

REVIEW

The Involvement of Protein Kinases in the Cardioprotective Effect of Chronic Hypoxia

Natalia V. NARYZHNAYA¹, Hui-Jie MA², Leonid N. MASLOV¹

¹Cardiology Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia, ²Hebei Medical University, Shijiazhuang, Hebei Province, P.R. China

Received January 21, 2020

Accepted September 29, 2020

Epub Ahead of Print November 2, 2020

Summary

The purpose of this review is to analyze the involvement of protein kinases in the cardioprotective mechanism induced by chronic hypoxia. It has been reported that chronic intermittent hypoxia contributes to increased expression of the following kinases in the myocardium: PKC δ , PKC α , p-PKC ϵ , p-PKC α , AMPK, p-AMPK, CaMKII, p-ERK1/2, p-Akt, PI3-kinase, p-p38, HK-1, and HK-2; whereas, chronic normobaric hypoxia promotes increased expression of the following kinases in the myocardium: PKC ϵ , PKC β II, PKC η , CaMKII, p-ERK1/2, p-Akt, p-p38, HK-1, and HK-2. However, CNH does not promote enhanced expression of the AMPK and JNK kinases. Adaptation to hypoxia enhances HK-2 association with mitochondria and causes translocation of PKC δ , PKC β II, and PKC η to the mitochondria. It has been shown that PKC δ , PKC ϵ , ERK1/2, and MEK1/2 are involved in the cardioprotective effect of chronic hypoxia. The role of other kinases in the cardioprotective effect of adaptation to hypoxia requires further research.

Key words

Heart • Adaptation • Chronic hypoxia • Ischemia/reperfusion • Kinases

Corresponding author

Natalia V. Naryzhnaya, Principal Investigator of Laboratory Experimental Cardiology, Cardiology Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Kievskaya str, 111A, 634012, Russia. E-mail: natalynar@yandex.ru

Introduction

It is well known that adaptation to hypoxia increases resistance of the heart to ischemia/reperfusion (I/R) (Meerson *et al.* 1989, Kolar and Ostádal 2004, Gu *et al.* 2018, Tsibulnikov *et al.* 2018, Naryzhnaya *et al.* 2019, Prokudina *et al.* 2019). A cardioprotective effect has been observed in various models of chronic hypoxia. The most studied cardioprotective model has been that of intermittent hypobaric hypoxia (IHH), which partially mimics high altitude hypoxia and continuous normobaric hypoxia (CNH), and there are many variations of this model; for example, IHH is modeled at altitudes of 5000 m (Li *et al.* 2016, Gu *et al.* 2018), 5500 m (Morel *et al.* 2003), 7000 m (Neckar *et al.* 2005, Hlaváčková *et al.* 2010), and even 7620 m (Nehra *et al.* 2016), while normobaric hypoxia is modeled for O₂ contents of 8 % (Viganò *et al.* 2011), 10 % (Rafiee *et al.* 2002, Kolar *et al.* 2017, Zhao *et al.* 2008), and 12 % (Tsibulnikov *et al.* 2018, Naryzhnaya *et al.* 2016, Prokudina *et al.* 2019), with and without reoxygenation periods.

The study of the mechanisms of cardioprotection that occur during chronic hypoxia, particularly the intracellular kinase mechanisms, has attracted the attention of numerous researchers. It has been established that kinases play an important role in regulating cardiac tolerance to I/R (Heusch 2015, Yellon and Downey 2003). The activation of some kinases increases the resistance of the heart to I/R (Heusch 2015, Yellon and Downey 2003) while other kinases, in contrast, are involved in I/R heart damage (Milano *et al.* 2007, Ling

et al. 2013). The kinase mechanism of protective action has been studied extensively in various pre- and post-conditioning models (Heusch 2015). However, it is reasonable to believe that this mechanism is significantly different in chronic hypoxia, compared to that in the

conditioning models. The expression and activity of many kinases in the myocardium are altered after various hypoxic effects (Table 1). This review analyzes published data on the role of kinases in implementing the infarction-limiting effect of adaptation to hypoxia.

Table 1. The effect of chronic hypoxia on the expression of protein kinases in the myocardium

The name of the kinases	Localization	Hypoxia model	Authors
<i>PKCε</i> ↑, <i>PKCδ</i> ↑	RV	IHH (5500 m, 23 h/day, 2 weeks)	Morel <i>et al.</i> 2003
<i>PKCδ</i> ↑	LV	IHH (7000 m, 8 h/day, 5 days/week, 24–32 exposure)	Neckar <i>et al.</i> 2005
<i>p-PKCε</i> ↑	LV	IHH (7000 m, 8 h/day, 5 days/week, 24–32 exposure)	Hlavackova <i>et al.</i> 2007
<i>PKCa</i> , <i>p-PKCa</i> ↑	LV	IHH (7000 m, 8 h/day, 5 weeks)	Micova <i>et al.</i> 2016
<i>PKCε</i> ↑	LV	CNH (SaO ₂ 85 %, 10-days, infant rabbit)	Rafiee <i>et al.</i> 2002
<i>PKCε</i> ↑	LV	CNH (10 % O ₂ , 21 days)	Holzerova <i>et al.</i> 2015
<i>AMPK</i> ↑, <i>p-AMPK</i> ↑	Total	IHH (5000 m, 6 h/day 28 days)	Li <i>et al.</i> 2016
	LV		Gu <i>et al.</i> 2018
<i>p-AMPK</i> ↓	Total	INH (8 % O ₂ , 120 s) : (21 %, 300 s.) cycles, 8 h/day, 7 days/week, 10 weeks	Xie <i>et al.</i> 2016
<i>AMPK</i> no effect	Total	CNH (18 % O ₂ , 24 h)	Mohammed Abdul <i>et al.</i> 2015
<i>p-AMPK</i> ↑	Total	CNH (48 h at 8 % O ₂)	Viganò <i>et al.</i> 2011
<i>p-AMPK</i> no effect		CNH (10 days at 8 % O ₂)	
<i>p-AMPK</i> ↓	LV	CNH (10 % O ₂ , 21 days)	Kolar <i>et al.</i> 2017
<i>p-AMPK</i> ↓	RV	Cyanotic heart disease	Zhang <i>et al.</i> 2018
<i>CaMKIIγ</i> ↑ <i>CaMKIIδ</i> ↑	Total	CNH (10 % O ₂ , 3 weeks)	Zhao <i>et al.</i> 2008
<i>CaMKII</i> ↑	RV	IHH (7620 m, 21 days)	Nehra <i>et al.</i> 2016
<i>ERK2</i> ↑	RV	IHH (7000 m, 8 h/day, 25 exposures)	Strnisková <i>et al.</i> 2006
<i>p-ERK2</i> no effect	RV, LV		
<i>p-ERK1/2</i> ↑	LV	IHH (7000 m, 8 h/day, 5 weeks)	Micova <i>et al.</i> 2016
<i>p-ERK1/2</i> ↑ to CNH	LV	INH (10 % O ₂ , 2 weeks)	Milano <i>et al.</i> 2010
<i>p-ERK1/2</i> ↑	Atriums	INH (8 % O ₂ , 6 h/day, 30 days)	Zhang <i>et al.</i> 2018b
<i>p-Akt</i> ↑	LV	IHH (7000 m, 8 h/day, 25 exposures)	Strnisková <i>et al.</i> 2006
<i>p-Akt</i> ↑ to CNH	LV	INH (10 % O ₂ , 2 weeks)	Milano <i>et al.</i> 2010
<i>p-Akt</i> ↑	LV	CNH (10 % O ₂ , 21 days)	Kolar <i>et al.</i> 2017
<i>PI3K</i> ↑	Total	INH (4 daily cycles, each composed of 2-min at 6–8 % O ₂ followed by 3-min reoxygenation for 5 times, for 14 days)	Milano <i>et al.</i> 2013
<i>PI3K</i> ↓, <i>p-Akt</i> ↓	RV	INH (8 % O ₂ , 6 h/day, 30 days)	Zhang <i>et al.</i> 2018b
<i>PKA</i> no effect	Total	CNH (10 % O ₂ , 14 days)	Larsen <i>et al.</i> 2008
<i>PKA</i> ↑	LV	IHH (7000 m, 8 h/day, 35 exposures)	Kohutova <i>et al.</i> 2019

Table 1. (continued)

The name of the kinases	Localization	Hypoxia model	Authors
<i>p38 no effect</i>	RV, LV	IHH (7000 m, 8 h/day, 35 exposures)	Strnisková <i>et al.</i> 2006
<i>p-p38</i> ↓	RV		
<i>p-p38</i> ↑	LV		
<i>p-p38</i> ↑	LV	IHH (7000 m, 8 h/day, 5 weeks)	Micova <i>et al.</i> 2016
<i>p-p38</i> ↑	LV	CNH (10 % O ₂ , 2 weeks)	Morel <i>et al.</i> 2006, Milano <i>et al.</i> 2010
<i>p-p38</i> ↑	RV	CNH, cyanotic heart defects	Quing <i>et al.</i> 2007
<i>p-JNK no effect</i>	LV	CNH (10 % O ₂ , 2 weeks)	Morel <i>et al.</i> 2006
<i>JNK particular fraction</i> ↓	LV	IHH (7000 m, 8 h/day, 25 exposures)	Strnisková <i>et al.</i> 2006
<i>JNK particular fraction</i> ↑	RV		
<i>p-JNK</i> ↑	H ₉ C ₂ cells	Acute hypoxia (1 % O ₂ , 12–72 h)	He <i>et al.</i> 2016
<i>p-JNK/JNK</i> ↑	Total	INH (9 % O ₂ , 20 times/h for 8 h/day, 35 days)	Zhao <i>et al.</i> 2019
<i>PKG</i>	LV	IHH (7000m, 8 h/day, 35 exposures)	Kohutova <i>et al.</i> 2019
<i>HK-1</i> ↑, <i>HK-2</i> ↑	LV	IHH (7000 m, 8 h/day, 5 weeks)	Waskova-Arnostova <i>et al.</i> 2015
<i>HK-1</i> ↑, <i>HK-2</i> ↑	LV	CNH (10 % O ₂ , 3 weeks)	Kolar <i>et al.</i> 2017
<i>HK-2</i> ↑	LV	CNH (10 % O ₂ , 3 weeks)	Nedvedova <i>et al.</i> 2018

IHH – intermittent hypobaric hypoxia, CNH – continuous normobaric hypoxia, INH – intermittent normobaric hypoxia, PKC – protein kinase C, AMPK – AMP-activated protein kinase, CaMKII – Ca²⁺-calmodulin kinase II, ERK – extracellular signal-regulated kinase, Akt – Akt-kinase, PI3K – phosphatidylinositol-3-kinase, PKA – protein kinase A, p38 – p38-kinase, JNK – c-Jun N-terminal kinase, PKG – protein kinase G, GSK3β – glycogen synthase kinase 3β, HK – hexokinase, LV – left ventricular, RV – right ventricular

Protein kinase C

Protein kinase C, commonly abbreviated as PKC (EC 2.7.11.13), is a family of protein kinase enzymes involved in controlling the function of several other proteins by phosphorylating the hydroxyl groups of the serine and threonine amino acid residues in them. PKC enzymes, in turn, are activated by signals such as increases in the concentration of diacylglycerol (DAG) or calcium ions (Ca²⁺) (Endoh 1995). Hence, PKC enzymes play important roles in several signal transduction cascades, including the protective cascade of different types of conditioning (Okubo *et al.* 2003, Gao *et al.* 2013).

Intermittent hypoxia

In adult rats that were exposed to IHH, 23 h/day at a 5500 m simulated altitude with 1 h reoxygenation for 2 weeks, there was an increase in PKCδ and PKCε levels in the right myocardial ventricle (Morel *et al.* 2003). It has been found that the PKCδ protein level in the left ventricle of chronically hypoxic rats is elevated in more

severe IHH (8h/day at a 7000 m simulated altitude, 5 days/week, 24–32 exposure, Neckar *et al.* 2005, Hlaváčková *et al.* 2010). In a study by Neckar, it was established that the infarction-reducing effect of IHH does not appear after PKC inhibition with chelerythrine and the selective PKCδ blocker rottlerin reduced, but did not eliminate, the infarction-limiting effect of IHH (Neckar *et al.* 2005). In a later study, it was found that the PKCδ level in the particulate fraction of the left ventricle negatively correlates with infarct size after adaptation to intermittent hypoxia under the same conditions (Hlavackova *et al.* 2007). At the same time, there is evidence that an increase in PKCδ activity could be the result of the oxidative stress observed after exposure to IHH. Thus, it has been found that daily administration of the antioxidant N-acetylcysteine to rats eliminates the infarction-reducing effect of IHH and eliminates translocation of PKCδ to the particulate fraction (Kolar *et al.* 2007).

It has been found that IHH (8 h/day, 5 weeks at a 7000 m simulated altitude) contributes to an increase in PKCα expression and phosphorylation (activation) p-PKCα

in the myocardium (Micova *et al.* 2016), as well as an increase in p-PKC ϵ expression (Hlaváčková *et al.* 2010).

Notably, in transgenic murine (aPKC ϵ) hearts with constitutively active PKC ϵ , a shift from fatty acid to glucose oxidation was observed after 14-days of hypobaric hypoxia and enhancement of mitochondrial respiration, when compared to wild type mice (McCarthy *et al.* 2011). After 14-days of exposure to hypobaric hypoxia, the protein levels of phospho-GSK3 β , PGC1 α , and HIF-1 α were elevated in aPKC ϵ mice.

It has been established that IHH increases cardiomyocyte resistance to anoxia/reoxygenation and reduces Ca²⁺ overload in cardiomyocytes after anoxia/reoxygenation, and the PKC inhibitor chelerythrine eliminates these protective effects (Ma *et al.* 2014).

Continuous hypoxia

After 10-days of exposure to normobaric hypoxia at SaO₂ 85 %, translocation (activation) of PKC ϵ to the particulate fraction has been reported in the right ventricle of infants with cyanotic heart defects and in both ventricles of newborn rabbits (Rafiee *et al.* 2002). It has also been found that CNH (10 % O₂, 21 days) promotes an increase in PKC ϵ expression and its content in the particulate fraction of the left ventricle homogenates (Holzerova *et al.* 2015). Thus, CNH induced the translocation of PKC ϵ to the mitochondria and nucleus of cells. The selective inhibitor PKC ϵ KP-1633 eliminated the cytoprotective effect of chronic hypoxia (Holzerova *et al.* 2015).

Afterward, we found that chelerythrine eliminates the infarct-sparing effect of CNH (12 % O₂, 21 days) (Tsibulnikov *et al.* 2018), and both chelerythrine and rottlerin eliminate an adaptive increase in cardiomyocyte tolerance to anoxia/reoxygenation under the same hypoxic training (Naryzhnaya *et al.* 2016).

Although rats subjected to more severe CNH (10 % O₂ for 3-7 days) contributed to altered translocation of PKC δ from the cytosol to the particular fraction in the right ventricle, the translocation of PKC β II and PKC η increased in the left ventricle at 14-21 days of this hypoxic condition (Zeng *et al.* 2017).

The presented data convincingly indicates the involvement of PKC δ and PKC ϵ in the cardioprotective effect of both chronic hypoxia regimes; however, at least with regard to continuous hypoxia, the changes in PKC disappeared with more severe hypoxic exposure.

AMPK kinase

AMPK or 5' adenosine monophosphate-activated protein kinase is the enzyme (EC 2.7.11.31), which plays a role in cellular energy homeostasis, largely to activate glucose and fatty acid uptake and oxidation when cellular energy is low. It is involved in the cardioprotective effects of preconditioning (Khaliulin *et al.* 2007) and postconditioning (Hermann *et al.* 2012).

Intermittent hypoxia

It has been shown that IHH (5,000m for 28 days, 6 h/day) contributed to an increase in the p-AMPK level in the left myocardial ventricle (Li *et al.* 2016) and in isolated left ventricular cardiomyocytes (Gu *et al.* 2018).

However, it has been demonstrated a decrease in the p-AMPK level in the myocardium of rats subjected to severe intermittent normobaric hypoxia (INH, 8 % O₂, 120 s : 21 %, 300 s. cycles, 8 h/day, 7 days/week, 10 weeks) (Xie *et al.* 2016).

Continuous hypoxia

Short-term moderate CNH (24h, 18 % O₂) does not induce a change in AMPK content in myocardial tissue (Mohammed Abdul *et al.* 2015). Moreover, the authors report on the infarction-limiting effect of this regimen of adaptation to hypoxia. Acute normobaric hypoxia lasting 48 h at 8 % O₂ in mice causes an increase in the p-AMPK (activated AMPK) level, but chronic continuous hypoxia - 10 days at 8 % O₂ - does not cause such significant changes (Viganò *et al.* 2011). Chronic continuous hypoxia (10 % O₂ 21 days) causes a decrease in the p-AMPK level in the rat left ventricle during ischemia, but there are no differences after the reperfusion phase (Kolar *et al.* 2017). Contrary in the myocardium of patients with congenital heart disease, which corresponds to severe hypoxic conditions, the amount of p-AMPK was higher than that in patients with acyanotic heart disease (Zhang *et al.* 2018a).

These data showed positive changes in AMPK at moderate intermittent hypoxia and downregulation of AMPK under continuous severe intermittent hypoxia. It should be noted that there is no data on the elimination of the cardioprotective effect of chronic hypoxia by AMPK blockers. There is no evidence that a decrease or an increase in AMPK phosphorylation is associated with cardioprotection in conditions of chronic hypoxia. Therefore, we cannot evaluate the role of this kinase.

CaMKII kinase

Ca²⁺/calmodulin-dependent protein kinase II (CaM kinase II or CaMKII, EC 2.7.11.17) is a serine/threonine-specific protein kinase that is regulated by the Ca²⁺/calmodulin complex. CaMKII is necessary for Ca²⁺ homeostasis and reuptake in cardiomyocytes (Anderson 2005).

It is known that the activation of Ca²⁺-calmodulin kinase II (CaMKII kinase) exacerbates I/R injury of the heart (Ling *et al.* 2013). It has been found that the expression of mRNA encoding calmodulin, CaMKII γ , and CaMKII δ in the rat myocardium was enhanced after exposure to severe continuous hypoxia (10 % O₂, 3 weeks) (Zhao *et al.* 2008). An increased CaMKII expression in the right ventricle after exposure to severe IHH at 7620 m (21 days) was noted by other investigators (Nehra *et al.* 2016). Cardioprotection under chronic hypoxia has not been identified in the last two articles. However, under mild IHH (5000 m, 6 h/day, 6 week) preservation of contractility function and cardioprotection under Ca²⁺ overload depended on increased CaMKII expression, because a selective CaMKII inhibitor KN93 prevented protection (Xie *et al.* 2004). These data are the reason for a consideration of CaMKII as a candidate to one of regulating mechanisms at chronic hypoxia. However, after the course of chronic INH (10 % O₂, 6 h/day, 7 days) elevated ⁴⁵Ca²⁺ uptake via sarcoplasmic reticulum Ca²⁺-ATPase (SERCA), release via ryanodine receptor (RyR) or extrusion by the Na⁺/Ca²⁺ exchanger (NCX) is not dependent on CaMKII (Yeung *et al.* 2007).

PI3 and protein kinase B/Akt kinase

PI3Ks (EC 2.7.1.-) are a family of related intracellular signal transducer enzymes capable of phosphorylating the 3 position hydroxyl group of the inositol ring of phosphatidylinositol. PI3K/AKT is an intracellular signaling pathway that is important in regulating the cell cycle. PI3K activation promotes phosphorylation and stimulation of AKT, localizing it in the plasma membrane (Mockridge *et al.* 2000). Protein kinase B/Akt kinase (AKT, EC 2.7.11-12), can have a number of downstream effects, such as activating mTOR (Jonassen *et al.* 2001) and largely involved in preconditioning phenomenon (Rossello *et al.* 2018).

It has been shown that IHH (7000 m, 8 h/day, 25 exposures) causes an increase in p-Akt expression in the left ventricle of the rat heart (Strnisková *et al.* 2006).

It was established that the PI3K/Akt inhibitor LY294002 eliminates the infarction-limiting effect of IHH (Ravingerová *et al.* 2007).

In Milano's study (2013), it has been found that severe INH (4 daily cycles, each composed of 2-min at 6-8 % O₂ followed by 3-min reoxygenation for 5 times, for 14 days) contributes to the infarct-sparing effect and shows elevation of a p-Akt amount (Milano *et al.* 2013). The PI3-kinase inhibitor wortmannin eliminated the infarct-reducing effect of INH, as well as elevation of the level of HIF-1 α and Nrf2 transcription factors. However, other authors report that similar hypoxic conditioning – INH – lead to a decrease in the PI3K and p-Akt levels in the rat right ventricle (Zhang *et al.* 2018b).

Severe CNH (10 % O₂, 2 weeks) promotes an increase in a p-Akt amount (and a corresponding increase in the p-Akt/Akt ratio) in rat left ventricles only if daily 1 h reoxygenation was performed, but not in continuous hypoxia model (Milano *et al.* 2010). The Akt/PI3 kinase inhibitor LY-294002 abolished enhancement of contractility and decreased of infarct size caused by hypoxia/reoxygenation episodes compared to non-reoxygenated animals. In addition, it was shown important facts: Akt/PI3 inhibition by LY-294002 had no effect on the p-ERK_{1/2}/ERK_{1/2} ratio, but ERK1/2 inhibition by PD-98059 contributed to a decrease in the p-Akt/Akt ratio. These facts indicate that ERK1/2 is an upstream kinase with respect to Akt (Milano *et al.* 2010).

Milder continuous hypoxia without a reoxygenation phase (10 % O₂ 21 days) causes an increase in the p-Akt level in the rat left ventricle (Kolar *et al.* 2017). However, we failed to confirm the involvement of PI3K in the infarct-limiting effect of similar hypoxic adaptation (12 % O₂, 21 days) (Tsubulnikov *et al.* 2018). In addition, we found that PI3K blockade by wortmannin does not affect increased tolerance of isolated cardiomyocytes to anoxia/reoxygenation in rats adapted for CNH (Naryzhnaya *et al.* 2016). Thus, the role of PI3K and Akt in the cardioprotective effect of chronic hypoxia is controversial.

MEK and ERK kinases

The mitogen-activated protein kinases (the MAPK/ERK kinases; MKKs or MEKs; EC 2.7.11.24) and subsequent downstream kinase Extracellular signal-Regulated Kinases (ERKs, EC 2.7.11.24) are widely

expressed protein kinase. These kinases are intracellular signaling molecules that are involved in functions including the regulation of meiosis, mitosis, and post-mitotic functions in differentiated cells. Many different stimuli, including growth factors, cytokines, ligands for heterotrimeric G- protein-coupled receptors, transforming agents activate the MEK/ERK pathway (Sugden and Clerk 1997). It is known that these kinases are involved in cardioprotection at ischemic preconditioning (Rossello *et al.* 2018).

It has been established that chronic IHH (7000 m, 8 h/day, 25 exposures) contributes to an increase in ERK2 expression in the right, but not left myocardium ventricle (Strnisková *et al.* 2006). However, it was not found phosphorylation (activation) of ERK2 in both ventricles. Other investigators demonstrated increased phosphorylation of p-ERK1/2 in the myocardium at IHH in the same conditions (Micova *et al.* 2016). In addition, an increase in the p-ERK1/2 level has been found in rat atrium after INH performed in severe hypoxia (6 h/day for 30 days 8 % O₂) (Zhang *et al.* 2018b).

It was established that excessive chronic CNH (two weeks, 10 % O₂) exacerbates reperfusion myocardial contractility dysfunction of the isolated heart model and increases infarct size (Milano *et al.* 2010). However, if a similar hypoxia was modeled with daily periods of reoxygenation, this negative effect was avoided; there was no a reduction in contractility and an increase in infarct size. This method of modeling chronic hypoxia, an increase in phosphorylated ERK1/2 (p-ERK1/2) content in the myocardium was noted, and inhibition of ERK1/2 PD-98059 or MEK1/2 U0126 prevented the protective effect of reoxygenation episodes (Milano *et al.* 2010). These data suggest that ERK and MEK are involved in cardioprotection at chronic hypoxia.

P38 kinase

The p38 mitogen-activated protein kinases (EC 2.7.11.24) are a class of the mitogen-activated protein kinases (MAPKs) that are responsive to stress stimuli, such as cytokines, ultraviolet irradiation, heat shock, and osmotic shock, and are involved in cell differentiation, apoptosis and autophagy (Liu *et al.* 2015). Phosphorylation of p38 kinase is necessary to realize cardioprotection due to ischemic preconditioning (Weinbrenner *et al.* 1997) and postconditioning (Sun *et al.* 2006).

According to Strnisková's data (Strnisková *et al.* 2006), after IHH (7000 m, 8 h/day, 35 exposures) the total p38 level does not change in both ventricles. The p-p38 level in the right ventricle was reduced, and was elevated in the left ventricle. An increase in the p-p38 level in the myocardium of the left ventricle after IHH was noted in a later study (Micova *et al.* 2016).

It has been established that CNH (two weeks, 10 % O₂) causes an increase in the p-p38 kinase level in the myocardium (Morel *et al.* 2006, Milano *et al.* 2010). Normobaric hypoxia with daily reoxygenation did not have a similar effect (Morel *et al.* 2006, Milano *et al.* 2010). In these studies, it was observed negative inotropic effect of CNH and enlarged of infarct size, which are prevented by the p-38 kinase inhibitors SB203580 or SB202190 (Morel *et al.* 2006). It was shown that the p-p38 level is increased in the myocardium of infants with cyanotic heart defects, but not in patients with acyanotic heart defects (Rafiee *et al.* 2002, Quing *et al.* 2007). However, in a study of the isolated heart of neonatal rabbit it was established that blocking p38 with SB203580 eliminates the cardioprotective effect of CNH (Rafiee *et al.* 2002). This is the only article about a positive role of p-p38 in the cardioprotective effect of chronic hypoxia.

JNK kinase

The c-Jun N-terminal kinases (JNKs, CE 2.7.11.24) were originally identified as the kinases that bind and phosphorylate c-Jun on Ser-63 and Ser-73 within its transcriptional activation domain. They belong to the mitogen-activated protein kinase family and are responsive to stress stimuli, such as cytokines, ultraviolet irradiation, heat shock, and osmotic shock. It is generally accepted that JNK plays a negative role in the regulation of cardiac tolerance to I/R (Milano *et al.* 2007). However, there is evidence that this enzyme is involved in the cardioprotective effect of remote preconditioning (Heidbreder *et al.* 2008).

Changes in the total JNK and p-JNK levels were not detected in the right and left ventricles of rats adapted for IHH (IHH 7000 m, 8 h/day, 35 exposures); however, it has been found that JNK content in the particulate fraction of the right ventricle was increased, but decreased in the particulate fraction of the left ventricles (Strnisková *et al.* 2006). It has been established that INH contributes to an increase in the p-JNK/JNK ratio in the myocardium (Zhao *et al.* 2019).

It has been found that curcumin, a JNK inhibitor, eliminates the cardioprotective effect of CNH in rabbit infants (Rafiee *et al.* 2002). However, an increase in the p-JNK level has not been found in the left ventricle of adult rats after CNH (Morel *et al.* 2006), as it was shown at cell H₉C₂ culture (He *et al.* 2016). The presented data indicate that chronic hypoxia could promote an increase in the active p-JNK level in the myocardium. At the same time, data on the involvement of this kinase in the cardioprotective effect of adaptation is limited to one study so far (Rafiee *et al.* 2002). Therefore, the role of JNK in the protective effect of chronic hypoxia remains to be elucidated.

Protein kinase A

Protein kinase A is a family of enzymes whose activity is dependent on cellular levels of cyclic AMP (cAMP). PKA is also known as cAMP-dependent protein kinase (EC 2.7.11.11). Protein kinase A has several functions in the cell, including regulation of glycogen, sugar, and lipid metabolism. This kinase is activated in β -adrenergic receptor agonists induced preconditioning (Robinet *et al.* 2005) and pharmacological preconditioning, but not in ischemic preconditioning (Lange *et al.* 2006).

At IHH (7000 m, 8 h/day, 35 exposures) PKA expression was increased in left ventricular myocardium (Kohutova *et al.* 2019).

In mice which were exposed to CNH (10 % O₂, 14 days), change in PKA isoforms' amount in left and right heart ventricle was not detected, as well as the total number of β -adrenergic receptors and adenylyl cyclase activity (Larsen *et al.* 2008). However, this is so far the only work in which the effect of CNH on the PKA level has been evaluated.

Therefore, it is too early to conclude a role of PKA in the cardioprotective effect of adaptation to hypoxia.

Protein kinase G

Protein kinase G (PKG, EC 2.7.11.12) or cGMP-dependent protein kinase is a serine/threonine-specific protein kinase that is activated by cGMP. Protein kinase G type I (PKG I) is involved in survival signaling of preconditioning (Heusch 2015) and postconditioning (Inserre *et al.* 2013).

It has been found that IHH (7620 m, 21 days)

causes an increase in the level of cGMP, PKG activator, in the myocardium (Nehra *et al.* 2016). In a later study it has been found an increase in PKG expression in the left ventricular myocardium at IHH (7000 m, 8 h/day, 35 exposures) (Kohutova *et al.* 2019). It is not known what is a role of this kinase in the infarction-limiting effect of chronic hypoxia.

Hexokinase

Hexokinase (HK, EC 2.7.1.1) is the enzyme that phosphorylates hexoses (six-carbon sugars), forming hexose phosphate. Hexokinase 1 and 2 in cardiomyocytes are associated with voltage-dependent anion channel (VDAC) in the outer mitochondrial membrane (Shoshan-Barmatz *et al.* 2009) and involved in the regulation of mitochondrial permeability transition pore (mPTP) opening (Tanaka *et al.* 2018). It is believed that the binding of hexokinase 2 (HK-2) to the mitochondria prevents apoptosis of cardiomyocytes (Majewski *et al.* 2004). It has been established that IHH (7000 m, 8 h/day, 5 wk) causes an increase in HK-1 and HK-2 expression in the myocardium and enhancement of translocation of hexokinase to the mitochondria (Waskova-Arnostova *et al.* 2015). CNH also increased HK-1 and HK-2 expression in the myocardium, enhanced the association of HK-2 with the mitochondria (Kolar *et al.* 2017). Similar effects of CNH were in SHR rats (Nedvedova *et al.* 2018). These findings are agreed with our results indicated on the stability of mPTP to opening inducing by I/R of the heart after CNH (Prokudina *et al.* 2019).

Conclusion

This study is devoted to an analysis of published data on the role of kinases in implementing the cardioprotective effect of adaptation to hypoxia (Fig. 1). It has been established that chronic intermittent hypoxia promotes increased expression of the following kinases in the myocardium: PKC δ , PKC α , p-PKC ϵ , p-PKC α , AMPK, p-AMPK, CaMKII, p-ERK1/2, p-Akt, PI3-kinase, p-p38, HK-1, and HK-2.

Chronic continuous hypoxia contributes to increased expression of the following kinases in the myocardium: PKC ϵ , PKC β II, PKC η , CaMKII, p-ERK1/2, p-Akt, p-p38, HK-1, and HK-2. However, enhanced expression of the AMPK and JNK kinases has not been detected.

Adaptation to hypoxia enhances the association

of HK-2 with the mitochondria and causes translocation of PKC δ , PKC β II, and PKC η to the mitochondria. It has been proven that PKC δ , PKC ϵ , ERK1/2, and MEK1/2 are involved in the cardioprotective effect of chronic hypoxia. The role of other kinases in the cardioprotective effect of adaptation to hypoxia requires further research. Possible relationships between the listed kinases have been studied in several works (Fig. 1). Thus, it has been found that chelerythrine significantly inhibits the translocation of both PKC ϵ and p38 MAPK from the

cytosolic to the particulate fraction in hypoxic rabbit heart, but p38 blockade does not alter PKC ϵ phosphorylation (Rafiee *et al.* 2002). These data establish a relationship between PKC and p38 under continuous hypoxia. Inhibition of Akt/PI3 by LY-294002 did not affect the p-ERK1/2/ERK1/2 ratio, but inhibition of ERK1/2 by PD-98059 promoted a decrease in the p-Akt/Akt ratio under intermittent hypoxia (Milano *et al.* 2010). These facts indicate that ERK1/2 is an upstream kinase in relation to Akt.

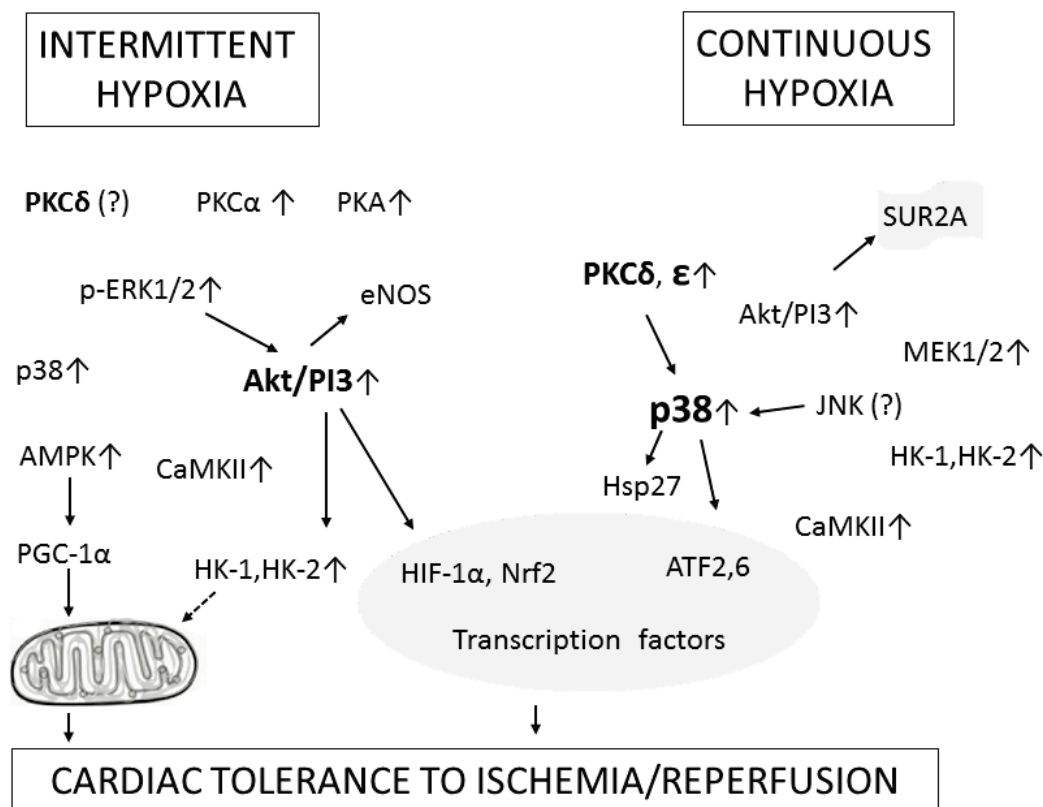


Fig. 1. Involvement of kinases in the cardioprotective effect of chronic hypoxia.

SUR2A – KATP channels regulatory subunits, MEK1/2 – mitogen-activated protein kinase kinase, ERK1/2 – extracellular signal-regulated kinase, PKC – protein kinase C, PKA – protein kinase A, PGC1 α – peroxisome proliferator-activated receptor- γ coactivator 1- α , JNK – c-Jun N-terminal kinase, p38 – p38 kinase, PI3K – phosphatidylinositol-3-kinase, Akt – Akt kinase, CaMKII – Ca²⁺-calmodulin kinase II, HK – hexokinase, HSP70 – heat shock protein 70, HIF1 α – hypoxia induced factor 1 α , Nrf2 – nuclear factor-E2-related factor 2, ATF 2,6 – activating transcription factors 2 and 6. **Bold type** indicates kinases, the inhibition of which leads to the elimination of the infarction-limiting effect of chronic hypoxia.

Protection corresponding to activation of kinases may be developed through any of the studied pathway (Fig. 1). Thus, the relationship between p38-kinase and the activation of transcription factor ATF6 was established under continuous hypoxia (Rafiee *et al.* 2002). Activation of HIF-1 α and Nrf2 transcription factors after INH is depended on PI3-kinase (Milano *et al.* 2013). A relationship was also established between

AKT and the sarcolemmal K_{ATP} channel, and the PI3K inhibitor prevented an increase in subunit SUR2A expression in the left ventricle during normobaric hypoxia (Mohammed Abdul, *et al.* 2015). It was established that activation of AMPK promoted peroxisome proliferator-activated receptor- γ coactivator 1- α (PGC-1 α) expression, which is involved in the cytoprotective effect of chronic hypoxia (Gu *et al.* 2018).

The PI3-kinase inhibitor wortmannin prevented endothelial NO-synthase (eNOS) phosphorylation in cardiomyocytes under intermittent hypoxia (4 daily cycles, each composed of 2-min at 6-8 % O₂ followed by 3-min reoxygenation for 5 times, for 14 days), (Milano *et al.* 2013). Activation of eNOS and enhancement of NO synthesis may prevent endothelial dysfunction during reperfusion.

The present data eloquently suggests that the study of the mechanisms cardioprotection formation in chronic hypoxia needs further development.

Acknowledgements

The authors are grateful for the technical support of Nikita S. Voronkov.

The work was supported by the Russian Science Foundation, Grant 16-15-10001. Section devoted to PKA and PKG, is carried out within the framework of state task AAAA-A15-115120910024-0

Conflict of Interest

There is no conflict of interest.

References

- ANDERSON M: Calmodulin kinase signaling in heart: an intriguing candidate target for therapy of myocardial dysfunction and arrhythmias. *Pharmacol Ther* 106: 39-55, 2005. <https://doi.org/10.1016/j.pharmthera.2004.11.002>
- ENDOH M: The effects of various drugs on the myocardial inotropic response. *Gen Pharmacol* 26: 1-31, 1995. [https://doi.org/10.1016/0306-3623\(94\)00144-C](https://doi.org/10.1016/0306-3623(94)00144-C)
- GAO Q, HU J, HU J, YU Y, YE H, LI Z, GUAN S: Calcium activated potassium channel and protein kinase C participate in the cardiac protection of remote post conditioning. *Pak J Pharm Sci* 26:285-90, 2013.
- GU S, HUA H, GUO X, JIA Z, ZHANG Y, MASLOV LN, ZHANG X, MA H: PGC-1 α participates in the protective effect of chronic intermittent hypobaric hypoxia on cardiomyocytes. *Cell Physiol Biochem* 50: 1891-1902, 2018. <https://doi.org/10.1159/000494869>
- HE S, LIU S, WU X, XIN M, DING S, XIN D, OUYANG H, ZHANG J: Protective role of downregulated MLK3 in myocardial adaptation to chronic hypoxia. *J Physiol Biochem* 73: 371-380, 2016. <https://doi.org/10.1007/s13105-017-0561-5>
- HEIDBREder M, NAUMANN A, TEMPEL K, DOMINIak P, DENDORFER A: Remote vs. ischaemic preconditioning: the differential role of mitogen-activated protein kinase pathways. *Cardiovasc Res* 78: 108-115, 2008. <https://doi.org/10.1093/cvr/cvm114>
- HERMANN R, MARINA PRENDES MG, TORRESIN ME, VÉLEZ D, SAVINO EA, VARELA A: Effects of the AMP-activated protein kinase inhibitor compound C on the postconditioned rat heart. *J Physiol Sci* 62: 333-341, 2012. <https://doi.org/10.1007/s12576-012-0209-8>
- HEUSCH G: Molecular basis of cardioprotection: signal transduction in ischemic pre-, post-, and remote conditioning. *Circ Res* 116: 674-699, 2015. <https://doi.org/10.1161/CIRCRESAHA.116.305348>
- HLAVÁČKOVÁ M, KOŽICHOVÁ K, NECKÁŘ J, KOLÁŘ F, MUSTERS RJ, NOVÁK F, NOVÁKOVÁ O: Up-regulation and redistribution of protein kinase C- δ in chronically hypoxic heart. *Mol Cell Biochem* 345: 271-282, 2010. <https://doi.org/10.1007/s11010-010-0581-8>
- HLAVÁČKOVÁ M, NECKÁŘ J, JEZKOVÁ J, BALKOVÁ P, STANKOVÁ B, NOVÁKOVÁ O, KOLÁŘ F, NOVÁK F: Dietary polyunsaturated fatty acids alter myocardial protein kinase C expression and affect cardioprotection induced by chronic hypoxia. *Exp Biol Med (Maywood)* 232: 823-832, 2007.
- HOLZEROVA K, HLAVÁČKOVÁ M, ŽURMANOVÁ J, BORCHERT G, NECKÁŘ J, KOLÁŘ F, NOVÁK F, NOVÁKOVÁ O: Involvement of PKC ϵ in cardioprotection induced by adaptation to chronic continuous hypoxia. *Physiol Res* 64: 191-201, 2015. <https://doi.org/10.33549/physiolres.932860>
- INSERTE J, HERNANDO V, VILARDOSA Ú, ABAD E, PONCELAS-NOZAL M, GARCIA-DORADO D: Activation of cGMP/protein kinase G pathway in postconditioned myocardium depends on reduced oxidative stress and preserved endothelial nitric oxide synthase coupling. *J Am Heart Assoc* 2:e005975, 2013. <https://doi.org/10.1161/JAHA.112.005975>

- JONASSEN AK, SACK MN, MJOS OD, YELLON DM: Myocardial protection by insulin at reperfusion requires early administration and is mediated via Akt and p70s6 kinase cell-survival signaling. *Circ Res* 89:1191-1198, 2001. <https://doi.org/10.1161/hh2401.101385>
- KHALIULIN I, CLARKE SJ, LIN H, PARKER J, SULEIMAN MS, HALESTRAP AP: Temperature preconditioning of isolated rat hearts-a potent cardioprotective mechanism involving a reduction in oxidative stress and inhibition of the mitochondrial permeability transition pore. *J Physiol* 581: 1147-1161, 2007. <https://doi.org/10.1113/jphysiol.2007.130369>
- KOHUTOVA J, ELSNICOVA B, HOLZEROVA K, NECKAR J, SEBESTA O, JEZKOVA J, VECKA M, VEBR P, HORNIKOVA D, SZEIFFOVA BACOVA B, EGAN BENOVA T, HLAVACKOVA M, TRIBULOVA N, KOLAR F, NOVAKOVA O, ZURMANOVA JM: Anti-arrhythmic cardiac phenotype elicited by chronic intermittent hypoxia is associated with alterations in connexin-43 expression, phosphorylation, and distribution. *Front Endocrinol (Lausanne) [Internet]* 9: 1-10, 2019. <https://doi.org/10.3389/fendo.2018.00789>
- KOLAR D, GRESIKOVA M, WASKOVA-ARNOSTOVA P, ELSNICOVA B, KOHUTOVA J, HORNIKOVA D, VEBR P, NECKAR J, BLAHOVA T, KASPAROVA D, NOVOTNY J, KOLAR F, NOVAKOVA O, ZURMANOVA JM: Adaptation to chronic continuous hypoxia potentiates Akt/HK2 anti-apoptotic pathway during brief myocardial ischemia/reperfusion insult. *Mol Cell Biochem* 432: 99-108, 2017. <https://doi.org/10.1007/s11010-017-3001-5>
- KOLAR F, JEZKOVÁ J, BALKOVÁ P, BREH J, NECKÁR J, NOVÁK F, NOVÁKOVÁ O, TOMÁSOVÁ H, SRBOVÁ M, OSTÁDAL B, WILHELM J, HERGET J: Role of oxidative stress in PKC- δ upregulation and cardioprotection induced by chronic intermittent hypoxia. *Am J Physiol Heart Circ Physiol* 292: H224-H230, 2007. <https://doi.org/10.1152/ajpheart.00689.2006>
- KOLAR F, OSTÁDAL B: Molecular mechanisms of cardiac protection by adaptation to chronic hypoxia. *Physiol Res* 53: S3-S13, 2004.
- LANGE M, SMUL TM, BLOMEYER CA, REDEL A, KLOTZ KN, ROEWER N, KEHL F: Role of the β 1-adrenergic pathway in anesthetic and ischemic preconditioning against myocardial infarction in the rabbit heart in vivo. *Anesthesiology* 105: 503-510, 2006. <https://doi.org/10.1097/00005542-200609000-00014>
- LARSEN KO, LYGREN B, SJAASTAD I, KROBERT KA, ARNKVAERN K, FLORHOLMEN G, LARSEN AK, LEVY FO, TASKÉN K, SKJØNSBERG OH, CHRISTENSEN G: Diastolic dysfunction in alveolar hypoxia: a role for interleukin-18-mediated increase in protein phosphatase 2A. *Cardiovasc Res* 80: 47-54, 2008. <https://doi.org/10.1093/cvr/cvn180>
- LI X, LIU Y, MA H, GUAN Y, CAO Y, TIAN Y, ZHANG Y: Enhancement of glucose metabolism via PGC-1 α participates in the cardioprotection of chronic intermittent hypobaric hypoxia. *Front Physiol* 7: 1-8, 2016. <https://doi.org/10.3389/fphys.2016.00219>
- LING H, GRAY CB, ZAMBON AC, GRIMM M, GU Y, DALTON N, PURCELL NH, PETERSON K, BROWN JH: CaMKII δ mediates myocardial ischemia/reperfusion injury through nuclear factor- κ B. *Circ Res* 112: 935-944, 2013. <https://doi.org/10.1161/CIRCRESAHA.112.276915>
- LIU J, CHANG F, LI F, FU H, WANG J, ZHANG S, ZHAO J, YIN D: Palmitate promotes autophagy and apoptosis through ROS-dependent JNK and p38 MAPK. *Biochem Biophys Res Commun* 463: 262-267, 2015. <https://doi.org/10.1016/j.bbrc.2015.05.042>
- MA HJ, LI Q, MA HJ, GUAN Y, SHI M, YANG J, LI DP, ZHANG Y: Chronic intermittent hypobaric hypoxia ameliorates ischemia/reperfusion-induced calcium overload in heart via Na⁺/Ca²⁺ exchanger in developing rats. *Cell Physiol Biochem* 34: 313-324, 2014. <https://doi.org/10.1159/000363001>
- MAJEWSKI N, NOGUEIRA V, BHASKAR P, COY PE, SKEEN JE, GOTTLÖB K, CHANDEL NS, THOMPSON CB, ROBEY RB, HAY N: Hexokinase-mitochondria interaction mediated by Akt is required to inhibit apoptosis in the presence or absence of Bax and Bak. *Mol Cell* 16: 819-830, 2004. <https://doi.org/10.1016/j.molcel.2004.11.014>
- MCCARTHY J, LOCHNER A, OPIE LH, SACK MN, ESSOP MF: PKC ϵ promotes cardiac mitochondrial and metabolic adaptation to chronic hypobaric hypoxia by GSK3 β inhibition. *J Cell Physiol* 226: 2457-2468, 2011. <https://doi.org/10.1002/jcp.22592>

- MEERSON FZ, USTINOVA EE, MANUKHINA EB: Prevention of cardiac arrhythmias by adaptation to hypoxia: regulatory mechanisms and cardiotropic effect. *Biomed Biochim Acta* 48: S83-S88, 1989.
- MICOVA P, HAHNOVA K, HLAVACKOVA M, ELSNICOVA B, CHYTILOVA A, HOLZEROVA K, ZURMANOVA J, NECKAR J, KOLAR F, NOVAKOVA O, NOVOTNY J: Chronic intermittent hypoxia affects the cytosolic phospholipase A2 α /cyclooxygenase 2 pathway via β 2-adrenoceptor-mediated ERK/p38 stimulation. *Mol Cell Biochem* 423: 151-163, 2016. <https://doi.org/10.1007/s11010-016-2833-8>
- MILANO G, MOREL S, BONNY C, SAMAJA M, VON SEGESSER LK, NICOD P, VASSALLI G: A peptide inhibitor of c-Jun NH2-terminal kinase reduces myocardial ischemia-reperfusion injury and infarct size in vivo. *Am J Physiol Heart Circ Physiol* 292: H1828-H1835, 2007. <https://doi.org/10.1152/ajpheart.01117.2006>
- MILANO G, VON SEGESSER LK, MOREL S, JONCIC A, BIANCIARDI P, VASSALLI G, SAMAJA M: 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, 2010. <https://doi.org/10.1258/ebm.2009.009153>
- MILANO G, ABRUZZO PM, BOLOTTA A, MARINI M, TERRANEO L, RAVARA B, GORZA L, VITADELLO M, BURATTINI S, CURZI D, FALCIERI E, VON SEGESSER LK, SAMAJA M: Impact of the phosphatidylinositol 3-kinase signaling pathway on the cardioprotection induced by intermittent hypoxia. *PLoS ONE* 8: e76659, 2013. <https://doi.org/10.1371/journal.pone.0076659>
- MOHAMMED ABDUL KS, JOVANOVIĆ S, DU Q, SUKHODUB A, JOVANOVIĆ A: Mild hypoxia in vivo regulates cardioprotective SUR2A: A role for Akt and LDH. *Biochim Biophys Acta - Mol Basis Dis*, 1852: 709-719, 2015. <https://doi.org/10.1016/j.bbadis.2015.01.001>
- MOCKRIDGE JW, MARBER MS, HEADS RJ: Activation of Akt during simulated ischemia/reperfusion in cardiac myocytes. *Biochem Biophys Res Commun* 270: 947-952, 2000. <https://doi.org/10.1006/bbrc.2000.2522>
- MOREL OE, BUVRY A, LE CORVOISIER P, TUAL L, FAVRET F, LEÓN-VELARDE F, CROZATIER B, RICHALET JP: Effects of nifedipine-induced pulmonary vasodilatation on cardiac receptors and protein kinase C isoforms in the chronically hypoxic rat. *Pflugers Arch* 446: 356-364, 2003. <https://doi.org/10.1007/s00424-003-1034-y>
- MOREL S, MILANO G, LUDUNGE KM, CORNO AF, SAMAJA M, FLEURY S, BONNY C, KAPPENBERGER L, VON SEGESSER LK, VASSALLI G: Brief reoxygenation episodes during chronic hypoxia enhance posthypoxic recovery of LV function: role of mitogen-activated protein kinase signaling pathways. *Basic Res Cardiol* 101: 336-345, 2006. <https://doi.org/10.1007/s00395-006-0596-1>
- NARYZHNYA NV, KHALIULIN I, LISHMANOV YB, SULEIMAN MS, TSIBULNIKOV SY, KOLAR F, MASLOV LN: Participation of opioid receptors in the cytoprotective effect of chronic normobaric hypoxia. *Physiol Res* 68: 245-253, 2019. <https://doi.org/10.33549/physiolres.933938>
- NARYZHNYA NV, MASLOV LN, KHALIULIN IG, ZHANG Y, PEI JM, TSEPOKINA AV, KHUTORNAYA MV, KUTIKHIN AG, LISHMANOV YB: Chronic continuous normobaric hypoxia augments cell tolerance to anoxia/reoxygenation: the role of protein kinases. *Russ Fiziol Zh Im I M Sechenova* 102: 1462-1471, 2016.
- NECKAR J, MARKOVÁ I, NOVÁK F, NOVÁKOVÁ O, SZÁRSZOI O, OSTÁDAL B, KOLÁR F: Increased expression and altered subcellular distribution of PKC- δ in chronically hypoxic rat myocardium: involvement in cardioprotection. *Am J Physiol Heart Circ Physiol* 288: H1566-H1572, 2005. <https://doi.org/10.1152/ajpheart.00586.2004>
- NEDVEDOVA I, KOLAR D, ELSNICOVA B, HORNIKOVA D, NOVOTNY J, KALOUS M, PRAVENEC M, NECKAR J, KOLAR F, ZURMANOVA JM: Mitochondrial genome modulates myocardial Akt/Glut/HK salvage pathway in spontaneously hypertensive rats adapted to chronic hypoxia. *Physiol Genomics* 50: 532-541, 2018. <https://doi.org/10.1152/physiolgenomics.00040.2017>
- NEHRA S, BHARDWAJ V, KAR S, SARASWAT D: Chronic hypobaric hypoxia induces right ventricular hypertrophy and apoptosis in rats: therapeutic potential of nanocurcumin in improving adaptation. *High Alt Med Biol* 17: 342-352, 2016. <https://doi.org/10.1089/ham.2016.0032>
- OKUBO S, TANABE Y, FUJIOKA N, TAKEDA K, TAKEKOSHI N: Differential activation of protein kinase C between ischemic and pharmacological preconditioning in the rabbit heart. *Jpn J Physiol* 53: 173-80, 2003. <https://doi.org/10.2170/jjphysiol.53.173>

- PROKUDINA ES, NARYZHNAYA NV, MUKHOMEDZIANOV AV, GORBUNOV AS, ZHANG Y, YAGGI AS, TSIBULNIKOV SY, NESTEROV EA, LISHMANOV YB, SULEIMAN MS, OELTGEN PR, MASLOV LN: Effect of chronic continuous normobaric hypoxia on functional state of cardiac mitochondria and tolerance of isolated rat heart to ischemia and reperfusion: Role of μ and delta2. *Physiol Res* 30: 68: 909-920, 2019. <https://doi.org/10.33549/physiolres.933945>
- QUING M, GÖRLACH A, SCHUMACHER K, WÖLTJE M, VAZQUEZ-JIMENEZ JF, HESS J, SEGHAIE MC: The hypoxia-inducible factor HIF-1 promotes intramyocardial expression of VEGF in infants with congenital cardiac defects. *Basic Res Cardiol* 102: 224-232, 2007. <https://doi.org/10.1007/s00395-007-0639-2>
- RAFIEE P, SHI Y, KONG X, PRITCHARD KA, TWEDDELL JS, LITWIN SB, MUSSATTO K, JAQUISS RD, SU J, BAKER JE: Activation of protein kinases in chronically hypoxic infant human and rabbit hearts: role in cardioprotection. *Circulation* 106: 239-245, 2002. <https://doi.org/10.1161/01.CIR.0000022018.68965.6D>
- RAVINGEROVÁ T, MATEJÍKOVÁ J, NECKÁR J, ANDELOVÁ E, KOLÁR F: Differential role of PI3K/Akt pathway in the infarct size limitation and antiarrhythmic protection in the rat heart. *Mol Cell Biochem* 297: 111-120, 2007. <https://doi.org/10.1007/s11010-006-9335-z>
- ROBINET A, HOIZEY G, MILLART H: PI 3-kinase, protein kinase C, and protein kinase A are involved in the trigger phase of beta1-adrenergic preconditioning. *Cardiovasc Res* 66: 530-542, 2005. <https://doi.org/10.1016/j.cardiores.2005.02.010>
- ROSSELLO X, RIQUELME JA, DAVIDSON SM, YELLON DM: Role of PI3K in myocardial ischaemic preconditioning: mapping pro-survival cascades at the trigger phase and at reperfusion. *J Cell Mol Med* 22:926-935, 2018. <https://doi.org/10.1111/jcmm.13394>
- SHOSHAN-BARMATZ V, ZAKAR M, ROSENTHAL K, ABU-HAMAD S: Key regions of VDAC1 functioning in apoptosis induction and regulation by hexokinase. *Biochim Biophys Acta* 1787: 421-430, 2009. <https://doi.org/10.1016/j.bbabi.2008.11.009>
- STRNISKOVÁ M, RAVINGEROVÁ T, NECKÁR J, KOLÁR F, PASTOREKOVÁ S, BARANČÍK M: Changes in the expression and/or activation of regulatory proteins in rat hearts adapted to chronic hypoxia. *Gen Physiol Biophys* 25: 25-41, 2006.
- SUN HY, WANG NP, HALKOS M, KERENDI F, KIN H, GUYTON RA, VINTEN-JOHANSEN J, ZHAO ZQ: Postconditioning attenuates cardiomyocyte apoptosis via inhibition of JNK and p38 mitogen-activated protein kinase signaling pathways. *Apoptosis* 11: 1583-1593, 2006. <https://doi.org/10.1007/s10495-006-9037-8>
- SUGDEN PH, CLERK A: Regulation of the ERK subgroup of MAP kinase cascades through G protein-coupled receptors. *Cell Signal* 9:337-51, 1997. [https://doi.org/10.1016/S0898-6568\(96\)00191-X](https://doi.org/10.1016/S0898-6568(96)00191-X)
- TANAKA T, SAOTOME M, KATO H, SATOH T, HASAN P, OHTANI H, SATOH H, HAYASHI H, MAEKAWA Y: Glycogen synthase kinase-3 β opens mitochondrial permeability transition pore through mitochondrial hexokinase II dissociation. *J Physiol Sci* 68: 865-871, 2018. <https://doi.org/10.1007/s12576-018-0611-y>
- TSIBULNIKOV SY, MASLOV LN, NARYZHNAYA NV, MA H, LISHMANOV YB, OELTGEN PR, GARLID K: Role of protein kinase C, PI3 kinase, tyrosine kinases, NO-synthase, KATP channels and MPT pore in the signaling pathway of the cardioprotective effect of chronic continuous hypoxia. *Gen Physiol Biophys* 37: 537-547, 2018. https://doi.org/10.4149/gpb_2018013
- VIGANÒ A, VASSO M, CARETTI A, BRAVATÀ V, TERRANEO L, FANIA C, CAPITANIO D, SAMAJA M, GELFI C: Protein modulation in mouse heart under acute and chronic hypoxia. *Proteomics* 11: 4202-4217, 2011. <https://doi.org/10.1002/pmic.201000804>
- WASKOVA-ARNOSTOVA P, ELSNICOVA B, KASPAROVA D, HORNIKOVA D, KOLAR F, NOVOTNY J, ZURMANOVA J: Cardioprotective adaptation of rats to intermittent hypobaric hypoxia is accompanied by the increased association of hexokinase with mitochondria. *J Appl Physiol* (1985) 119: 1487-1493, 2015. <https://doi.org/10.1152/jappphysiol.01035.2014>
- WEINBRENNER C, LIU GS, COHEN MV, DOWNEY JM: Phosphorylation of tyrosine 182 of p38 mitogen-activated protein kinase correlates with the protection of preconditioning in the rabbit heart. *J Mol Cell Cardiol* 29: 2383-91, 1997. <https://doi.org/10.1006/jmcc.1997.0473>

- XIE S, DENG Y, PAN Y, REN J, JIN M, WANG Y, WANG Z, ZHU D, GUO X, YUAN X, SHANG J, LIU H: Chronic intermittent hypoxia induces cardiac hypertrophy by impairing autophagy through the adenosine 5'-monophosphate-activated protein kinase pathway. *Arch Biochem Biophys* 606: 41-52, 2016. <https://doi.org/10.1016/j.abb.2016.07.006>
- XIE Y, ZHU WZ, ZHU Y, CHEN L, ZHOU ZN, YANG HT: Intermittent high altitude hypoxia protects the heart against lethal Ca^{2+} overload injury. *Life Sci* 76: 559-772, 2004. <https://doi.org/10.1016/j.lfs.2004.09.017>
- YELLON DM, DOWNEY JM: Preconditioning the myocardium: from cellular physiology to clinical cardiology. *Physiol Rev* 83: 1113-1151, 2003. <https://doi.org/10.1152/physrev.00009.2003>
- YEUNG HM, KRAVTSOV GM, NG KM, WONG TM, FUNG ML: Chronic intermittent hypoxia alters Ca^{2+} handling in rat cardiomyocytes by augmented $\text{Na}^+/\text{Ca}^{2+}$ exchange and ryanodine receptor activities in ischemia-reperfusion. *Am J Physiol Cell Physiol* 292: C2046-56, 2007. <https://doi.org/10.1152/ajpcell.00458.2006>
- ZENG C, LIANG B, JIANG R, SHI Y, DU Y: Protein kinase C isozyme expression in right ventricular hypertrophy induced by pulmonary hypertension in chronically hypoxic rats. *Mol Med Rep* 16: 3833-3840, 2017. <https://doi.org/10.3892/mmr.2017.7098>
- ZHANG H, LIU B, LI T, ZHU Y, LUO G, JIANG Y, TANG F, JIAN Z, XIAO Y: AMPK activation serves a critical role in mitochondria quality control via modulating mitophagy in the heart under chronic hypoxia. *Int J Mol Med* 41: 69-76, 2018a. <https://doi.org/10.3892/ijmm.2017.3213>
- ZHANG K, MA Z, WANG W, LIU R, ZHANG Y, YUAN M, LI G: Beneficial effects of tolvaptan on atrial remodeling induced by chronic intermittent hypoxia in rats. *Cardiovasc Ther* 36: e12466, 2018b. <https://doi.org/10.1111/1755-5922.12466>
- ZHAO PJ, PAN J, LI F, SUN K: Effects of chronic hypoxia on the expression of calmodulin and calcicium/calmodulin-dependent protein kinase II and the calcium activity in myocardial cells in young rats (In Chinese). *Zhongguo Dang Dai Er Ke Za Zhi* 10: 381-385, 2008.
- ZHAO YS, AN JR, YANG S, GUAN P, YU FY, LI W, LI JR, GUO Y, SUN ZM, JI ES: Hydrogen and oxygen mixture to improve cardiac dysfunction and myocardial pathological changes induced by intermittent hypoxia in rats. *Oxid Med Cell Longev* 2019: 7415212, 2019. <https://doi.org/10.1155/2019/7415212>
-