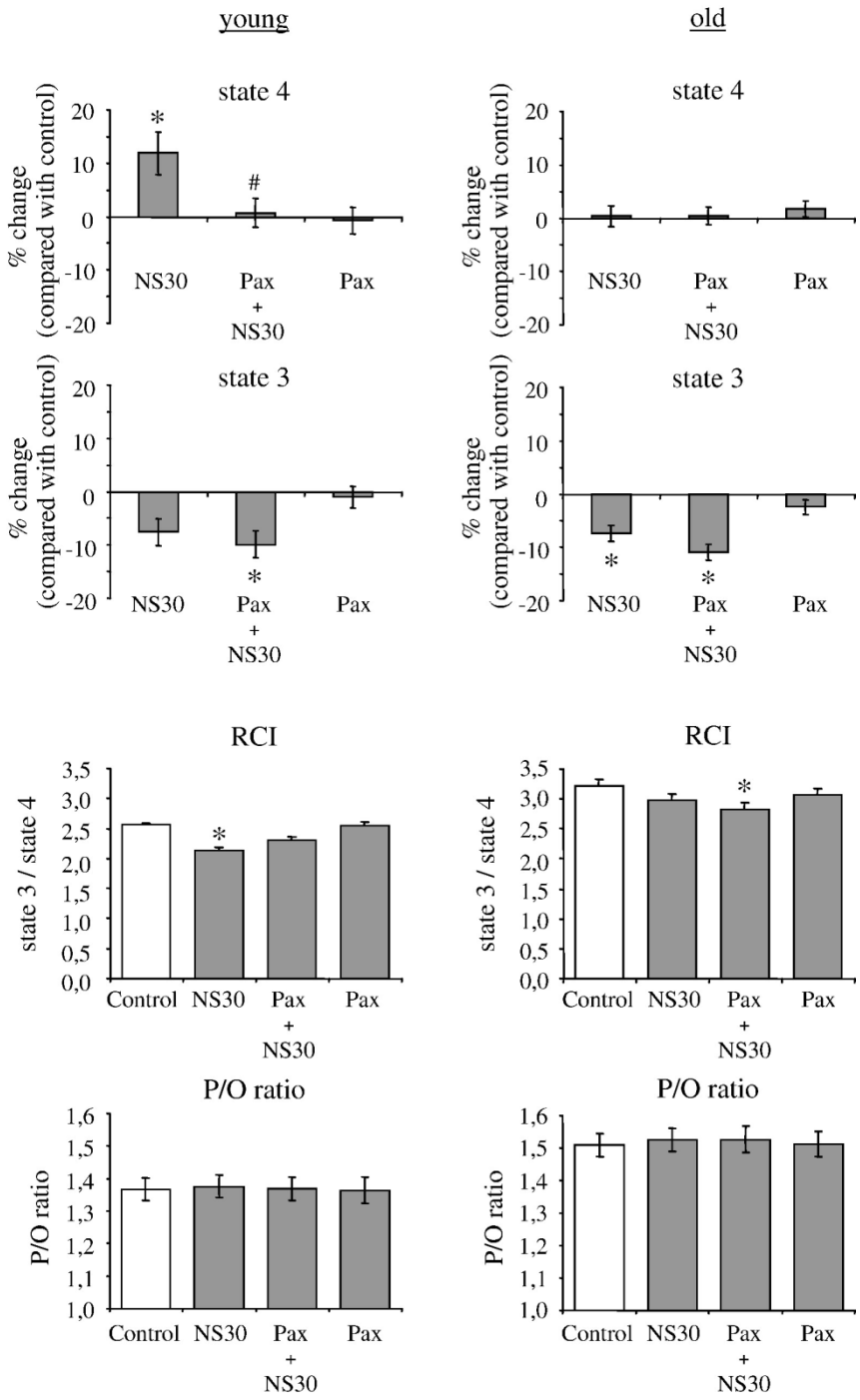

Figure 3. *Summarized data of the effects of 30 µmol/L NS1619 (NS30) on mitochondrial respiration and the blocking effects of mKCa channel antagonist paxilline (5 µmol/L, Pax) on mitochondrial respiration in young (left panel, n = 12 for all groups) or old (right panel, n = 12 for all groups) rat heart mitochondria. Data are mean ± S.E.M., ANOVA followed by Tukey's post hoc test; *P < 0.05 vs. control, #P < 0.05 vs. NS1619. RCI = respiratory control index, a parameter for the coupling between mitochondrial respiration and oxidative phosphorylation. P/O ratio = ratio between phosphate incorporated into ATP and oxygen consumed; a parameter for the efficiency of oxidative phosphorylation.*

4. Discussion

The major findings of this study are that a) mitochondrial respiration is depressed in mitochondria from aged rat hearts in comparison with those from young hearts, and b) the regulation of mitochondrial respiration by opening of mK_{Ca} channels is age-dependent.

Mitochondria and alterations in mitochondrial function are deeply involved in the aging process (Lenaz, 1998). Investigations on age-dependent changes in mitochondrial bioenergetics have produced conflicting results, showing significant changes (Chen et al., 1972; Hansford, 1978; Chiu and Richardson, 1980) or no differences (Takasawa et al., 1993) (for review see

(Lesnefsky and Hoppel, 2006)). Fannin et al. demonstrated that these conflicting results can be explained by the finding that aging selectively decreases oxidative capacity in interfibrillar mitochondria, while respiration rates of subsarcolemmal mitochondria remains unchanged (Fannin et al., 1999). Palmer et al. showed that a brief exposure to protease during the isolation procedure is required to isolate interfibrillar mitochondria (Palmer et al., 1977). In this study, we investigated respiration rates of interfibrillar mitochondria (or a mixed population), and confirmed that in this mitochondrial population the respiratory capacity of mitochondria from old rat hearts is reduced compared tomitochondria from young rat hearts.

Mitochondrial respiration and oxidative phosphorylation can be regulated by activation of K^+ channels in the inner mitochondrial membrane. It was suggested that activation of mitochondrial K^+ channels causes potassium influx from the intermembrane space into the mitochondrial matrix. Several recent studies have demonstrated a stimulating effect of matrix K^+ influx through mK_{ATP} channels on mitochondrial respiration (Holmuhamedov et al., 1998; Debska et al., 2002; Minners et al., 2001). Furthermore, there is strong evidence for the existence of another class of ion channels in the IMM that promote K^+ influx into the mitochondrial matrix: the Ca^{2+} dependent K^+ channel (mK_{Ca}). Siemen et al. (Siemen et al., 1999) first reported mK_{Ca} channels in the IMMof glial cells. Xu et al. (Xu et al., 2002) very recently discovered these channels in cardiac myocyte mitochondria. Patch-clamp recordings from mitoplasts of these cells showed Ca^{2+} dependent, large K^+ conductance channels in the IMM and immunoblots of cardiac mitochondria with antibodies against the C terminal part of K_{Ca} channel

identified a 55 kDa protein as part of this putative channel (Ohya et al., 2005). O'Rourke suggested that mitochondrial K^+ channels function as energy dissipating channels (energy stored as the proton gradient, ΔμH) by expending ΔμH, in part to eject K^+ via an electroneutral K^+/H^+ exchanger. The resulting decrease in ΔμH in turn enhances electron flow. The bioenergetic consequence of K^+ channel opening would be accelerated cycling of K^+ ions between the matrix and the intermembrane space (i.e. matrix K^+ inflow through K^+ channel, K^+ extrusion via K^+/H^+ exchanger) and an increase in mitochondrial respiration (O'Rourke, 2004).

The regulation of mitochondrial function by mitochondrial K^+ channel activation is a key step to trigger ischemic and pharmacological preconditioning (O'Rourke, 2004; Weber et al., 2006). It was shown that pharmacological blockade of K^+ channel abrogates the cardioprotective effects of ischemic preconditioning (Shintani et al., 2004; Auchampach et al., 1992; Cao et al., 2005). Furthermore, a preconditioning effect can be mimicked by administration of a K^+ channel opener (Garlid et al., 1997; Loubani et al., 2005; Cao et al., 2005). The exact mechanism by which K^+ channel opening triggers and/or mediates preconditioning is incompletely understood. Most studies investigating the agedependent effect of ischemic or anesthetic preconditioning found that the protective potency of this phenomenon is diminished or abolished in the aged heart (Juhaszova et al., 2005; Abete et al., 1996; Fenton et al., 2000; Sniecinski and Liu, 2004; Tani et al., 1997). Furthermore, Lee et al. demonstrated that preconditioning significantly enhances the tolerance of the heart to subsequent ischemia in adult but not in senescent patients (Lee et al., 2002). Since a prolonged period of ischemia and the mK_{ATP} channel activator nicorandil were able to (re)initiate a preconditioning state, the authors concluded that the impaired preconditioning response is due to some defects in signal transduction of activation of K^+ channels in the aged heart. For this loss of efficiency of preconditioning in the aged heart, age-dependent alterations in the regulation of mitochondrial function by ion cycling might be a possible reason.

The aim of this study was to investigate, whether the bioenergetic consequences of mK_{Ca} channel opening by NS1619 on mitochondrial function are age-dependent. Here we show that opening of mK_{Ca} channels by NS1619 increases state 4 respiration only in young rat heart mitochondria and not in mi-

tochondria isolated from old rat hearts. The finding that NS1619 accelerates mitochondrial respiration under resting conditions is in agreement with a previous study from Sato et al., who demonstrated a dose dependent increase in flavoprotein oxidation by mK_{Ca} channel activation (Sato et al., 2005). Recently, Cancherini et al. described that NS1619 inhibited mitochondrial respiration (Cancherini et al., 2007). The inhibitory effects of NS1619 on mitochondrial state 3 respiration were described before by Debska et al. (Debska et al., 2003) and our group (Heinen et al., 2007a,b) and are confirmed by the present study. Cancherini et al. suggested that NS1619 promotes nonselective permeabilization of the inner mitochondrial membrane to ions (Cancherini et al., 2007). In a previous study (Heinen et al., 2007b), we discovered in isolated guinea pig heart mitochondria that opening of mK_{Ca} channels by NS1619 accelerated mitochondrial state 4 respiration while maintaining mitochondrial membrane potential (ψm); conditions that were capable to increase generation of reactive oxygen species (ROS), a key trigger of preconditioning (Heinen et al., 2007b; Becker, 2004; Stowe et al., 2006). In the present study, mK_{Ca} channel activation also increased state 4 respiration in a dose dependent manner in young rat heart mitochondria. Furthermore, the effect of 30 µmol/L NS1619 was completely reversible by paxilline. We conclude that the effect of NS1619 on state 4 respiration is mKCa channel mediated. Furthermore, our results demonstrate for the first time that this effect is age-dependent, since NS1619 had no effect on state 4 respiration in old rat heart mitochondria.

It is interesting to note that activation of mK_{Ca} channels by NS1619 had no effect on the efficiency of oxidative phosphorylation as seen by no change in P/O ratios while state 4 respiration was increased. A possible explanation for this observation is that K^+ channel opening regulates mitochondrial metabolism due to regulation of the matrix volume. It is proposed that the mitochondrial matrix contracts under state 3 conditions due to a reduced mitochondrial membrane potential, which is the driving force for cation and water uptake (Kowaltowski et al., 2001). Opening of K^+ channels may reverse this matrix contracture to preserve oxidative phosphorylation (Halestrap, 1989; Kowaltowski et al., 2001).

From the results of this study it is hard to conclude whether a decreased density of mK_{Ca} channels in the inner mitochondrial membrane or a reduced sensitivity (or a combination) is responsible for the reduced effect of NS1619 on mito-

chondrial respiration. It is interesting to note that the high concentration of NS1619 accelerated state 4 respiration in young and old heart mitochondria in a comparable magnitude (approx. 20 nmol O2/mg/min). This finding supports the possibility that a decreased sensitivity of mK_{Ca} channels causes the age-dependent difference in the effect of 30 µmol/L NS1619 (a concentration that has been shown to induce cardioprotection in young hearts).

We demonstrated recently that mK_{Ca} channel opening by NS1619 increased state 4 respiration independent if complex I substrate pyruvate or complex II substrate succinate (with or without rotenone) was used (Heinen et al., 2007a,b). Based on this finding, we conducted all experiments in isolated mitochondria respiring on complex II substrate succinate (+ rotenone). Nevertheless, this is a limitation of the present study.

From the observation of this study that the bioenergetic consequences of mK_{Ca} channel opening on mitochondrial respiration are age-dependent, we speculate that the aging related reduction in mK_{Ca} channel activation and the resulting effects on mitochondrial function might contribute to the decreased protective potency of ischemic preconditioning in the aged myocardium. Whether NS1619 does indeed not confer protection to the older hearts against ischemia–reperfusion injury needs further investigation in a functional correlate study.

Acknowledgements

The authors wish to thank Matthias L. Riess, Amadou K.S. Camara, David F. Stowe and Mohammed Aldakkak for their support with this study.

This study was funded by a young investigator grant (9772270) of the research committee of the Heinrich-Heine-University Düsseldorf to Andre Heinen.

References

Abete, P., Ferrara,N., Cioppa, A., Ferrara, P., Bianco, S., Calabrese, C., Cacciatore, F., Longobardi, G., Rengo, F., 1996. Preconditioning does not prevent postischemic dysfunction in aging heart. J. Am. Coll. Cardiol. 27, 1777–1786.

Auchampach, J.A., Grover, G.J., Gross, G.J., 1992. Blockade of ischaemic preconditioning in dogs by the novel ATP dependent potassium channel antagonist sodium 5-hydroxydecanoate. Cardiovasc. Res. 26, 1054–1062.

Becker, L.B., 2004. New concepts in reactive oxygen species and cardiovascular reperfusion physiology. Cardiovasc. Res. 61, 461–470.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Cancherini, D.V., Queliconi, B.B., Kowaltowski, A.J., 2007. Pharmacological and physiological stimuli do not promote Ca^{2+}-sensitive K^{+} channel activity in isolated heart mitochondria. Cardiovasc. Res. 73, 720–728.

Cao, C.M., Xia, Q., Gao, Q., Chen, M., Wong, T.M., 2005. Calcium-activated potassium channel triggers cardioprotection of ischemic preconditioning. J. Pharmacol. Exp. Ther. 312, 644–650.

Chen, J.C., Warshaw, J.B., Sanadi, D.R., 1972. Regulation of mitochondrial respiration in senescence. J. Cell. Physiol. 80, 141–148.

Chiu, Y.J., Richardson, A., 1980. Effect of age on the function of mitochondria isolated from brain and heart tissue. Exp. Gerontol. 15, 511–517.

Debska, G., Kicinska, A., Skalska, J., Szewczyk, A., May, R., Elger, C.E., Kunz, W.S., 2002. Opening of potassium channels modulates mitochondrial function in rat skeletal muscle. Biochim. Biophys. Acta 1556, 97–105.

Debska, G., Kicinska, A., Dobrucki, J., Dworakowska, B., Nurowska, E.,

Skalska, J., Dolowy, K., Szewczyk, A., 2003. Large-conductance K^{+} channel openers NS1619 and NS004 as inhibitors of mitochondrial function in glioma cells. Biochem. Pharmacol. 65, 1827–1834.

Devlin, W., Cragg, D., Jacks, M., Friedman, H., O'Neill, W., Grines, C., 1995. Comparison of outcome in patients with acute myocardial infarction aged >75 years with that in younger patients. Am. J. Cardiol. 75, 573–576.

Fannin, S.W., Lesnefsky, E.J., Slabe, T.J., Hassan, M.O., Hoppel, C.L., 1999. Aging selectively decreases oxidative capacity in rat heart interfibrillar mitochondria. Arch. Biochem. Biophys. 372, 399–407.

Fenton, R.A., Dickson, E.W., Meyer, T.E., Dobson Jr., J.G., 2000. Aging reduces the cardioprotective effect of ischemic preconditioning in the rat heart. J. Mol. Cell Cardiol. 32, 1371–1375.

Garlid, K.D., Paucek, P., Yarov-Yarovoy, V., Murray, H.N., Darbenzio, R.B., D'Alonzo, A.J., Lodge, N.J., Smith, M.A., Grover, G.J., 1997. Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitiveK^+ channels. Possible mechanism of cardioprotection. Circ. Res. 81, 1072–1082.

Halestrap, A.P., 1989. The regulation of the matrix volume of mammalian mitochondria in vivo and in vitro and its role in the control of mitochondrial metabolism. Biochim. Biophys. Acta 973, 355–382.

Hansford, R.G., 1978. Lipid oxidation by heart mitochondria from young adult and senescent rats. Biochem. J. 170, 285–295.

Heinen, A., Aldakkak, M., Stowe, D.F., Rhodes, S.S., Riess, M.L., Varadarajan, S.G., Camara, A.K., 2007a. Reverse electron flow-induced ROS production is attenuated by activation of mitochondrial Ca^{2+} sensitive K^+ channels. Am. J. Physiol, Heart Circ. Physiol. 293, H1400–H1407.

Heinen, A., Camara, A.K., Aldakkak,M., Rhodes, S.S., Riess, M.L., Stowe, D.F., 2007b. Mitochondrial Ca^{2+}-induced K^+ influx increases respiration and enhances ROS production while maintaining membrane potential. Am. J. Physiol., Cell Physiol. 292, C148–C156.

Holmuhamedov, E.L., Jovanovic, S., Dzeja, P.P., Jovanovic, A., Terzic, A., 1998. Mitochondrial ATP-sensitive K^+ channels modulate cardiac mitochondrial function. Am. J. Physiol. 275, H1567–H1576.

Juhaszova, M., Rabuel, C., Zorov, D.B., Lakatta, E.G., Sollott, S.J., 2005. Protection in the aged heart: preventing the heart-break of old age? Cardiovasc. Res. 66, 233–244.

Kowaltowski, A.J., Seetharaman, S., Paucek, P., Garlid, K.D., 2001. Bioenergetic consequences of opening the ATP-sensitive K^+ channel of heart mitochondria. Am. J. Physiol, Heart Circ. Physiol. 280, H649–H657.

Lakatta, E.G., Levy, D., 2003. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part II: the aging heart in health: links to heart disease. Circulation 107, 346–354.

Lee, T.M., Su, S.F., Chou, T.F., Lee, Y.T., Tsai, C.H., 2002. Loss of preconditioning by attenuated activation of myocardial ATP-sensitive potassium channels in elderly patients undergoing coronary angioplasty. Circulation 105, 334–340.

Lenaz, G., 1998. Role of mitochondria in oxidative stress and ageing. Biochim. Biophys. Acta 1366, 53–67.

Lesnefsky, E.J., Hoppel, C.L., 2006. Oxidative phosphorylation and aging. Ageing Res. Rev. 5, 402–433.

Loubani, M., Fowler, A., Standen, N.B., Galinanes, M., 2005. The effect of gliclazide and glibenclamide on preconditioning of the human myocardium. Eur. J. Pharmacol. 515, 142–149.

Minners, J., Lacerda, L., McCarthy, J., Meiring, J.J., Yellon, D.M., Sack, M.N., 2001. Ischemic and pharmacological preconditioning in Girardi cells and C2C12 myotubes induce mitochondrial uncoupling. Circ. Res. 89, 787–792.

Murphy, E., 2004. Primary and secondary signaling pathways in early preconditioning that converge on the mitochondria to produce cardioprotection. Circ. Res. 94, 7–16.

Murphy, E., Steenbergen, C., 2007. Preconditioning: the mitochondrial connection. Annu. Rev. Physiol. 69, 51–67.

Murry, C.E., Jennings, R.B., Reimer, K.A., 1986. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 74, 1124–1136.

O'Rourke, B., 2004. Evidence for mitochondrial K^+ channels and their role in cardioprotection. Circ. Res. 94, 420–432.

Ohya, S., Kuwata, Y., Sakamoto, K., Muraki, K., Imaizumi, Y., 2005. Cardioprotective effects of estradiol include the activation of largeconductance Ca^{2+}-activated K^+ channels in cardiac mitochondria. Am. J. Physiol, Heart Circ. Physiol. 289, H1635–H1642.

Palmer, J.W., Tandler, B., Hoppel, C.L., 1977. Biochemical properties of subsarcolemmal and interfibrillar mitochondria isolated from rat cardiac muscle. J. Biol. Chem. 252, 8731–8739.

Riess, M.L., Eells, J.T., Kevin, L.G., Camara, A.K., Henry, M.M., Stowe, D.F., 2004. Attenuation of mitochondrial respiration by sevoflurane in isolated cardiac mitochondria is mediated in part by reactive oxygen species. Anesthesiology 100, 498–505.

Sato, T., Saito, T., Saegusa, N., Nakaya, H., 2005. Mitochondrial Ca^{2+}-activated K^+ channels in cardiac myocytes: a mechanism of the cardioprotective effect and modulation by protein kinase A. Circulation 111, 198–203.

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., Kitakaze, M., 2004. Opening of Ca^{2+}-activated K^+ channels is involved in ischemic preconditioning in canine hearts. J. Mol. Cell. Cardiol. 37, 1213–1218.

Siemen, D., Loupatatzis, C., Borecky, J., Gulbins, E., Lang, F., 1999. Ca^{2+}-activated K channel of the BK-type in the inner mitochondrial membrane of a human glioma cell line. Biochem. Biophys. Res. Commun. 257, 549–554.

Sniecinski, R., Liu, H., 2004. Reduced efficacy of volatile anesthetic preconditioning with advanced age in isolated rat myocardium. Anesthesiology 100, 589–597.

Stowe, D.F., Aldakkak, M., Camara, A.K., Riess, M.L., Heinen, A., Varadarajan, S.G., Jiang, M.T., 2006. Cardiac mitochondrial preconditioning by Big Ca^{2+}-sensitive K^+ channel opening requires superoxide radical generation. Am. J. Physiol, Heart Circ. Physiol. 290, H434–H440.

Takasawa, M., Hayakawa, M., Sugiyama, S., Hattori, K., Ito, T., Ozawa, T., 1993. Age-associated damage in mitochondrial function in rat hearts. Exp. Gerontol. 28, 269–280.

Tani, M., Suganuma, Y., Hasegawa, H., Shinmura, K., Ebihara, Y., Hayashi, Y., Guo, X., Takayama, M., 1997. 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.

Weber, N.C., Toma, O., Damla, H., Wolter, J.I., Schlack, W., Preckel, B., 2006. Upstream signaling of protein kinase C-epsilon in xenon-induced pharmacological preconditioning. Implication of mitochondrial adenosine triphosphate dependent potassium channels and phosphatidylinositol-dependent kinase-1. Eur. J. Pharmacol. 539, 1–9.

Xu, W., Liu, Y., Wang, S., McDonald, T., Van Eyk, J.E., Sidor, A., O'Rourke, B., 2002. Cytoprotective role of Ca^{2+}-activated K^+ channels in the cardiac inner mitochondrial membrane. Science 298, 1029–1033.