

Role of protein kinase C, PI3 kinase, tyrosine kinases, NO-synthase, K_{ATP} channels and MPT pore in the signaling pathway of the cardioprotective effect of chronic continuous hypoxia

Sergey Y. Tsibulnikov¹, Leonid N. Maslov¹, Natalia V. Naryzhnaya¹, Huijie Ma², Yury B. Lishmanov^{1,3}, Peter R. Oeltgen⁴ and Keith Garlid⁵

¹ Laboratory of Experimental Cardiology, Cardiology Research Institute, Tomsk National Research Medical Center, Tomsk 634012, Russia

² Department of Physiology, Hebei Medical University, Shijiazhuang 050017, China

³ Laboratory of Nuclear Medicine, National Research Tomsk State Polytechnical University, 634050 Tomsk, Russia

⁴ Department of Pathology, University of Kentucky College of Medicine, Lexington, KY, USA

⁵ UCLA Cardiovascular Research Laboratory, David Geffen School of Medicine, Los Angeles, CA, USA

Abstract. It was established that adaptation to chronic continuous normobaric hypoxia (CCNH) increases cardiac tolerance to ischemia and reperfusion. Coronary artery occlusion (20 min) and reperfusion (3 h) was performed in Wistar rats. CCNH promoted a decrease in the infarct size/area at risk ratio in 2-fold. CCNH promoted an increase in the nitrite/nitrate levels in blood serum and myocardium. Pretreatment with protein kinase C (PKC) inhibitor chelerythrine, NO-synthase (NOS) inhibitor L-NAME, iNOS inhibitor S-methylisothiourea, K_{ATP} channel blocker glibenclamide, mito K_{ATP} channel blocker 5-hydroxydecanoic acid abolished the infarct-reducing effect of CCNH. The non-selective tyrosine kinase inhibitor genistein attenuated but not eliminated infarct-sparing effect of CCNH. The nNOS inhibitor 7-nitroindazole, sarc K_{ATP} channel blocker HMR 1098, MPT pore inhibitor atractyloside, PI3 kinase inhibitor wortmannin did not reverse infarct-limiting effect of CCNH. It was concluded that infarct-reducing effect of CCNH is mediated *via* PKC, iNOS activation and mito K_{ATP} channel opening. While nNOS, PI3 kinase, sarc K_{ATP} channel, MPT pore are not involved in the development of CCNH-induced cardiac tolerance to impact of ischemia-reperfusion.

Key words: Chronic hypoxia — Myocardial infarction — Kinases — K_{ATP} channels — MPT pore

Abbreviations: AAR, area at risk; AIH, intermittent hypoxia; AMI, acute myocardial infarction; CCNH, chronic continuous normobaric hypoxia; DAG, diacylglycerol; ECG, electrocardiogram; iNOS, inducible NOS; IS, infarct size; mito K_{ATP} channel, mitochondrial ATP-dependent K^+ channel; MPT pore, mitochondrial permeability transition pore; nNOS, neuronal NOS; NOS, NO-synthase; ORs, opioid receptors; PKC, protein kinase C; PLC, phospholipase C; PLD, phospholipase D; PVCs, ventricular complexes; RT, receptor tyrosine kinase; sarc K_{ATP} channel, sarcolemmal K_{ATP} channel.

Introduction

In-hospital mortality from acute myocardial infarction (AMI) in Israel was 1–4% (according to Buber et al. 2015).

In Denmark, during 30 days after hospitalization 7.9% patients with acute myocardial infarction died (Pedersen et al. 2014). In Germany, patients with AMI with ST segment elevation had a 30-day mortality rate of 5.6% (Zimmermann et al. 2013). However, in patients with cardiac arrest in the prehospital phase, the mortality rate was 26.6% (Zimmermann et al. 2013). Most of these patient lives could have been saved if they had been treated in advance with drugs which would increase cardiac resistance to ischemia/reperfu-

Correspondence to: Leonid N. Maslov, Laboratory of Experimental Cardiology, Cardiology Research Institute, Tomsk National Research Medical Center, Kievskaya 111A, Tomsk 634012, Russia
E-mail: maslov@cardio-tomsk.ru

sion which occurs during acute coronary syndrome. In our opinion, the study of cardioprotective effect of adaptation to chronic hypoxia could provide invaluable assistance in the creation of such drugs.

Fitzpatrick et al. (2005) demonstrated that the cardioprotective effect of adaptation to chronic hypoxia in newborn rabbits can be maintained for 30 days after the last treatment of hypoxia. In another study, Ostadal et al. (1995) showed that the protective effect of adaptation to chronic hypoxia may persist for as long as 4 months. No pharmacological agent has ever been shown to induce such a prolonged increase in tolerance of the heart to the action of ischemia/reperfusion. Understanding the molecular mechanism(s) of adaptive increase of cardiac resistance to ischemia/reperfusion may have pharmacological implications and opens up the prospect for the creation of fundamentally new drugs that mimic the cardioprotective effect of adaptation to chronic hypoxia.

Our studies and those of others indicate that an adaptation to intermittent hypoxia (AIH) provides increased cardiac tolerance to ischemia and reperfusion (Neckar et al. 2002; Kolar et al. 2005; Naryzhnaya et al. 2009; Estrada et al. 2016; Míčová et al. 2017). The number of publications devoted to the cardioprotective effect of continuous chronic normobaric hypoxia is also large (Tajima et al. 1994; Maslov et al. 2013, 2015; Alanova et al. 2015, 2017; Holzerova et al. 2015; Kasparova et al. 2015; Naryzhnaya et al. 2015; Kolar et al. 2017; Neckář et al. 2017). Some studies suggest that the adaptation of rats to chronic continuous normobaric hypoxia (CCNH) reduces tolerance of heart to the action of hypoxia/reoxygenation and allows for increasing cardiac resistance to ischemia and reperfusion and represents only adaptation to AIH (Milano et al. 2002, 2004, 2010, 2011). However, we and others have found that adaptation of the rats to CCNH has the infarct-limiting effect during coronary artery occlusion and reperfusion *in vivo* reducing myocardial infarct size by 1.5–2-fold (Maslov et al. 2013; Alanova et al. 2015, 2017; Kasparova et al. 2015; Neckář et al. 2017). CCNH increases an isolated heart resistance to global ischemia-reperfusion injury (Tajima et al. 1994; Maslov et al. 2015), and enhances tolerance isolated cardiomyocytes to the action of hypoxia/reoxygenation (Naryzhnaya et al. 2015). However, despite the large number of publications, the signal mechanism of cardioprotective action of CCNH remains poorly studied.

In planning this study, we assumed that the signaling mechanism of the infarct-limiting effect of adaptation to hypoxia is similar to the molecular mechanism of delayed ischemic preconditioning that it is involved protein kinase C, PI3 kinase, tyrosine kinases, NO-synthase isoforms, K_{ATP} channels, MPT pore (Kis et al. 2003; Rajesh et al. 2003; Yellon et al. 2003). However, it is not possible to assert a priori that the signaling pathways of the infarct-limiting effect of adap-

tation to chronic hypoxia and preconditioning are identical. This is because the adaptive increase in cardiac tolerance to ischemia persists 1–4 months (Ostadal et al. 1995; Fitzpatrick et al. 2005) while that of delayed preconditioning lasts for only 72 hours (Baxter et al. 1997).

The main goal of the study is to evaluate the significance of protein kinase C, PI3 kinase, tyrosine kinases, NO-synthase, K_{ATP} channels and MPT pore in the signal mechanism of the infarct reducing effect of chronic continuous normobaric hypoxia.

Material and Methods

Animals

The experimental protocol was approved by the Ethical Committee of the Research Institute of Cardiology and it conformed to the EU Directive 2010/63/EU. Male Wistar rats weighing 250–300 g were housed at $23 \pm 1^\circ\text{C}$ with a relative humidity of 60–70% and a light/dark cycle of 12 h with free access to water and standard rat chow. Further processing was performed as described previously (Maslov et al. 2013). Briefly, rats were exposed to continuous normobaric hypoxia for three weeks in a chamber (1.5 m³) equipped with a hypoxia generator Bio-Nova-204G4R1 (NTO Bio-Nova Company, Moscow, Russia). Concentrations of O₂ and CO₂ in the chamber were continuously measured by TCO₂-IR and OLC 20 sensors, and maintained at 11.75–12.25% and 0.03%, respectively, by a MX32 controller (Oldham, France). Normoxic rats were used as a control group. It has been shown early that chronic hypoxia led to the right ventricle hypertrophied as a consequence of hypoxic pulmonary hypertension. While the relative right ventricle weight increased by 64% in these animals, the relative left ventricle weight did not differ from controls. CCNH resulted in a marked polycythemia as documented by increases in the number of red blood cells, hematocrit and the concentration of hemoglobin (Kolar et al. 2005)

Myocardial ischemia/reperfusion

The rats were anesthetized with pentobarbital sodium (60 mg/kg) 1 h after completion of the adaptation. After tracheotomy lungs were ventilated by SAR-830 Series device (Central Wisconsin Engineers Inc., Schofield, USA) with room air. Atelectasis was prevented by maintaining a positive end-expiratory pressure of 5–10 mm H₂O. Arterial pH, PCO₂, and PO₂ were monitored throughout the experiment by a blood gas analyzer (Stat Profile M, Nova Biomedical Corporation, Waltham, MA, USA) and maintained within a normal physiological range by adjusting the respiratory rate and/or tidal volume. Body temperature was maintained at

37°C by a heating pad PhysioSuite (non-invasive monitoring system for mice and rats, Kent Scientific Corporation, Torrington, USA).

The left femoral artery was cannulated for blood pressure, heart rate, and blood gases measurements. Blood pressure and standard peripheral lead electrocardiogram (ECG) recordings were performed with a MP35 apparatus (Biopac Systems, Inc., Goleta, CA) and a computer using the BSL PRO 3.7.3 software (Biopac Systems Inc., Goleta, CA). The right femoral vein was cannulated for the administration of pharmacological agents or vehicles.

Regional myocardial I/R was induced as described by Neckar et al. (2005). Left thoracotomy was performed and after 10-min stabilization, regional myocardial ischemia was induced by tightening a ligature (6-0 Prolene) placed around the left anterior descending coronary artery near to

its origin. Characteristic changes in the configuration of the ECG and a transient decrease in blood pressure verified the coronary artery occlusion. After a 20-min occlusion period, the ligature was released and reperfusion of previously ischemic tissue continued.

Infarct size determination

At the end of 3-h reperfusion, the hearts were excised and perfused with saline through the cannulated aorta. The area at risk and the infarct size were delineated by staining with 5% potassium permanganate and 1% 2,3,5-triphenyltetrazolium chloride, respectively (Neckar et al. 2005). The right ventricle was separated and the left ventricle was cut perpendicularly to its long axis into slices 1 mm thick and stored overnight in 10% neutral formaldehyde solution. The infarct

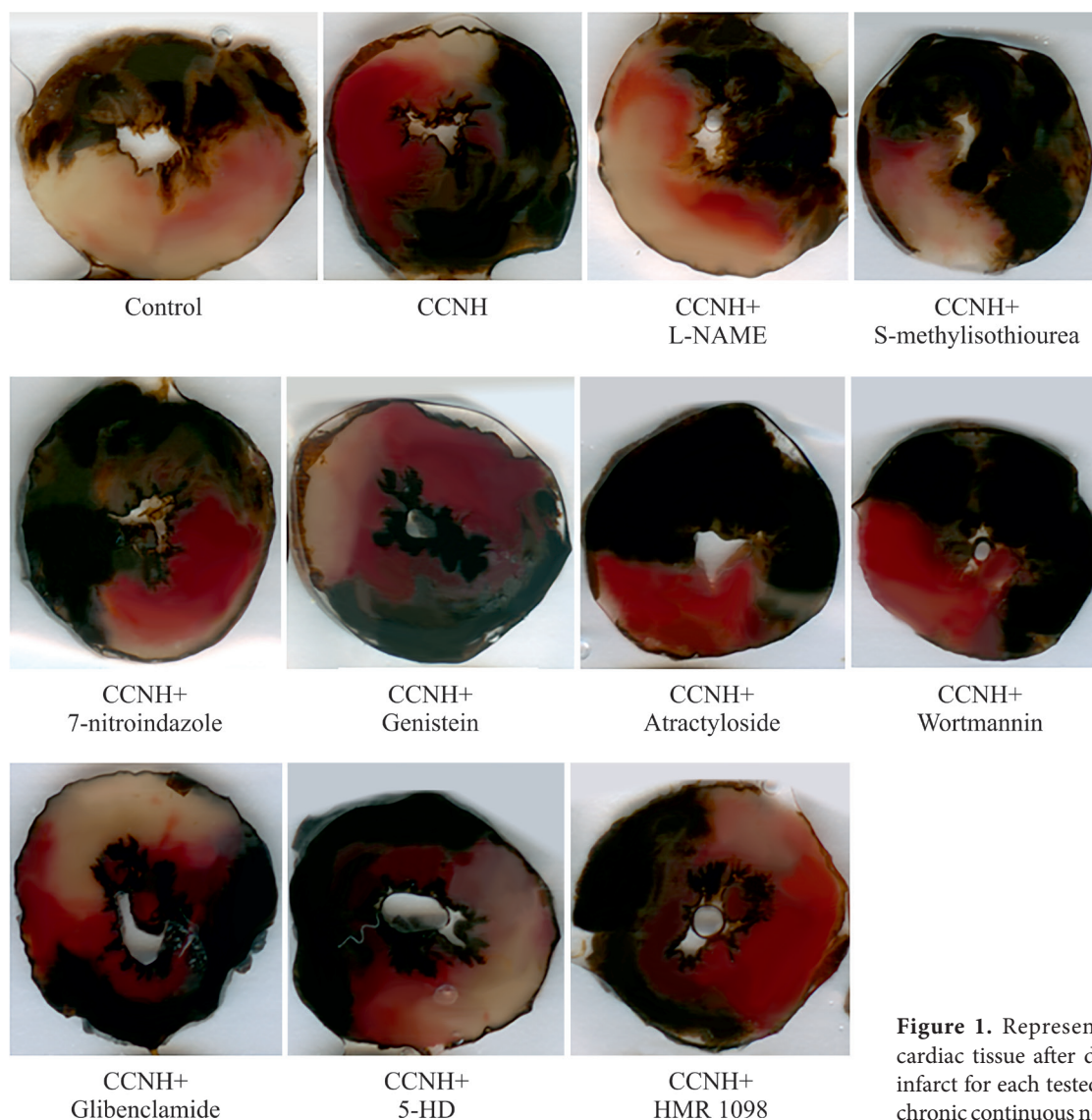


Figure 1. Representative pictures of cardiac tissue after damage induced by infarct for each tested inhibitor. CCNH, chronic continuous normobaric hypoxia.

size (IS), the size of the area at risk (AAR) and the size of the left ventricle were determined by a planimetric method using Scanjet G4050 scanner (Hewlett-Packard, Palo Alto, USA). The IS was normalized to the AAR (IS/AAR) and the size of the AAR was normalized to the left ventricle (AAR/LV).

Incidence of arrhythmias

Ventricular arrhythmias were recorded from the ECG signal during 20-min coronary artery occlusion. The incidences of single premature ventricular complexes including salvos, ventricular tachycardia and ventricular fibrillation were evaluated separately.

Protocol

All compounds were administered intravenously 25 min before coronary artery occlusion with the exception of glibenclamide, hydroxydecanoic acid sodium salt (5-HD), atractyloside and S-methylisothiouraea (Figs. 1, 2). Glibenclamide was given 45 min before ischemia as recommended by Schultz et al. (1997). 5-Hydroxydecanoate was injected 5 min before ischemia as recommended by Fryer et al. (2000). Atractyloside also was administered 5 min before ischemia (Rajesh et al. 2003). S-methylisothiouraea was administered intraperitoneally 25 min before coronary artery occlusion (Jiang et al. 2004). The inhibitor protein kinase C (PKC) chelerythrine was administered at a dose 5 mg/kg (Maslov et al. 2009). The K_{ATP} channel blocker glibenclamide was used at a dose 0.3 mg/kg (Schultz et al. 1997). The mitochondrial K_{ATP} channel (mito K_{ATP} channel) blocker 5-HD was applied at a dose of 5 mg/kg and sarcolemmal K_{ATP} channel (sarc K_{ATP} channel) HMR 1098 was used at a dose of 3 mg/kg (Fryer et al. 2000). An inhibitor of all isoenzymes of NO-synthase (NOS) L-NAME was given at a dose of 10 mg/kg (Maslov et al. 2009). The specific inducible NOS (iNOS) inhibitor S-methylisothiouraea was administered at a dose of 3 mg/kg (Jiang et al. 2004). The neuronal NOS (nNOS) blocker 7-nitroindazole was used at a dose 30 mg/kg (MacKenzie et al. 1994). The tyrosine kinase inhibitor genistein was given at a dose of 5 mg/kg (Maslov et al. 2009). The PI3 kinase

blocker wortmannin was injected at a dose of 0.015 mg/kg (Gross et al. 2004; Tong et al. 2011). The mitochondrial permeability transition (MPT) pore opener atractyloside was administered at a dose of 5 mg/kg (Rajesh et al. 2003).

2,3,5-Triphenyltetrazolium, potassium permanganate, HMR 1098, pentobarbital, L-NAME, 5-HD, S-methylisothiouraea, atractyloside were dissolved in isotonic saline. Other compounds were preliminary dissolved in 0.1 ml DMSO then dissolved in 0.9 ml 20% hydroxypropyl- β -cyclodextrin solution that was used for intravenous administration. This 20% hydroxypropyl- β -cyclodextrin solution was administered to control animals. Our previous investigation showed that this solution had no effect of the cardiac tolerance to impact of ischemia and reperfusion (Maslov et al. 2009).

Determination of NO metabolites

In order to estimate nitric oxide concentration in the blood and myocardial tissue, we had to determine its stable metabolites, the nitrites and nitrates (Bryan and Grisham 2007). Blood and myocardial tissue were taken 5 minutes after the end of ischemia (i.e. during reperfusion) for measurement of nitrite and nitrate concentrations. The resulting blood samples were centrifuged 10 minutes at $1100 \times g$. Serum samples were deproteinized by centrifugation in tubes Amicon Ultra-10k Merck Millipore (Billerica, USA) for 30 min at $14000 \times g$. Deproteinized serum were frozen and stored at -70°C (Bryan and Grisham 2007).

The heart was removed from the chest and was rinsed through the aorta with 5 ml of phosphate buffer containing 0.01 M KH_2PO_4 , 0.01 M K_2HPO_4 , 2.7 mM KCl, 137 mM NaCl, 2.5 mM EDTA, pH 7.4. 100-150 mg. Dissected tissue of the left ventricle from the area at risk was immediately frozen and stored in liquid nitrogen. Frozen myocardial tissue samples were homogenized in 1.5 ml of the same buffer and centrifuged for 15 minutes at $12000 \times g$. The supernatant was deproteinized and stored in the same way as described above for the serum (Bryan et al. 2007).

To determine the total content of nitrates and nitrites in deproteinized blood and tissue extracts, we used a colorimetric Nitrite/Nitrate Assay Kit, 23479-1KT-F from

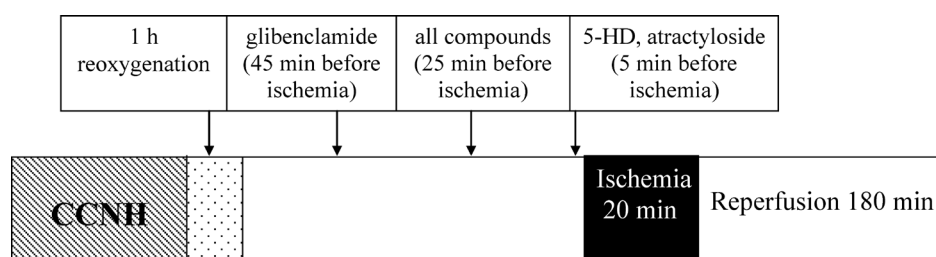


Figure 2. Protocol of experimental research. CCNH, chronic continuous normobaric hypoxia.

Sigma-Aldrich (USA). Absorbance measurement was performed with a spectrophotometer INFINITE 200M Tecan (Salzburg, Austria).

Chemicals used

Pentobarbital sodium was purchased from Sanofi-Aventis (France). 2,3,5-Triphenyltetrazolium chloride, N^ω-nitro-L-arginine methyl ester hydrochloride (L-NAME), atractyloside sodium salt, 5-5-HD, glibenclamide, genistein, wortmannin, S-methylisothiourea hemisulfate salt, 7-nitroindazole were purchased from Sigma-Aldrich (USA). HMR 1098 was a gift of Sanofi-Aventis Deutschland GmbH (Frankfurt am Main, Germany). Hydroxypropyl-β-cyclodextrin was purchased from Tocris Bioscience (Bristol, UK). Chelerythrine chloride was purchased from LC Laboratories (USA).

Statistical analysis

Results were expressed as means ± SEM from indicated number of experiments. One-way analysis of variance with Newman-Keuls *post hoc* test was used to detect differences in parametric

variables among groups. The Chi squared test was used to detect differences in the incidence of arrhythmias among groups. Differences were considered significant at $p < 0.05$.

Results

Hemodynamics and ventricular arrhythmias

Mean blood pressure and heart rate did not differ between normoxic and CCNH groups. The blood pressure and heart rate values during ischemia and reperfusion were relatively stable (Tables 1, 2). None of the inhibitors used in this study had any significant effect on these variables (data not show).

All normoxic control rats exhibited premature ventricular complexes (PVCs) during 20-min coronary artery occlusion. Ventricular tachycardia was registered in 95% of rats, ventricular fibrillation was detected in 76% of rats. These heart rhythm disturbances were reversible and ended with the restoration of normal sinus rhythm. The incidence and severity of ischemic arrhythmias were significantly affected neither by CCNH, nor by the administration of inhibitors (data not shown).

Table 1. Mean blood pressure of rats after chronic continuous normobaric hypoxia course

| | Mean BP | | | | | | |
|---------|-----------------|---------|----------------|---------|---------|-------------------|---------|
| | Before ischemia | | After ischemia | | | After reperfusion | |
| | 10 min | 0 min | 5 min | 10 min | 20 min | 5 min | 45 min |
| Control | 129 ± 2 | 127 ± 2 | 124 ± 3 | 125 ± 2 | 123 ± 2 | 124 ± 1 | 125 ± 3 |
| CCNH | 126 ± 1 | 124 ± 2 | 126 ± 2 | 123 ± 3 | 124 ± 3 | 126 ± 2 | 123 ± 2 |

Values are means ± SEM; $n = 15$ (in each group). CCNH, chronic continuous normobaric hypoxia; BP, blood pressure.

Table 2. Heart rate of rats after chronic continuous normobaric hypoxia course

| | Heart rate | | | | | | |
|---------|-----------------|----------|----------------|----------|----------|-------------------|----------|
| | Before ischemia | | After ischemia | | | After reperfusion | |
| | 10 min | 0 min | 5 min | 10 min | 20 min | 5 min | 45 min |
| Control | 371 ± 22 | 370 ± 18 | 382 ± 22 | 368 ± 17 | 369 ± 24 | 373 ± 18 | 366 ± 26 |
| CCNH | 374 ± 15 | 379 ± 14 | 380 ± 24 | 371 ± 21 | 369 ± 20 | 369 ± 21 | 364 ± 23 |

Values are means ± SEM; $n = 15$ (in each group). CCNH, chronic continuous normobaric hypoxia.

Table 3. The content of nitrates and nitrites in blood serum and myocardium of rats after chronic continuous normobaric hypoxia course

| Groups | Before ischemia | | After ischemia | |
|-----------------------|-----------------|--------------------|-----------------|------------------------|
| | Serum (mM/l) | Myocardium (mM/mg) | Serum (mM/l) | Myocardium AAR (mM/mg) |
| Normoxia ($n = 12$) | 3.75 ± 0.49 | 0.20 ± 0.058 | 3.68 ± 0.376 | 0.17 ± 0.044 |
| CCNH ($n = 10$) | 5.53 ± 0.65 | 0.43 ± 0.068 | 6.10 ± 1.34 | 0.367 ± 0.07 |
| | ($p = 0.045$) | ($p = 0.021$) | ($p = 0.027$) | ($p = 0.03$) |

Values are means ± SEM. Blood and myocardial tissue were taken before and 5 minutes after the beginning of reperfusion. CCNH, chronic continuous normobaric hypoxia; AAR, area at risk zone; p is significance of differences with respect to normoxia rats.

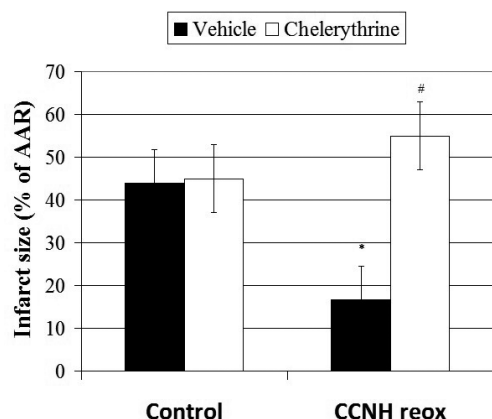


Figure 3. Effects of inhibitor protein kinase C chelerythrine on myocardial infarct size in normoxic (Control) and chronically hypoxic (CCNH reox) rats. Values are means \pm SEM; $n = 10$ (in each group). CCNH, chronic continuous normobaric hypoxia; AAR, area at risk; reox, reoxygenation. * $p < 0.05$ vs. corresponding normoxic group; # $p < 0.05$ vs. corresponding hypoxic group.

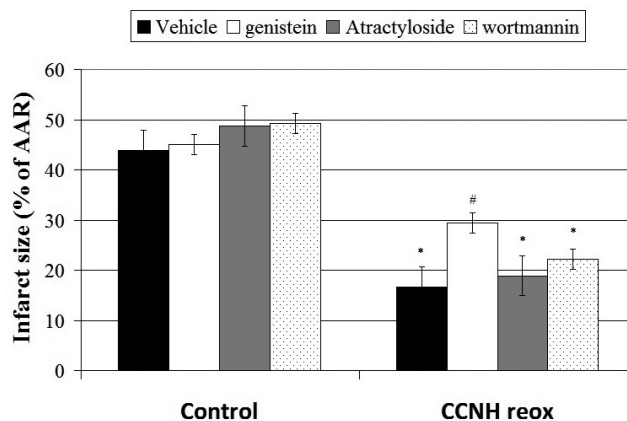


Figure 5. Effects of the tyrosine kinase inhibitor genistein, the PI3 kinase blocker wortmannin and the mitochondrial permeability transition pore opener atractyloside on myocardial infarct size in normoxic and chronically hypoxic rats. Values are means \pm SEM; $n = 10$ (in each group). CCNH, chronic continuous normobaric hypoxia; AAR, area at risk; reox, reoxygenation. * $p < 0.05$ vs. corresponding normoxic group; # $p < 0.05$ vs. corresponding hypoxic group.

The content of nitrates and nitrites in blood serum and myocardium

Chronic hypoxia resulted in a 1.5-fold increase in nitrite/nitrate in blood and in 2-fold increase in myocardial tissue (Table 3). Our data indicate that 5 min after start of reperfusion nitrite/nitrate content was approximately 2-fold higher

in blood and myocardium (area at risk) of adapted rats as compared with non-adapted animals (Table 3).

Infarct size

Mean values of the normalized AAR ranged from 37.3% to 45.4% of left ventricle and did not differ significantly

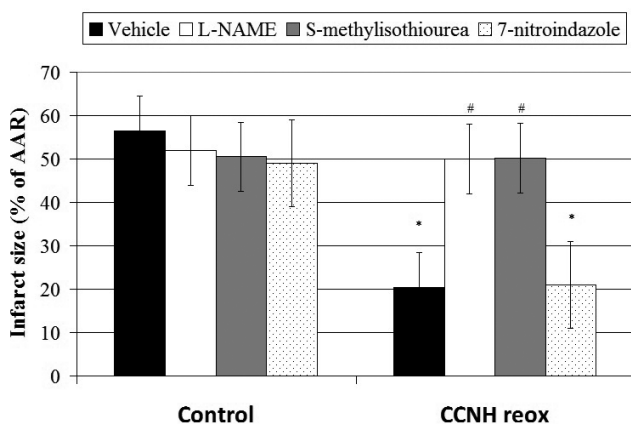


Figure 4. Effects of inhibitor of all isoenzymes of NO-synthase L-NAME, the specific inducible NOS inhibitor S-methylisothiourrea and the neuronal NOS blocker 7-nitroindazole on myocardial infarct size in normoxic and chronically hypoxic rats. Values are means \pm SEM; $n = 10-12$ (in each group). CCNH, chronic continuous normobaric hypoxia; AAR, area at risk; reox, reoxygenation. * $p < 0.05$ vs. corresponding normoxic group; # $p < 0.05$ vs. corresponding hypoxic group.

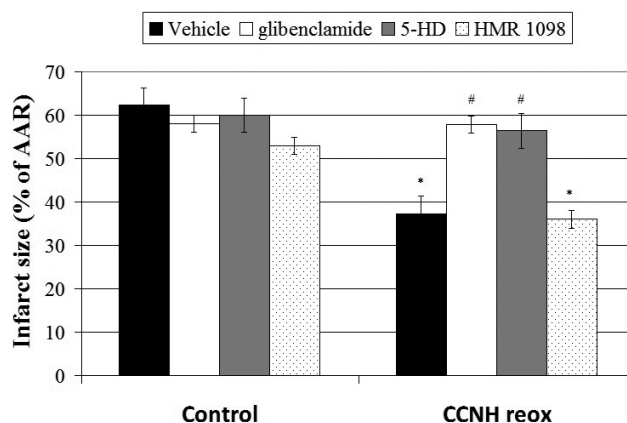


Figure 6. Effects of the K_{ATP} channel blocker glibenclamide, the mitochondrial K_{ATP} channel blocker 5-HD and sarcolemmal K_{ATP} channel HMR 1098 on myocardial infarct size in normoxic and chronically hypoxic rats. Values are means \pm SEM; $n = 10$ (in each group). CCNH, chronic continuous normobaric hypoxia; AAR, area at risk; reox, reoxygenation. * $p < 0.05$ vs. corresponding normoxic group; # $p < 0.05$ vs. corresponding hypoxic group.

among the groups. Mean values of infarct size normalized to AAR were similar in normoxic controls from three series of experiments, and the adaptation to CCNH resulted in a marked infarct-limiting effect as showed in Figs. 1, 3–6.

Our studies indicate that CCNH decreases the IS/AAR ratio approximately by 2-fold (Figs. 1 and 3). Pretreatment with chelerythrine completely abolished the infarct-reducing effect of CCNH (Figs. 1 and 3). The inhibition of NO synthesis with L-NAME completely eliminated the infarct-sparing effect of CCNH (Figs. 1 and 4). As shown in Figure 4, the selective iNOS inhibitor S-methylisothiourea also blocked the infarct-limiting effect of CCNH. In contrast the selective nNOS inhibitor 7-nitroindazole did not reverse adaptation-induced enhancement of cardiac tolerance to ischemia and reperfusion (Figs. 1 and 4). As shown in Figure 5, the selective tyrosine kinase inhibitor partially eliminated the infarct-reducing effect of chronic hypoxia. In contrast the selective PI3 inhibitor, wortmannin, did not alter the effect on the IS/AAR ratio in both adapted and non-adapted rats (Figure 5). Pretreatment with MPT pore opener, atractyloside, had no effect on the CCNH-induced enhancement of cardiac tolerance to ischemia and reperfusion (Figure 5). Pretreatment with non-selective K_{ATP} channel inhibitor, glibenclamide, completely reversed infarct-sparing effect of chronic hypoxia (Figs. 1 and 6). As shown in Figure 6 the selective mito K_{ATP} channel blocker, 5-HD, exhibited the same effect. Pretreatment with the selective sarc K_{ATP} channel blocker, HMR 1098, did not abolish infarct reducing effect of CCNH (Figs. 1 and 6).

Discussion

We have previously shown that chronic continuous normobaric hypoxia causes adaptation to hypoxia and enhances cardiac tolerance to impact of ischemia/reperfusion due to activation of opioid receptors (ORs) by endogenous opioid peptides (Maslov et al. 2013). Our current studies demonstrate that PKC plays an important role in cardioprotective effect of CCNH. This result is consistent with our previously published data indicating that PKC is involved in the infarct-limiting effect of adaptation to chronic hypoxia (Hlavackova et al. 2010; Holzerova et al. 2015). PKC plays an important role in cardioprotective effect of ischemic preconditioning (Yellon and Downey 2003). It has also been shown that this kinase is involved in the infarct-sparing effect of opioids (Fryer et al. 2001; Zhang et al. 2005; Maslov et al. 2009). Therefore, the participation of PKC in the cardioprotective effect of adaptation to hypoxia is not surprising. Previous studies have shown that the PI3 kinase is involved in the cardioprotective effect of “second window” of preconditioning (Kis et al. 2003). The same enzyme mediates the

infarct-limiting effect of exogenous opioids (Peart et al. 2008, 2011). However, our studies indicate that the PI3 kinase is not involved in the infarct-reducing effect of adaptation to hypoxia.

Prior studies have shown that PKC and K_{ATP} channel play an important role in the signaling pathway of the infarct-limiting effect of preconditioning (Yellon and Downey 2003). Our studies indicate that pretreatment with the non-selective K_{ATP} channel blocker glibenclamide completely abrogated the infarct-sparing effect of CCNH. The selective mito K_{ATP} channel inhibitor 5-HD exerted the same effect. In contrast, the selective sarc K_{ATP} channel blocker HMR 1098 did not alter CCNH-induced tolerance to ischemia and reperfusion. This result is consistent with published data indicating the participation of mito K_{ATP} channel in the infarct-limiting effect of ischemic preconditioning (Yellon and Downey 2003) and adaptation to intermittent hypoxia (Kolar et al. 2005; Bu et al. 2015). Our studies and those of other investigators have documented that mito K_{ATP} channel is involved in the signaling pathway of cardioprotective effect of exogenous opioids (McPherson and Yao 2001; Peart et al. 2008; Maslov et al. 2009; Gross et al. 2012). This result is consistent with published data on participation of opioid receptors in the infarct reducing effect of CCNH (Maslov et al. 2013) and chronic intermittent hypoxia (Estrada et al. 2016). It has also been shown that PKC ϵ can activate mito K_{ATP} channel (Costa et al. 2005; Jaburek et al. 2006; Budas and Mochly-Rosen 2007). In turn, mito K_{ATP} channel opening may promote an increase in production of reactive oxygen species which activates PKC (Costa et al. 2008).

The results of our experiments with atractyloside were somewhat unexpected since we had previously demonstrated that adaptation to chronic continuous hypoxia blocks the opening of MPT pore in response to ischemia/reperfusion of the isolated rat heart (Maslov et al. 2015). It has been shown that atractyloside abolished infarct reducing effect chronic intermittent hypoxia (Bu et al. 2015). Prior studies have shown that MPT pore plays an important role in the pathogenesis of apoptotic cell death (Halestrap 2010). However, its involvement in the mechanism of necrotic cell death has not been proved. We hypothesize that the MPT pore may be involved in the antiapoptotic effect of adaptation to hypoxia. To date this is our assumption because the antiapoptotic effect of adaptation to hypoxia has yet to be studied. It is possible that MPT pore participates in the infarct limiting effect of chronic intermittent hypoxia but it are not involved in the cardioprotective effect of CCNH.

Our studies indicate that iNOS is involved in the infarct-sparing effect of CCNH. This finding is consistent with our previously published data on the participation of NO/cGMP signaling pathway in the infarct-limiting effect of pre- and postconditioning (Costa et al. 2008). In addition, our data are consistent with other studies that have shown that the

cardioprotective effect of opioids is depend upon an activation of NOS (Patel et al. 2004; Maslov et al. 2009; Karlsson et al. 2011; Gross et al. 2012).

Our follow-up experiments confirmed the important role of NO-synthase in the infarct reducing effect of adaptation to hypoxia. We demonstrated that chronic continuous normobaric hypoxia causes a 1.5-fold increase in the total content of nitrates and nitrites in the blood, compared to intact animals ($p < 0.05$, Table 3). The content of nitrates and nitrites in myocardial tissue after CCNH was twice as large as that in unadapted rats (Table 3). We observed similar changes following 5 min after exposure to ischemia. Nitrate and nitrite levels were elevated 1.65-fold in the blood and 2.1-fold in myocardium of rats subjected to CCNH, compared to control rats after ischemia (Table 3). These data indicate a significant increase in nitrite and nitrate content in rats with chronic hypoxia and can serve as an indirect measure of the intensity of nitric oxide synthesis with NO-synthase. Our next experiments were designed to identify the significance of these changes in the implementation of the infarct-limiting effect CCNH. We found that the selective inhibition of iNOS completely abolished infarct sparing effect of CCNH. This result demonstrates that this isoenzyme plays an important role in the adaptation increase in cardiac tolerance to the impact of the heart ischemia/reperfusion.

Signal transmission from the opioid receptor to PKC involves phospholipase C (PLC) (Chen et al. 1991; Jin et al. 1992; Okajima et al. 1993), phospholipase D (PLD) (Mangoura and Dawson 1993) and diacylglycerol (DAG) (Mangoura and Dawson 1993) which activates PKC. While the transmission signal from the opioid receptor, NO-synthase, involves a transactivation of receptor tyrosine kinase (RT), epidermal growth factor receptor and the following signaling pathway OR/RT/PI3K/AKT/nNOS/NO/K_{ATP} (Cunha et al. 2010; Cohen and Downey 2011).

It should be noted that Downey and coworkers (Qin et al. 2004) showed that cardioprotective signaling from GPCR receptors terminates with protein kinase G (PKG) and that the signaling pathway contains NOS, to synthesize NO, and guanylyl cyclase, to synthesize cGMP, which is necessary to activate PKG. In a later work, this group showed that activated PKG opens mitoK_{ATP} when added to isolated mitochondria (Costa et al. 2005). However, we found that the PI3 kinase is not involved in the infarct-limiting effect of CCNH. Apparently there is an alternative pathway of adaptation increase in cardiac tolerance to ischemia/reperfusion. One of tyrosine kinases is involved in this pathway but this pathway plays a minor role in the increase in cardiac tolerance to ischemia because genistein reduces the cardioprotective effect of adaptation but does not eliminate it.

In 2011, Karlsson et al. (2011) reported that infarct-reducing effect of the opioid Eribis peptide 94 is accom-

panied by phosphorylation of eNOS Ser1177. Based on the aforementioned study our hypothesis is that endogenous opioid peptides can induce phosphorylation of eNOS by activating PKC. Indeed there is evidence that PKC can phosphorylate eNOS (Chen et al. 2014; Li et al. 2016). There is also evidence that PKC may be involved in the upregulation of iNOS (Ahmed et al. 2010; Bhatt et al. 2010). At the same time we cannot exclude a mechanism of action in which iNOS activation occurs utilizing an opioid-independent mechanism relying on de novo synthesis of iNOS.

Costa et al. found evidences that the increase in NO production can induce the signaling pathway for NO/GC/cGMP/PKG/K_{ATP} (Costa and Garlid 2008) which may serve as the other signaling pathway for OR/PLC, PLD/DAG/PKC/K_{ATP} and also leads to enhancement of cardiac tolerance to ischemia/reperfusion.

Thus, our results indicate that the infarct-reducing effect of chronic continuous hypoxia may be the result of the enhancement in NO production as a result of the activation of iNOS. Nitric oxide may promote mitoK_{ATP} channel opening which in turn can lead to increased tolerance to cardiac ischemia and reperfusion. Our data indicate that the PKC plays an important role in the infarct-limiting effect of continuous adaptation to hypoxia. While the role of the tyrosine kinase in the cardioprotective effect of CCNH may be of lesser importance. Our experiments indicate that the MPT pore and PI3 kinase do not play a significant role in the infarct-reducing effect of CCNH.

Acknowledgement. This project was supported by the Russian Science Foundation grant 16-15-10001. Experiments with chelerythrine were performed within the framework of the state task AAAA-A15-115120910024-0. The authors are grateful to Dr. Norbert Krass and Dr. Juergen Punter for HMR 1098. The authors are grateful to Dr. I. Khaliulin for discussing the results.

Conflict of interest. The authors declare that there are no conflicts of interest.

References

- Ahmed RP, Haider KH, Shujia J, Afzal MR, Ashraf M (2010): Sonic Hedgehog gene delivery to the rodent heart promotes angiogenesis via iNOS/netrin-1/PKC pathway. *PLoS One* **5**, e8576 <https://doi.org/10.1371/journal.pone.0008576>
- Alanova P, Kolar F, Oštádal B, Neckar J (2015): Role of NO/cGMP signaling pathway in cardiac ischemic tolerance of chronically hypoxic rats. *Physiol. Res.* **64**, 783–787
- Alanova P, Chytilová A, Neckar J, Hrdlička J, Míčová P, Holzerová K, Hlaváčková M, Macháčková K, Papoušek F, Vašinová J, et al. (2017): Myocardial ischemic tolerance in rats subjected to endurance exercise training during adaptation to chronic hypoxia. *J. Appl. Physiol.* **122**, 1452–1461

- <https://doi.org/10.1152/japplphysiol.00671.2016>
- Baxter GF, Goma FM, Yellon D (1997): Characterisation of the infarct-limiting effect of delayed preconditioning: timecourse and dose-dependency studies in rabbit myocardium. *Basic Res. Cardiol.* **92**, 159–167
<https://doi.org/10.1007/BF00788633>
- Bhatt KH, Pandey RK, Dahiya Y, Sodhi A (2010): Protein kinase C δ and protein tyrosine kinase regulate peptidoglycan-induced nuclear factor- κ B activation and inducible nitric oxide synthase expression in mouse peritoneal macrophages in vitro. *Mol. Immunol.* **47**, 861–870
<https://doi.org/10.1016/j.molimm.2009.10.029>
- Bryan NS, Grisham MB (2007): Methods to detect nitric oxide and its metabolites in biological samples. *Free. Radic. Biol. Med.* **43**, 645–657
<https://doi.org/10.1016/j.freeradbiomed.2007.04.026>
- Bu HM, Yang CY, Wang ML, Ma HJ, Sun H, Zhang Y (2015): KATP channels and MPTP are involved in the cardioprotection bestowed by chronic intermittent hypobaric hypoxia in the developing rat. *J. Physiol. Sci.* **65**, 367–376
<https://doi.org/10.1007/s12576-015-0376-5>
- Buber J, Laish-Farkash A, Koren-Morag N, Fefer P, Segev A, Hod H, Matetzky S (2015): Cardiac troponin elevation pattern in patients undergoing a primary percutaneous coronary intervention for ST-segment elevation myocardial infarction: characterization and relationship with cardiovascular events during hospitalization. *Coron. Artery Dis.* **26**, 503–509
<https://doi.org/10.1097/MCA.0000000000000276>
- Budas GR, Mochly-Rosen D (2007): Mitochondrial protein kinase C ϵ (PKC ϵ): emerging role in cardiac protection from ischaemic damage. *Biochem. Soc. Trans.* **35**, 1052–1054
<https://doi.org/10.1042/BST0351052>
- Chen F, Kumar S, Yu Y, Aggarwal S, Gross C, Wang Y, Chakraborty T, Verin AD, Catravas JD, Lucas R, Black SM, Fulton DJ (2014): PKC-dependent phosphorylation of eNOS at T495 regulates eNOS coupling and endothelial barrier function in response to G $+$ -toxins. *PLoS One* **9**, e99823
<https://doi.org/10.1371/journal.pone.0099823>
- Chen L, Marine LMH (1991): Sustained potentiation of NMDA receptor mediated glutamate responses through activation of protein kinase C by a m opioid. *Neuron* **7**, 319–326
[https://doi.org/10.1016/0896-6273\(91\)90270-A](https://doi.org/10.1016/0896-6273(91)90270-A)
- Cohen MV, Downey JM (2011): Is it time to translate ischemic preconditioning's mechanism of cardioprotection into clinical practice? *J. Cardiovasc. Pharmacol. Ther.* **16**, 273–280
<https://doi.org/10.1177/1074248411407071>
- Costa AD, Garlid KD, West IC, Lincoln TM, Downey JM, Cohen MV, Critz SD (2005): Protein kinase G transmits the cardioprotective signal from cytosol to mitochondria. *Circ. Res.* **97**, 329–336
<https://doi.org/10.1161/01.RES.0000178451.08719.5b>
- Costa AD, Pierre SV, Cohen MV, Downey JM, Garlid KD (2008): cGMP signalling in pre- and post-conditioning: the role of mitochondria. *Cardiovasc. Res.* **77**, 344–352
<https://doi.org/10.1093/cvr/cvm050>
- Costa AD, Garlid KD (2008): Intramitochondrial signaling: interactions among mitoKATP, PKC ϵ , ROS, and MPT. *Am. J. Physiol. Heart Circ. Physiol.* **295**, H874–882
<https://doi.org/10.1152/ajpheart.01189.2007>
- Cunha TM, Roman-Campos D, Lotufo CM, Duarte HL, Souza GR, Verri WA, Funez MI, Dias QM, Schivo IR, Domingues AC, et al. (2010): Morphine peripheral analgesia depends on activation of the PI3Kgamma/AKT/nNOS/NO/KATP signaling pathway. *Proc. Natl. Acad. Sci. USA* **107**, 4442–4447
<https://doi.org/10.1073/pnas.0914733107>
- Estrada JA, Williams AG, Sun J, Gonzalez L, Downey HF, Caffrey JL, Mallet RT (2016): δ -Opioid receptor (DOR) signaling and reactive oxygen species (ROS) mediate intermittent hypoxia induced protection of canine myocardium. *Basic Res. Cardiol.* **111**, 17
<https://doi.org/10.1007/s00395-016-0538-5>
- Fitzpatrick CM, Shi Y, Hutchins WC, Su J, Gross GJ, Ostadal B, Tweddell JS, Baker JE (2005): Cardioprotection in chronically hypoxic rabbits persists on exposure to normoxia: role of NOS and KATP channels. *Am. J. Physiol. Heart Circ. Physiol.* **288**, H62–68
<https://doi.org/10.1152/ajpheart.00701.2004>
- Fryer RM, Hsu AK, Nagase H, Gross GJ (2000): Opioid-induced cardioprotection against myocardial infarction and arrhythmias: mitochondrial versus sarcolemmal ATP-sensitive potassium channels. *J. Pharmacol. Exp. Ther.* **294**, 451–457
- Fryer RM, Wang Y, Hsu AK, Gross GJ (2001): Essential activation of PKC- δ in opioid-initiated cardioprotection. *Am. J. Physiol. Heart Circ. Physiol.* **280**, H1346–1353
<https://doi.org/10.1152/ajpheart.2001.280.3.H1346>
- Gross ER, Hsu AK, Gross GJ (2004): Opioid-induced cardioprotection occurs via glycogen synthase kinase β inhibition during reperfusion in intact rat hearts. *Circ. Res.* **94**, 960–966
<https://doi.org/10.1161/01.RES.0000122392.33172.09>
- Gross GJ, Hsu A, Nithipatikom K, Pfeiffer AW, Bobrova I, Bissessar E (2012): Acute and chronic cardioprotection by the enkephalin analogue, Eribis peptide **94**, is mediated via activation of nitric oxide synthase and adenosine triphosphate-regulated potassium channels. *Pharmacology* **90**, 110–116
<https://doi.org/10.1159/000340058>
- Halestrap AP (2010): A pore way to die: the role of mitochondria in reperfusion injury and cardioprotection. *Biochem. Soc. Trans.* **38**, 841–860
<https://doi.org/10.1042/BST0380841>
- Hlavackova M, Kořichová K, Neckář J, Kolar F, Musters RJ, Novák F, Nováková O (2010): Up-regulation and redistribution of protein kinase C- δ in chronically hypoxic heart. *Mol. Cell. Biochem.* **345**, 271–282
<https://doi.org/10.1007/s11010-010-0581-8>
- Holzerova K, Hlaváčková M, Žurmanová J, Borchert G, Neckar J, Kolar F, Novák F, Nováková O (2015): Involvement of PKC ϵ in cardioprotection induced by adaptation to chronic continuous hypoxia. *Physiol. Res.* **64**, 191–201
- Jaburek M, Costa AD, Burton JR, Costa CL, Garlid KD (2006): Mitochondrial PKC ϵ and mitochondrial ATP-sensitive K $+$ channel copurify and coreconstitute to form a functioning signaling module in proteoliposomes. *Circ. Res.* **99**, 878–883
<https://doi.org/10.1161/01.RES.0000245106.80628.d3>
- Jiang X, Shi E, Nakajima Y, Sato S (2004): Inducible nitric oxide synthase mediates delayed cardioprotection induced by morphine in vivo: evidence from pharmacologic inhibition and gene-knockout mice. *Anesthesiology* **101**, 82–88

- <https://doi.org/10.1097/00000542-200407000-00014>
- Jin W, Lee N, Loh HH, Thayer SA (1992): Dual excitatory and inhibitory effects of opioids on intracellular calcium in neuroblastoma x glioma hybrid NG108-15 cells. *Mol. Pharmacol.* **42**, 1083–1089
- Karlsson LO, Grip L, Bissessar E, Bobrova I, Gustafsson T, Kavianipour M, Odenstedt J, Wikström G, Gonon AT (2011): Opioid receptor agonist Eribis peptide 94 reduces infarct size in different porcine models for myocardial ischaemia and reperfusion. *Eur. J. Pharmacol.* **651**, 146–151
<https://doi.org/10.1016/j.ejphar.2010.10.069>
- Kasparova D, Neckar J, Dabrowska L, Novotny J, Mraz J, Kolar F, Zurmanova J (2015): Cardioprotective and nonprotective regimens of chronic hypoxia diversely affect the myocardial antioxidant systems. *Physiol. Genomics.* **47**, 612–620
<https://doi.org/10.1152/physiolgenomics.00058.2015>
- Kis A, Yellon DM, Baxter GF (2003): Second window of protection following myocardial preconditioning: an essential role for PI3 kinase and p70S6 kinase. *J. Mol. Cell. Cardiol.* **35**, 1063–1071
[https://doi.org/10.1016/S0022-2828\(03\)00208-6](https://doi.org/10.1016/S0022-2828(03)00208-6)
- Kolar D, Gresikova M, Waskova-Arnostova P, Elsnicova B, Kohutova J, Hornikova D, Vebr P, Neckar J, Blahova T, Kasparova D, et al. (2017): Adaptation to chronic continuous hypoxia potentiates Akt/HK2 anti-apoptotic pathway during brief myocardial ischemia/reperfusion insult. *Mol. Cell. Biochem.* **432**, 99–108
<https://doi.org/10.1007/s11010-017-3001-5>
- Kolar F, Neckar J, Ostadal B (2005): MCC-134, a blocker of mitochondrial and opener of sarcolemmal ATP-sensitive K⁺ channels, abrogates cardioprotective effects of chronic hypoxia. *Physiol. Res.* **54**, 467–471
- Li P, Zhang L, Zhang M, Zhou C, Lin N (2016): Uric acid enhances PKC-dependent eNOS phosphorylation and mediates cellular ER stress: A mechanism for uric acid-induced endothelial dysfunction. *Int. J. Mol. Med.* **37**, 989–997
<https://doi.org/10.3892/ijmm.2016.2491>
- MacKenzie GM, Rose S, Bland-Ward PA, Moore PK, Jenner P, Marsden CD (1994): Time course of inhibition of brain nitric oxide synthase by 7-nitroindazole. *Neuroreport.* **5**, 1993–1996
<https://doi.org/10.1097/00001756-199410000-00039>
- Mangoura D, Dawson G (1993): Opioid peptides activate phospholipase D and protein kinase C- ϵ in chicken embryo neuron culture. *Proc. Natl. Acad. Sci. USA* **90**, 2915–2919
<https://doi.org/10.1073/pnas.90.7.2915>
- Maslov LN, Lishmanov YuB, Oeltgen PR, Barzakh EI, Krylatov AV, Govindaswami M, Brown SA (2009): Activation of peripheral δ 2 opioid receptors increases cardiac tolerance to ischemia/reperfusion injury: Involvement of protein kinase C, NO-synthase, KATP channels and the autonomic nervous system. *Life Sci.* **84**, 657–663
<https://doi.org/10.1016/j.lfs.2009.02.016>
- Maslov LN, Naryzhnaya NV, Tsibulnikov SY, Kolar F, Zhang Y, Wang H, Gusakova AM, Lishmanov YB (2013): Role of endogenous opioid peptides in the infarct size-limiting effect of adaptation to chronic continuous hypoxia. *Life Sci.* **93**, 373–379
<https://doi.org/10.1016/j.lfs.2013.07.018>
- Maslov LN, Naryzhnaya NV, Prokudina ES, Kolar F, Gorbunov AS, Zhang Y, Wang H, Tsibulnikov SY, Portnichenko AG, Lasukova TV, Lishmanov YB (2015): Preserved cardiac mitochondrial function and reduced ischemia/reperfusion injury afforded chronic continuous hypoxia: role of opioid receptors. *Clin. Exp. Pharmacol. Physiol.* **42**, 496–501
<https://doi.org/10.1111/1440-1681.12383>
- McPherson BC, Yao Z (2001): Signal transduction of opioid-induced cardioprotection in ischemia-reperfusion. *Anesthesiology* **94**, 1082–1088
<https://doi.org/10.1097/00000542-200106000-00024>
- Míčová P, Klevstig M, Holzerová K, Vecka M, Žurmanová J, Neckář J, Kolář F, Nováková O, Novotný J, Hlaváčková M (2017): Antioxidant tempol suppresses heart cytosolic phospholipase A2 α stimulated by chronic intermittent hypoxia. *Can. J. Physiol. Pharmacol.* **95**, 920–927
<https://doi.org/10.1139/cjpp-2017-0022>
- Milano G, Corno AF, Lippa S, Von Segesser LK, Samaja M (2002): Chronic and intermittent hypoxia induce different degrees of myocardial tolerance to hypoxia-induced dysfunction. *Exp. Biol. Med. (Maywood)* **227**, 389–397
<https://doi.org/10.1177/153537020222700604>
- Milano G, Bianciardi P, Corno AF, Raddatz E, Morel S, von Segesser LK, Samaja M (2004): Myocardial impairment in chronic hypoxia is abolished by short aeration episodes: involvement of K⁺ATP channels. *Exp. Biol. Med. (Maywood)* **229**, 1196–1205
<https://doi.org/10.1177/153537020422901115>
- Milano G, von Segesser LK, Morel S, Joncic A, Bianciardi P, Vassalli G, Samaja M (2010): Phosphorylation of phosphatidylinositol-3-kinase-protein kinase B and extracellular signal-regulated kinases 1/2 mediate reoxygenation-induced cardioprotection during hypoxia. *Exp. Biol. Med. (Maywood)* **235**, 401–410
<https://doi.org/10.1258/ebm.2009.009153>
- Milano G, Bianciardi P, Rochemont V, Vassalli G, Segesser LK, Corno AF, Guazzi M, Samaja M (2011): Phosphodiesterase-5 inhibition mimics intermittent reoxygenation and improves cardioprotection in the hypoxic myocardium. *PLoS One* **6**, e27910
<https://doi.org/10.1371/journal.pone.0027910>
- Naryzhnaya NV, Maslov LN, Vychuzhanova EA, Sementsov AS, Podoksyonov YK, Portnichenko AG, Lishmanov YB (2015): Effect of hypoxic preconditioning on stress reaction in rats. *Bull. Exp. Biol. Med.* **159**, 450–452
<https://doi.org/10.1007/s10517-015-2988-4>
- Naryzhnaya NV, Neckar J, Maslov LN, Lishmanov YuB, Kolar F, Lasukova TV (2009): The role of sarcolemmal and mitochondrial KATP-channels in realization of the cardioprotection and antiarrhythmic effect of different regimens of hypoxic adaptation. *Russ. Fiziol. Zh. Im. I. M. Sechenova.* **95**, 837–849 (in Russian)
- Neckar J, Papousek F, Novakova O, Ostadal B, Kolar F (2002): Cardioprotective effects of chronic hypoxia and ischaemic preconditioning are not additive. *Basic Res. Cardiol.* **97**, 161–167
<https://doi.org/10.1007/s003950200007>
- Neckář J, Svatoňová A, Weissová R, Drahotová Z, Zajíčková P, Brabcová I, Kolář D, Alánová P, Vašinová J, Šilhavý J, et al. (2017): Selective replacement of mitochondrial DNA increases the cardioprotective effect of chronic continuous hypoxia in spontaneously hypertensive rats. *Clin. Sci. (Lond)* **131**, 865–881
<https://doi.org/10.1042/CS20170083>

- Okajima F, Tomura H, Kondo Y (1993): Enkephalin activates the phospholipase C/Ca²⁺ system through cross-talk between opioid receptors and P2-purinergic or bradykinin receptors in NG 108-15 cells. *Biochem. J.* **290**, 241–247
<https://doi.org/10.1042/bj2900241>
- Ostadal B, Kolar F, Pelouch V, Widimsky J (1995): Ontogenetic differences in cardiopulmonary adaptation to chronic hypoxia. *Physiol. Res.* **44**, 45–51
- Patel HH, Hsu AK, Gross GJ (2004): COX-2 and iNOS in opioid-induced delayed cardioprotection in the intact rat. *Life Sci.* **75**, 129–140
<https://doi.org/10.1016/j.lfs.2003.10.036>
- Pearl JN, Gross ER, Reichelt ME, Hsu A, Headrick JP, Gross GJ (2008): Activation of kappa-opioid receptors at reperfusion affords cardioprotection in both rat and mouse hearts. *Basic Res. Cardiol.* **103**, 454–463
<https://doi.org/10.1007/s00395-008-0726-z>
- Pearl JN, Hoe LE, Gross GJ, Headrick JP (2011): Sustained ligand-activated preconditioning via δ -opioid receptors. *J. Pharmacol. Exp. Ther.* **336**, 274–281
<https://doi.org/10.1124/jpet.110.172593>
- Pedersen F, Butrymovich V, Kelbæk H, Wachtell K, Helqvist S, Kasstrup J, Holmvang L, Clemmensen P, Engstrøm T, Grande P, et al. (2014): Short- and long-term cause of death in patients treated with primary PCI for STEMI. *J. Am. Coll. Cardiol.* **64**, 2101–2108
<https://doi.org/10.1016/j.jacc.2014.08.037>
- Qin Q, Yang XM, Cui L, Critz SD, Cohen MV, Browner NC, Lincoln TM, Downey JM (2004) Exogenous NO triggers preconditioning via a cGMP- and mitoKATP-dependent mechanism. *Am. J. Physiol. Heart Circ. Physiol.* **287**, H712–718
<https://doi.org/10.1152/ajpheart.00954.2003>
- Rajesh KG, Sasaguri S, Zhitian Z, Suzuki R, Asakai R, Maeda H (2003): Second window of ischemic preconditioning regulates mitochondrial permeability transition pore by enhancing Bcl-2 expression. *Cardiovasc. Res.* **59**, 297–307
[https://doi.org/10.1016/S0008-6363\(03\)00358-4](https://doi.org/10.1016/S0008-6363(03)00358-4)
- Schultz JE, Yao Z, Cavero I, Gross GJ (1997): Glibenclamide-induced blockade of ischemic preconditioning is time dependent in intact rat heart. *Am. J. Physiol.* **272**, H2607–2615
<https://doi.org/10.1152/ajpheart.1997.272.6.H2607>
- Tajima M, Katayose D, Bessho M, Isoyama S (1994): Acute ischaemic preconditioning and chronic hypoxia independently increase myocardial tolerance to ischaemia. *Cardiovasc. Res.* **28**, 312–329
<https://doi.org/10.1093/cvr/28.3.312>
- Tong G, Sun Z, Wei X, Gu C, Kaye AD, Wang Y, Li J, Zhang Q, Guo H, Yu S, Yi D, Pei J (2011): Nitric oxide mediates the anti-apoptotic effect of U50,488H after myocardial ischemia and reperfusion-roles of PI3-Kinase, Akt, and endothelial nitric oxide synthase phosphorylation. *Life Sci.* **88**, 31–38
<https://doi.org/10.1016/j.lfs.2010.10.018>
- Yellon DM, Downey JM (2003): Preconditioning the myocardium: from cellular physiology to clinical cardiology. *Physiol. Rev.* **83**, 1113–1151
<https://doi.org/10.1152/physrev.00009.2003>
- Zhang Y, Chen ZW, Girwin M, Wong TM (2005): Remifentanyl mimics cardioprotective effect of ischemic preconditioning via protein kinase C activation in open chest of rats. *Acta Pharmacol. Sin.* **26**, 546–550
<https://doi.org/10.1111/j.1745-7254.2005.00100.x>
- Zimmermann S, Flachskampf FA, Alff A, Schneider R, Dechant K, Klinghammer L, Stumpf C, Zopf Y, Loehr T, Brand G, et al. (2013): Out-of-hospital cardiac arrest and percutaneous coronary intervention for ST-elevation myocardial infarction: long-term survival and neurological outcome. *Int. J. Cardiol.* **166**, 236–241
<https://doi.org/10.1016/j.ijcard.2011.11.029>

Received: February 5, 2018

Final version accepted: April 5, 2018