Molecular Mechanisms of Cardiac Protection by Adaptation to Chronic Hypoxia

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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
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 et 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 countered 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<sub>ATP</sub> and sarcK<sub>ATP</sub> 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<sub>ATP</sub> 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<sub>ATP</sub> 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<sub>ATP</sub> channels are not involved in protection by chronic hypoxia (Forkel et al. 2004).

It appears that cardioprotective mechanism and selectivity of K<sub>ATP</sub> modulators are species-dependent. It has been demonstrated that both diazoxide and preconditioning mediate their protective effects by activation of sarcK<sub>ATP</sub> but not mitoK<sub>ATP</sub> 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<sub>ATP</sub> 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<sub>ATP</sub> modulators, independent of mitochondrial K<sup>+</sup> influx, should be taken into consideration (Hanley et al. 2002). Thus, the involvement of K<sub>ATP</sub> 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<sub>ATP</sub> 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<sub>ATP</sub> 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<sub>ATP</sub> opening and downstream signaling pathways is formation of ROS (Pain et al. 2000, Forbes et al. 2001). Moreover, although the mechanism of mitoK<sub>ATP</sub> 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<sub>ATP</sub> 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 (Szászsoi 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-Benzeineb 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 isofrom 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 (Szászsozi 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 (Strmisková 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 phosphatidylinositol, the substrate of PKC-activating signaling cascade (Ježková et al. 2002), and the abundance of PKCδ (but not PKCe 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 KATP 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.

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References


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/I**: 46, 2003b.


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