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Ischaemic preconditioning: involvement of adenosine, G_i-proteins, protein kinase C and K_{ATP}-channels

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Ischaemic preconditioning is known as the phenomenon where a brief episode of myocardial ischaemia renders the myocardium resistant to severe injury caused by a subsequent longer period of ischaemia. The protective effect of preconditioning has been shown in various mammalian species. Since the discovery of this phenomenon a lot of studies have been performed to identify the underlying mechanisms. It is well established that adenosine A₁-receptors and ATP-sensitive potassium channels play an essential role in ischaemic preconditioning. More recent studies prove evidence that apart from A₁-receptors, A₃-receptors could also be involved, and that the activation of G-proteins, especially the alpha subunit of the pertussis-sensitive inhibitory G-protein (G_i-protein), as well as activation of the protein kinase C might constitute important steps in intracellular signalling. The exact links among these factors, however, remain to be elucidated. *J Clin Basic Cardiol* 1999; 2: 19–21.

Key words: ischaemia, preconditioning, adenosine, G_i-proteins, protein kinase C, K_{ATP}-channels

Short episodes of myocardial ischaemia protect the myocardium against severe damage resulting from a subsequent longer period of ischaemia [1, 2, 3]. The efficacy of preconditioning has been demonstrated in dogs [4], rabbits [5], rats [6] and pigs [2] and there is also evidence that it occurs in humans [7, 8]. Preconditioning improves the restoration of function after reperfusion, reduces the extent of necrosis caused by a long period of ischaemia [2, 6] and furthermore prevents life threatening ventricular arrhythmias like ventricular tachycardia or ventricular fibrillation during ischaemia and/or after reperfusion [6, 9–12]. The basic mechanisms of preconditioning, however, vary among different species and some pathways of intracellular information-transduction are yet unknown.

Aim of this article is to summarize the basic mechanisms, which have been shown to be crucial in ischaemic preconditioning in different species, pointing at some controversial questions and lack of experimental work, and, conclusively also to look at possible therapeutical implications.

Adenosine-receptors

The involvement of adenosine-receptors in preconditioning of the myocardium is widely accepted throughout the literature [13–18]. Liu et al. were the first who demonstrated the protective effect of adenosine on the ischaemic myocardium. This hypothesis is based upon the observation that specific adenosine A₁-receptor agonists mimic the effect of preconditioning, while adenosine receptor antagonists block its effect. These particular effects could be demonstrated in almost every species tested. However, in rats the question of an involvement of adenosine in preconditioning remains controversial. Li and Kloner, for example, showed a lack of effect of adenosine [19]. In contrast to the findings of Li and Kloner, Grover et al. proved evidence that the A₁-receptor agonist R-PIA was effective in preconditioning of the rat heart. The protective properties were a concentration dependent reduction of the heart rate and enhanced recovery of left ventricular pressure on reperfusion [13]. The protective effect of adenosine is generally thought to depend on the activation of ATP-sensitive potassium channels. Thus the protection by adenosine itself, as well as adenosine receptor agonists can be completely abolished by the

blockade of the K_{ATP}-channel (eg, [14, 18]). In the experiments of Grover et al., preconditioning could not be abolished by glibenclamide, a specific blocker of K_{ATP}-channels [20]. These results suggest that the protection of the myocardium by adenosine does not only rely on the activation of K_{ATP}-channels. While Grover et al. could not show a link between adenosine receptors and K_{ATP}-channels, Kirsch et al. demonstrated in neonatal rat ventricular myocytes the opening of K_{ATP}-channels by adenosine and the A₁-receptor agonist N⁶-cyclohexyladenosine [21] proposing a link of adenosine A₁-receptors and K_{ATP}-channels via the pertussis-toxin sensitive inhibitory G-protein (G_i).

Adenosine A₁-receptors are not the only adenosine receptors which were found to be involved in ischaemic preconditioning. Recently, Armstrong and Ganote provided evidence that A₃-receptors might also play an important role in protecting the heart against ischaemic damage. In isolated rabbit myocytes they showed that preconditioning could be brought about by the selective A₁/A₃-agonist APNEA or glucose-free preincubation. Preconditioning could not be blocked by the highly selective A₁-antagonist DPCPX but was largely inhibited by BW 433U83, which has affinity to A₁-receptors and A₃-receptors respectively. However, more studies will be needed to assure the involvement of A₃-receptors.

Common target for the activated adenosine receptors in most of the studies are the K_{ATP}-channels. The question which remains unsolved is the link between the adenosine receptors and the K_{ATP}-channels. Proposed mechanisms are G_i-proteins and/or protein kinase C.

G_i-proteins

Kirsch et al. were the first who proposed the coupling of K_{ATP}-channels to adenosine A₁-receptors by G_i-proteins. Their main finding was that the alpha 1, alpha 2 and alpha 3 subunits of the inhibitory G-protein, which is known to be sensitive to the pertussis-toxin, activated the K_{ATP}-channel, whereas neither the beta or gamma subunit of the G_i-protein, nor the G_s-protein showed the same effect. Subsequent studies confirmed these findings. Ito et al. [22] and Ito, Tung et al. [23] proved the role of this particular G-protein in linking adenosine receptors to the K_{ATP}-channel. These findings,

however, were made in guinea-pigs, whereas Kirsch et al. used ventricles of neonatal rats. Ito, Tung et al. detected in inside-out-patches that the alpha subunit of the G_i-protein was effective in eliciting bursting openings of a K-channel with a unitary conductance of about 90 pS. This channel activity could be suppressed by 2 mM ATP and glibenclamide, which confirmed that the particular channel was the ATP-sensitive K-channel. They further found that adenosine also increased the open state probability of the K_{ATP}-channel after application of GTP, probably via a native G-protein in the membrane of the patch. The beta and gamma subunits of the G_i-protein did not elicit any current, confirming the results of Kirsch et al., that these subunits are not able to activate the channel. The G_i-protein, however, can not only be activated by adenosine, but also by activators of alpha 1-adrenoceptors, suggesting that alpha-adrenergic agonists like norepinephrine might also be involved in ischaemic preconditioning. This would not be surprising because during an episode of myocardial ischaemia the plasma levels of adenosine (released from the cardiac myocytes) and alpha-adrenergic agonists are raised and, hence, could constitute a physiological protection against ischaemia. In fact, there is indeed some experimental evidence that the stimulation of alpha-adrenoceptors by endogenous catecholamines is one pathway of ischaemic preconditioning [24, 25]. Hu et al. used for their experiments isolated Langendorff-perfused rat hearts and measured both recovery of left ventricular pressure and the occurrence of ventricular arrhythmias, namely ventricular tachycardia and ventricular fibrillation. In their paper they propose the involvement of alpha 1-adrenoceptors in preconditioning through the activation of protein kinase C via the PTX-sensitive G-protein. However, they did not investigate the possible involvement of K_{ATP}-channels as final step in the intracellular cascade. Their conclusion is that preconditioning depends on the enhancement of the activity of PKC through G_i-proteins. But as stated below, the activation of PKC does not necessarily require the involvement of G_i-proteins and they did not investigate the direct interaction between G_i-proteins and the PKC at all. The question of the effect of PKC does not seem to be answered sufficiently either. In rat heart, the possible involvement of G_i-proteins is still a matter of discussion. Lawson et al. [26] as well as Liu and Downey could not detect an abolition of preconditioning by PTX, strongly arguing against G_i-proteins as mediators of preconditioning. Hu and Nattel in contrast describe the involvement of the G_i-protein, though activated by alpha 1-adrenoceptors, and Piacentini et al. reported that pretreatment with PTX abolished the antiarrhythmic effect of ischaemic preconditioning in isolated Langendorff-perfused rat hearts. The discrepancies in the findings of Hu and Nattel, Piacentini et al. on one side and Lawson et al. and Liu and Downey on the other side could partially be explained by the use of different parameters in determining the effects of preconditioning, but another explanation could be the direct activation of PKC without involvement of G_i-proteins. It is not unlikely that stimulated alpha 1-adrenoceptors or stimulated adenosine receptors activate both, the G_i-protein and PKC, respectively, resulting in an increased open state probability of the K_{ATP}-channel. This hypothesis, of course, needs further investigation, but there is no evidence against it so far. We shall return to this point later.

Protein kinase C

Another important pathway concerning ischaemic preconditioning is the activation of the protein kinase C. It is well known that the stimulation of a variety of receptors involved

in ischaemic preconditioning, including alpha 1-adrenoceptors [24] and adenosine receptors [3, 13, 14, 15, 27] is capable of activating PKC. Other studies also demonstrate that an active PKC elicits a K_{ATP}-current in rabbit ventricular myocytes [17, 28] and in humans [28]. In humans these findings were confirmed performing studies on contractile function after simulated ischaemia [29]. Furthermore, ischaemic preconditioning could also be brought about in rats by activation of PKC with phorbol 12-myristate 13-acetate, a specific activator of PKC [24]. While in rabbits and humans the effect of the activated PKC clearly is the activation of K_{ATP}-channels, this issue remains controversial in rats. Grover et al. presented evidence that the specific blocker of K_{ATP}-channels, glibenclamide, did not abolish the effects of preconditioning (see above). Thus, referring to this paper Hu and Nattel did not investigate a possible involvement of K_{ATP}-channels as final target for the PKC.

In rabbits, adenosine was shown to bring about preconditioning by activation of K_{ATP}-channels and that a constitutively active PKC also protects the myocardium via eliciting a K_{ATP}-current. Interestingly, no studies have been performed so far investigating a possible direct activation of PKC via adenosine, neither in rabbits, nor in rats. Kirsch et al. observed the activation of K_{ATP}-channels through adenosine via PTX-sensitive G-protein in a membrane delimited pathway in rat heart, but did not elucidate the involvement of the PKC. Further studies seem to be necessary investigating a possible direct activation of K_{ATP}-channels by PKC via adenosine. This could possibly solve the controversial questions about the involvement of G_i-proteins and PKC in the rat heart (see above).

Therapeutic implications

The opening of K_{ATP}-channels was detected to be the most important factor in ischaemic preconditioning in almost every species and data available for humans confirm the importance of these channels in protecting the myocardium [28, 29]. The opening of these particular K-channels seems to be mediated by protein kinase C. Therapeutically it seems to be sensitive to mimic ischaemic preconditioning by the use of substances which are known to open the K_{ATP}-channels, such as cromakalim or diazoxide. These drugs are already used as vasodilative drugs to lower the blood pressure. In vascular smooth muscle, the opening of K_{ATP}-channels leads to a hyperpolarisation of the cell membrane, which reduces the open state probability of the voltage-gated calcium channel and decreases the calcium entry into the cell. Thus, the contractile apparatus is not sufficiently supplied with calcium for contraction and the smooth muscles relax [30]. In cardiac muscle, however, the effect of preconditioning can be mimicked and K_{ATP}-channel openers thereby constitute one way of cardioprotection. The opening of K_{ATP}-channels via activation of protein kinase C remains impossible so far, since the substances which enhance the activity of protein kinase C, like phorbol 12-myristate 13-acetate, are known to be irritant and furthermore tumor promoting. Another therapeutical possibility to protect the myocardium is the use of adenosine. Adenosine has a variety of effects on the heart, especially the AV-node, and is a potent antiarrhythmic drug (mainly used to stop supraventricular tachycardias) and is also a strong dilator of the coronary arteries [31].

On the other hand care should be taken in prescribing drugs inactivating K_{ATP}-channels, such as the sulphonylurea compounds glibenclamide, tolbutamide or glipizide. The sulphonylureas are mainly used in the treatment of diabetes mellitus Type II and the blockade of K_{ATP}-channels in patients with

ischaemic heart disease could, on the one hand prevent the cardioprotective effects of ischaemic preconditioning in cardiac muscle; on the other hand hypoxia and simulated ischaemia have been shown to dilate the coronaries via opening of K_{ATP}-channels [32, 33, 34], hence the blockade of the K_{ATP}-channels also prevents the reflexory dilation of the coronary arteries and thereby oxygen delivery to the heart which is precisely matched by its metabolic needs.

Conclusions

Ischaemic preconditioning is an important mechanism to protect the heart against ischaemic injury. It is mainly induced by the opening of ATP-sensitive potassium channels. These channels can be activated by the stimulation of adenosine receptors and alpha 1-adrenoceptors via G_i-proteins and protein kinase C. In this paper we have shown that despite all experimental work that has been performed so far, the precise intracellular mechanisms remain partially unclear and hence more studies are required to identify the pathways of preconditioning.

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