Physiol. Res. 53 (Suppl. 1): S3-S13, 2004

Molecular Mechanisms of Cardiac Protection by Adaptation to Chronic Hypoxia

F. KOLÁŘ, B. OŠŤÁDAL

Department of Developmental Cardiology, Institute of Physiology, Academy of Sciences of the Czech Republic, and Center for Experimental Cardiovascular Research, Prague, Czech Republic

Received February 10, 2004
Accepted March 1, 2004

Summary

Effective protection of the heart against ischemia/reperfusion injury is one of the most important goals of experimental and clinical research in cardiology. Besides ischemic preconditioning as a powerful temporal protective phenomenon, adaptation to chronic hypoxia also increases cardiac tolerance to all major deleterious consequences of acute oxygen deprivation such as myocardial infarction, contractile dysfunction and ventricular arrhythmias. Although many factors have been proposed to play a potential role, the detailed mechanism of this long-term protection remains poorly understood. This review summarizes current limited evidence for the involvement of ATP-sensitive potassium channels, reactive oxygen species, nitric oxide and various protein kinases in cardioprotective effects of chronic hypoxia.

Key words
Chronic hypoxia • Ischemia • Cardiac protection

Introduction

Hypoxic states of the heart belong to the most frequent and dangerous diseases of modern times. They result from disturbed oxygen supply to cardiac cells, which is insufficient to meet their metabolic demands. Among these states, acute coronary occlusion is the leading cause of morbidity and mortality in the Western world and according to the World Health Organization will be the major cause of death in the world as a whole by the year 2020 (Murray and Lopez 1997). As pointed out by Yellon and Downey (2003), the impact of newly developed strategies in primary prevention of ischemic heart disease may be rather limited. There is, therefore, a need for effective forms of secondary prevention and treatments, which will be able to preserve myocardial viability during acute ischemia/reperfusion (I/R) insult. It is not surprising that the interest of many experimental and clinical cardiologists during the past 40 years has been focused on the question of how cardiac tolerance to oxygen deprivation might be increased.

Already in the late 1950s, the first observations appeared (summarized by Hurtado 1960), showing that the incidence of myocardial infarction is lower in people living at high altitude. These epidemiological observations on the protective effect of high altitude were confirmed in experimental studies (Kopecký and Daum 1958, Poupa *et al.* 1966) using a model of high altitude hypoxia simulated in a hypobaric chamber. In the early 1970s the interest was concentrated on the possibilities of pharmacological limitations of infarct size. Maroko *et al.* (1971) proposed that a variety of interventions during

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

acute ischemia could reduce the extent of tissue injury in an animal model, but none of these interventions proved to be effective in humans. After the period of scepticism, the discovery of the short-lasting adaptation of the myocardium by Murry *et al.* (1986) opened the door of the new era of cardiac protection. They demonstrated in a dog model that four cycles of 5-min ischemia separated by reperfusion markedly limited infarct size induced by subsequent prolonged ischemia. This phenomenon termed ischemic preconditioning has been recognized as the most powerful form of *in vivo* protection against myocardial injury other than early reperfusion (Kloner *et al.* 1998).

Adaptation to chronic hypoxia and various forms of preconditioning represent well-defined and reproducible means to improve cardiac ischemic tolerance. Unfortunately, no satisfactory explanation of the mechanism associated with the protective effects of these phenomena has yet been found. It appears that the identification of detailed molecular pathways involved in the preservation of myocardial viability is a prerequisite for the development of effective pharmacological agents, which could be used in clinical practice. Whereas a lot of data are available concerning the mechanism of preconditioning (for review see Yellon and Downey 2003, Zaugg and Schaub 2003), much less is known on the protective mechanism of adaptation to chronic hypoxia. The purpose of this paper is, therefore, to summarize current knowledge on molecular pathways, which may be involved in increased ischemic tolerance of chronically hypoxic hearts.

Cardioprotective effects of chronic hypoxia

It is interesting to note that the history of cardioprotection mediated by chronic hypoxia is quite different from that of preconditioning. Whereas the latter phenomenon was discovered in the laboratory (Murry *at al.* 1986), experimental investigation of the protective effect of adaptation to high altitude was stimulated by clinical-epidemiological observations (Hurtado 1960). In this connection, it should be pointed out that Kopecký and Daum carried out the first experimental studies on the protective effect of chronic hypoxia on cardiac muscle in Prague in 1958. They have found that cardiac muscle isolated from rats exposed every other day for six weeks to an altitude of 7000 m recovered its contractile function during reoxygenation following a period of acute anoxia to a higher level than that of the control animals.

These findings were later repeatedly confirmed in studies using various experimental models, adaptation protocols, and different end points of injury. It has been reported (McGrath *et al.* 1973, Widimský *et al.* 1973) that protective effect can be induced by a relatively short intermittent exposure of rats to simulated high altitude (4 h a day, a total of 24 exposures). Moreover, a significant sex difference was demonstrated in the susceptibility of the isolated cardiac muscle to acute anoxia: the myocardium of female control rats proved to be more tolerant to oxygen deficiency. Chronic hypoxia resulted in enhanced tolerance in both sexes, yet the sex difference was maintained (Ošťádal *et al.* 1984). The majority of studies demonstrated that the hearts of adult chronically hypoxic animals develop smaller myocardial infarction (Meerson *et al.* 1973, Turek *et al.* 1980), and exhibit better functional recovery (McGrath *et al.* 1973, Widimský *et al.* 1973, Tajima *et al.* 1994) following ischemia as compared to controls. These hearts are also less susceptible to necrogenic effect of isoprenaline (Poupa *et al.* 1966, Faltová *et al.* 1987). Of the three major end points of acute myocardial I/R injury (lethal cell damage, contractile dysfunction and ventricular arrhythmias), the incidence and severity of arrhythmias have been the least studied (Meerson *et al.* 1987, 1989). It appears that the antiarrhythmic protection is critically dependent on experimental model and the degree and duration of hypoxic exposure (Asemu *et al.* 2000).

Chronic hypoxia is also the main pathophysiological feature of hypoxemic congenital heart disease. Understanding the mechanisms by which hypoxemia modifies the immature heart and how these modifications impact on the protective mechanisms during acute ischemia may provide the insight into treatments for limiting myocardial damage during cardiac surgery in children (Baker *et al.* 1998). In this connection it is necessary to mention that healthy immature myocardium is more tolerant to ischemia than that of adults (for review see Ošťádal *et al.* 1999). However, only a few authors have compared the tolerance to the oxygen deprivation in chronically hypoxic versus normoxic immature heart. We have observed (Ošťádal *et al.* 1995) that chronic hypoxia, simulated in the barochamber, results in the similarly enhanced recovery of contractile function in rats exposed to chronic hypoxia either from the 4th day of postnatal life or in adulthood. Similarly, Baker *et al.* (1995) demonstrated that adaptation to hypoxia increased the tolerance of the developing rabbit heart (day 7 to day 28 of postnatal life). Our recent experiments (Ošťádalová *et al.* 2002) have shown that the protective effect of chronic hypoxia is absent in newborn rats: prenatal exposure (i.e. pregnant mothers) to hypoxia fails to further increase ischemic tolerance in 1-day-old hearts. This protective phenomenon develops – similarly as ischemic preconditioning (Ošťádalová *et al.* 1998) – during the first week of life. Decreasing tolerance to ischemia during early postnatal life is thus counteracted by the development of endogenous protection. As far as the clinical relevance of this developmental approach is concerned, metabolic adaptation to chronic hypoxia and activation of protective pathways have been observed in the myocardium of children with cyanotic congenital cardiac malformations (Šamánek *et al.* 1989, Ferreiro *et al.* 2001, Rafiee *et al*. 2002).

Molecular mechanisms

As cardiac protection against acute I/R injury by chronic hypoxia lasts markedly longer than any form of preconditioning (Ošťádal *et al.* 1994), its molecular mechanism is of particular interest for potential clinical exploitation in the future. However, chronic hypoxia has been much less studied and the understanding of its protective signaling is still far behind that of preconditioning. A number of diverse factors were proposed to play a role (for review see Kolář 1996, Ošťádal *et al*. 1998), but only a few of them have been examined experimentally so far. Direct experimental evidence supporting their involvement in cardioprotective mechanism of chronic hypoxia is limited to several pathways, which are briefly reviewed in the following paragraphs. Interestingly, most of these factors are also involved in the mechanism of preconditioning, suggesting that both short- and long-lasting protective phenomena may share, at least in part, the same signaling pathway or its components. This view is supported by our observation that infarct size-limiting effects of chronic hypoxia and classic ischemic preconditioning do not add up in adult rats (Neckář *et al.* 2002a).

ATP-sensitive potassium channels (K_{ATP})

Cardiomyocytes contain two distinct subtypes of K_{ATP} channels, the sarcolemmal (sarcK_{ATP}) and mitochondrial (mitoK_{ATP}). They are composed of two types of subunits, the pore forming subunit K_{ir} (inwardly rectifying potassium channel) and the regulatory subunit SUR (sulfonylurea receptor). Whereas the molecular structure of cardiac sarcK_{ATP} has been already determined (octameric complex of K_{ir}6.2 and SUR2A), the identity of mitoK_{ATP} remains elusive. However, the two channel subtypes can be distinguished on a basis of their sensitivity to pharmacological modulators; this approach has been the main source of data on the involvement of mitoK_{ATP} and sarcK_{ATP} in cardioprotection. A number of reports suggest that these channels, in particular those which are localized in the mitochondrial membrane, play essential role in various forms of early and delayed preconditioning (Oldenburg *et al.* 2002, Garlid *et al.* 2003, Gross and Peart 2003).

Exposure to chronic hypoxia leads to the activation of K_{ATP} channels in various tissues (Cameron and Baghdady 1994). Embryonic rat heart-derived H9c2 cells exhibited an increased transcription of the channel regulatory subunit SUR2A already after 24 h of mild hypoxia in culture (Crawford *et al.* 2003). Several recent studies point to the involvement of K_{ATP} channels in the mechanism of increased tolerance of chronically hypoxic hearts to acute I/R injury. However, like with preconditioning, a certain controversy exists as to whether sarcK_{ATP} or mitoK_{ATP} subtype is important (Kolář *et al.* 2003a). In our studies, the mitoK_{ATP}-selective blocker, 5-hydroxydecanoate (5-HD), completely abolished both the improvement of post-ischemic recovery of myocardial contractility and the reduction of infarct size in chronically hypoxic rats, but it had no effect in normoxic controls. In addition, mitoK_{ATP}-selective openers, diazoxide or BMS-191095, reduced contractile dysfunction and infarct size in normoxic hearts, but no additive protection occurred in the hypoxic group (Neckář *et al.* 2002b). Likewise, both 5-HD and the non-selective K_{ATP} blocker, glibenclamide, prevented the decrease in severity of ventricular arrhythmias induced by I/R insult in chronically hypoxic animals, while diazoxide had antiarrhythmic effect in normoxic but not in hypoxic hearts (Asemu *et al.* 1999, Neckář *et al.* 2001). 5-HD or glibenclamide also abolished protective effect of chronic hypoxia on intracellular calcium overload induced by hypoxia/reoxygenation in rat isolated cardiomyocytes (Zhu *et al.* 2003). MitoK_{ATP} channels also appear to play a role in increased cardiac ischemic tolerance of neonatal rats kept hypoxic for 10 days after birth as the improved recovery of contractility was blocked by 5-HD (Ošťádalová *et al.* 2002). These results suggest that mitoK_{ATP} channels are involved in the protective effects of chronic hypoxia against all major manifestations of acute I/R insult. It has been proposed that chronic hypoxia leads to a sustained, tonic activation of mitoK_{ATP} (Asemu *et al.* 1999, Eells *et* *al.* 2000) that may explain the inability of the openers to further increase ischemic tolerance of hypoxic hearts.

However, not all experimental data are in full concordance with this view. Unlike in rats, both mitoK_{ATP} and sarcK_{ATP} appear to contribute to increased ischemic tolerance in hearts of chronically hypoxic neonatal rabbits: improved post-ischemic recovery of contractility was completely abolished by co-administration of 5-HD and HMR 1098 (selective sarcK_{ATP} blocker), whereas 5-HD alone exhibited only a partial inhibitory effect (Kong *et al.* 2001). Moreover, chronic hypoxia protected H9c2 cultured cells against intracellular calcium loading during acute hypoxia/reoxygenation and this cytoprotective effect was abolished by the sarcK_{ATP} blockade (Crawford *et al.* 2003). In contrast, glibenclamide had no effect on increased tolerance of the right ventricle of chronically hypoxic rats to I/R-induced contractile dysfunction in blood-perfused working heart preparation, which is the only study suggesting that K_{ATP} channels are not involved in protection by chronic hypoxia (Forkel *et al.* 2004).

It appears that cardioprotective mechanism and selectivity of K_{ATP} modulators are species-dependent. It has been demonstrated that both diazoxide and preconditioning mediate their protective effects by activation of sarcK_{ATP} but not mitoK_{ATP} in the mouse heart (Suzuki *et al.* 2002, 2003). Moreover, the drugs that are generally believed to target only one channel subtype might not be sufficiently selective under certain conditions. For example, 5-HD blocks sarcK_{ATP} activated by high ADP level and low pH, or by metabolic inhibition (Notsu *et al.* 1992a,b) and these channels are more readily opened by diazoxide under the same conditions (D'Hahan *et al.* 1999, Matsuoka *et al.* 2000). Last but not least, other effects of mitoK_{ATP} modulators, independent of mitochondrial K^+ influx, should be taken into consideration (Hanley *et al.* 2002). Thus, the involvement of K_{ATP} channel and its subtypes in increased ischemic tolerance of chronically hypoxic hearts remains incompletely resolved; new methodical approaches yielding other than pharmacological evidence are needed to disclose their precise role.

Reactive oxygen species (ROS)

A number of reports assumed that mitoK_{ATP} only acts as the end-effector of various cardioprotective phenomena. Recent experiments, however, strongly support the view that this channel plays a dual role both as a trigger (acting prior to the prolonged ischemia) and mediator (acting during ischemia) of cardioprotection (Liu and O'Rourke 2001, Patel and Gross 2001, Oldenburg *et al.* 2002). It implies that the activation of the mitoK_{ATP} by openers or by preconditioning triggers a signaling cascade with a positive feedback to keep the channel open during prolonged ischemia (Oldenburg *et al.* 2002). Emerging evidence indicates that the link between mitoK_{ATP} opening and downstream signaling pathways is formation of ROS (Pain *et al.* 2000, Forbes *et al.* 2001). Moreover, although the mechanism of mitoK_{ATP} opening as a mediator of protection has not been elucidated, alterations of ROS production during ischemia and reperfusion also appear to play a role (Narayan *et al.* 2001).

Chronic hypoxia, in particular that of intermittent nature, is associated with increased oxidative stress as evidenced by lipid peroxidation and the induction of antioxidant enzyme response in various tissues and organs (Yoshikawa *et al.* 1982, Nakanishi *et al.* 1995). Increase of ROS production and oxidative injury of tissue appear to be involved in the pathogenesis of hypoxic pulmonary hypertension (Herget *et al.* 2000). However, the hypothesis that ROS signaling may be implicated in cardioprotection induced by chronic hypoxia and possibly linked to mitoK_{ATP} activation has not been sufficiently examined. Our preliminary data suggest that, on one hand, ROS contribute to I/R injury in normoxic rat hearts, but, on the other hand, they are involved in protective mechanisms induced by chronic hypoxia. ROS scavenger melatonin and tempol (superoxide dismutase mimetic) reduced the incidence and severity of reperfusion arrhythmias, but abolished protective antiarrhythmic effect of chronic hypoxia (Szárszoi *et al.* 2003). In contrast, the size of myocardial infarction was slightly reduced by these compounds in both normoxic and chronically hypoxic groups. Unlike in the acute experiment, a chronic antioxidative treatment with N-acetylcysteine during the adaptation to hypoxia led to a significant attenuation of the improvement in tolerance to lethal myocardial injury (Kolář *et al.* 2003b). Similar blunting effect on cardioprotection was observed in chronically hypoxic rats exposed simultaneously to hypercapnia, which is known to reduce oxidative stress (Neckář *et al.* 2003).

Nitric oxide (NO)

The role of NO in I/R injury and cardioprotection is complex and not fully understood. This molecule can increase cardiac ischemic tolerance by a number of cyclic GMP-dependent and independent mechanisms (Ferdinandy and Schulz 2003). Whereas its involvement in early preconditioning is not clearly established, except for its antiarrhythmic effect (Végh *et al.* 1992), there is a consensus that NO participates in triggering the late preconditioning (Bolli 2000). On the other hand, under certain conditions excess NO concentrations may exert detrimental rather than beneficial effects. These effects are not caused directly by NO itself but are most likely mediated by peroxynitrite, the reaction product of NO and superoxide (Beckman and Koppenol 1996, Ferdinandy and Schulz 2003). Beside its toxic action, peroxynitrite is considered an important upstream event upon triggering mechanism of late preconditioning (Bolli 2000).

Reports concerning the effect of chronic hypoxia on NO and its role in hypoxia-induced protection are not sufficiently conclusive. Chronic hypoxia in neonatal rabbits increased basal myocardial NO production by activation of constitutive NO synthase (eNOS) due to down-regulation of caveolin-3, whereas inducible NOS (iNOS) was undetectable (Baker *et al.* 1999, Shi *et al.* 2000). It has been suggested that the association of eNOS with heat shock protein 90 helps to produce NO and to limit superoxide generation in this model (Shi *et al.* 2002). The expression of eNOS was also increased in the chronically hypoxic myocardium of adult rats (Forkel *et al.* 2004). In contrast, our preliminary data on rats suggest that chronic hypoxia up-regulates myocardial iNOS, whereas the abundance of eNOS is reduced (Kolář *et al.* 2003c). Rouet-Benzineb *et al.* (1999) and Grilli *et al.* (2003) also detected higher abundance and enzyme activity of iNOS in chronically hypoxic rats. Similarly, hypoxia increased this isoform activity and expression in atrial myocardium of children with cyanotic congenital heart defects; on the other hand, eNOS was down-regulated (Ferreiro *et al.* 2001).

It has been proposed that endogenous NO plays a positive role in increased ischemic tolerance of chronically hypoxic neonatal rabbit hearts by a mechanism which involves activation of soluble guanylyl cyclase, accumulation of cyclic GMP, possible activation of cGMP-dependent protein kinase and phosphorylation of sarcK_{ATP} (Baker *et al.* 1999). Acute inhibition of NOS activity by L-NAME led to a complete abolition of improved post-ischemic recovery of contractility by chronic hypoxia, whereas the NO donor, *S*-nitroso-glutathione (GSNO), had protective effect in normoxic but not in hypoxic hearts (Baker *et al.* 1999). Similarly, NOS inhibition blocked completely myocardial protection afforded by chronic hypoxia in neonatal rats (Ošťádalová *et al.* 2002). In contrast, our preliminary experiments in adult hypoxic rats have not confirmed this hypothesis as L-NAME had no effect on the improvement of post-ischemic recovery of contractility in isolated perfused hearts (Szárszoi *et al.* 2002). Moreover, L-NAME markedly reduced the incidence and severity of reperfusion arrhythmias in normoxic hearts but not in chronically hypoxic hearts, whereas GSNO completely abolished the antiarrhythmic effect of chronic hypoxia (Kolář *et al.* 2003c). These results suggest toxic rather than beneficial effect of endogenous NO in I/R injury of isolated perfused normoxic adult rat hearts and the inhibitory effect of exogenous NO on antiarrhythmic protection in chronically hypoxic hearts. Further focused studies are needed to resolve this discrepancy, which might possibly reflect ontogenic differences.

Protein kinases

Important role in the mechanism of increased cardiac ischemic tolerance is played by various protein kinases that are involved in several parallel protective signaling pathways. Protein kinase C (PKC) appears to be a key player in signal transduction of preconditioning (Ytrehus *et al.* 1994, Speechly-Dick *et al.* 1994, Mitchel *et al.* 1995). It has been originally postulated that PKC exerts its protective effect by activating mitoK_{ATP} (Speechly-Dick *et al.* 1995). However, it appears that this enzyme is downstream of the mitoK_{ATP} activation and ROS production because blocking PKC with chelerythrine abrogated the ability of diazoxide to precondition the rat heart (Wang *et al.* 2001), but it did not block diazoxide-induced ROS signal (Krenz *et al.* 2002). Superoxide of mitochondrial origin may be an important activator of PKC (Nishikawa *et al.* 2000). Recently, it has been shown that extracellular signal-regulated kinases (ERK) are also activated in response to mitochondria-derived superoxide secondary to the mitoK_{ATP} opening (Samavati *et al.* 2002). Activation of ERK, which belong to a large family of mitogen-activated protein kinases (MAPKs), is involved in cell survival pathways induced by preconditioning (Strohm *et al.* 2000, Fryer *et al.* 2001). Although the role of other major members of the MAPKs family, p38 MAPK and SAPK/JNK, in ischemic injury is rather controversial (Strnisková *et al.* 2002), the protection by preconditioning may also require their activation (Mocanu *et al.* 2000, Fryer *et al.* 2001) which, at least in case of p38 MAPK, has been shown to be secondary to ROS production (Yue *et al.* 2002).

Role of PKC and MAPKs in the mechanism by which chronic hypoxia protects the hearts against acute

I/R injury remains elusive. Limited information is available suggesting that PKC is up-regulated and permanently activated under the conditions of chronic hypoxia (Rouet-Benzineb *et al.* 1999, Morel *et al.* 2003). Our data suggest that the adaptation to hypoxia increases myocardial concentration of phoshatidylinositol, the substrate of PKC-activating signaling cascade (Ježková *et al.* 2002), and the abundance of PKCδ (but not PKCε which is important in preconditioning) in particulate fractions of rat ventricular myocardium. Rottlerin, a selective inhibitor of PKCδ isoform, attenuated the infarct-size limiting effect of chronic hypoxia, suggesting that protection was partially mediated by this isoform (Neckář *et al.* 2004). Another report (Rafiee *et al.* 2002) demonstrated that PKCε, p38 MAPKs and JNK are activated and translocated from the cytosolic to particulate fractions in chronically hypoxic infant human and rabbit myocardium. Inhibitors of these kinases (chelerythrine, SB203580 or curcumin, respectively) abolished cardioprotection by chronic hypoxia, but had no effects on normoxic rabbit hearts. It seems again that the role of various protein kinases and their particular isoforms in chronically hypoxic hearts is species-dependent, but the available data are not sufficient to resolve this issue.

Other protective pathways

Cai *et al.* (2003) demonstrated that the increased tolerance of chronically hypoxic mouse heart to I/R injury depends on hypoxia-inducible factor 1 (HIF-1) and erythropoietin. The increased expression and plasma level of erythropoietin as well as cardioprotection due to chronic hypoxia were lost in mice heterozygous for a knockout allele at the locus encoding HIF-1α; the administration of erythropoietin to rats protected their hearts against I/R injury 24 h later. Chronic hypoxia increases expression of HIF-1α in rat myocardium that mediates adaptive expression of other potentially protective proteins such as NOS (Rouet-Benzineb *et al.* 1999, Forkel *et al.* 2004), heme oxygenase or vascular endothelial growth factor (Deindl *et al.* 2003).

Another protective pathway of chronic hypoxia has been proposed by Dong *et al.* (2003). They demonstrated that the rate of cardiac myocyte apoptosis induced by I/R insult was reduced in chronically hypoxic rat hearts, together with increased expression of antiapoptotic factor Bcl-2 and decreased expression of proapoptotic factor Bax. However, it should be noted that chronic hypoxia itself might induce apoptosis (Weiland *et al.* 2001).

Blockers of various opioid receptor subtypes can abolish protective effect of chronic hypoxia against ventricular arrhythmias induced by adrenaline in rats. This observation suggests that endogenous opioids play a role in antiarrhythmic effect of chronic hypoxia (Lishmanov *et al.* 1998).

Conclusions

It may be concluded that adaptation to chronic hypoxia increases cardiac tolerance to acute oxygen deprivation in both adult and immature hearts. Although many potential factors have been proposed to play a role in this cardioprotective phenomenon, the available data are not sufficiently conclusive and its detailed molecular mechanism remains unknown. Limited evidence exists for the involvement of K_{ATP} channels, ROS, NO, protein kinases, opioids and erythropoietin, but potential contributions of other factors cannot be excluded at present. Most of these factors also participate in the mechanism of preconditioning, suggesting the activation of common protective pathways. As compared with the temporal character of preconditioning, cardiac protection by adaptation to hypoxia may persist even after the regression of other hypoxia-induced changes, such as polycythemia, pulmonary hypertension and right ventricular hypertrophy. This fact offers a more optimistic view of the future of effective protection of the ischemic myocardium.

As far as the clinical relevance of the adaptation to chronic hypoxia is concerned, it is necessary to stress that chronic hypoxia and hypoxemia are not confined to life at high altitude, but can be found in common and important cardiopulmonary diseases, i.e. chronic ischemic heart disease and chronic obstructive lung disease. In addition, increased cardiac tolerance to acute anoxia was described in children operated for cyanotic congenital heart disease. Unfortunately, epidemiological data on the incidence of myocardial infarction in populations suffering from chronic obstructive lung disease or other manifestations of chronic hypoxia are not available.

Acknowledgements

These studies were supported by research grants of MŠMT ČR LN 00A069, GA ČR 305/01/0279, GA ČR 305/04/0465, IGA MH ND/7607-3 and AVOZ 5011922.

References

ASEMU G, PAPOUŠEK F, OŠŤÁDAL B, KOLÁŘ F: Adaptation to high altitude hypoxia protects the rat hearts against ischemia-induce arrhythmias. Involvement of mitochondrial K_{ATP} channels. *J Mol Cell Cardiol* **31**: 1821-1831, 1999.

ASEMU G, NECKÁŘ J, SZÁRSZOI O, PAPOUŠEK F, OŠŤÁDAL B, KOLÁŘ F: Effects of adaptation to intermittent high altitude hypoxia on ischemic ventricular arrhythmias in rats. *Physiol Res* **49**: 597-606, 2000.

BAKER EJ, BOERBOOM LE, OLINGER GN, BAKER JE: Tolerance of the developing heart to ischemia: impact of hypoxemia from birth. *Am J Physiol* **268**: H1165-H1173, 1995.

BAKER JE, BOERBOOM LE, OLINGER GN: Age related changes in the ability of hypothermia and cardioplegia to protect ischemic rabbit myocardium. *J Thorac Cardiovasc Surg* **96**: 717-724, 1998.

BAKER JE, HOLMAN P, KALYANARAMAN B, GRIFFITH OW, PRITCHARD KA: Adaptation to chronic hypoxia confers tolerance to subsequent myocardial ischemia by increased nitric oxide production. *Ann N Y Acad Sci* **874**: 236-253, 1999.

BECKMAN JS, KOPPENOL WH: Nitric oxide, superoxide, and peroxynitrite: the good, the bad and ugly. *Am J Physiol* **271**: C1424-C1437, 1996.

BOLLI R: The late phase of preconditioning. *Circ Res* **87**: 972-983, 2000.

CAI Z, MANALO DJ, WEI G, RODRIGUEZ ER, FOX-TALBOT K, LU H, ZWEIER JL, SEMENZA GL: Hearts from rodents exposed to intermittent hypoxia or erythropoietin are protected against ischemia-reperfusion injury. *Circulation* **108**: 79-85, 2003.

CAMERON JS, BAGHDADY R: Role of ATP sensitive potassium channels in long term adaptation to metabolic stress. *Cardiovasc Res* **28**: 788-796, 1994.

CRAWFORD RM, JOVANOVIĆ S, BUDAS GR, DAVIES AM, LAD H, WENGER RH, ROBERTSON KA, ROY DJ, RANKI HJ, JOVANOVIĆ A: Chronic mild hypoxia protects heart-derived H9c2 cells against acute hypoxia/reoxygenation by regulating expression of the SUR2A subunit of the ATP-sensitive K^+ channel. *J Biol Chem* **278**: 21444-31455, 2003.

D´HAHAN N, MOREAU C, PROST AL, JACQUET H, ALEKSEEV AE, TERZIC A, VIVAUDOU M: Pharmacological plasticity of cardiac ATP-sensitive potassium channels towards diazoxide revealed by ADP. *Proc Natl Acad Sci USA* **96**: 12162-12167, 1999.

DEINDL E, KOLÁŘ F, NEUBAUER E, VOGEL S, SCHAPER W, OŠŤÁDAL B: Effect of intermittent high altitude hypoxia on gene expression in rat heart and lung. *Physiol Res* **52**: 147-157, 2003.

DONG JW, ZHU HF, ZHU WZ, DING HL, MA TM, ZHOU ZN: Intermittent hypoxia attenuates ischemia/reperfusion induced apoptosis in cardiac myocytes via regulating Bcl-2/Bax expression. *Cell Res* **13**: 385-391, 2003.

EELLS JT, HENRY MM, GROSS GJ, BAKER JE: Increased mitochondrial K_{ATP} channel activity during chronic myocardial hypoxia: is cardioprotection mediated by improved bioenergetics? *Circ Res* **87**: 915-921, 2000.

FALTOVÁ E, MRÁZ M, PELOUCH V, PROCHÁZKA J, OŠŤÁDAL B: Increase and regression of the protective effect of high altitude acclimatization on the isoprenaline-induced necrotic lesions in the rat myocardium. *Physiol Bohemoslov* **36**: 43-52, 1987.

FERDINANDY P, SCHULZ R: Nitric oxide, superoxide, and peroxynitrite in myocardial ischaemia-reperfusion injury and preconditioning. *Br J Pharmacol* **138**: 532-543, 2003.

FERREIRO CR, CHAGAS AC, CARVALHO MH, DANTAS AP, JATENE MB, BENTO DE SOUZA LC, LEMOS DA LUZ P: Influence of hypoxia on nitric oxide synthase activity and gene expression in children with congenital heart disease: a novel pathophysiological adaptive mechanism. *Circulation* **103**: 2272-2276, 2001.

FORBES RA, STEENBERGEN C, MURPHY E: Diazoxide-induced cardioprotection requires signaling through a redox-sensitive mechanism. *Circ Res* **88**: 802-809, 2001.

FORKEL J, CHEN X, WANDINGER S, KESER F, DUSCHIN A, SCHWANKE U, FREDE S, MASSOUDY P, SCHULZ R, JACOB H, HEUSCH G: Responses of chronically hypoxic rat hearts to ischemia: K_{ATP} channel blockade does not abolish increased RV tolerance to ischemia. *Am J Physiol* **286**: H545-H551, 2004.

FRYER RM, HSU AK, GROSS GJ: Erk and p38MAP kinase activation are components of opioid induced delayed cardioprotection. *Basic Res Cardiol* **96**: 136-142, 2001.

GARLID KD, DOS SANTOS P, XIE Z-J, COSTA ADT, PAUCEK P: Mitochondrial potassium transport: the role of the mitochondrial ATP-sensitive K^+ channels in cardiac function and cardioprotection. *Biochim Biophys Acta* **1606**: 1-21, 2003.

GRILLI A, DE LUTIIS AM, PATRUNO A, SPERANZA L, GIZZI F, TACCARDI AA, DI NAPOLI P, DE CATERINA R, CONTI P, FELACO M: Inducible nitric oxide synthase and heme oxygenase-1 in rat heart: direct effect of chronic exposure to hypoxia. *Ann Clin Lab Sci* **33**: 208-215, 2003.

GROSS GJ, PEART JN: K_{ATP} channels and myocardial preconditioning: an update. *Am J Physiol* **285**: H921-H930, 2003.

HANLEY PJ, MICKEL M, LOFFLER M, BRANDT U, DAUT J. K_{ATP} channel-independent targets of diazoxide and 5-hydroxydecanoate in the heart. *J Physiol Lond* **542**: 735-741, 2002.

HERGET J, WILHELM J, NOVOTNÁ J, ECKHARDT A, VYTÁŠEK R, MRÁZKOVÁ L, OŠŤÁDAL M: A possible role of the oxidant tissue injury in the development of hypoxic pulmonary hypertension. *Physiol Res* **49**: 493-501, 2000.

HURTADO A: Some clinical aspects of life at high altitudes. *Ann Intern Med* **53**: 247-258, 1960.

JEŽKOVÁ J, NOVÁKOVÁ O, KOLÁŘ F, TVRZICKÁ E, NECKÁŘ J, NOVÁK F: Chronic hypoxia alters fatty acid composition of phospholipids in right and left ventricular myocardium. *Mol Cell Biochem* **232**: 49-56, 2002.

KLONER RA, PRZYKLENK K, SHOOK T, CANNON CP: Protection conferred by preinfarct angina is manifest in the aged heart: evidence from the TIMI 4 trial. *J Thromb Thrombolysis* **6**: 89-92, 1998.

KOLÁŘ F: Cardioprotective effects of chronic hypoxia: relation to preconditioning. In: *Myocardial Preconditioning,* CL WAINWRIGHT, JR PARRATT (eds), Springer, Berlin, 1996, pp 261-275.

KOLÁŘ F, OŠŤÁDALOVÁ I, OŠŤÁDAL B, NECKÁŘ J, SZÁRSZOI O: Role of mitochondrial K_{ATP} channels in improved ischemic tolerance of chronically hypoxic adult and immature hearts. In: *Signal Transduction and Cardac Hypertrophy,* NS DHALLA, LV HRYSHKO, E KARDAMI, PK SINGAL (eds), Kluwer Academic Publishers, Boston, 2003a, pp 69-83.

KOLÁŘ F, SZÁRSZOI O, NECKÁŘ J, OŠŤÁDAL B: Improved cardiac ischemic tolerance in rats adapted to chronic hypoxia is reduced by simultaneous treatment with N-acetylcysteine. *Eur J Heart Failure* Suppl **2/1**: 46, 2003b.

KOLÁŘ F, SZÁRSZOI O, NECKÁŘ J, PECHÁŇOVÁ O, MIKOVÁ D, HAMPL V, OŠŤÁDAL B: Role of nitric oxide and reactive oxygen species in reperfusion-induced arrhythmias and cardioprotection in chronically hypoxic rat hearts. *Physiol Res* **52**: 52P, 2003c.

KONG X, TWEDDELL JS, GROSS GJ, BAKER JE: Sarcolemmal and mitochondrial K_{ATP} channels mediate cardioprotection in chronically hypoxic hearts. *J Mol Cell Cardiol* **33**: 1041-1045, 2001.

KOPECKÝ M, DAUM S: Tissue adaptation to anoxia in rat myocardium (in Czech). *Čs Fyziol* **7**: 518-521, 1958.

KRENZ M, OLDENBURG O, WIMPEE H, COHEN MV, GARLID KD, CRITZ S, DOWNEY JM, BENOIT JM: Opening of ATP-sensitive potassium channels causes generation of free radicals in vascular smooth muscle cells. *Basic Res Cardiol* **97**: 365-373, 2002.

LISHMANOV YB, USKINA EV, KRYLATOV AV, KONDRATIEV BY, UGDYZHEKOVA DS, MASLOV LN: A modulated effect of endogenous opioids in antiarrhythmic effect of hypoxic adaptation (in Russian). *Russian J Physiol* **84**: 363-372, 1998.

LIU Y, O´ROURKE B: Opening of mitochondrial K_{ATP} channels triggers cardioprotection. Are reactive oxygen species involved? *Circ Res* **88**: 750-752, 2001.

MAROKO PR, KJEKSHUS JK, SOBEL BE, WATANABE T, COVELL JW, ROSS J, BRAUNWALD E: Factors influencing infarct size following experimental coronary artery occlusions. *Circulation* **43**: 67-82, 1971.

MATSUOKA T, MATSUSHITA K, KATAYAMA Y, FUJITA A, INAGEDA K, TANEMOTO M, INANOBE A, YAMASHITA S, MATSUZAWA Y, KURACHI Y: C-terminal tails of sulfonylurea receptors control ADP-induced activation and diazoxide modulation of ATP-sensitive potassium channels. *Circ Res* **87**: 873-880, 2000.

McGRATH JJ, PROCHÁZKA J, PELOUCH V, OŠŤÁDAL B: Physiological response of rats to intermittent high altitude stress: effect of age. *J Appl Physiol* **34**: 289-293, 1973.

MEERSON FZ, GOMAZKOV GA, SHIMKOVICH MV: Adaptation to high altitude hypoxia as a factor preventing development of myocardial ischemic necrosis. *Am J Cardiol* **31**: 30-34, 1973.

MEERSON FZ, USTINOVA EE, ORLOVA EH: Prevention and elimination of heart arrhythmias by adaptation to intermittent high altitude hypoxia. *Clin Cardiol* **10**: 783-789, 1987.

MEERSON FZ, USTINOVA EE, MANUKHINA EB: Prevention of cardiac arrhythmias by adaptation: regulatory mechanisms and cardiotropic effect. *Biomed Biochim Acta* **48**: 583-588, 1989.

MITCHELL MB, MENG X, AO L, BROWN JM, HARKEN AH, BANERJEE A: Preconditioning of isolated rat heart is mediated by protein kinase C. *Circ Res* **76**: 73-81, 1995.

MOCANU MM, BAXTER GF, YUE Y, CRITZ SD, YELLON DM: The p38 MAPK inhibitor, SB203580, abrogates ischaemic preconditioning in rat heart but timing of administration is critical. *Basic Res Cardiol* **95**: 472-478, 2000.

MOREL O-E, BURVY A, LE CORVOISIER P, TUAL L, FAVRET F, LEON-VELARDE F, CROZATIER B, RICHALET J-P: Effects of nifedipine-induced pulmonary vasodilatation on cardiac receptors and protein kinase C isoforms in the chronically hypoxic rats. *Pflügers Arch* **446**: 356-364, 2003.

MURRAY CJ, LOPEZ AD: Alternate projections of mortality and disability by cause 1990-2020: global burden of disease study. *Lancet* **349**: 1498-1504, 1997.

MURRY CE, JENNINGS RB, REIMER KA: Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* **74**: 1124-1136, 1986.

NAKANISHI K, TAJIMA F, NAKAMURA A, YAGURA S, OOKAWARA T, YAMASHITA H, SUZUKI K, TANIGUCHI N, OHNO H: Effect of hypobaric hypoxia on antioxidant enzymes in rats. *J Physiol Lond* **489**: 869-876, 1995.

NARAYAN P, MENTZER RM, LASLEY RD: Adenosine A1 receptor activation reduces reactive oxygen species and attenuates stunning in ventricular myocytes. *J Mol Cell Cardiol* **33**: 121-129, 2001.

NECKÁŘ J, PAPOUŠEK F, OŠŤÁDAL B, NOVÁKOVÁ O, KOLÁŘ F: Antiarrhythmic effect of adaptation to high altitude hypoxia is abolished by 5-hydroxydecanoate. *J Mol Cell Cardiol* **31**: A51, 2001.

NECKÁŘ J, PAPOUŠEK F, NOVÁKOVÁ O, OŠŤÁDAL B, KOLÁŘ F: Cardioprotective effects of chronic hypoxia and preconditioning are not additive. *Basic Res Cardiol* **97**: 161-167, 2002a.

NECKÁŘ J, SZÁRSZOI O, KOTEN L, PAPOUŠEK F, OŠŤÁDAL B, GROVER GJ, KOLÁŘ F: Effects of mitochondrial K_{ATP} modulators on cardioprotection induced by chronic high altitude hypoxia in rats. *Cardiovasc Res* **55**: 567-575, 2002b.

NECKÁŘ J, SZÁRSZOI O, HERGET J, OŠŤÁDAL B, KOLÁŘ F: Cardioprotective effect of chronic hypoxia is blunted by concomitant hypercapnia. *Physiol Res* **52**: 171-175, 2003.

NECKÁŘ J, SZÁRSZOI O, MARKOVÁ I, NOVÁKOVÁ O, NOVÁK F, OŠŤÁDAL B, KOLÁŘ F. Is protein kinase C important for cardioprotection conferred by adaptation to chronic hypoxia in rats? *Physiol Res* 2004 (in press).

NISHIKAWA T, EDELSTEIN D, DU XL, YAMAGISHI S, MATSUMURA T, KANEDA Y, YOREK MA, BEEBE D, OATES PJ, HAMMES HP, GIARDINO I, BROWNLEE M: Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* **104**: 787-790, 2000.

NOTSU T, OHHASHI K, TANAKA I, ISHIKAWA H, NIHO T, FUKUTAKE K, MIZOTA M: 5-Hydroxydecanoate inhibits ATP-sensitive K^+ channel currents in guinea-pig single ventricular myocytes. *Eur J Pharmacol* **220**: 35-41, 1992a.

NOTSU T, TANAKA I, TAKANO M, NOMA A: Blockade of the ATP-sensitive K^+ channel by 5-hydroxydecanoate in guinea pig ventricular myocytes. *J Pharmacol Exp Ther* **260**: 702-708, 1992b.

OLDENBURG O, COHEN MV, YELLON DM, DOWNEY JM: Mitochondrial K_{ATP} channels: role in cardioprotection. *Cardiovasc Res* **55**: 429-437, 2002.

OŠŤÁDAL B, PROCHÁZKA J, PELOUCH V, URBANOVÁ D, WIDIMSKÝ J: Comparison of cardiopulmonary responses of male and female rats to intermittent high altitude hypoxia. *Physiol Bohemoslov* **33**: 129-138, 1984.

OŠŤÁDAL B, KOLÁŘ F, PELOUCH V, PROCHÁZKA J, WIDIMSKÝ J: Intermittent high altitude and the cardiovascular system. In: *The Adapted Heart,* M NAGANO, N TAKEDA, NS DHALLA (eds), Raven Press, New York, 1994, pp 173-182.

OŠŤÁDAL B, KOLÁŘ F, PELOUCH V, WIDIMSKÝ J: Ontogenetic differences in cardiopulmonary adaptation to chronic hypoxia. *Physiol Res* **44**: 45-51, 1995.

OŠŤÁDAL B, OŠŤÁDALOVÁ I, KOLÁŘ F, PELOUCH V, DHALLA NS: Cardiac adaptation to chronic hypoxia. In*:* *Advances in Organ Biology,* Vol. 6, *Myocardial Preservation and Cellular Adaptation,* EE BITTAR, DK DAS (eds), JAI Press, Stamford, London, 1998, pp 46-60.

OŠŤÁDAL B, OŠŤÁDALOVÁ I, DHALLA NS: Development of cardiac sensitivity to oxygen deficiency: comparative and ontogenetic aspects. *Physiol Rev* **73**: 635-659, 1999.

OŠŤÁDALOVÁ I, OŠŤÁDAL B, KOLÁŘ F, PARRATT JR, WILSON S: Tolerance to ischaemia and ischaemic preconditioning in neonatal rat heart. *J Mol Cell Cardiol* **30**: 857-865, 1998.

OŠŤÁDALOVÁ I, OŠŤÁDAL B, JARKOVSKÁ D, KOLÁŘ F: Ischemic preconditioning in chronically hypoxic neonatal rat heart. *Pediatr Res* **52**: 561-567, 2002.

PAIN T, YANG X-M, CRITZ SD, YUE Y, NAKANO A, LIU GS, HEUSCH G, COHEN MV, DOWNEY JM: Opening of mitochondrial K_{ATP} channels triggers the preconditioned state by generating free radicals. *Circ Res* **87**: 460-466, 2000.

PATEL HH, GROSS GJ: Diazoxide induced cardioprotection: what comes first, K_{ATP} channels or reactive oxygen species? *Cardiovasc Res* **51**: 633-636, 2001.

POUPA O, KROFTA K, PROCHÁZKA J, TUREK Z: Acclimatization to simulated high altitude and acute cardiac necrosis. *Fed Proc* **25**: 1243-1246, 1966.

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.

ROUET-BENZINEB P, EDDAHIBI S, RAFFESTIN B, LAPLACE M, DEPOND S, ADNOT S, CROZATIER B: Induction of cardiac nitric oxide synthase 2 in rats exposed to chronic hypoxia. *J Mol Cell Cardiol* **31**: 1697-1708, 1999.

SAMAVATI L, MONICK MM, SANLIOGLU S, BUETTNER GR, OBERLEY LW, HUNNINGHAKE GW: Mitochondrial K_{ATP} channel openers activate the ERK kinase by an oxidant-dependent mechanism. *Am J Physiol* **283**: C273-C281, 2002.

SHI Y, PRITCHARD KA, HOLMAN P, RAFIEE P, GRIFFITH OW, KALYANARAMAN B, BAKER JE: Chronic myocardial hypoxia increases nitric oxide synthase and decreases caveolin-3. *Free Radic Biol Med* **29**: 695-703, 2000.

SHI Y, BAKER JE, ZHANG C, TWEDDELL JS, SU J, PRITCHARD KA: Chronic hypoxia increases endothelial nitric oxide synthase generation of nitric oxide by increasing heat shock protein 90 association and serin phosphorylation. *Circ Res* **91**: 300-306, 2002.

SPEECHLY-DICK ME, MOCANU M, YELLON DM: Protein kinase C: its role in ischemic preconditioning in the rat. *Circ Res* **75**: 586-590, 1994.

STRNISKOVÁ M, BARANČÍK M, RAVINGEROVÁ T: Mitogen-activated protein kinases and their role in regulation of cellular processes. *Gen Physiol Biophys* **21**: 231-255, 2002.

STROHM C, BARANČÍK M, BRUEHL ML, KILIAN SAR, SCHAPER W: Inhibition of the ER-kinase cascade by PD98059 and UO126 counteracts ischemic preconditioning in pig myocardium. *J Cardiovasc Pharmacol* **36**: 218-229, 2000.

SUZUKI M, SASAKI N, MIKI T, SAKAMOTO N, OHMOTO-SEKINE Y, TAMAGAWA M, SEINO S, MARBAN E, NAKAYA H: Role of sarcolemmal K_{ATP} channels in cardioprotection against ischemia/reperfusion injury in mice. *J Clin Invest* **109**: 509-516, 2002.

SUZUKI M, SAITO T, SATO T, TAMAGAWA M, MIKI T, SEINO S, NAKAYA H: Cardioprotective effect of dazoxide is mediated by activation of sarcolemmal but not mitochondrial ATP-sensitive potassium channels in mice. *Circulation* **107**: 682-685, 2003.

SZÁRSZOI O, ASEMU G, OŠŤÁDAL B, KOLÁŘ F: Cardioprotection by chronic hypoxia: role of nitric oxide. *Physiol Res* **51**: 69P, 2002.

SZÁRSZOI O, ASEMU G, OŠŤÁDAL B, KOLÁŘ F: The role of reactive oxygen species and nitric oxide in ischemia/reperfusion injury of chronically hypoxic rat heart. *Eur J Heart Failure* Suppl 2/1: 53, 2003.

ŠAMÁNEK M, BASS A, OŠŤÁDAL B, HUČÍN B, STEJSKALOVÁ M: Effect of hypoxaemia on enzymes supplying myocardial energy in children with congenital heart disease. *Int J Cardiol* **25**: 265-270, 1989.

TAJIMA M, KATAYOSE D, BESSHO M, ISOYAMA S: Acute ischaemic preconditioning and chronic hypoxia independently increase myocardial tolerance to ischaemia. *Cardiovasc Res* **28**: 312-319, 1994.

TUREK Z, KUBAT K, RINGNALDA BEM: Experimental myocardial infarction in rats acclimated to simulated high altitude. *Basic Res Cardiol* **75**: 544-553, 1980.

VÉGH A, SZEKERES L, PARRATT JR: Preconditioning of the ischaemic myocardium; involvement of the L-arginine nitric oxide pathway. *Br J Pharmacol* **107**: 648-652, 1992.

WANG Y, TAKASHI E, XU M, AYUB A, ASHRAF M: Downregulation of protein kinase C inhibits activation of mitochondrial K_{ATP} channels by diazoxide. *Circulation* **104**: 85-90, 2001.

WEILAND JF, JOHNS RA, IHLING C, DIMMELER S: Chronic hypoxia induces apoptosis in cardiac myocytes: a possible role for Bcl-like proteins. *Biochem Biophys Res Commun* **286**: 419-425, 2001.

WIDIMSKÝ J, URBANOVÁ D, RESSL J, OŠŤÁDAL B, PELOUCH V, PROCHÁZKA J: Effect of intermittent altitude hypoxia on the myocardium and lesser circulation in the rat. *Cardiovasc Res* **7**: 798-808, 1973.

YELLON DM, DOWNEY JM: Preconditioning the myocardium: from cellular physiology to clinical cardiology. *Physiol Rev* **83**: 1113-1151, 2003.

YOSHIKAWA T, FURIKAWA Y, WAKAMATSU Y, TAKEMURA S, TANAKA H, KONDO M: Experimental hypoxia and lipid peroxide in rats. *Biochem Med* **27**: 207-213, 1982.

YTREHUS K, LIU Y, DOWNEY JM: Preconditioning protects ischemic rabbit heart by protein kinase C activation. *Am J Physiol* **266**: H1145-H1152, 1994.

YUE Y, QIN Q, COHEN MV, DOWNEY JM, CRITZ SD: The relative order of mK_{ATP} channels, free radicals and p38 MAPK in preconditioning's protective pathway in rat heart. *Cardiovasc Res* **55**: 681-689, 2002.

ZAUGG M, SCHAUB MC: Signaling and cellular mechanisms in cardiac protection by ischemic and pharmacological preconditioning. *J Muscle Res Cell Motil* **24**: 219-249, 2003.

ZHU HF, DONG JW, ZHU WZ, DING HL, ZHOU NZ: ATP-dependent potassium channels involved in the cardiac protection induced by intermittent hypoxia against ischemia/reperfusion injury. *Life Sci* **73**: 1275-1287, 2003.

Reprint requests

Dr. František Kolář, Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic. FAX: +420 241 062 125. E-mail: kolar@biomed.cas.cz