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Mechanism of Cardioprotection by Early Ischemic Preconditioning

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Abstract

A series of brief ischemia/reperfusion cycles (termed ischemic preconditioning, IPC) limits myocardial injury produced by a subsequent prolonged period of coronary artery occlusion and reperfusion. Over the last 2 decades our understanding of IPC's mechanism has increased exponentially. Hearts exposed to IPC have a better metabolic and ionic status during prolonged ischemia compared to naïve hearts. However, this difference is not thought to be the main mechanism by which IPC protects against infarction. Signaling pathways that are activated by IPC distinguish IPC hearts from naïve hearts. During the trigger phase of IPC, adenosine, bradykinin and opioid receptors are occupied. Although these three receptors trigger signaling through divergent pathways, the signaling converges on protein kinase C. We have proposed that at the end of the index ischemia the activated PKC sensitizes the low-affinity A_{2b} adenosine receptor (A_{2b}AR) through phosphorylation of either the receptor or its coupling proteins so that A_{2b}AR can be activated by endogenous adenosine released by the previously ischemic cardiomyocytes. The sensitized A_{2b}AR would then be responsible for activation of the survival kinases including PI3 kinase, Akt and ERK which then act to inhibit lethal mitochondrial permeability transition pore formation which normally uncouples mitochondria and destroys many myocytes in the first minutes of reperfusion. Herein we review the evidence for the above mechanisms and their functional details.

Keywords

A_{2b} adenosine receptor; G protein-coupled receptor; Ischemic preconditioning; Mitochondrial permeability transition pore; PKC; Myocardial infarction; Signal transduction; Cardioprotection

Introduction

Cardiovascular disease is the leading cause of death in Western countries. In 2007, almost 8 million individuals were affected by acute myocardial infarction (AMI). Clinically the goal is

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to reestablish blood flow to the ischemic area as soon as possible to salvage cells that would be damaged by the ischemia. While reperfusion is necessary for tissue survival, it is worth noting that reperfusion itself can also cause tissue damage, termed reperfusion injury. As more tissue is irreversibly injured, the prognosis becomes worse because terminally differentiated cardiac myocytes cannot regenerate. Loss of contractile mass puts an inordinate load on surviving tissue causing the remaining cells to hypertrophy resulting in adverse remodeling of the ventricle ending ultimately in heart failure. Finding a way to render heart cells resistant to death from ischemia/reperfusion injury would greatly improve the prognosis of AMI. In the 1970s and 1980s many interventions were tested based on the prevailing theories of how ischemia caused cell death, but none unambiguously prevented infarction in animal or clinical models [1].

In 1986, Murry, Jennings and Reimer [2] studying myocardial infarction caused by a prolonged period of coronary artery occlusion (hereafter termed the index ischemia) in dogs discovered that if the index ischemia was preceded by 4 brief episodes of 5 min of ischemia each followed by 5 min of reperfusion infarct size was greatly reduced to only 25% of that in the control group. Unlike the case with previously reported interventions, this one could be easily reproduced by all investigators who tested it. This phenomenon was termed ischemic preconditioning (IPC). Subsequently, this powerful protective function of IPC was shown to occur in all species tested including mouse, rat, rabbit, feline, canine, sheep, monkey and even human hearts. Although IPC is not amenable for treating AMI since those patients present with ischemia already in progress, its discovery proved once and for all that myocardial protection was possible. All that remained was to understand its mechanism so it or a derivative could be translated into a clinical procedure. Elucidating that mechanism, however, turned out to be more difficult than ever imagined so that 2½ decades later, our understanding is still incomplete.

IPC causes two phases of protection. The first phase, termed “early” or “first window” IPC, protects the heart for an hour or two and then wanes; the second phase, “delayed” or “late” IPC or “second window of protection” (SWOP), appears 24 h after the IPC protocol and can last for 3 days. This review will focus on the protective mechanism of the first phase, while another review in this same focused issue will expand on SWOP.

Metabolic and ionic effects of IPC

In the first few years after the discovery of IPC the most prominent hypothesis was that IPC protected by improving the metabolic balance during the ischemic insult. Several studies examined the metabolic and ionic effects of IPC on hearts. It was found that after the IPC protocol the myocardium had a smaller adenine nucleotide pool, a creatine phosphate overshoot, excess intracellular glucose, and a contractile deficit termed stunning [3,4]. In addition, during the prolonged ischemic insult preconditioned myocardium displayed a slower rate of ATP depletion as well as lactate and H^+ accumulation [4–7]. The milder acidosis reduces sodium loading of the myocardium through the sodium/hydrogen exchanger. That would both maintain the transmembrane sodium gradient to prevent intracellular edema and reduce calcium influx at reperfusion via the sodium/calcium exchanger. During these early years an effect on reperfusion injury was not seriously considered as most investigators were convinced that IPC’s protection occurred during ischemia.

However, none of the above mechanisms seemed to explain IPC’s profound protection. For example, one study [8] showed that application of adenosine and bradykinin B_2 receptor antagonists could block the protection of IPC on infarct size without eliminating the suppression of purine accumulation during ischemia caused by IPC. Therefore, suppression of adenine nucleotide degradation is not essential for IPC’s anti-infarct effect. Depletion of

glycogen was also proposed as the mechanism of IPC's protective action. Application of adenosine which can pharmacologically precondition hearts did indeed deplete myocardial glycogen. However, bradykinin which also preconditions the heart did not affect tissue glycogen levels [9]. Finally the adenosine receptor antagonist 8-(p-sulfophenyl)theophylline (SPT) which actually blocks IPC's protection did not prevent glycogen depletion. Therefore, loss of glycogen *per se* did not cause the protection of IPC.

It is difficult to determine whether IPC's metabolic and ionic effects during ischemia contribute anything to IPC's protective mechanism. As will be seen in the next section the major effect of IPC is to prevent cell death from a reperfusion type injury. Of course one can't have reperfusion injury without first having ischemic injury. Unfortunately, rats and rabbits which have been most widely used in studies of IPC develop very large infarcts with an index ischemia of relatively short duration. In pigs, dogs, and especially primates [10] a much longer index ischemia appears to be required to trigger mitochondrial permeability transition pore (mPTP) formation. As the duration of the index ischemia increases, a greater portion of the total cell death may occur during the ischemic period and in macaque hearts there is some evidence that a significant portion of the salvage caused by IPC may indeed occur during the index ischemia [10].

IPC exerts its protection in the first minutes of reperfusion

Most of the evidence suggests that IPC exerts its protection in the first minutes of reperfusion. That was first demonstrated when Hausenloy and Yellon [11] blocked PI3 kinase or ERK in the first minutes of reperfusion and found that IPC's protection in rat hearts was blocked. Shortly thereafter we saw a similar effect in rabbits [12]. Rapid cycles of coronary occlusion/reperfusion at the end of the index ischemia (postconditioning) have almost the same protective effect as IPC [13], but obviously postconditioning's protective effect could only have happened during reperfusion. Finally, several pharmacologic interventions known to activate IPC's signal transduction pathway applied during the first minutes of reperfusion mimic the protection of IPC [14,15].

IPC is receptor-mediated through signal transduction pathways

The first insight into IPC's mechanism occurred in 1991 when Liu et al. [16] discovered that activation of the G_i-coupled adenosine A_1 receptor triggered IPC's protection. They reported that an adenosine receptor antagonist could block IPC's protection and also showed that infusion of adenosine or the A_1-selective agonist R(−)-N^6-(2-phenyl-isopropyl) adenosine (PIA) in lieu of brief ischemia could duplicate IPC's protection. It was proposed that endogenous adenosine released during the brief ischemia of the IPC protocol activated A_1 adenosine receptors leading to a preconditioned phenotype. It became clear that IPC was the result of signal transduction pathways in the heart. In the following years those pathways were extensively studied and have been found to be surprisingly complex. IPC's signaling pathway can be divided into 2 phases: pre-ischemic trigger phase and post-ischemic mediator phase.

IPC's trigger pathway

Two other autacoids, bradykinin [17] and opioids [18], are also released during an IPC protocol, and their receptors appear to work in parallel with the adenosine receptors. Blockade of any one of these three agonist receptors inhibits IPC's protection from a single preconditioning cycle. However, because blockade of any one receptor type could not abolish protection from multiple cycles of IPC, it was proposed that the three receptors had additive effects [19]. The additional preconditioning cycles were assumed to have produced more ligands for the two remaining receptors that were not inhibited so they could successfully reach the hypothesized protective threshold without contribution from the third blocked receptor.

The 3 triggers implicated in IPC bind to G_i protein-coupled receptors (G_i-PCR). The additive trigger theory requires that binding of the 3 triggers by their respective receptors results in signaling that converges on a common target. It is thought that protein kinase C (PKC) is the common target because protection afforded by IPC and all of the triggers could be eliminated by PKC inhibitors. Adenosine, bradykinin and opioids activate very divergent pathways despite the fact that their signaling converges on a single target. Adenosine receptors are thought to activate PKC through the phospholipases making diacylglycerol from membrane phospholipid [20]. Opioid receptors are proposed to depend on metalloproteinase-mediated transactivation of the epidermal growth factor receptor (EGFR) which activates PI3 kinase [21]. This receptor tyrosine kinase auto-phosphorylates its tyrosine residues when bound to its triggering growth factor. Bradykinin also triggers through PI3 kinase activation but that appears to be independent of EGFR. The steps downstream of PI3 kinase for both opioids and bradykinin appear to be similar. PI3 kinase causes phosphorylation of Akt through the phospholipid-dependent kinases. Phosphorylated Akt subsequently activates endothelial nitric oxide synthase (eNOS) to produce nitric oxide (NO), which then stimulates guanylyl cyclase (GC) to produce cGMP which in turn stimulates PKG [20,22].

All G_i-PCR tested so far seem to have the ability to mimic IPC through PKC activation. That includes catecholamines [23], angiotensin II [24], endothelin [25], and sphingosine 1-phosphate [26]. This indicates that virtually all G_i-PCR in the heart can trigger the IPC phenotype. However, inhibition of the receptors of any of these additional ligands does not raise the threshold for IPC, probably because ligands for these receptors are not released by a preconditioning protocol in a quantity large enough to contribute to IPC.

Role of reactive oxygen species (ROS) in IPC

There is agreement that ROS production plays an essential role in the protective mechanism of IPC. As early as 1988, Murry et al. [27] had proposed that ROS signaling might be involved in IPC because the intravenous administration of the free radical scavengers superoxide dismutase and catalase abolished preconditioning with ischemia in some, but not all, of their dog hearts. IPC's protection can be mimicked by transient exposure to an oxygen radical generating system, and, conversely, a ROS scavenger can abolish protection from IPC [28, 29]. In a cell model brief exposure to oxidants preconditioned cardiomyocytes [30]. Protection from a ROS generator could be blocked by a PKC inhibitor indicating that the ROS signal occurred upstream of PKC [31]. Conversely, a direct activator of PKC which mimicked IPC's protection could not be blocked by a ROS scavenger [32] indicating again that ROS are upstream of PKC. Because ROS can directly activate PKC through sulfhydryl oxidation [33], we have assumed that the ROS are acting directly on PKC. However, this has not been proven.

We have examined when redox signaling actually occurs. Both superoxide and hydrogen peroxide production in the heart begins very early during ischemia/anoxia and actually ceases with the reintroduction of oxygen [34,35]. MPG is a cell-permeant ROS scavenger that eliminates the protective effect of IPC when administered during an IPC protocol [20,36–38]. By administering MPG during either the ischemic or the reperfusion phases of IPC, we showed that protective redox signaling occurs when oxygen is reintroduced following the brief occlusion rather than during it [32]. MPG is a fairly selective scavenger and reportedly does not scavenge either superoxide or hydrogen peroxide [39]. MPG should scavenge peroxynitrite and hydoxyl radical. Exactly what ROS species is responsible for the redox signaling is unknown, but the responsible species is obviously targeted by MPG.

The source of ROS appears to be mitochondria where mK_{ATP} channels play an essential role. It is proposed that PKG activity opens mK_{ATP} channels on the inner mitochondrial membrane permitting K^+ to enter the matrix along its electrochemical gradient [40]. The influx is balanced

by electrogenic H^+ efflux driven by the respiratory chain. This mK_{ATP} channel-dependent matrix alkalinization causes complex I and/or III to generate increased amounts of superoxide and its products, H_2O_2 and hydroxyl radical [41]. Blocking site III electron transport with myxothiazol abolishes the ROS burst and any maneuver that lets potassium into the mitochondria seems to produce ROS [42]. Little is known about either the structure of mK_{ATP} channels or the detailed mechanism by which mitochondria produce ROS. Mitochondria contain connexin 43 on their inner membranes [43] which also seems to be involved in ROS production since connexin 43-deficient hearts cannot be preconditioned and myocytes from those hearts produce much less ROS in response to diazoxide, a direct opener of mK_{ATP} channels [44]. It is possible that there is some interaction between mK_{ATP} channels and connexin 43.

Costa et al. [40] showed that application of exogenous PKG and cGMP to isolated mitochondria resulted in opening of mK_{ATP}, and this PKG-dependent channel opening could be blocked by mK_{ATP} inhibitors 5-hydroxydecanoate, glibenclamide, and tetraphenylphosphonium. The mK_{ATP} channels are localized on the inner mitochondrial membrane which is not accessible to cytosolic PKG. This suggests that required intermediate steps transmit the signal between a PKG target on the outer membrane and the K_{ATP} on the inner membrane. Accordingly Costa et al. [40] found that channel opening was dependent on PKC-ε in the mitochondria. It is not known how many other steps might be involved within the mitochondria.

Although mitochondria have received most of the attention, NADPH oxidase, another source of ROS in the heart, might also be involved. One study reported that NADPH oxidase-deficient mice could not be protected by 2 cycles of IPC [45]. It is possible that there are several different sources which contribute ROS that trigger IPC.

Role of protein kinase C

In 1994, Ytrehus et al. [46] found that a PKC inhibitor abolished IPC's protection in a rabbit model, and that experiment has been widely reproduced. However, it is still controversial which PKC isozyme mediates this protection. It seems that PKC-ε is both required and sufficient to induce cardioprotection. Cardiac-specific overexpression of PKC-ε provides cardioprotection against ischemia/reperfusion damage [47,48]. Also a PKC-ε-selective peptide activator protected hearts and peptide inhibitors of PKC-ε abolished ischemic/hypoxic or pharmacologic preconditioning in mice, rats, rabbits and pigs [47,49–52]. Conversely, IPC failed to decrease infarct size in mice in which cardiac PKC-ε had been deleted, although functional recovery was still improved by IPC [53].

The function of PKC-δ in IPC is controversial. Cardiac-specific expression of PKC-δ-activating peptide worsens ischemia/reperfusion-mediated injury [51] and administration of a PKC-δ-inhibitory peptide lessens it [54,55]. In contrast, other studies show that PKC-δ has a cardioprotective role in ischemic/hypoxic preconditioning [56–58] and PKC-δ knockout mice exhibit increased injury following IPC [58]. Although a PKC-δ-selective peptide activator infused immediately before ischemia increased cardiac damage, PKC-δ activation an hour prior to the ischemic event resulted in cardioprotection in mouse hearts [51,59]. Furthermore a PKC-δ inhibitor administered during reperfusion prevented reperfusion injury in both pig [59] and rat [55] hearts. In addition, faster recovery of ATP levels in hearts treated with a PKC-δ inhibitor can be detected during reperfusion [59]. Therefore, PKC-ε activation is required for IPC. Although activation of PKC-δ during reperfusion is detrimental, transient activation of PKC-δ prior to ischemia may induce cardioprotection [60].

Another unresolved issue is determination of the target of PKC. It shouldn't be surprising that an important kinase like PKC has many targets. And it is known that PKC can directly or indirectly modulate components associated with mitochondrial membranes such as mPTP,

mK_{ATP}, BAX/BAD and Bcl-2 [38,40,61] which are important molecules or structures that determine the live-or-die fate of myocytes.

There are several hypotheses about how PKC elicits its protective function:

1. Garlid's group [41] proposed that the occupied G_i-PCR activated by IPC concentrate in caveolae, where signaling enzymes are scaffolded into signalosomes which migrate to mitochondria. The signalosomes interact with mitochondria through PKG (by unknown steps and molecules) by activating two pools of PKC-ε1 and 2. PKC-ε1 near the inner mitochondrial membrane phosphorylates and opens mK_{ATP} resulting in K^+ uptake, elevated matrix pH, and increased ROS production. The ROS produced by mK_{ATP} opening diffuse to and activate both PKC-ε1 and PKC-ε2. PKC-ε 1 maintains ROS production in a positive feedback loop while PKC-ε 2 inhibits mPTP and protects the heart. In this theoretical construct PKC-ε 2 is the end-effector that determines the cell's fate. If the activity of PKC-ε 2 were blocked, IPC's effects would be abolished regardless of which upstream molecules were activated. However, this proposal is not supported by data obtained in our rabbit hearts since chelerythrine, a non-isoform selective and potent PKC inhibitor, could not abolish the protective effect of an A_{2b}AR agonist [62].
2. Kitakaze et al. [63] proposed that IPC activates 5′-nucleotidase (CD73) which would generate more protective adenosine from adenosine monophosphate and further hypothesized that PKC was responsible [64]. Eltzchig's group [65] recently expanded this theory by proposing that the increased extracellular adenosine activated the low-affinity A_{2b}AR which in turn protected the cells. A key feature of this hypothesis is that IPC hearts should have a higher adenosine concentration than the naïve hearts. However, this has been hard to demonstrate. Whereas some studies have shown increased adenosine levels after IPC [66], most researchers either have not detected a difference of adenosine concentration in IPC and non-IPC hearts [67] or actually have documented lower adenosine levels in IPC hearts [68,69].
3. We have proposed that PKC activation acts to increase A_{2b}AR's affinity to adenosine, probably by phosphorylation of A_{2b}AR or its coupling proteins. This receptor has a very low affinity for adenosine such that even during ischemia when tissue adenosine levels reach 1–4 μM, this level would still be well below the A_{2b}AR's K_d of 5–15 μM. Because protection from a direct PKC activator can be aborted by an A_{2b}AR blocker [70] and a PKC inhibitor does not affect protection from an A_{2b} agonist [62], we believe that the A_{2b}AR clearly resides downstream of PKC. Kuno et al. [62] noted that PKC activation lowered the A_{2b}AR's threshold for adenosine to induce signaling in the heart. Thus it is possible that A_{2b}AR can respond to the heart's endogenous adenosine only after they have been sensitized by PKC. If this is true, that would make the primary difference between an IPC heart and a naïve one the changing affinity state of the A_{2b}AR. Because it is only a question of sensitivity, our theory explains why a potent A_{2b} agonist can protect even a naïve heart.

IPC exerts its protection at reperfusion (mediator phase)

In 2005, Hausenloy et al. [71] proposed that IPC protects by inducing activation of PI3 kinase/Akt and the MEK1/2/ERK1/2 cascades at reperfusion. Pharmacological inhibition of either of these cascades early in reperfusion abolished IPC-induced protection. This led them to the conclusion that IPC actually exerts its protection early in reperfusion following the lethal ischemic insult. This revolutionary paradigm shift provided enormous hope for the clinical translation of IPC. Although the protection of ischemic or pharmacological preconditioning is powerful, it could not be effectively employed in patients with AMI since preconditioning has to be introduced before the lethal ischemia. Only after the onset of AMI do patients present to

the hospital. But if IPC exerts its protection at reperfusion, then therapeutic salvage could still be possible even after ischemia had begun by intervening at reperfusion.

Indeed, in the past several years researchers have found that many reagents can protect the myocardium when given in the first minutes of reperfusion, e.g., insulin [72], the adenosine A_1/A_2 agonist AMP 579 [73], the A_{2b}AR-selective agonist Bay 60-6583 [74], transforming growth factor-β1 [75], urocortin [76], cardiotrophin-1 [77], adenosine agonist 5′-(N-ethylcarboxamido) adenosine (NECA) [78], bradykinin [78], natriuretic peptides [79], erythropoietin [80,81], and cyclosporin A [82]. Like IPC, all of these reagents except cyclosporin A depend on the activation of PI3 kinase and/or ERK for protection to occur. Also, it is worth noting that atrial natriuretic peptide [83] and cyclosporin A [84] have already been successful in clinical trials.

GSK-3β and mPTP

As mentioned above the end-effector for IPC may be PKC-*ε* 2 which acts to inhibit formation of lethal mPTP. mPTP was first described by Hunter and Haworth [85]. In 1988 Crompton's group [86] postulated the possible involvement of mPTP in reoxygenation-injured hearts. Also in 1988, the same group discovered that the immunosuppressant drug cyclosporin A could inhibit mPTP opening induced by calcium, phosphate and oxidative stress [87]. This provided an important pharmacological tool for investigating the function of mPTP in cardioprotection. Three years later they reported that pretreating anoxic rat myocytes with cyclosporin A improved their survival [88]. Griffiths and Halestrap [89] claimed that cyclosporin A at reperfusion improved ATP levels in perfused rat hearts. Then the same authors [90] discovered that mPTP remain closed during ischemia and open only in the first few minutes of reperfusion, a convenient time-point for clinical therapeutic intervention. In 2002, Yellon's group [91] made the association between mPTP inhibition and IPC at reperfusion. It is currently unresolved how IPC actually inhibits opening of mPTP at reperfusion, but several mechanisms have been proposed and will be discussed below. The molecular identity of mPTP remains a mystery. The pore originally was thought to be assembled from the voltage-dependent anion channel (VDAC) in the outer membrane, the adenine nucleotide translocase (ANT) in the inner membrane, and cyclophilin-D (CyP-D). New evidence based on genetic knockout studies now suggests that these proteins are not core components of the pore but rather act as pore regulators. A more detailed discussion of this subject is available in recent reviews [92,93].

In 2002, Murphy's group showed that IPC leads to GSK-3β inhibition by Ser9 phosphorylation and that pharmacologic inhibition of GSK-3β mimics IPC by reducing infarct size [94]. Since then extensive evidence implicating GSK-3β as a critical element in IPC has emerged [94–99]. Inactivation of the signaling kinase GSK-3β could strongly inhibit mPTP formation in myocytes [100] but a GSK-3β inhibitor has no effect on mPTP opening in isolated mitochondria [101], indicating that the GSK-3β isoform that is involved with cardioprotection must reside outside of mitochondria. In one study the mK_{ATP} blocker 5-HD blocked cardioprotection by GSK-3β inhibition, suggesting a GSK-3β-mK_{ATP} interaction [102].

Recently, a mouse model was used to explore the role of GSK-3 in cardioprotection [103]. In these experiments a genetically modified mouse with a knock-in of signal-resistant GSK-3α and GSK-3β was used. In contrast to the extensive evidence summarized above, both IPC and postconditioning protocols protected hearts of the homozygous GSK-3 double knock-in mice. Therefore, in this particular mouse model, phosphorylation of GSK-3β on Ser9 was apparently not required for induction of protection. The reason for this apparent discrepancy is not clear, and further studies will be required to resolve the controversy. One possibility is that while GSK-3β inhibition can inhibit mPTP, that is not the mechanism used by IPC.

How do mPTP kill?

The high conductance mPTP dissipates the transmembrane proton/electrochemical gradient that drives ATP generation. That leads to ATP depletion, further ROS production, solute entry, and ultimately swelling and rupture of the organelle. If enough of the cell's mitochondria are thus destroyed, necrotic death will quickly follow. If only a fraction of the mitochondria are lost, apoptotic cell death can result from mitochondrial release of cytochrome c. Ischemia somehow injures the mitochondria to promote mPTP formation. However mPTP formation is inhibited by the low pH that occurs during ischemia. But restoration of pH coupled with rapid elevation in mitochondrial calcium and ROS upon reperfusion leads to a rapid opening of the pore. This scenario has been confirmed in isolated myocytes and whole heart preparations by a variety of techniques.

Survival kinases control mPTP inhibition

Hausenloy and colleagues [71] termed PI3 kinase, Akt and ERK "reperfusion injury survival kinases" (RISK). Juhasova et al [100] demonstrated the tight coupling between these kinases and mPTP formation in an elegant isolated cardiomyocyte model. It is believed that RISK act to prevent mPTP formation in the reperfused heart. The dynamics can be illustrated by transiently inhibiting one RISK. When a 20-min pulse of wortmannin, a PI3 kinase antagonist, was administered at the onset of reperfusion in IPC hearts, protection was eliminated [12]. If the pulse was delayed for 30 min after the start of reperfusion, protection was still abolished. However, if the wortmannin infusion was started after an hour of reperfusion, protection was no longer affected indicating that the hearts had recovered from the injury incurred during ischemia and no longer needed the support of RISK.

While the importance of RISK has been clearly demonstrated in rat and rabbit hearts, their involvement may not be universal. In a recent study using a well established pig heart model activation of RISK was not increased by ischemic postconditioning over that seen in control hearts without postconditioning [104]. Furthermore wortmannin could not abort postconditioning's protection.

A_{2b}AR are essential for IPC

In rabbit hearts RISK appear to be under direct control of A_{2b}AR. At the beginning of reperfusion PKC activity driven by redox signaling is thought to decrease the threshold needed for adenosine to activate A_{2b}AR. In IPC hearts endogenous adenosine would activate RISK through A_{2b}AR. We have proposed that A_{2b}AR binding at reperfusion is needed for protection since MRS 1754, a highly selective A_{2b}AR antagonist, can block both ischemic postconditioning [70] as well as IPC [12]. Until the discovery of Bay 60-6583 in 2006 [74] there were few tools for study of A_{2b}AR. Bay 60-6583 is an agonist with better than 1,000:1 selectivity for A_{2b}AR over A_1, A_{2a} and A_3 receptors. Bay 60-6583 administered at reperfusion for 1 h had the same cardiac protective effect as IPC and this effect could be blocked by MRS 1754 [74]. Additionally A_{2b}AR knockout mice could not be preconditioned, while A_1, A_{2a} or A_3 knockout mice could still be protected by IPC [65].

A_{2b}AR agonists given in the first minutes of reperfusion could elevate the level of phosphorylated survival kinases Akt and ERK1/2 similar to IPC [62]. Blocking either of the RISK abolished the protection of either IPC or A_{2b}AR agonists. Also the A_{2b} adenosine receptor antagonist MRS 1754 blocked phosphorylation of survival kinases by Bay 60-6583. However, there is still some controversy surrounding identity of the adenosine receptor subtype involved in activation of RISK at reperfusion to produce IPC's protection. Xi et al. [105] proposed that both A_{2a}AR and A_{2b}AR occupation is required at reperfusion in a rat heart model. Since A_{2a}AR have such a high affinity for adenosine, they would indeed be occupied at the

end of ischemia in most species explaining why Bay 60-6583 administration alone can protect. Vinten-Johansen's group [106,107] suggested that A_{2a}AR are involved in the protection at reperfusion by inhibiting endothelial-neutrophil interactions. Unfortunately, the selective A_{2a}AR antagonists that would be needed to resolve the controversy are still not available.

Conclusions and future directions

The mechanism of IPC's powerful anti-infarct effect is becoming more and more clear. The discovery that much of IPC's protective effect occurs at reperfusion has opened the door to the treatment of AMI. Already several IPC-based interventions, including ischemic postconditioning, cyclosporin A [108], and atrial natriuretic peptide [83], have produced very encouraging results in clinical trials, and at the time of this writing several large-scale trials are being organized. There are still many details to be worked out for the surprisingly complex mechanism of IPC. For example, the identity of the memory whereby IPC hearts stay in a protected phenotype for hours after only a brief period of ischemia is unknown. Nor is the link between RISK and mPTP understood. We personally think that investigating these details would be time well spent as many novel signaling modalities have already been revealed by these studies such as mitochondrial redox signaling. More importantly these future studies should reveal simpler and even more effective therapeutic interventions for protecting the reperfused heart.

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