Ischemic Preconditioning: Its Role in Cardioprotection

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Ischemic preconditioning is a phenomenon in which single or multiple brief periods of myocardial ischemia-hypoxia with following reperfusion result in a marked reduction in myocardial infarct size, severity of stunning or incidence of cardiac arrhythmias. Since IPC is a strong endogenous protective mechanism, the triggering factors and effectors of IPC have been under intense investigation, with an eye to developing a new treatment for acute myocardial infarction. The goal of the present study was to examine whether the positive inotropic agents such as milrinone, 5-bromisatin, derivative of indole-2, 3-dione, and the benzimidazole compound 1-acethyl-5, 6-dimethoxy-2-methylthiobenzimidazole may lead to cardioprotection from hypoxic damage. The experiments were carried out on the isolated isometricaly contracted guinea pig papillary muscles perfused by low oxygenated (pO_2 = 13–18 mmHg), glucose free Tyrode solution. The protocols of the experiment were as follows: after a 20-min perfusion with normal (pO_2 = 560–580 mmHg) oxygenated saline containing one of the tested agents or vehicle (control) followed a 60-min perfusion with low-oxygenated saline. The data have shown that milrinone, a benzimidazole derivative, and 5-bromisatin in an inotropic concentration resulted in a less hypoxic injury of papillary muscle in comparison with control. In control papillary muscles the contraction force during 40 min was fully depressed. In milrinone and benzimidazole groups the contraction force during the same period sustained 30.5 and 40.7 percent of control, respectively. The hypoxic contracture in milrinone and benzimidazole groups appeared significantly ($p < .05$) later and its magnitude was about 34.0 percent smaller than in control. The similar results were obtained with 5-bromisatin. The latter compound decreased the hypoxic contracture of papillary muscles about 40.0 percent as compared to control. Thus, a 20-min pretreatment of papillary muscles with milrinone, benzimidazole derivative, or 5-bromisatin mimicked the IP by increasing Ca^{2+} ions in the myocardium, and this most likely served as a trigger for induction of IPC, leading to protection of the myocardium from hypoxic injury.

Key words: guinea pig papillary muscles, positive inotropic agents, milrinone, 5-bromisatin, 1-acethy -5,6- dimethoksi-2-methylthiobenzimidazole, hypoxic damage

INTRODUCTION

Single or multiple brief periods of myocardial ischemia (hypoxia as well) with following reperfusion have been shown to protect the heart against a more prolonged ischemic insult, the result of which is a marked reduction in myocardial infarct size (1), severity of stunning (2) or incidence of cardiac arrhythmias (3, 4). This phenomenon was discovered in 1986 by Murry et al. (5, 6) and has been known as ischemic preconditioning (IPC). IPC has been demonstrated in a wide variety of animals, from dogs to rats, in human myocardium as well (7–10). Since IPC is a strong endogenous protective mechanism, the triggering factors and effectors of IPC have been under intense investigation (11–13) with an eye to developing new treatments for acute myocardial infarction, moreover, evidence now exists suggesting that ischemic preconditioning is clinically beneficial (14). At present, several endogenous agonists such as adenosine (15–17), norepinephrine (18–20) or bradykinin (21, 22) have been shown to be triggers for IPC. Activation of protein kinase C (PKC), which is known to be a key player in numerous intracellular signal transduction pathways (23–27), is believed to be a central mechanism in the protection of IPC across various species.

In 1983, the adenosine triphosphate sensitive potassium channels (K_{ATP} channel) were discovered, and in the 1990s it become clear that one of the possible mechanisms through which the preconditioning phenomenon worked was via the opening of the K_{ATP} channel (12, 28, 29). Recently there have been two

K_{ATP} channels demonstrated in the heart, the sarcolemmal channel (sarc K_{ATP}) and the mitochondrial channel (mito K_{ATP}) (30–32). According to Gross and Fryer (35), subsequent evidence suggested that the recently identified mitochondrial K_{ATP} channel might be the fundamental factor mediating IPC-induced cardioprotection.

The goal of this study was (1) to examine whether the positive inotropic agents such as milrinone, 5-bromisatin, derivative of indole-2, 3-dione, and benzimidazole compound 1-acethyl-5, 6-dimethoxy-2-methylthiobenzimidazole may lead to cardioprotection from hypoxic damage; (2) to review the experimental studies analysing the possible mechanisms of ischemic preconditioning for cardioprotection in the absence and presence of some mimics of IPC.

Role of adenosine, alfa$_1$-adrenoceptors, protein-kinase C and Ca^{2+} in IPC. The first experimental studies, which were carried out with dog's heart, have shown that the occlusion of circumflex artery for one, two or four 5–10 min episodes (each separated by 5–20 min of reperfusion) with the following sustained 40 min occlusion prevented cumulative metabolic deficits accumulation (adenine nucleotide, lactate), myocardial ischemic cell death, and reduced ATP depletion. Changes in hemodynamics or collateral blood flow did not account for the reduction in infarct size (5, 6). As a potential subcellular mechanism of ICP was proposed the hypothesis that reduced ATP depletion plays an essential role in cardioprotection. In another study (34), the mechanism by which IPC improves recovery of function and reduces enzyme release after a subsequent 30-min period of ischemia in perfused rat hearts was related to ionic alterations in the myocardium. Thus, during reperfusion the recovery of contractile function was significantly better preconditioned hearts than in untreated hearts (71 ± 9% vs. 36 ± 8% initial left ventricular developed pressure), and after 60 min of ischemia the preconditioned hearts had a significantly less (1.6 time) release of the intracellular enzyme creatine kinase. Furthermore, IPC resulted in less expressed ionic alterations, pH_i and ATP depletion. These findings supported the hypothesis that the preconditioning attenuates an increase in $[Ca^{2+}]_i$, $[Na^+]_i$ and $[H^+]$ during ischemia, most likely because of a reduced stimulation of Na^+-H^+ and Na^+-Ca^{2+} exchange. In a short run Liu et al. (35) and Thornton et al. (36) have demonstrated experimentally that adenosine can mimic IPC, because a 5-min intracoronary infusion of adenosine was as effective as 5 min of ischemia in perfused isolated rabbit hearts against infarction from 45-min ischemic insult. At that time, exposure of these ones to 8-sulfophenyltheophylline, an antagonist of adenosine A_1 receptors, blunts the infarct size-limiting effect of ICP. They concluded that cardiac like peripheral adenosine receptors are involved in the ischemic protection and that adenosine released during the preconditioning occlusion stimulates cardiac A_1 receptors. Other investigators have come to the same conclusion (37–39). For instance, Cohen et al. (40) have shown that a sustained 30 min coronary occlusion in open-chest rabbits led to a 10- to 20-fold increase of the interstitial adenosine level. The major role of endogenous adenosine is to increase the coronary blood flow. Adenosine-induced vasodilatation is also enhanced by increasing H^+ and opening K_{ATP} channels, which occurs in the ischemic myocardium (17). However, coronary vasodilatation is not the only effect of adenosine in the ischemic myocardium. Stimulation of adenosine A_1 receptors coupled to G_i proteins attenuates beta-adrenoceptor mediated increases in myocardial contractility, Ca^{2+} influx into myocytes and noradrenaline release from the presynaptic nerves (41). Adenosine is produced by the enzymatic dephosphorylation of 5'-AMP by 5'-nucleotidase and by the hydrolysis of S-adenosylhomocysteine (SAH) by SAH-hydrolase. 5'-Nucleotidase activity is thought to contribute to adenosine production aside from the accumulation of 5'-AMP in the ischemic myocardium, while the hydrolysis of SAH plays a major role in the adenosine production in the normoxic myocardium. Furthermore, Kitakaze et al. (17, 42) have found that alpfa$_1$-adrenergic receptors, activated in ischemic hearts, increase both 5'-nucleotidase activity and adenosine production. In the other studies (11), it has been strongly suggested that alpfa$_1$-adrenoceptor stimulation mediates cardioprotection seen in IPC, which is attributable to activation of ectosolic 5'-nucleotidase. Thus, a four-fold artery occlusion for 5 min separated by 5 min of reperfusion (ischemic preconditioninig) in dogs contributes to a significant ($p < 0.001$) rise of adenosine concentration in coronary venous blood during the first cycle of reperfusion and continued to increase gradually with subsequent cycles of reperfusion, remaining higher than in control group. IPC significantly increased both ectosolic and cytosolic 5'-nucleotidase activity in the myocardium. The sequent 90 min coronary occlusion by followed 6 h of reperfusion showed that IPC markedly attenuated the infarct size compared with control, although the collateral flow did not differ in the two groups. The infarct size normalized by risk area was in control group 40.6 ± 2.3% and in IPC group 6.7 ± 2.0%. Methoxamine, an agonist of alfa$_1$-adrenoreceptors, mimicked the infarct-size limiting effect

of IPC and simultaneously increased the ectosolic and cytosolic 5'-nucleotidase activity to the levels obtained with IPC. At that time, prazozin, an antagonist of alfa$_1$-adrenoreceptors, completely abolished the infarct size-limiting effect of ICP, as well as ectosolic and cytosolic 5'-nucleotidase activity. Ectosolic and cytosolic 5'-nucleotidases are different enzymes, and it has been shown that activation of ectosolic 5'-nucleotodase plays a more important role for cardioprotection in IPC (20). Activity of ectosolic 5'-nucleotodase produces adenosine using extracellular AMP, and this conversion of extracellular AMP to adenosine may allow to convey endogenous adenosine into cardiomyocytes for the substrates of resynthesis of ATP.

Although at first sight activation of 5'-nucleotidase and alpfa$_1$-adrenoreceptors seems to be independent, they appear to be tightly linked, since there are a lot of reports to show that activation of alpfa$_1$-adrenoreceptors increases 5'-nucleotidase activity through activation of protein kinase C (PKC) (24, 26, 38, 43, 44). For example, the augmentation of ecto 5'-nucleotidase activity in the preconditioned canine myocardium was strongly attenuated by inhibitors of PKC, polymyxin B and GF109203X (45). Both inhibitors blunted the infarct size-limiting effect of ischemic preconditioning as well (infarct size 33.1 ± 6.9% and 35.1 ± 6.4% in control and preconditioning groups, respectively). An inhibitor of ecto 5'-nucleotidase AMP-CP (α, β-methyleneadenosine 5'-diphosphate, 1.0 and 5 x 10^{-5} mol/l) blunted the infarct size-limiting effect of IPC and reduced the increased ecto 5'-nucleotidase activity from 74.8 ± 2.2 to 22.9 ± 3.4 and 5.0 ± 1.7 nmol/mg protein per minute, respectively ($p < 0.001$). Transient administration of methoxamine mimicked the increase in ecto 5'-nucleotidase activity and the infarct size-limiting effect. At the same time, it was observed that IPC translocates PKC from cytosolic fractions to membrane fractions, indicating that IPC activates PKC in the canine myocardium as well.

Thus, these and other studies suggest that activation of ecto-5'-nucleotidase caused by activated PKC is a primary mediator for the infarct size-limiting effect of IPC (45). On the other hand, there is a linkage of alfa$_1$-adrenoceptor activation to protein kinase C with the following activation of ecto-5'-nucleotidase in a preconditioned myocardium. These observations indicate that endogenous norepinephrine, an agonist of alfa-adrenoceptors, can trigger the infarct-size limiting effect of IPC in the heart (46). Alfa$_1$-adrenoceptor activation has been shown to stimulate phospholipase C and to increase the production of inositol-1,4,5- triphosphate in a variety of organs and cells. For example, alpha$_1$-adrenoceptor activation in the myocardium is associated with a rapid increase in inositol-1,4,5-triphosphate production and a sustained increase in 1,2-DAG production, as well as activation of calcium channels and Na^+–H^+ and Na^+–Ca^{2+} exchange ((47, 48). DAG in turn activates PKC, which can phosphorylate a spectrum of cardiac proteins that control myocardial excitability and contraction (49–50). It is hypothesized that a PKC-induced modification of the Na^+–H^+ exchange turnover rate is responsible for cellular alkalisation (51), by either exchange in affinity of the allosteric site for H^+ and/or an increase in the maximal rate of ionic exchanger of the antiporter. Gambasi et al. (52) discovered a shift to alkaline pH, which was induced by phorbol ester and prevented by PKC inhibitors H-7. Moreover, it is now believed that the activation and translocation of PKC during the preconditioning stimulus accounts for the ability of cardiomyocytes to "remember" the ischemic episode (53). PKC stays associated with the membrane for about an hour after ischemia and can phosphorylate proteins early in a second ischemic period thus mediating the cardioprotection (54). In the other study (55), the question was examined whether direct stimulation of PKC can protect cardiomyocytes against an injury induced by simulated ischemia (Ca^{2+} overload) and reperfusion (hypercontracture). Cells of the rat heart were incubated in anoxic media at pH 6.4 until pCa of ≤ 5, intracellular pH [pH_i] of 6.5 and cytosolic [Na^+]$_i$ 50 μmol/l were reached. For stimulation of PKC, 1,2DOG (1,2-dioctanoyl-*sn*-glycerol), membrane-permeable diacylglycerol analogue was used. The main finding was the following: two-step treatment of cardiomyocytes with 1,2DOG (20 μmol/l) before and during simulated ischemia markedly reduced Ca^{2+} overload during oxygen depletion and prevented the development of hypercontracture during reoxygenation. Furthermore, application of the PKC inhibitor BIM (bisindolylmaleimide I) eliminated the protection of two-step treatment with 1,2DOG against Ca^{2+} overload. None of the beneficial effects of two-step treatment with 1,2DOG was observed when the PKC inhibitor BIM was present until just before reoxygenation. This observation also supports the conclusion that treatment with 1,2DOG acts through PKC activation.

According to Miyawaki et al. (56), the preconditioning achieved by repetitive Ca^{2+} depletion and repletion for short duration conferred cardioprotection against ischemia-reperfusion injury via the activation of PKC as well, elicited by a transient increase in [Ca^{2+}]$_i$. In other words, the crucial role of

Ca^{2+} as an important intracellular trigger or mediator for IPC was demonstrated. Thus, in this study it was tested whether (1) elevation of $[Ca^{2+}]_i$ by a brief augmentation of $[Ca^{2+}]_o$ (2.3 mol/l) mimics IPC, (2) administration of a Ca^{2+} antagonist verapamil (8 x x 10^{-3} μmol/l for 5 min each before / after IPC) attenuates the protective effects of IPC, and (3) specific isoforms of PKC are involved with "Ca^{2+}-mediated" preconditioning. A significant functional recovery and a decreased lactate dehydrogenase release (7.0 ± 0.7 *vs*. 21.9 ± 1.4 U/g in control) were observed in high-Ca^{2+} preconditioning and IPC hearts compared with ischemic control hearts i.e., the protective effect of high-Ca^{2+} preconditioning was similar to that of IPC. The ATP content in preconditioned hearts was significantly higher than in ischemic control hearts (13.8 ± 0.8 *vs*. 6.1 ± 0.8 μmol/g dry wt). The cellular structure in the high-Ca^{2+} hearts was also well preserved, similar by to that in IPC hearts. Treatment with chelerythrine chloride (specific PKC inhibitor) during IPC or high-Ca^{2+} period also abolished their beneficial effects on the cellular structure. The activation and translocation of PKC from the cytoplasm to the sarcolemma were observed in the preconditioned hearts as well. Translocation of PKC from the cytoplasm to the plasma membrane has been recognized as an index of PKC activation (24, 45, 57). Verapamil administered during IPC significantly attenuated the salutary effects of IPC, while pretreatment with verapamil alone did not exert a significant effect on the ishemic injury compared with no pre-treatment in ischemic control hearts. Chelerythrine completely blocked the preconditioning-induced increase in PKC activity of the membrane fraction. Treatment with verapamil also significantly reduced the PKC activity of membrane fraction in IPC hearts. These findings indicate that intracellular Ca^{2+}, which increases after induction of myocardial ischemia, plays an important role in activating intracellular pathways leading to protection (56).

In our study, which was carried out on the isolated isometricaly contracted guinea pig papillary muscles under hypoxic conditions (perfusion with low-oxygenated, pO_2 = 13–18 mmHg, glucose-free Tyrode solution) the positive inotropic agents, milrinone and benzimidazole derivative (1-acethyl-5,6-dimethoxy-2-methylthiobenzimidazole), in a sense mimicked IPC. The protocols of the experiment were as follows: after 20-min perfusion with normal (pO_2 = 560–580 mmHg) oxygenated saline containing one of the test agents or vehicle (control) followed a 60 min perfusion with low-oxygenated saline (58). The data have shown that milrinone and benzimidazole derivative in an inotropic concentration (according to their EC_{50} value, 83.0 μmol/l and 240.0 μmol/l, respectively) resulted in a less hypoxic injury of papillary muscle (in comparison with control). Thus, in control papillary muscles the contraction force dramatically decreased and during 40 min was completely depressed, while in milrinone and benzimidazole groups the contraction force during the same period decreased by 70.0 and 60.0 percent of control, respectively ($p < 0.05$). The hypoxic contracture in milrinone and benzimidazole groups appeared significantly ($p < 0.05$) later (4.88 ± 1.5 min in control, 10.8 ± 1.8 min in milrinone, 8.75 ± 2.4 min in benzimidazole) and its magnitute was about 34.0 percent smaller than in control. Similar results were obtained with bromisatin (derivative of indolin-2,3-dione) possessing positive inotropic properties as well (59) The latter compound decreased the hypoxic contracture of papillary muscles by about 40.0 percent compared with control. Milrinone, a well-known phosphodiesterase inhibitor, increases the contraction force of papillary muscles through the cyclic AMP-dependent way, *i.e*., in response to milrinone in the myocardium more calcium ions accumulate (60). The fact that carbachol, an antagonist of muscarinic receptors, completely antagonizes the positive inotropic effect of benzimidazole derivative suggests the cyclic AMP-dependent mechanisms in the action of this agent (61, 62). Besides, the results obtained in the benzimidazole groups pretreated with the beta-adrenergic blocking agent dl-propranolol, supported the hypothesis that activation of beta-adrenergic receptors as a possible action mechanism might be involved in the positive inotropic action of test agents. Stimulation of beta-adrenergic signaling pathway or inhibition of phosphodiesterase in cardiac myocytes result in accumulation of cyclic AMP and in activation of cyclic AMP-dependent protein kinase A, as well as in phosphorylation of several PKA substrates. These include the sarcolemmal L-type Ca^{2+}-channel, the ryanodine receptor and phospholamban of the sarcoplasmic reticulum, the myofibrillar protein troponin I as well. Phosphorylation of these substrates contributes to an increase in the cytosolic uptake of Ca^{2+} ions, enhanced contractility and accelerated relaxation in response to positive inotropic agents acting via cAMP-dependent way. Our experimental studies with guinea pig papillary muscles confirmed that in the positive inotropic action of benzimidazole derivative the sarcolemmal L-type Ca^{2+}-channels are involved (63) and this compound increases the rate of tension development and the relaxation-onset index (64). So, we could hypothesise that the influence of the test agents augments the Ca^{2+} content, in the myo-

cardium preconditioned papillary muscles for the following injury of hypoxia.

More intimate studies have shown that alfa$_1$-adrenoreceptors and endogenous catecholamines (noradrenaline) are involved in the action mechanism of bromisatin, othewise, bromisatin, differently than milrinone and benzimidazole, thoroughly acts via a cyclic AMP-independent way (65). Resent studies have indicated that norepinephrine (2, 9, 26, 46) is strongly implicated in IPC of the isolated heart of the rat and other species. This endogeneous substance, as indicated above, stimulates phosphatidylinositol cyclase and produces DAG and inositol-1,4,5-triphosphate, causing elevation in cytosolic free $[Ca^{2+}]_i$ through Ca^{2+} release from the sarcoplasmic reticulum (47, 48, 50, 66–67). Therefore it is possible to conclude that transient increase in $[Ca^{2+}]_i$ during IPC serves as a trigger for induction of IPC and that $[Ca^{2+}]_i$ effectively of the activates PKC, which modulates different ion channels (34, 68) leading to protection myocardium.

So, a short-term application of the positive inotropic agents elevates the resistance of the myocardium to the following long-lasting ischemic-hypoxic reperfusion injury, and, on the contrary, the drugs exhibiting a negative inotropic action (for instance, verapamil) display a deleterious influence on cardioprotection.

Role of ATP-sensitive potassium channels in IPC. A better understanding of the mechanism of IPC showed that PKC can activate the sarcolemmal K_{ATP} channels, followed by a shortening of the cardiac action potential duration (APD) and thus exerts an energy-sparing effect by reducing Ca^{2+} influx in myocardium (69–72). K_{ATP} channels are presented in vascular smooth muscle cells (73–75) and in the sarcolemma of ventricular myocytes, in the pancreatic α- and B-cells (76), as well as in the inner mitochondrial membrane (77–80). K_{ATP} channel activity is essentially voltage-independent, but is modulated by intracellular nucleotides, particularly ATP, which inhibit channel activity, while Mg^{2+}ADP stimulate their activity. Consequently, K_{ATP} channel activity is regulated by cell metabolism and provides a means of linking the electrical activity of a cell to its metabolic rate. In the study by Light et al. (81) it is shown that PKC is able to alter the stoichiometry of ATP binding to K_{ATP}, resulting in PKC-mediated activation of K_{ATP} at a more physiological (millimolar) ATP concentration. This finding has an important implication for preconditioning, since no significant myoplasmic reduction of ATP levels, which triggers the opening of K_{ATP}, has been observed during a brief period of ischemic preconditioning.

The involvement of K_{ATP} channels in preconditioning was first suggested by Gross and Auchampach (82) and Auchampach et al. (83) in the canine hearts. To test the hypothesis that preconditioning is the result of opening ATP-sensitive potassium channels, the selective K_{ATP} channel antagonists, glibenclamide and sodium 5-hydroxydecanoate (5-HD), were administered before or immediately after preconditioning in barbital-anesthetized open-chest dogs subjected to a 60 min left coronary occlusion followed by 5 hours of reperfusion. IPC was elicited by a 5-minute artery occlusion followed by 10 min of reperfusion before a 60 min occlusion period. Preconditioning as usual, produced a marked reduction in infarct size, whereas glibenclamide and 5-HD administered before or immediately after preconditioning completely abolished the protective effect of IPC. At the same time these authors have shown that the selective potassium channel opener RP 52891 contributed to a significant reduction in the infarct size of preconditioned hearts. These results further strengthen the hypothesis that activation of myocardial K_{ATP} channels is involved in the mechanism of IPC in dogs. Indeed, in the subsequent studies numerous investigators have confirmed that pharmacological K_{ATP} openers contribute to the protection of ischemic-reperfused myocardium in a number of models and species (77, 84–87) and, on the contrary, the blockers of these channels abolish protection from IPC (88–93).

It is a well known that opening the K_{ATP} channel on the surface membrane leads to membrane hyperpolarization and shortening of phase 3 of the cardiac action potential (AP). Both these effects resulted in a consequent closure of voltage-dependent ion channels and reduction of free intracellular calcium ions, which would theoretically produce a cardioprotective effect (94). However, it was observed that enhanced shortening of the cardiac AP was probably not the end-effector in the mechanism responsible for the protective effect of the K_{ATP} openers. Thus, in 1994 Yao and Gross (93, 95) have shown that bimakalim, an opener of K_{ATP} channels, at a low dose did not significantly affect the ischemia-related shortening of the AP during the initial 5 min of occlusion of the descending coronary artery in dogs, though it markedly reduced the infarct size (12.6 ± 3.3% *vs*. 27.2 ± 5.7% in controls) equivalent to two higher doses of bimakalin which enhanced AP shortening. Similarly, in other studies it was established that there was no relationship between APD shortening and the cardioprotective effect of cromakalim in dogs. Moreover, dofetilide, a class III antiarrhythmic agent which abolishes the

APD-shortening effect of cromakalim, at a dose that prevented the cromakalim-induced shortening of APD in ischemic tissue did not attenuate the cardioprotective effects of cromakalim (76, 96). In addition, dofetilide had no effect on infarct size when given to non-preconditioned hearts and did not alter the protective effect of preconditioning. These findings suggested that the APD shortening observed with the surface K_{ATP} channel openers is not correlated with their cardioprotective effects for IPC (85).

Recently, Garlid et al. (97) in reconstituted bovine hearts have shown that the K_{ATP} opener diazoxide was a week cardiac sarcK_{ATP} opener, but it was a potent (about 1000-fold) opener of mitoK_{ATP}, making it a useful tool for determining the importance of this mitochondrial site. Myocyte studies showed that diazoxide was significantly less potent than cromakalim in increasing sarcolemmal K^+ currents. Diazoxide shortened ischemic APD also significantly less than did cromakalim at equicardioprotective doses. The studies on reconstituted rat mitochondrial cardiac K_{ATP} demonstrated that diazoxide more potently activated the K^+ flux in this preparation. Glibenclamide and 5-HD inhibited the protective effect of diazoxide as well as K^+ flux through the diazoxide-opened mitochondrial $K_{ATP.}$ These findings suggested that openers of the ATP-sensitive potassium channels protect ischemic hearts in a manner consistent with an interaction with mitoK_{ATP}. Liu et al. (79, 98) using intact isolated rabbit ventricular myocytes simultaneously measured flavoprotein fluorescence as an index of mitochondrial redox state and sarcK_{ATP} currents and showed that diazoxide induced a reversible oxidation of flavoproteins at the doses that correlated with its cardioprotective effects but did not activate sarcolemmal K_{ATP} channels. The K_{ATP} channel blocker 5-HD inhibited both the redox changes and cardioprotection. It was concluded that diazoxide targets mitochondrial but not sarcolemmal K_{ATP} channels and implies that mitochondrial K_{ATP} channels may be effectors for ischemic preconditioning.

It has been reported that K_{ATP} channels also play a role in the protection of preconditioning in human myocardium, since IPC consisting of 3 min of simulated ischemia (90 min of hypoxic substrate-free superfusion paced at 3 Hz and than by 120 min of reperfusion) as well as during brief repeated coronary occlusions is completely abolished by pretreatment with glibenclamide, thus suggesting that it is mainly mediated by K_{ATP} channels. Recently, in the study of Ghosh et al. (77), it was been confirmed that mitoK_{ATP} channels are involved in human preconditioning as well. The authors used a model of simulated ischemia with right atrial trabeculae obtained from patients undergoing elective cardiac surgery, and investigated the effects of the mitoK_{ATP} channel opener diazoxide, the blockers 5-HD and glibenclamide, and the novel sulfonylthiourea sarcK_{ATP} blocker HMR 1883. In this study, also a dose-response analysis of the test agents was carried out. So, it was shown that glibenclamide (10 μM), which blocks K_{ATP} channels both in the sarcolemma and the mitochondrial inner membrane, 5-HD (1 mM) which shows selectivity in blocking for mitoK_{ATP} channels over sarcoK_{ATP} (99), abolished the protective effect of IPC on creatine kinase leakage in human myocardium. HMR 1883, which is thought to have the reciprocal selectivity to 5-HD, preferentially blocking the sarcK_{ATP} (85), in this study did not block the protective effect of IPC. In the absence of an IPC stimulus, the non-selective K_{ATP} channel opener pinacidil and the selective opener of mitoK_{ATP} channels diazoxide were protective, reducing creatine kinase leakage to the levels obtained with IPC. So, this study provides evidence that mitochondrial rather than sarcolemmal K_{ATP} channels are involved in human myocardium for ischemic preconditioning.

Sarcolemmal K_{ATP} channels are an octameric complex composed of two protein subunits (76). The pore is formed from four Kir6.2 subunits, each of which is associated with a sulfonylurea (SUR) receptor subunit. Kir6.2 belongs to a family of inwardly rectifying K^+ channels (100), whereas SUR is a member of the ATP-binding cassette subunit. It is suggested that SUR2A serves as a regulatory subunit in cardiac and skeletal muscles (99, 101, 102). Unfortunately, the molecular identity of the mitochondrial K_{ATP} channel remains unknown as yet. However, there are suggestions, by Suzuki et al. (103), that of the inwardly rectifying K^+ channel subunit Kir6.1 might be a subunit of the ATP-sensitive K^+ channel in the mitochondria as well as in the plasma membrane. Certainly, the mitoK_{ATP} and sarcK_{ATP} channels appear to exhibit minor differences in their structure, but the function of the mitoK_{ATP} channel appears to be involved in the regulation of matrix volume, while sarcK_{ATP} channels contribute to the electrical activity of the cellular membrane. Therefore, opening the mitoK_{ATP} channels leads to mitochondria membrane depolarisation, K^+ ions influx through mitochondrial K_{ATP} channels and changes in matrix volume (104–106). According to Kowaltowski (105), the opening of mitoK_{ATP} channels would help preserve the architecture of the inner membrane space with a subsequent slowing of ATP hydrolysis and preservation of the ability to use cre-

atine as a substrate of respiration. However, one should bear in mind that in acute myocardial ischemia (hypoxia as well), in non-preconditioned hearts, in the massive efflux of K^+ ions via K_{ATP} channels slows conduction and shortens the duration of the AP as well as an effective refractory period. These factors predispose to cardiac arrhythmias and ventricular fibrillation. Moreover, the spatial dispersion in these parameters, in part determined by inhomogeneity in extracellular potassium concentration, is considered highly arrhythmogenic (107, 108, 109). In such cases, the experimental studies have shown that pharmacological agents that enhance the loss of potassium ions in the myocardium (K_{ATP} channel openers as cromakalim, pinacidil) are distinguished by an arrhythmogenic action, and agents that interfere with tissue potassium loss (K_{ATP} channels blockers, as glibenclamide) show antiarrhythmic effects, prolonging the action potential duration and effective refractory period (110, 111).

In conclusion, it has been established that when the heart of various animal species, including human, is subjected to a short-term non-lethal period of ishemia, it quickly adapts itself to become resistant to infarction from a subsequent ischemic insult. This transient ischemic cardioprotection has been shown to be mediated by stimulation of various receptors linked to PKC, for instance, by Ca^{2+} ions, norepinephrine, adenosine, bradykinin, opioids, nitric oxide or endotelin-1 (27, 79, 107, 112–114). The combined effect of the release of these substances on G proteins and cell phospholipases of the isozymes of PKC, which appears to be the first element of a complex kinase cascade is activated during the prolonged ischemia in the preconditioned hearts (115). Recent studies have implied a possible existence of some protein kinase, whose activation carries the signal from PKC to the mitochondrial K_{ATP} channels, causing them to open and thus protect the myocardium from ischemic or hypoxic damage. It is expected that with the action mechanism of IPC more essentially understood, pharmacological preconditioning (112) will become practical for clinical use.

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References

1. Miller DL, Van Winkle DM. Ischemic preconditioning limits infarct size following regional ischemia-reperefusion *in situ* in mouse hearts. Cardiovasc Res 1999; 42: 680–4.
2. Rehring TF, Brew EC, Friese RS, Banerjee A, Harken AN. Cardiac preconditioninig protects against irreversible injury rather than attenuating stunning. J Surg Res 1995; 59: 111–4.
3. Tosaki A, Szerdahelyi P, Engelman RM, Das DK. Potassium channel openers and blockers: do they possess proarrthythmic or antiarrhythmic activity in ischemic and reperfused rat hearts? J Pharmacol Exp Ther 1993; 267: 1355–62.
4. Vegh A, Komori S, Szekeres L, Parratt JR. Antiarrhythmic effects of preconditioning in anesthetized dogs and rats. Cardiovasc Res 1992; 26: 487–95.
5. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 1986; 74: 1124–36.
6. Reimer KA, Murry CE, Yamasawa I, Hill ML, Jennings RB. Four brief periods of myocardial ischemia cause no cumulative ATP loss or necrosis. Am J Physiol 1986; 251: H1306–15.
7. Takano H, Tang XL, Boili R. Differential role of K_{ATP} channels in late preconditioning against myocardial stunning and infarction in rabbits. Am J Physiol Heart Circ Physiol 2000; 279: H2350–9.
8. Sanada S, Kitakaze M, Asanuma H, harada K, Ogita H, Node K, Takashima S, Sakata Y, Asakura M, Shinozaki Y, Mori H, Kuzuya T, Hori M. Role of mitochondrial and sarcolemmal K_{ATP} channels in ischemic preconditioning of the canine heart. Am J Physiol Heart Circ Physiol 2001; 280: H256–63.
9. Cleveland JC, Wollmering MM, Meldrum DR, Rowland RT, Rehring TF, Sheridan BC, Harken AH, Banerjee A. Ischemic preconditioning in human and rat ventricle. Am J Physiol 1996; 271 (5 Pt. 2): H1786–94.
10. Kloner R, Yellon D. Does ischemic preconditioning occure in patients? J Am Cell Cardiol 1994; 24: 1133–42.
11. Carroll R, Yellon DM. Myocardial adaptation to ischemia – the preconditioning phenomenon. Int J Cardiol 1999; 68, Suppl. 1: S93–101.
12. Carr CS, Grover GJ, Pugsley WB, Yellon DM. Comparison of the protective effects of a highly selective ATP-sensitive potassium channel opener and ischemic preconditioning in isolated human atrial muscle. Cardiovasc. Drugs Ther 1997; 11: 473–8.
13. Huh J, Gross GJ, Nagase H, Liang BT. Protection of cardiac myocytes via delta(1)-opioid receptors, protein kinase C, and mitochondrial K_{ATP} channels. Am J Physiol Heart Circ Physiol 2001; 280: H377–83.
14. Ottani F, Galvanic M, Ferrini D, Sorbello F, Limonetti P, Pantoli D, Rusticali F. Prodromal angina limits infarct size. A role for ischemic preconditioning. Circulation 1995; 91: 291–7.
15. Mei DA, Nithipatikom K, Lasley RD, Gross GI. Myocardial preconditioning produced by ischemia, hypoxia, and a K_{ATP} channel opener: effects of interstitial adenosine in dogs. J Mol Cell Cardiol 1998; 30: 1225–36.
16. Baxter GF, Marber MS, Patel VC, Yellon DM. Adenosine receptor involvement in a delayed phase of myocardial protection 24 hours after ischemic preconditioning. Circulation 1994; 90: 2993–3000.
17. Kitakaze M, Hori M, Kamada T. Role of adenosine and its interaction with alpha adrenoceptor activity

in ischemic and reperfusion injury of myocardium. Cardiovasc Res 1993; 27: 18–27.
18. Banerjee A, Locke-Winter C, Rogers KB, Mitchell MB, Brew EC, Cairns CB, Bensard DD, Harken AH. Preconditioning against myocardial dysfunction after ischemia and reperfusion by alpha$_1$-adrenergic mechanism. Circ Res 1993; 73: 656–70.
19. Tsuchida A., Liu Y, Liu GS, Cohen MV, Downey JM. α_1-Adrenergic agonists precondition rabbit ischemic myocardium independent of adenosine by direct activation of protein kinase C Circ Res 1994; 75: 576–85.
20. Kitakaze M, Hori M, Morioka T, Minamino T, Takashima S, Sato H, Shinozaki Y, Chujo M, Mori H, Inoue M, Kamada T. Alpha$_1$-adrenoceptor activation mediates the infarct size-limiting effect of ischemic preconditioning through augmentation of 5'-nucleotidase activity. J Clin Invest 1994; 93: 2197–205.
21. Brew EC, Mitchell MB, Rehring TF, Gamboni-Robertson F, McIntyre RC, Harken AH, Banerjee A. Role of bradykinin in cardiac functional protection of global ischemia-reperfusion in rat heart. Am J Physiol 1995; 269: H1370–8.
22. Goto M, Liu Y, Yang XM, Ardell JL, Cohen MV, Downey JM. Role of bradykinin in protection of ischemic preconditioning in rabbit hearts. Circ Res 1995; 77: 611–21.
23. Kitakaze M, Node K, Minamino T, Komamura K, Funaya H, Shinazaki Y, Chujo M, Mori H, Inoue M, Hori M, Kamada T. Role of activation of protein kinase C in the infarct size-limiting effect if ischemic preconditioning trough activation of ecto-5'-nucleotidase. Circulation 1996; 93: 781–91.
24. Speechly-Dick ME, Mocanu MM, Yellon DM. Protein kinase C. Its role in ischemic preconditioning in the rat. Circ Res 1994; 75: 586–90.
25. Liu Y, Ytrehus K, Downey JM. Evidence that translocation of protein kinase C is a key event during ischemic preconditioning of rabbit myocardium J Mol Cell Cardiol 1994; 26: 661–8.
26. Ytrehus K, Liu Y, Downey JM. Preconditioning protects ischemic rabbit heart by protein kinase C activation. Am J Physiol 1994; 266: H1145–52.
27. Sato T, O'Rourke B, Marban E. Modulation of mitochondrial ATP-dependent K^+ channels by protein kinase C. Circ Res 1998; 83: 110–4.
28. Schulz R, Rose J, Heusch G. Involvement of activation of ATP-dependent potassium channels in ischemic preconditioning in swine. Am J Physiol 1994; 267 (4 Pt 2): H1341–52.
29. Liang BT, Gross GJ. Direct preconditioning of cardiac myocytes via opioid receptors and K_{ATP} channels. Circ Res 1999; 84: 1396–400.
30. Garlid KD, Paucek P, Yarov-Yarovoy V, Murray HN, Darbenzio RB, D'Alonzo AJ, Lodge NJ, Smith MA, Grover GJ. Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K^+ channels. Possible mechanism of cardioprotection. Circ Res 1997; 81: 1072–82.
31. Sato T, Sasaki N, Seharaseyon J, O' Rourke B, Marban E. Selective pharmacological agents implicate mitochondrial but not sarcolemmal K_{ATP} channels in ischemic cardioprotection. Circulation 2000; 101: 2418–23.
32. Gross GJ. The role of mitochondrial K_{ATP} channels in cardioprotection. Basic Res Cardiol 2000; 95: 280–4.
33. Gross GJ, Fryer RM. Sarcolemmal *versus* mitochondrial ATP-sensitive K^+ channels and myocardial preconditioning. Circ Res 1999; 84: 973–8.
34. Steenbergen C, Perlman ME, London RE, Murphy E. Mechanism of preconditioning. Ionic alterations. Circ Res 1993; 72: 112–25.
35. Liu GS, Thornton J, Van Winkle DM, Stanley AWH, Olson RA, Downey JM. Protection against infarction afforded by preconditioning is mediated by A_1 adenosine receptors in rabbit heart. Circulation 1991; 84: 350–6.
36. Thornton JD, Liu GS, Olson RA, Downey JM. Intravenous pre-treatment witn A_1-selevtive adenosine analogues protects the heart against infarction. Circulation 1992; 85: 659–65.
37. Cohen MV, Walsh RS, Goto M, Downey JM. Hypoxia preconditions rabbit myocardium via adenosine and catecholamine release. J Mol Cell Cardiol 1995; 27: 1527–34.
38. Headrick JP. Ischemic preconditioning: bioenergetic and metabolic changes and the role of endogenous adenosine. J Mol Cell Cardiol 1996; 28: 1227–40.
39. Cohen MV, Snell KS, Tsuchida A, Van Vylen DG, Downey JM. Effects of anesthesia and K^+ ATP channel blockade on interstitial adenosine accumulation in ischemic rabbit myocardium Basic Res Cardiol 1995; 90: 410–7.
40. Sato H, Hori M, Kitakaze M, Takashima S, Inoue M, Kitabatake A, Kamada T. Endogenous adenosine blunts beta-adrenoceptor-mediated inotropic response in hypoperfused canine myocardium. Circulation 1992; 85: 1594–603.
41. Kitakaze M, Hori M, Takashima S, Sato H, Inoue M, Kamada T. Ischemic preconditioning increases adenosine release and 5'-nucleotidase activity during myocardial ischemia and reperfusion in dogs. Circulation 1993; 87: 208–15.
42. Tsuchida A, Liu Y, Liu GS, Cohen MV, Downey JM. Alpha$_1$-adrenergic agonists precondition rabbit ischemic myocardium independent of adenosine by direct activation of protein kinase C. Circ Res 1994; 75: 576–785.
43. Mittchell MB, Meng X, Ao L, Brown JM, Harken AN, Banerjee A. Preconditioning of isolated rat heart is mediated by protein kinase C. Circ Res 1995; 76: 73–81.
44. Kitakaze M, Node K, Minamino T, Komamura K et al. Role of activation of protein kinase C in the infarct size-limiting effect of ischemic preconditioning through activation of ecto-5'-nucleotidase. Circulation 1996; 93: 781–91.
45. Takasaki Y, Adachi N, Dote K, Tsubota S, Yorozuya T, Arai T. Ischemic preconditioning suppresses the noradrenaline turnover in the rat heart. Cardiovasc Res 1998; 39: 373–80.

46. De Jonge HW, Van Heugten HAA, Lamers JMJ. Signal transduction by the phosphotidylinositol cycle in myocardium. J Mol Cell Cardiol 1995; 27: 93–106.
47. Ruffolo RR, Stadel JM, Hieble JP. α-Adrenoceptors: recent developments. Med Res Rev 1994; 14: 229–70.
48. Sugden PH, Bogoyevitch MA. Intracellular signalling through protein kinases in the heart. Cardiovasc. Res. 1995; 30: 478–92.
49. Pi Ye Q, Sreekumar R, Huang Xu P, Walker JW. Positive inotropy mediated by diacylglycerol in rat ventricular myocytes. Circ Res 1997; 81: 92–100.
50. Terzic A, Puceat A, Clement O, Scamps F, Vassort G. α_1-Adrenergic effects on intracellular pH and calcium and on myofilaments in single rat cardiac cells. J Physiol 1992; 447: 275–92.
51. Gambassi G, Spurgeon HA, Lakatta EG, Blank PS, Capogrossi MC. Different effects of α- and β-adrenergic stimulation on cytosolic pH and myofilament responsiveness to Ca^{2+} in cardiac myocytes. Circ Res 1992; 71: 870–82.
52. Cohen MV, Downey JM. Ischemic preconditioning: can the protection be bottled? Lancet 1993; 342: 6.
53. Parrat JR. Protection of the heart by ischemic preconditioning: mechanisms and possibilities for pharmacological exploitation. Trends Pharmacol Sci 1994; 15: 19–25.
54. Ladilov YV, Balser C, Piper HM. Protection of rat cardiomyocytes against simulated ischemis and reoxygenation by treatment with protein kinase C activator. Circ Res 1998; 82: 451–7.
55. Miyawaki H, Ashraf M. Ca^{2+} as a mediator of ischemic preconditioning. Circ Res 1997; 80: 790–9.
56. Ping P, Zhang J, Pierce WM, Bolli Jr R. Fuctional proteomic analysis of protein kinase C ε signalling complexes in the normal heart and during cardioprotection. Circ Res 2001; 88: 59–62.
57. Gendvilienė V, Mačianskienė R, Garalienė V. Naujo kardiotoninio preparato AcV-1 ir milrinono įtaka jūrų kiaulytės miokardo elektromechaniniam aktyvumui normoje ir hipoksijoje. Medicina 1995; priedas 2: 290–3.
58. Гаралене Вида. Положительно-инотропная активность 5-бромизатина (5-броминдолин-2,3-диона. Экспериментальная и клиническая фармакология 1999; 62: 20–4.
59. Young RA, Ward A. Milrinone: A preliminary review of its pharmacological properties and therapeutic use. Drugs 1988; 36: 158–92.
60. Garalienė V, Labanauskas L, Brukštus A, Daukšas V. Synthesis and positive inotropic effects of 1-acyl-5, 6-diethoxy-2-methylthiobenzimidazoles. Arzneimittel-Forschung/Drug Res 1998; 48 (II): 1137–42.
61. Garalienė V. 1-Acil-2-metiltio-5, 6-dimetoksibenzimidazolų ir karbacholo įtaka jūrų kiaulytės kraujagyslių lygiesiems raumenims bei papiliarinių raumenėlių susitraukimui. Medicina 1997; 33: 821–5.
62. Garalienė V. 1-Acil-2-metiltio-5,6-dimetoksibenzimidazolai: jų poveikis jūrų kiaulyčių papiliarinių raumenėlių susitraukimui. Medicina 1998; 34: 738–44.
63. Garalienė V, Navalinskas A. Positive inotropic agents: their influence on contraction–relaxation coupling and sodium–calcium exchange in guinea pig papillary muscle. Acta Medica Lituanica 1999; 6 (4): 255–60.
64. Garalienė V, Prieveliene R. The effect of reserpine-induced sympathectomy on the response to benzimidazole, pyrazolylpyrimidine and indolindione derivatives in the isolated guinea pig papillary muscles and aorta strips. Lith J Cardiol 2000; 7 (2): 33–8.
65. Endoh M. The effects of various drugs on the myocardial inotropic response. Gen Pharmacol 1995; 26: 1–31.
66. Schmitz W, Kohl C, Neumann J, Scholz H, Scholz J. On the mechanism of positive inotropic effects of alpha-adrenoceptor agonist. Basic Res Cardiol 1989; 84 (Suppl. 1): 23–33.
67. Hogue ANE, Haist JV, Karmazyn M. Na^+-H^+ exchange inhibition protects against mechanical, ultrastructural, and biochemical impairment induced by low concentrations of lysophosphatidylcholine in isolated rat hearts. Circ Res 1997; 80: 95–102.
68. Light PE, Allen BG, Walsh MP, French RJ. Regulation of adenosine triphosphate-sensitive potassium channels from rabbit ventricular myocytes by protein kinase C and type 2A protein phosphatase. Biochemistry 1995; 34: 7252–7.
69. Day YJ, Gao Z, Tan PC, Linden J. ATP-sensitive potassium channel and myocardial preconditioning. Acta Anaesthesiol Sin 199; 37: 121–31.
70. Light PE. Cardiac K_{ATP} channels and ischemic preconditioning: current perspectives. Can J Cardiol 1999; 15: 1123–30.
71. Hu K, Duan D, Li GR, Nattel S. Protein kinase C activates ATP-sensitive K^+ current in human and rabbit ventricular myocytes. Circ Res 1996; 78: 492–8.
72. Quayle JM, Nelson MT, Standen NB. ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. Physiol Rev 1997; 77: 1165–232.
73. Nuttle LC, Farly JM. Muscarinic receptors inhibit ATP-sensitive K^+ channels in swine tracheal smooth muscle. Am J Physiol 1997; 273: (2 Pt 1): L478–84.
74. Bonev AD, Nelson MT. Vasoconstrictors inhibit ATP-sensitive K^+ channels in arterial smooth muscle through protein kinase C. J Gen Physiol 1996; 108: 315–23.
75. Ashcroft FM, Gribble FM. New windows on the mechanism of action of K_{ATP} channel openers. Trends Pharmacol Sci 2000; 21: 409–51.
76. Ghosh S, Standen NB, Galinanes. Evidence for mitochondrial K_{ATP} channels as effectors of human myocardial preconditioning. Cardiovasc Res 2000; 45: 934–40.
77. Gross GJ. The role of mitochondrial K_{ATP} channels in cardioprotection. Basic Res Cardiol 2000; 95: 280–4.
78. Liu Y, Sato T, Seharaseyon J, Szewczyk A, O'Rourke B, Marban E. Mitochondrial ATP-dependent potassium channels. Viable candidate effectors of ischemic preconditioning. Ann NY Acad Sci 1999; 30: 27–37.
79. Wang Y, Ashraf M. Role of protein kinase C in mitochondrial K_{ATP} channel-mediated protection against Ca^{2+} overload injury in rat myocardium Circ Res 1999; 84: 1156–65.

80. Light PE, Sabir AA, Allen BG, Walsh MP, French RJ. Protein kinase C-induced changes in the stoichiometry of ATP binding activate cardiac ATP-sensitive K^+ channels. A possible mechanistic link to ischemic preconditioning. Circ Res 1996; 79: 399–406.
81. Gross GI, Auchampach JA. Blockade of ATP-dependent potassium channels prevents myocardial preconditioning. Circ Res 1992; 70: 223–33.
82. Auchampach J, Grover G, Gross G. Blockade of ischemic preconditioning in dogs by novel ATP-dependent potassium channel antagonist sodium-5-hydroxydecanoate. Cardiovasc Res 1992; 26: 1054–62.
83. Grover GJ. Pharmacology of ATP-sensitive potassium channel (K_{ATP}) openers in models of myocardial ischemia and reperfusion. Can J Physiol Pharmacol 1997; 75: 309–15.
84. Grover GJ, D'Alonzo AJ, Parham CS, Darbenzio RB. Cardioprotection with the K_{ATP} opener cromacalim is not correlated with ischemic myocardium action. J Cardiovasc Pharmacol 1995; 26: 145–52.
85. D'Alonzo AJ, Darbenzio RB, Hess TA, Sewter JC, Sleph PG, Grover GJ. Effect of potassium on the action of the K_{ATP} modulators cromacalim, pinacidil, or glibenclamide on arrhythmias in isolated perfused rat heart subjected to regional ischemia. Cardiovasc Res 1994; 28: 881–7.
86. Carr CS, Grover GJ, Pugsley WB, Yellon DM. Comparison of the protective effects of a highly selective ATP-sensitive potassium channel opener and ischemic preconditioning in isolated human atrial muscle. Cardiovasc Drugs Ther 1997; 11: 473–8.
87. Gogelein H, Hartung J, Englert HC, Scholkens BA. HMR 1883, a novel cardioselective inhibitor of the ATP-sensitive potassium channel. Part I: effects on cardiomyocytes, coronary flow and pancreatic beta-cells. J Pharmacol Exp Ther 1998; 286: 1453–64.
88. Tomai F, Crea F, Gaspardone A, Versaci F, De Paulis R, Penta de Peppo A, Chiariello L, Gioffre PA. Ischemic preconditioning during coronary angioplasty is prevented by glibenclamide, a selective ATP-sensitive K^+ channel blocker. Circulation 1994; 90: 700–5.
89. Yao Z, Gross GJ. A comparison of adenosine-induced cardioprotection and ischemic preconditioning in dogs. Efficacy, time course, and role of K_{ATP} channels. Circulation 1994; 89: 1229–36.
90. Misler S, Giebisch G. ATP-sensitive potassium channels in physiology, pathophysiology and pharmacology. Curr Opin Nephrol Hypertens 1992; 1: 21–33.
91. Yao Z, Gross GJ. Effects of the K_{ATP} channel opener bimakalim on coronary blood flow, monophasic action potential duration, and infarct size in dogs. Circulation 1994; 89: 1769–75.
92. Grover GJ, D'Alonzo AJ, Dzwonczyk S, Parham CS, Darbenzio RB. Preconditioning is not abolished by the delayed rectifier K^+ blocker dofetilide. Am J Physiol 1996; 271 (3 Pt 2): H1207–14.
93. Liu Y, Sato T, O'Rourke B, Marban E. Mitochondrial ATP-dependent potassium channels: novel effectors of cardioprotection? Circulation 1998; 97: 2463–9.
94. Jaburek M, Yarov-Yarovoy V, Paucek P, Garlid KD. State-dependent inhibition of mitochondrial K_{ATP} channel by glyburide and 5-hydroxydecanoate. J Biol Chem 1998; 273: 13578–82.
95. Inagaki N, Gonoi T, Clement JP, Namba N, Inazawa J, Gonzalez G, Aguilar-Bryan L, Seino S, Bryan J. Reconstitution of K_{ATP}: an inward rectifier subunit plus the sulfonylurea receptor. Science 1995; 270: 1166–9.
96. Inagaki N, Gonoi T, Clement JP, Wang CZ, Aguilar-Bryan L, Bryan J, Seino S. A family of sulfonylurea receptor forms with BIR (Kir6.2) a smooth muscle type ATP-sensitive K^+ channel. J Biol Chem 1996; 271: 24321–4.
97. Babenko AP, Gonzalez G, Aguilar-Bryan L, Bryan J. Reconstituted human cardiac KATP channels. Functional identity with the native channels from the sarcolemma of human ventricular cells. Circ Res 1998; 83: 1132–43.
98. Suzuki M, Kotake K, Fujikura K, Inagaki N, Suzuki T, Gonoi T, Seino S, Takata K. Kir6.1: a possible subunit of ATP-sensitive K^+ channels in mitochondria. Biochem Biophys Res Commun 1997; 24: 693–7.
99. Gross GJ, Fryer RM. Sarcolemmal *versus* mitochondrial ATP-sensitive K^+ channels and myocardial preconditioning. Circ Res 1999; 84: 973–9.
100. Kowaltowski AJ, Seetharaman S, Paucek P, Garlid KD. Bioenergetic consequences of opening the ATP-sensitive K^+ channel of heart mitochondria. Am J Physiol Heart Circ Physiol 2001; 280: H649–57.
101. Garlid KD. Opening mitochondrial K_{ATP} in the heart – what happens, and what does not happen. Basic Res Cardiol 2000; 95: 275–9.
102. Bugge E, Ytrehus K. Endotelin-1 can reduce infarct size through protein kinase C and K_{ATP} channels in the isolated rat hearts. Cardiovasc Res 1996; 32: 920–9.
103. Wilde AA. Role of ATP-sensitive K^+ channel current in ischemic arrhythmias. Cardiovasc Drugs Ther 1993; Suppl. 3: 521–6.
104. Kantor PF, Coetzee WA, Carmeliet EE, Dennis SC, Opie LH. Reduction of ischemic K^+ loss and arrhythmias in rat hearts. Effects of glibenclamide, a sulfonylurea. Circ Res 1990; 66: 478–85.
105. Bellemin-Baurreau J, Poizot A, Hicks PE, Rochette L, Amstrong JM. Effects of ATP-dependent K^+ channel modulators on an ischemia-reperfusion rabbit isolated heart model with programmed electrical stimulation. Eur J Pharmacol 1994; 256: 115–24.
106. Tosaki A, Hellegouarch A. Adenosine triphosphate-sensitive potassium channel blocking agent ameliorates, but the opening agent aggravates, ishemia/reperfusion-induced injury. Heart function studies in non-fibrillating isolated hearts. J Am Coll Cardiol 1994; 23: 487–96.
107. Nakano A, Cohen MV, Downey JM. Ischemic preconditioning: from basic mechanism to clinical application. Pharmacol Ther 2000; 86: 263–75.

108. Sasaki N, Sato T, Ohler A, O'Rourke B, Marban E. Activation of mitochondrial ATP-dependent potassium channels by nitric oxide. Circulation 2000; 101: 439–45.
109. Headrick JP, Gauthier NS, Morrison R, Matherne GP. Cardioprotection by K_{ATP} channels in wild-type hearts and hearts overexpressing A_1-adenosine receptors. Am J Physiol Heart Circ Physiol 2000; 279: H1690–7.
110. Speechly-Dick ME, Grover GJ, Yellon DM. Does ischemic preconditioning in the human involve protein kinase C and the ATP-dependent K^+ Channels? Studies of contractile function after simulated ischemia in an atrial *in vitro* model. Circ Res 1995; 77: 1030–5.

V. Garlienė

ISCHEMINIS PRIPRATINIMAS IR JO REIKŠMĖ MIOKARDO APSAUGAI

S a n t r a u k a

Trumpalaikis vienkartinis arba daugkartinis ischeminis-hipoksinis poveikis miokardui saugo jį nuo ilgai trunkančio ischeminio-reperfuzinio pakenkimo, pasireiškiančio miokardo infarkto apimties sumažėjimu ar mažiau ryškiais ritmo sutrikimais. Šis fenomenas atskleistas 1986 m. ir vadinamas ischeminiu audinio pripratinimu (IP). Kadangi IP yra vidinis ląstelių apsaugos mechanizmas, veiksniai, sukeliantys šiuos mechanizmus, šiuo metu intensyviai tyrinėjami, kad būtų gauta naujų vaistų ūmiam chroniniam širdies pakenkimui gydyti. Šio darbo tikslas buvo tirti teigiamų inotropinių medžiagų, milrinono, 5-bromizatino indolin-2,3-diono darinio ir benzimidazolo darinio 1-acetil-5,6-dimetoksi-2-metiltiobenzimidazolo poveikį miokardo susitraukimo jėgai ir ramybės įtempimui normos ir hipoksijos sąlygomis. Tyrimai atlikti su izoliuotais izometriškai susitraukinėjančiais jūrų kiaulyčių papiliariniais raumenėliais, kurie buvo perfuzuojami bedeguoniniu ir neturinčiu gliukozės Tyrode fiziologiniu tirpalu (pO_2 13–18 mm Hg, kai normoje pO_2 560–580 mm Hg). Tyrimo eiga buvo tokia: 60 min. papiliarinius raumenėlius perfuzavome prisotintu deguonimi normaliu Tyrode tirpalu, toliau 20 min. perfuzija buvo atliekama su tiriamųjų junginių atitinkamų koncentracijų tirpalais. Po to vėl 60 min. perfuzija hipoksiniu tirpalu su tomis pačiomis junginių koncentracijomis. Eksperimentai parodė, kad papiliarinių raumenėlių susitraukimo jėga mažėjo žymiai lėčiau, kai jie buvo veikiami hipoksiniu tirpalu su milrinonu (83 μmol/l) arba benzimidazolo dariniu (240 μmol/l). Hipoksija susitraukimo jėgą kontrolinėje grupėje visai blokavo per 40 min., tuo tarpu milrinono ir benzimidazolo grupėse ji sudarė atitinkamai 30,5 ir 40,7%. kontrolės atžvilgiu. Hipoksinė raumenėlių kontraktūra eksperimentinėse grupėse pradėjo vystytis gerokai ($p < 0,05$) vėliau ir buvo apie 34,0% mažesnė, jei lygintume su kontrole. Panašūs rezultatai gauti ir veikiant 5-bromizatinui. Dėl bromizatino poveikio kontraktūra buvo mažesnė apie 40%. Taigi preliminarinė 20 min. trukmės papiliarinių raumenėlių perfuzija su milrinonu, benzimidazolo dariniu ir 5-bromizatinu, padidino Ca^{2+} jonų koncentraciją miokardo audinyje, o tai tikriausiai ir tapo apsauginiu veiksniu (imituojančiu ischeminį pripratinimą) nuo hipoksijos sukelto pakenkimo.

Toliau straipsnyje diskutuojama apie adenozino, noradrenalino, C proteinkinazės bei K_{ATP} kanalų stimuliatorių ir blokatorių įtaką miokardo ischeminiam pripratinimui, jų veikimo mechanizmus ir klinikinio vartojimo galimybes.

Raktažodžiai: jūrų kiaulyčių papiliariniai raumenys, teigiamos inotropinės medžiagos milrinonas, 5-bromizatinas, 1-acetil-5,6-dimetoksi-2-metiltiobenzimidazolas, hipoksinis miokardo pakenkimas