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Ischemic preconditioning of myocardium

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ABSTRACT

Preconditioning of the myocardium with short episodes of sublethal ischemia will delay the onset of necrosis during a subsequent lethal ischemic insult. Ischemic preconditioning seems to involve a variety of stress signals which include activation of membrane receptors and signaling molecules such as protein kinase C, mitogen-activated protein kinases, opening of ATP-sensitive potassium channel, and expression of many protective proteins. The purpose of this review is to assess the current position in this field and to facilitate future research.

INTRODUCTION

Efforts to prevent ischemic injury have focussed on finding ways to block events associated with irreversible ischemic injury. In 1986**,** Murrey *et al* described a classic phenomenon termed ischemic preconditioning (IP) for the first time. It was originally thought that each ischemic episode caused cumulative ATP depletion while the intermittent reperfusion would wash out the ischemic catabolites. Surprisingly ATP levels were not depleted by subsequent ischemic challenges and no infarction occurred. This observation led the same group of scientists[1] to test the hypothesis that the preservation of high-energy phosphates was due to a slowing of consumption during ischemia associated with a rapid and protective adaptation of the myocyte. They tested this hypothesis by subjecting the myocardium to a series of four 5-min coronary branch occlusions; each separated by 5 min of reperfusion. This rendered the myocardium more resistant to the subsequent sustained 40-min ischemic insult. The infarct size was reduced to 25 % of that seen in control group. This phenomenon is called "preconditioning with ischemia." The classic IP is short lived and fast decayed with anti-ischemic effects disappearing completely within 2 h. However, a delayed resurgence of the IP-induced cardioprotection was demonstrated by Kuzuya *et al* in 1993[2]. They observed that a significant effect of IP reappeared when sustained ischemia was initiated 24 h later. This delayed effect of preconditioning has been refered to the "second window of protection[3]." The duration of delayed IP appears to be relatively long lasting and its effects may maintain for a few days. The evolution of necrosis is delayed but not prevented. Preconditioning will limit infarct size during a temporary coronary occlusion but not during a prolonged or permanent occlusion. The stimulus for preconditioning is a critical reduction in myocardial blood flow, and the end point is infarct size. The optimal duration of ischemia appears to be species dependent. Ischemic preconditioning has been demonstrated in rats after one to three cycles of ischemia/reperfusion(I/R)[4], a single 5-min cycle of I/R in rabbits[5] and a 2.5-min cycle of I/R in dogs[6].

TRIGGERS OF CLASSIC PRECONDITIONING

Classic ischemic preconditioning is not dependent

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on the existence of collateral vessels[1] and occurs in the presence of protein synthesis inhibitors[7]. The cellular basis of the mechanism underlying preconditioning is not fully understood. Preconditioning results in activation of a number of receptors such as adenosine[8], alpha-adrenergic[9], delta-opioid[10], and bradykinin.

Two individual stimuli, sublethal hypoxic ischemic insult and elevated bradykinin levels by ACE inhibition, were insufficient to induce a preconditioning response when administered separately, but in combination, a full protective effect was seen. Triggers must be distinguished from mediators of preconditioning. Bradykinin is a trigger but not useful during sustained ischemia but adenosine is a potential trigger and a mediator during sustained ischemia.

Adenosine After the onset of myocardial ischemia, ATP depletion occurs rapidly[11] and adenosine is released in large amounts in the interstitial space. Here it interacts with its own receptors. Activation of adenosine receptors along with an increase in adenosine receptor density during ischemic-preconditioning provides the basis for adenosine to exert its protective effect on the ischemic heart[12].

The A_1 adenosine receptor, which is located on cardiac myocytes, is involved in the cardioprotective effect of ischemic preconditioning. In isolated guinea-pig heart protection by IP could be reproduced by activation of A_1 receptors but could not be abolished by blockade of A_1 receptors[13]. The newly characterized A_3 receptor, which inhibits stimulated adenyl cyclase activity, has also been suggested to mediate ischemic preconditioning in the rabbit[14]. Exogenous adenosine is most protective when administered before ischemia, whereas during ischemia, it was only partially protective. Adenosine produced the protective effects of IP, preserved ATP, perhaps by stimulating glycolysis.

ATP-sensitive K^+ channels This channel is normally inhibited by intracellular ATP and opens during periods of energy depletion. In the heart K-ATP channels are present on the sarcolemma of cardiac myocytes where they were first described but their purpose remains unclear[15,16]. Reports that sulphonylurea receptor antagonists could diminish IP-induced protection, suggested that K-ATP channels might be effectors of protection and this idea was reinforced by the observation that K-ATP channel openers like cromakalin and pinacidil mimicked protection[17, 18]. On the basis of this, it was proposed that opening of surface K-ATP channels during ischemia was somehow facilitated by activation of signaling pathways such that action potential shortening that occurred in early phase of ischemia was enhanced. The result was better preservation of cellular energy stores and suppression of deleterious downstream events, such as cellular calcium overload. The opening of surface K-ATP channels in cardiomyocytes has little effect on membrane potential, but the outward current carried by K-ATP shortens the action potential and if large enough can render the cell inexcitable. Using flavoprotein flourescence method, the pharmacological profile of several K-ATP openers and inhibitors has been characterized, and compounds have been found that specifically act on either the mitochondrial or sarcolemmel K-ATP channels. Compounds capable of activating mitochondrial K-ATP mimicked protection against ischemia. However compounds selective for sarcolemmel channels have no such action and serve convincing evidence that sarcolemmel channels play a much less role in protection.

The mechanism of mitochondrial K-ATP protection may involve alterations in mitochondrial handling, optimization of energy production, and modulation of reactive oxygen species (ROS) production during ischemia or reperfusion. Mitochondrial K-ATP channel opening may not only be a common downstream effector leading to protection but also provide positive feedback by altering upstream components such as ROS or protein kinase C (PKC). Mitochondrial K-ATP channels are not only modulated by PKC and NO but also may trigger translocation and activation of tyrosine kinase.

Bradykinin and B_2 receptors It was shown in 1996 by Miki *et al* that captopril, potentiates IP without increasing kinin levels, and that the effect of captopril can be reversed by HOE140, a specific bradykinin receptor antagonist. This finding has been further extended using B_2 kinin receptor knockout mice as well as kininogen deficient rats, and demonstrated a loss of protective effect in these strains. The results obtained in these experiments suggest that activation of prekallikrein may contribute to the effect of IP and that an intact kallikrein-kinin system is necessary for the cardioprotective effect of IP[19]. Pretreatment with bradykinin resulted in cardiac protection against free radical injury through the activation of B_2 receptors, suggesting that endogenous generation of bradykinin may mediate IP in guinea pig heart[20]. But blockade of B_1 receptor not B_2[19] receptor prevented protection afforded

by IP, as seen in isolated rat heart following *in vivo* ischemia-reperfusion injury. This suggests that in coronary circulation an endogenously produced B_1 receptor agonist could itself be a trigger for IP. It is also known that all the vasomotor effects of kinins are mediated by B_2 receptors. However, inducible G-protein-coupled B_1 receptors are rapidly upregulated after tissue injury and damage[21]. So to ascertain whether the bradykinin receptor subtype involved in vascular preconditioning is the B_2 subtype or the subtype B_1 requires further research.

POST RECEPTOR SIGNALING CASCADE

Triggers mentioned above are coupled to G proteins, and pretreatment with pertussis toxin blocked the protective effect of ischemic preconditioning[22]. Activation and translocation of PKC play key roles in mediating both classic and delayed preconditioning. G protein coupled receptors lead to activation of phospholipase C and the generation of diacylglycerol (DAG), which activates and translocates PKC to cell membranes where it activates K-ATP channels at or near physiological levels of ATP.

Hypoxic preconditioning stimulated the activity of PKC and markedly enhanced the activity of K-ATP channels in the isolated rat cardiac myocytes[23]. PKC activation is also involved in the upregulation of K-ATP channels. Isolated cardiomyocytes and transgenic mice have identified PKC∈ as the isoform resposible for this protection[24]. Kinases other than PKC, such as tyrosine kinase (TK), also appear to be involved in the mechanisms of preconditioning because treatment with blockers of this kinase, such as genistein, before the IP stimulus can blunt protection[25]. Furthermore mitogen activated protein kinases (MAPKs) participate in the intracellular events that lead to cardioprotection in delayed preconditioning, are also involved in classical preconditioning. A specific p38 MAPK inhibitor SB203580 abolished protective effect of IP[26]. In conscious rabbit, IP elicited an increase of p44 and p42 MAPK cellular activity that was associated with translocation of both kinases from cytosol to the nuclear compartment[27].

GENERAL CHARACTERISTICS OF DELAYED PRECONDITIONING

Unlike the early phase of IP, which lasts 2 to 3 h and protects against infarction but not against stunning, the late phase of IP lasts 3 to 4 d and protects against both infarction and stunning, suggesting that it may have greater clinical relevance. The delayed protection extends to other indices of cardiac dysfunction-reperfusion induced tachyarrythmias. The prolonged duration of protection makes it particularly interesting. It is triggered by a variety of stimuli, such as heat stress, exercise, and cytokines. Thus, late IP appears to be a universal response of the heart to stress in general. It is now clear that late IP is a polygenic phenomenon that requires the simultaneous activation of multiple stress-responsive genes. Adenosine A_1 receptor activation during preconditioning is an important trigger of delayed protection against infarction. Since it is clear that delayed IP can be induced by means other than transient ischemia, it leads to the development of various practical therapeutic approaches. Bacterial endotoxin treatment is known to induce delayed myocardial protection, probably by upregulating various cytoprotective proteins, including antioxidants and inducible nitric oxide synthase. The endotoxin derivative monophosphoryl lipid A induces myocardial protection 24 h after administration and opening of the K-ATP channel may be integral to this late protective response.

TRIGGERS AND MEDIATORS OF LATE PHOSPHOLIPASE C (PC)

Nitric oxide appears to play a dual role in the pathophysiology of the phase of ischemic IP, acting initially as the trigger[28] and subsequently as the mediator[29] of this adaptive response. Direct measurement of NOS activity has shown biphasic regulation of NOS by IP, with an increase in calcium dependent NOS (eNOS) activity immediately after IP followed by an increase in calcium independent NOS (iNOS) activity 24 h later[30]. The finding that both IP and nitroglycerin induce a rapid increase in steady state levels of iNOS mRNA, which is abolished by administration of *L*-NA before IP, also supports this concept. Taken together these studies support the paradigm in which 2 different NOS isoforms are sequentially involved in the pathophysiological cascade of late PC, with eNOS generating the NO that initiates the development of IP response on d 1 and iNOS then generates the NO that protects against recurrent ischemia on d 2[31]. Monophosphoryl lipid A (MLA) repesents a novel agent capable of enhancing myocardial tolerance to ischemia/reperfusion injury[32]. Current evidence suggests that the cardio-protective ef-

fects of MLA involve myocardial inducible nitric oxide synthase [iNOS(s)] enzyme activation with NO coupled activation of myocardial K-ATP channels upon ischemic challenge. MLA is currently being evaluated in phase 2 clinical trials, in patients undergoing coronary artery bypass surgery. More recently it was determined that RC-552, a novel synthetic glycolipid related in chemical structure to MLA, could offer similar protection[33].

SIGNALLING ASPECTS OF DELAYED PRECONDITIONING

Activation of PKC appears to be a crucial intermediate step since inhibition of PKC during preconditioning abolishes protection 24 h later[34]. The involvement of other parallel downstream kinases including tyrosine kinase and MAPK kinases may also be involved. Delayed protection induced by adenosine A_1 agonist in rabbits is dependent on both PKC and tyrosine kinase activation, since it can be abolished by pretreatment with either chelerythrine (a PKC inhibitor) or lavendustin-A (a tyrosine kinase inhibitor)[35]. In addition other cascades are likely to be important, especially those in the MAPK families. Three major MAPK families exist in eukaryotic cells: the classic p42/p44 MAPKs[27], p38 kinase, and the stress activated c-jun N-terminal kinase (JNK). PKC is known to phosphorylate and activate raf-1 kinase, which provides a direct link to the p42/p44 MAPK family. Other studies suggest that activation by ischemia or reactive oxygen species of p38 kinase and JNK are known to phosphorylate factors that co-ordinate gene transcription. The activation of p38 is short lived. The activation of p44/p42 MAPKs and JNKs is abolished by chelerythrine, indicating that it is downstream of and dependent on activation of PKC. Selective overexpression of PKC∈ in adult rat myocytes induces activation p44/p42 MAPKs and protects against stimulated ischemia. The involvement of these kinases in delayed preconditioning is to become the focus of attention in the coming years.

PROTEIN EFFECTORS OF DELAYED PRECONDITIONING

The time course of delayed preconditioning is suggestive of a mechanism involving new protein synthesis. There is either increased activity of manganese superoxide dismutase (SOD) or elevation of myocardial content of the major inducible heat shock protein, HSP 72[3]. Both proteins are stress-induced proteins and have cytoprotective properties. Manganese-SOD is a mitochondrial antioxidant, which detoxifies superoxide anions. HSP 72 is a chaperone protein involved in regulation of protein folding, transport, and denaturation during the cellular response to injury. These relationships have been confirmed by gene transfection studies and transgenic mice constitutively over expressing human HSP72[36].

THERAPEUTIC APPROACHES BASED ON PRECONDITIONING

Even with the development of pharmacological agents that mimic preconditioning, the timing of administration will be critical. Patients with unstable angina are at high risk of myocardial infarction and such a treatment would "buy time" for the administration of other revascularisation techniques.

Preconditioning strategies could also be applied prior to a planned procedure involving a potentially injurious ischemic insult. An example is coronary artery bypass graft (CABG) surgery.

A_1 agonists represent a promising therapy, however down regulation of the receptor occurs with continued occupancy of the receptor. Intermittent administration with modest doses would circumvent this problem. Bradykinin synthesized by endothelial cells is involved as a trigger in preconditioning and may contribute to cardioprotective effect of ACE inhibitor[37]. K-ATP channel openers are also possibilities of therapeutic exploitation. The role of mitochondrial rather than sarcolemmal K-ATP channels in classical preconditioning suggests that targeting the organelle which is specifically invalid has theoretical advantages. This also avoids the unwanted side effects of sarcolemmal transmembrane potential[17].

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