Review

Intrinsic defensive mechanisms in the heart: a potential novel approach to cardiac protection against ischemic injury

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Abstract. Despite recent advances in pharmacotherapy of coronary artery disease and interventional cardiology, the management of myocardial ischemia still remains a major challenge for basic scientists and clinical cardiologists. An urgent need to combat ischemic heart disease, its forms, such as infarction, and complications including sudden cardiac death led to the development of an alternative strategy of myocardial protection based on the exploitation of the heart's own intrinsic protective mechanisms. A new concept relies on the evidence that the heart is able to protect itself by way of adaptation, either short-term or long-term, to transient episodes of stress (e.g., ischemia, hypoxia, free oxygen radicals, heat stress, etc.) preceding sustained ischemia. Preconditioning by brief episodes of ischemia (ischemic preconditioning, IP) represents the most powerful cardioprotective phenomenon. Apart from the short-lasting protection afforded by classical IP or its delayed ("second window") phase, adaptation to long-lasting physiological stimuli or pathological processes is also known to increase myocardial resistance to ischemic injury. Although molecular mechanisms of cardiac adaptation conferring a higher ischemic tolerance still remain not sufficiently elucidated, multiple cascades of intracellular signalization are suggested to be involved in this process. Experimental studies led to the observations that pharmacological modulations at different levels of signal transduction might mimic protective effects of the adaptive phenomena and thus provide a safer way of inducing cardioprotection in humans.

Key words: Myocardial ischemia — Endogenous cardioprotection — Ischemic preconditioning — Adaptation — Cell signaling

Introduction

Ischemic heart disease and its most severe form, myocardial infarction, as well as sudden cardiac death due to malignant ventricular arrhythmias, are major causes of cardiovascular morbidity and mortality in modern societies and still remain a great challenge for clinical cardiologists and basic scientists. Delivery of oxygen and metabolic substrates *via* coronary circulation is an essential prerequisite for normal cardiac function, and its cessation leads within minutes to irreversible cellular injury. The duration of ischemia and the extent of metabolic and structural alterations in the myocardium are the main factors that determine the fate of cardiomyocytes and progress towards cell death (by mechanisms of necrosis or apoptosis) or cell survival. Despite recent advances in reperfusion therapy – trombolysis, percutaneous coronary intervention and coronary artery bypass graft surgery – there is an urgent need for exploring other therapeutic strategies aimed at ultimate protection of the heart against the long-lasting ischemic insult.

The last decade has witnessed the development of a novel approach to myocardial protection against ischemic injury that exploits heart's own defensive mechanisms. A new concept of endogenous cardioprotection is based on the evidence that the heart (and probably other organs) is able to protect itself against detrimental consequences of ischemia, by way of adaptation, either short-term or long-term. Intrinsic cardioprotective mechanisms can be triggered by transient episodes of stress (e.g., ischemia, hypoxia, free oxygen radicals, heat stress, etc.) leading to attenuation of myocardial injury during subsequent sustained ischemia.

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One of the forms of endogenous cardioprotection, termed ischemic preconditioning (IP), can be defined as an adaptive response, whereby brief episodes of the ischemic stress render the heart more resistant to subsequent longer periods of ischemia (Murry et al. 1986). This short-lasting (hours) phenomenon has been described in all species including humans undergoing multiple aortic cross-clamping during coronary artery bypass graft surgery or balloon inflations during percutaneous coronary interventions (Yellon et al. 1993), as well as in patients with transient episodes of angina prior to surgery (Loubani et al. 2004). Although IP offers an extremely powerful protection exceeding the effectiveness of any pharmacological intervention (Schott et al. 1990), its molecular mechanisms still remain elusive. It is suggested that endogenous substances released from the heart (or other organs, so-called remote preconditioning) in the early phase of ischemia (e.g., adenosine, bradykinin, catecholamines), are involved in the protective mechanisms of IP by activating multiple cascades of intracellular signalization, from membrane receptors, *via* postreceptor signal transduction pathways, up to the final end-effector systems, such as ion transport systems (Parratt and Vegh 1999; Cohen et al. 2000). Experimental studies of the mechanisms of IP lead to the observations that pharmacological modulations at different levels of signal transduction may mimick cardioprotective effects of IP and thus provide a safer way of inducing the IP-like cardioprotection in humans without the harmful consequences of ischemia (Yao and Gross 1994; Ravingerova et al. 1996; Kitakaze et al. 1999; Nakano et al. 2000a).

Another form of endogenous cardioprotection was initially described by Szekeres et al. (1984) as a delayed and long-lasting cardiac adaptation induced by certain drugs such as prostacyclin and its stable derivatives, and later on defined as a late phase of preconditioning or the "second window" of protection (Kuzuya et al. 1993; Baxter et al. 1995). It appears as a delayed response to initial short stressful stimulus, lasts longer (up to 3 days) and is, therefore, more important from the clinical point of view (Bolli 2001; Loubani et al. 2004). Different from the early phase, delayed protection involves not only posttranslational modification of the existing proteins, but the changes in gene expression and synthesis of new proteins with antioxidative, antiadrenergic, cytoprotective and antiapoptotic properties (e.g., heat stress proteins, superoxide dismutase, catalase, phosphodiesterase isoforms, antiapoptotic protein Bcl-2) and of those ensuring production of nitric oxide (NO) (inducible NO synthase, iNOS) (Borchert et al. 1994; Imagawa et al. 1999; Bolli 2001; Sommerschield and Kirkeboen 2002). Similarly to the classical IP, the second phase of cardioprotection can be also induced by the IP-mimicking agents, such as bradykinin, adenosine, catecholamines, opioids (Vegh et al. 1992; Ravingerova et al. 1997; Fryer et al. 2001; Baxter 2002; Loubani et al. 2004).

Short-term endogenous cardioprotection: basic concept

Animal studies reveal that in the intact non-adapted myocardium, myocardial ischemia alters a balance between the formation of reactive oxygen species (ROS) and the availability of endogenous antioxidants, and functional deteriorations and severe arrhythmias upon reperfusion are related to a certain extent to an excessive generation of ROS during prolonged ischemia/reperfusion (I/R) and their deleterious effects on cardiac cell membranes and function of ion transport systems (Ravingerová et al. 1994, 1999; Goldhaber and Qayyum 2000; Fischer et al. 2003; Kaplan et al. 2003; Babušíková et al. 2004). This has been verified by the efficacy of antioxidants and scavengers in experimental settings of acute I/R (reviewed by Hoffman et al. 2004). Thus, it has been demonstrated that antioxidant N-acetylcysteine attenuated myocardial dysfunction associated with ischemic injury due to cardiopulmonary bypass/cardioplegic arrest in anesthetized dogs (Fischer et al. 2003). Similarly, pretreatment with melatonin prior to ischemia reduced the severity and duration of reperfusion-induced ventricular arrhythmias in isolated perfused rat hearts (Szarszoi et al. 2001; Važan et al. 2005), attenuated calcium overload of the heart (Važan et al. 2003) and improved postischemic recovery of the contractile function (Szarszoi et al. 2001).

On the other hand, the concept of IP has offered an alternative approach to combat deleterious effects of long-lasting ischemia by way of increasing the heart's own ischemic tolerance. The latter is manifested during prolonged ischemia by a delay of necrotic processes in the cardiac cells and, therefore, reduced size of myocardial infarction, attenuation of postischemic contractile dysfunction, as well as reduction of life-threatening I/R-induced ventricular arrhythmias (Murry et al. 1986; Vegh et al. 1992; Cave 1995).

Molecular mechanisms

Mechanisms of IP involve stimulation of a number of receptors in the early triggering phase followed by activation of multiple postreceptor pathways (mediating phase) leading to the final end-effector phase resulting in cardioprotection manifested by three major end-points.

Early phase of preconditioning

It has been shown that in the early phase, stimulation of Gi/q proteins-coupled receptors (GPCR; e.g., adenosine A1/A3, bradykinin B2, muscarinic, opioid or α1-adrenergic receptors) by their respective agonists or activation of receptors with tyrosin kinase activity (TKR) *via* their ligands including growth factors and hormones (insulin), increased production

of ROS, as well as enhanced activity of endothelial isoform of NO synthase (eNOS) and bioavailability of NO could play a role (Liu et al. 1991; Ravingerová et al. 1995; Bolli et al. 1997; Cohen et al. 2000; Yellon and Downey 2003; Krieg et al. 2004). In addition, it has been suggested (Das et al. 1992) that preconditioning of the heart may lead to strengthening of its oxidative defense system *via* stimulation of a number of endogenous antioxidative enzymes, which is likely to play a role in myocardial preservation during subsequent I/R injury.

The role of α1-adrenergic receptors stimulation

It has been demonstrated that stimulation of α1-adrenergic receptors in the early phase of IP preconditions the rat and rabbit heart against contractile dysfunction and myocardial infarction (Banerjee et al. 1993; Tsuchida et al. 1994). Moreover, α1-adrenergic receptors stimulation by endogenously released catecholamines coupled with upregulation of Gi proteins plays a key role in the antiarrhythmic protection in the rat heart (Ravingerová et al. 1995, 2002) as indicated by suppression of antiarrhythmic effect of IP after administration of α1-adrenergic blocker prazosin and a failure of β-adrenergic stimulation with isoproterenol to mimic the effect of IP simulated by exogenous application of norepinephrine. Furthermore, suppression of norepinephrine-induced antiarrhythmic protection by pretreatment of animals with pertussis toxin and inactivation of Gi proteins further supported the role of Gi/q-proteins-mediated signal transduction in postreceptor mechanisms of cardioprotection, in addition to their role in the infarct size-limiting effect of classical IP (Thornton et al. 1993; Yellon and Downey 2003). Cardioprotective effects were associated with activation of protein kinase C (PKC) (Mitchell et al. 1995). In accordance, our study also demonstrated that administration of norepinephrine in rats resulted in an immediate subcellular relocalization of PKCε to the membrane fraction lasting up to 4 h indicating its activation (Wilson et al. 1996).

The role of NO

The role of NO in I/R injury and cardioprotection is very complex and not completely understood. It has been characterized mostly for a delayed phase of IP and, in particular, in protection against stunning (Bolli et al. 1997; Bolli and Marban 1999) and arrhythmias (Vegh et al. 1992). NO has been shown to increase ischemic tolerance by multiple cyclic GMP-dependent and independent interactions (Ferdinandy and Schulz 2003), and although the role of NO in the classical IP is not clearly established, it is generally accepted that NO produced by eNOS in the early phase triggers a delayed phase of preconditioning through the up-regulation of iNOS in the second phase (Bolli 2001). However, under certain conditions, excess NO may exert detrimental rather than beneficial effects due to formation of its highly toxic metabolite peroxynitrite, the reaction product of NO and superoxide. This negative role of NO has been demonstrated in many experimental settings and for all manifestations of I/R injury including human pathology and supported by protective effects of limitation of NO production either pharmacologically or by genetical modulations of eNOS and iNOS expression (Mathies et al. 1992; Patel et al. 1993; Ferdinandy and Schulz 2003). It has been also shown in our studies in a setting of Langendorff-perfused rat hearts, that in the non-adapted hearts subjected to a test ischemic challenge, acute blockade of NO synthesis by NOS inhibitor L-NAME improved postischemic contractile recovery and suppressed reperfusion-induced arrhythmias (Andelova et al. 2005). On the other hand, NOS inhibition abrogated protective effects of preconditioning indicating that NO production in the early phase of IP is required for cardioprotection to occur, and that NO can play a dual role in the myocardium being deleterious in the non-adapted hearts and acting as a signaling molecule in the mechanisms of short-term adaptation. The latter points out to the controversial role of free radicals in the myocardium and addresses the issue of the effectiveness of antiradical interventions in the intact non-preconditioned (unstressed) myocardium and in the myocardium that has shifted to a defensive phenotype in response to stress.

Postreceptor mechanisms

Activation of intracellular enzymes (protein kinases) is considered as the mainstream process in signal transduction mechanisms mediating protective effect of IP (Cohen et al. 2000; Yellon and Downey 2003). They are involved in multiple parallel signaling pathways and modulate, by mechanisms of protein phosphorylation, the function of ion transport systems in different cellular compartments including those targets considered as hypothetical end-effector systems.

Protein kinase C

In particular, activation of PKC through phospholipase C- or phospholipase D-mediated pathway (Mitchell et al. 1995) appears to be a crucial step in the molecular mechanisms of IP and protection against myocardial infarction in rabbit myocardium (Ytrehus et al. 1994), as well as in the antiinfarct protection in the second phase of IP (Baxter et al. 1995). It has been originally proposed that PKC exerts its protective effect by activating mitochondrial ATP-dependent K^+ channels (mitoK_{ATP}) (Speechly-Dick et al. 1994). However, later on it has been shown that this enzyme might be also activated downstream of the mitoK_{ATP} opening and production of ROS since inhibition of PKC with chelerythrine blocked the

ability of mitoK$_{ATP}$ opener diazoxide to precondition the rat heart (Wang et al. 2001), but not the diazoxide-induced ROS signal (Krenz et al. 2002). Furthermore, Nishikawa et al. (2002) demonstrated that superoxide of mitochondrial origin may be an important activator of PKC.

Mitogen-activated protein kinases

Studies of myocardial response to I/R injury and cardioprotection revealed an important role of a large family of mitogen-activated protein kinases (MAPK) (Lazou et al. 1998; Nagarkatti and Sha'afi 1998; reviewed by Ravingerová et al. 2003b). In particular, activation of extracellular signal-regulated kinases (ERK) by mitochondria-derived superoxide due to mitoK$_{ATP}$ opening (Samavati et al. 2002) has been implicated in the mechanisms of both, early (Strohm et al. 2000) and delayed cardioprotection (Fryer et al. 2001). This cascade is associated with hypertrophic response on the one hand, and on the other hand, it is involved in cell survival mechanisms (Hausenloy et al. 2004). Although the role of other subfamilies of the MAPK family, so called "stress" kinases p38-MAPK and SAPK/JNK, in myocardial response to ischemic injury is rather controversial, cardioprotection afforded by preconditioning may also require activation of p38-MAPK (Maulik et al. 1996; Mocanu et al. 2000; Nakano et al. 2000b). The latter has been also shown to be secondary to ROS production in mitochondria since antiinfarct protection in rat hearts was blocked by p38-MAPK inhibitor SB203580 and by ROS scavenger mercaptopropionylglycine, and generation of ROS in mitochondria caused by menadione induced both infarct size limitation and p38-MAPK activation (Yue et al. 2002). Although there is some evidence that activation of SAPK/JNK cascade occurs in a setting of IP (Ping et al. 1999) and is an important component of preconditioning- or opioids-induced delayed antiinfarct protection in rats *in vivo* (Fryer et al. 2001), the exact mechanisms and the role of SAPK/JNK in cardioprotection are not completely understood.

Phosphatidylinositol 3-kinase/Akt

Phosphatidylinositol 3-kinase (PI3K) and its downstream effector, a serine-threonine protein kinase B (Akt), is a key signaling enzyme system implicated in the processes of normal physiological cell growth, as well in the mechanisms of cell survival and metabolic control (Matsui and Rosenzweig 2005). Many biological stimuli activating PI3K/Akt, such as GPCR agonists (phenylephrine, endothelin-1, angiotensin II), as well as ligands of TKR (e.g., insulin and insulin growth factor IGF-I) are involved in mediating of physiological, as well as pathological hypertrophic response of the heart (Naga Prasad et al. 2000; Friehs and del Nido 2003; El Jamali et al. 2004), but they have been also shown to reverse ROS-induced apoptotic effects in neonatal cardiomyocytes (Aikawa et al. 2000) and participate in cardioprotective effects of classical IP as well (Krieg et al. 2004). Recent observations indicate that activation of PI3K/Akt during IP occurs upstream of PKC activation (Tong et al. 2000) and further targets mitoK$_{ATP}$ channels by NO- or PKC-dependent mechanism that keeps them in the open state (Oldenburg et al. 2004). It is proposed that acute PI3K/Akt activation plays an important role in the preconditioning protection by mediating antiapoptotic effects *via* protein phosphorylation leading to either inhibition of proapoptotic pathways (e.g., of pro-apoptotic BAD proteins with a subsequent preservation of anti-apoptotic Bcl-2 proteins, inhibition of caspase-9 and/or glycogen synthase kinase-3β or activation of several antiapoptotic signaling mechanisms, e.g., eNOS, PKC, glucose transporter GLUT (Murphy 2004; Matsui and Rosenzweig 2005).

However, different from the beneficial effects of acute activation of Akt, its sustained activation may have a negative impact on the myocardium and contribute to the development of heart failure (Naga Prasad et al. 2000). Divergency between normal adaptive response with moderately increased Akt activation *versus* pathological hypertrophy with high-level Akt activation is also determined by different pathways of PI3K signaling: while growth factors (hormones)/TKR-activated p110α isoform is involved in the physiological adaptive response, prolonged activation of p110γ isoform *via* biomechanical stress/neurohormonal mediators/GPCR mechanisms is required for stress-induced pathological maladaptation (Naga Prasad et al. 2000; Oudit et al. 2004). The role of PI3K/Akt in ischemic myocardium is also not unequivocal with respect to the different end-points of injury. Whereas a positive role of PI3K/Akt activation has been demonstrated in the infarct size limitation by IP (Mocanu et al. 2002), its role in the mechanisms of arrhythmogenesis is practically unknown and has not been consistently investigated so far. In our recent study (Ravingerová et al. 2006) we have observed substantial differences in the effects of PI3K/Akt inhibition in the non-preconditioned rat heart, where PI3K/Akt inhibitor LY294002 exhibited no effect on the size of myocardial infarction, while the incidence of ischemic arrhythmias was markedly decreased (Fig. 1). These differential effects of PI3K/Akt in the ischemic heart on the size of infarction and arrhythmogenesis are important due to the fact that activation of this cascade occurs as a component of the well-known treatment with glucose-insulin-potassium in patients with myocardial infarction and most probably underlies the mechanism of this protective intervention.

End-effector mechanisms of preconditioning

K_{ATP} channels

Opening of K$_{ATP}$ channels has been suggested as a most likely end-effector mechanism in preconditioning cascade since

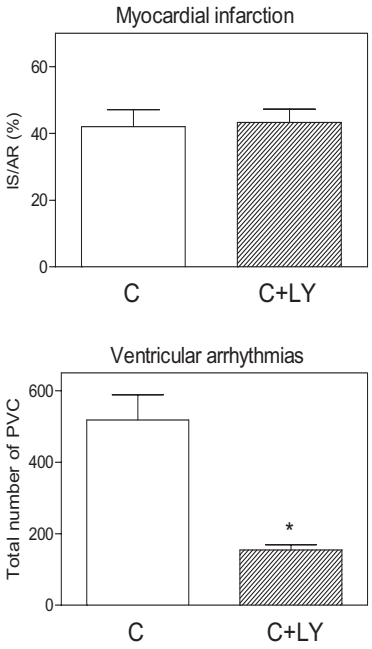

Figure 1. Differential effects of PI3K/Akt inhibition on the size of myocardial infarction and ventricular arrhythmias in the rat heart. C, control hearts exposed to test LAD (left anterior descending coronary artery) occlusion/reperfusion; LY, PI3K/Akt inhibitor LY294002; IS/AR, infarct size normalized to the size of area at risk ; PVC, premature ventricular contractions (extrasystoles); * $p < 0.05$ *vs.* C.

their blockade with K_{ATP} blocker, glibenclamide, abolished IP in dogs (Gross and Auchampach 1992). The role of these channels localized in different cell compartments has been demonstrated in various forms of cardioprotection, and in many animal species, strengthened by the finding that K_{ATP} openers could mimic the IP-induced protection (Grover et al. 1994; Gross and Fryer 1999). The role of K_{ATP} channels activation as a final step in cardioprotective signaling mechanisms is further supported by the findings that activation of PKC appears to phosphorylate sarcolemmal K_{ATP} channel (Hu et al. 1996), and there is some evidence that NO, PKC and MAPK-mediated pathways facilitate the opening of mitoK_{ATP} channels as well (Sato et al. 1998; Murphy 2004). On the other hand, mitoK_{ATP} opening could play not only the end-effector role in the preconditioning cascade, but it can also trigger this chain of events as well and act as an "upstream" mechanism of protein kinases activation mediated by an increased production of free oxygen radicals and NO (Yue et al. 2002).

However, K_{ATP} modulations may exert both anti- and proarrhythmic effects depending on the experimental conditions, animal species and mechanism of arrhythmias (Tosaki et al. 1992). Thus, in our study, antiarrhythmic protection by IP in isolated rat heart was not reversed by glibenclamide (Ravingerová et al. 2002). On the other hand, it has been revealed that cardioprotection occurs independently from the shortening of action potential duration, which is the main target of sarcolemmal K_{ATP} openers (Hamada et al. 1998), and that selective inhibitors of mitoK_{ATP} channels block myocardial protection afforded by IP (Sato et al. 2000). In accordance, we have demonstrated that a selective inhibitor of mitoK_{ATP} channels, 5-hydroxydecanoate, blocked the infarct size-limiting effect of IP in rabbits without affecting ischemia-induced shortening of action potential duration and blunted antiinfarct protection in rats (Munch-Ellingsen et al. 2000), as well as it abolished protection against contractile dysfunction in guinea pig papillary muscle conferred by hypoxic preconditioning (Ravingerova et al. 1998). Recently it was proposed that mitoK_{ATP} channel is 2000-fold more sensitive than the sarcolemmal one to K_{ATP} opener diazoxide, which has been shown to mimic the IP protection, and that it is the most likely end-effector involved with IP (Garlid et al. 2003). Exact mechanism by which opening of mitoK_{ATP} results in cardioprotection is not completely elucidated so far, although depolarization of the mitochondrial inner membrane and dissipation of membrane potential, in conjunction with limitation of calcium uptake by mitochondria (Holmuhamedov et al. 1999), regulation of mitochondrial volume and rate of respiration (Lim et al. 2002), as well as modulation of ROS production (Pain et al. 2000) could underlie protective effects. Another potential mechanism of attenuation of cell death by mitoK_{ATP} openers is related to the regulation of mitochondrial antiapoptotic proteins (Bcl-2) involved in the limitation of the mitochondrial permeability and release of apoptosis-inducing cytochrome c (Shimizu et al. 1999).

Fig. 2 shows schematic representation of molecular signaling mediated by "survival" cascades (ERK and PI3K/Akt) that cross-talk on different levels of cellular signal transduction, both being involved in hypertrophic response and converge on mitochondrial target proteins.

Long-term cardiac adaptation

Increased resistance to ischemic injury can also develop due to long-lasting adaptation of the heart to some more physiological stimuli like tachycardia (Domenech et al. 1998), physical excercise (Yamashita et al. 2001), or chronic hypoxia of a continuous and intermittent nature (Baker et al. 1999; Zhuang and Zhou 1999) including chronic high-altitude hypoxia (Meerson et al. 1972; Kolar 1996).

Enhanced ischemic tolerance has been also observed during adaptation to pathological conditions associated with myocardial hypoxia and remodeling, e.g., experimental diabetes mellitus (Feuvray and Lopaschuk 1997; Ravingerova et

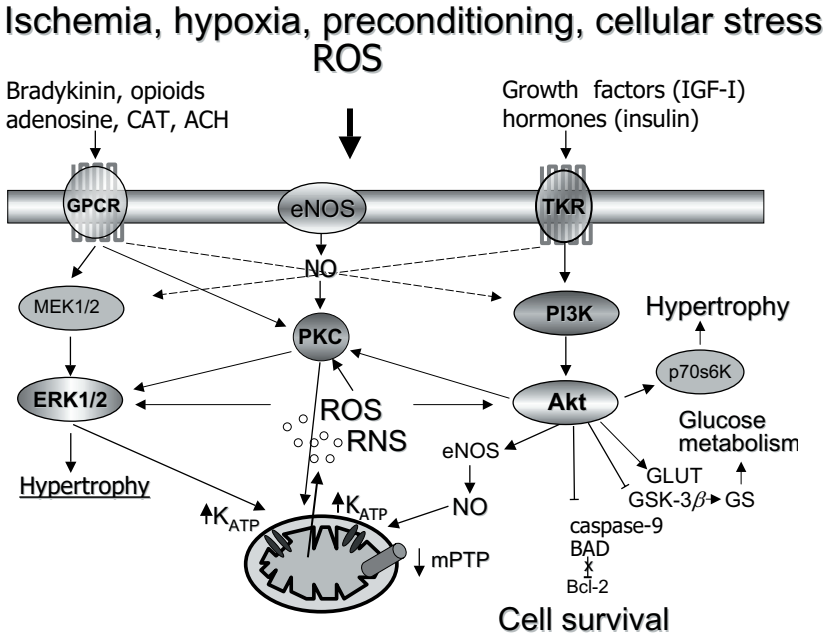

Figure 2. Schematic representation of mechanisms of molecular signaling activated by ischemia and other forms of cellular stress and mediated by "survival" cascades of protein kinases. GPCR, G proteins-coupled receptors; TKR, tyrosine kinase receptors; NO, nitric oxide; eNOS, endothelial NO synthase; PI3K, phosphatidylinositol 3-kinase; Akt, Akt kinase; ERK1/2, extracellular signal-regulated cascade of MAPK (mitogen-activated protein kinases); MEK1/2, upstream regulator of MAPK; PKC, protein kinase C; ROS, reactive oxygen species; RNS, reactive nitrogen species; K_{ATP}, ATP-sensitive K^+ channel; BAD, Bcl-2 pro- and antiapoptotic proteins; GS, glycogen synthase; GSK-3β, GS kinase-3β; GLUT, glucose transporter; mPTP, mitochondrial permeability transition pore; p70s6K, ribosomal S6 kinase; CAT, catecholamines; ACH, acetylcholine; IGF-I, insulin growth factor.

al. 2000, 2001), as well as in patients with episodes of stable angina pectoris prior to myocardial infarction (Loubani et al. 2004; Solomon et al. 2004). Molecular mechanisms of this form of cardioprotection are not clarified either, although there are certain indications that they might share some pathways with other adaptive phenomena.

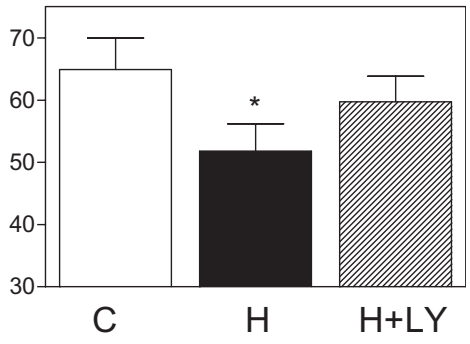

Figure 3. Effect of PI3K/Akt inhibition on anti-infarct protection in rats adapted to chronic intermittent hypobaric hypoxia. C, control normoxic rats exposed to test LAD occlusion/reperfusion; H, hypoxia-adapted rats; LY, PI3K/Akt inhibitor LY294002; IS/AR, infarct size normalized to the size of area at risk; * $p < 0.05$ *vs.* C.

Adaptation to chronic hypoxia

Compared with IP, cardioprotection due to chronic oxygen deprivation is not so robust, however, it persists for much longer periods of time than any other form of cardioprotection (Neckar et al. 2004) despite the development of cardiac hypertrophy and remodeling (Pelouch et al. 1997). Molecular mechanisms of adaptation by chronic hypoxia are not yet completely understood, and experimental evidence is limited to the involvement of mitoK_{ATP} channels activation, ROS, NO, opioids, as well as up-regulation of PKC-δ (reviewed by Kolar and Ostadal 2004; Neckar et al. 2005). Activation of p38- and JNK cascades of MAPK has been also reported to occur in chronically hypoxic infant human and neonatal rabbit myocardium (Rafiee et al. 2002). Recent studies revealed the changes in the levels of ERK in the hypertrophied rat myocardium adapted to chronic hypoxia (Strnisková et al. 2006). In addition, anti-infarct protection conferred by long-term adaptation of rats to hypoxic environment was attenuated by PI3K/Akt inhibitor LY294002 (Fig. 3) that completely abolished infarct size limitation induced by classical IP indicating that this cascade might also play an important role in the infarct size-limiting mechanism of chronic hypoxia in the rat heart (Ravingerová et al. 2007).

Susceptibility to ischemic injury in the diabetic heart

Whereas clinical and epidemiological studies have clearly demonstrated an increased incidence of myocardial infarction and its complications, as well as severe ventricular arrhythmias, in diabetic patients (Kannel and McGee 1979), experimental data are contradictory and suggest that susceptibility of the diabetic hearts to ischemia may be increased, unchanged or decreased (Feuvray and Lopaschuk 1997).

Thus, studies of infarct size indicate that diabetic dogs develop larger infarcts than normal ones (Forrat et al. 1993), whereas in the diabetic rabbits and rats, infarct size tends to be smaller (Liu et al. 1993; Hadour et al. 1998). Similarly, susceptibility to arrhythmias in the diabetic hearts has been found both enhanced and reduced (Hekimian et al. 1985; Kusama et al. 1992). Functional recovery upon reperfusion in the diabetic rats has been reported to be improved in the early stage and impaired in the later phase of the disease (Tosaki et al. 1996). Our *in vivo* studies demonstrated that in the open-chest rats with acute STZ-induced diabetes, their hearts exert a very powerful protection against myocardial infarction (Ravingerová et al. 2003a). Moreover, isolated rat hearts in the acute phase of diabetes exhibit a lower sensitivity to ischemia-induced arrhythmias and decreased accumulation of acid metabolites (Ravingerova et al. 2000).

It is suggested that differences between the species, duration and severity of the diabetic state, as well as different protocols of the studies may account for the above discrepancies. Under certain experimental conditions (e.g., global I/R) associated with Na^+ and Ca^{2+} gain, processes related to the alterations in glucose metabolism (lower rate of glycolysis) and to the regulation of the intracellular pH (decreased activity of Na^+/H^+-exchanger) might be responsible for the reduced sensitivity to ischemia in the diabetic hearts (Khandoudi et al. 1990; Pierce et al. 1990; Feuvray and Lopaschuk 1997). In addition, enhanced ischemic tolerance in the diabetic heart can be considered as an alternative form of intrinsic cardioprotection analogous to that induced by preconditioning in the normal heart or by adaptation to chronic myocardial hypoxia. Increased resistance to ischemia in an experimental model of diabetes can be triggered by numerous metabolic stimuli, in particular by those related to enhanced production of ROS (which is a common feature of a diabetic heart) and deteriorations in intracellular calcium signalling that by itself is known to induce preconditioning-like protection in the normal heart (Meldrum et al. 1996). Furthermore, PKC activation is also known to occur in the diabetic myocardium even in the early phase of the disease (Malhotra et al. 1997). Moreover, its translocation has been shown to mediate cardioprotection in the STZ-induced diabetic rat heart (Moon et al. 1999), whereas PKC inhibition abolished an increased resistance to I/R injury in the diabetic hearts (Ooie et al. 2003). In addition, we have observed an increased phosphorylation of ERK1/2 in the diabetic myocardium (Strnisková et al. 2003) suggesting its potential positive role in the response of the diabetic heart to acute ischemic challenge. Since PI3K/Akt cascade plays an important role in regulation of glucose metabolism, it could be expected to be involved in the changes in ischemic tolerance in diabetics, however, the latter still remains to be elucidated. Another mechanism of protection against myocardial infarction related to a limitation of an increased influx of calcium, is the opening of sarcolemmal K_{ATP} channels, which in the diabetic cardiomyocytes have been found to be much more sensitive and open at higher levels of ATP (Smith and Wahler 1996). Of major concern may be thus hypoglycemic therapy with sulphonylurea drugs (e.g., glibenclamide) in humans, since K_{ATP} inhibition as its consequence and suppression of clinical manifestations of preconditioning may be one of the causes of higher mortality in diabetic patients (Tomai et al. 1994; Brady and Terzic 1998). On the other hand, the role of mitoK_{ATP} channels opening regarded as a key component of myocardial adaptation to chronic oxidative stress (Kolar and Ostadal 2004), has not been sufficiently elucidated so far in the diabetic heart. Thus, it seems that in the diabetic heart, numerous factors might trigger adaptive mechanisms that successfully counteract the development of metabolic disorders leading to irreversible cell damage and arrhythmias at least in the early stage of the disease, and might share some common pathways with other forms of cardioprotection.

Conclusions

It appears that both, short-term cardioprotective phenomena and long-lasting adaptation to physiological stimuli and some pathological conditions associated with chronic oxygen deprivation might share certain common signalling routes, such as K_{ATP} channels opening and "survival" protein kinase pathways that have been implicated in cardioprotective mechanisms in many experimental settings. Their activation may thus represent a potential therapeutic target in order to confer endogenous protection to the myocardium by shifting the balance towards cell survival. The unravelling of these intrinsic defensive mechanisms mediating protective effects could almost certainly lead to their potential exploitation in the clinical practice.

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