

Theme Issue Article

Signalling pathways in ischaemic postconditioning

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Summary

Coronary heart disease (CHD) is the leading cause of death globally. Following an acute coronary artery occlusion, timely myocardial reperfusion using either primary percutaneous coronary intervention (PCI) or thrombolytic therapy remains the most effective treatment strategy for reducing myocardial infarct size, preventing left ventricular remodelling, preserving left ventricular systolic function and improving clinical outcomes. However, the full benefits of myocardial reperfusion are not realised, given that the actual process of reperfusing ischaemic myocardium can independently induce cell death – a phenomenon termed lethal reperfusion injury. Ischaemic postconditioning represents an innovative treatment strategy for limiting lethal myocardial reperfusion injury and further reducing myocardial infarct size for those patients undergoing primary PCI. It is achieved by interrupting the normal myocardial reperfusion

process, with several intermittent episodes of coronary myocardial ischaemia induced by low-pressure inflations of the angioplasty balloon in the infarct-related coronary artery. Experimental studies demonstrate that this stuttered form of myocardial reperfusion improves myocardial perfusion, maintains endothelial function, attenuates apoptotic cell death, reduces myocardial infarct size, preserves left ventricular systolic function and reduces mortality. The mechanisms underlying the cardioprotective effect of ischaemic postconditioning are the subject of intense investigation. In this article we review the signalling pathways which have been implicated as potential mediators of ischaemic postconditioning, the identification of which have provided novel pharmacological targets of cardioprotection capable of recapitulating the protective benefits of ischaemic postconditioning.

Keywords

Reperfusion injury, ischaemic postconditioning, protein kinases, mitochondrial permeability transition pore, myocardial infarction

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Introduction

The complete thrombotic occlusion of a coronary artery at the site of a ruptured atherosclerotic plaque, heralds the onset of an acute myocardial infarction (AMI), a major manifestation of coronary heart disease (CHD), the leading cause of death worldwide. The introduction of ischaemic postconditioning (IPost) as an interventional treatment strategy for limiting myocardial infarct size and preserving left ventricular (LV) systolic function in AMI patients undergoing primary percutaneous coronary intervention (PCI) (1, 2) has emerged as an exciting innovative treatment strategy. Importantly, it confirms the existence of lethal myocardial reperfusion injury in man, and has regenerated interest in the myocardial reperfusion phase as a viable target for cardioprotection (3).

Although Zhao et al. (4) were the first to demonstrate in 2003 that intermittent myocardial reperfusion, using four-30 second

(s) cycles of ischaemia/reperfusion was capable of reducing myocardial infarct size in the canine heart, the actual term ‘postconditioning’ was first used by Na et al. in 1996 (5). In this earlier study, it was reported that intermittent myocardial reperfusion, using repeated cycles of 5 s reperfusion and 35 s ischaemia for the first 20 minutes of myocardial reperfusion, was able to reduce reperfusion arrhythmias in the feline heart. However, the concept of interfering with the myocardial reperfusion phase by either stuttering or gradually reperfusing had been originally proposed in the 1980s as a treatment strategy for reducing myocardial reperfusion injury (6, 7). All things considered, the concept of IPost has given rise to an intensive search for the mechanism of protection, a crucial part of which can be attributed to the underlying signalling pathways. This review will focus primarily on the signalling pathways recruited during IPost. For more comprehensive accounts of IPost, the reader is kindly directed to the following articles (8–11).

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Signalling pathways underlying ischaemic postconditioning (IPost)

When originally described IPost appeared to be a relatively passive mechanical process, protecting the heart against the detrimental effects of lethal myocardial reperfusion injury by limiting oxidative stress, reducing calcium accumulation, maintaining endothelial function and reducing inflammation (12), in a similar manner to gradual or low pressure myocardial reperfusion (6, 7). However, subsequent studies have identified a number of signalling pathways which are recruited by IPost, many of which are fundamental to the cardioprotective effects elicited by IPost. These can be divided into three tiers, beginning with specific cell-surface receptors responsible for activating a number of signalling kinase cascades, many of which appear to converge on the mitochondria.

Cell-surface receptor activation

A number of experimental studies have linked the activation of specific cell-surface receptors at the time of reperfusion with IPost-protection, a property it shares with ischaemic preconditioning (IPC), its pre-ischaemic counterpart (13, 14). In general, the IPost stimulus results in the activation of a number of G-protein coupled receptors which in turn activate a complex array of intracellular signalling pathways, many of which are also recruited in the context of IPC (14).

Adenosine receptor activation

The first G-protein coupled receptor (GPCR) to be linked to IPost was the adenosine receptor. An experimental study by Downey's research group (15) was the first to observe that the reduction in myocardial infarct size elicited by IPost could be abolished in the presence of 8-p-(sulfophenyl) theophylline, a non-specific adenosine receptor blocker. Interestingly, some 14 years earlier, the same research group had been responsible for first implicating adenosine receptor activation with the phenomenon of IPC (16).

Following this important finding, it was not readily apparent why adenosine receptor activation should be critical for IPost protection, given that non-treated control hearts which were also bathed in adenosine were unprotected. In this regard, Vinten-Johansen's group (17) found that IPost delayed the washout of adenosine generated during ischaemia-reperfusion in murine hearts, and it was postulated that this may result in greater activation of adenosine receptors in the postconditioned hearts. Recent attention has now focused on the specific adenosine receptor subtypes underlying IPost protection. It has been postulated that the selective activation of a particular receptor subtype may be critical to protection, although the mechanism conferring such selectivity, in the presence of large amounts of extracellular adenosine, is still unclear. In this regard, the use of pharmacological inhibitors to the different receptor subtypes have implicated the A2A and A3 but not the A1 receptor subtypes in the *in vivo* postconditioned rat heart (17), whereas, in direct contrast, the A2B but not the A1 or A2A receptor subtypes have been implicated in another study using perfused postconditioned rabbit hearts (18). The results from studies using transgenic mice lacking the A1 (19) and A2A receptors (20), suggesting that these are the adeno-

sine receptor subtypes which are critical for IPost protection, has confused the field further. Whether, mice lacking the A2B or A3 receptors are amenable to IPost remains to be determined.

Downey's group have proposed that protein kinase G (PKG) via protein kinase C (PKC) may be responsible for sensitizing the A2B receptor subtype to endogenous adenosine in preconditioned hearts and in hearts administered PKG at the time of myocardial reperfusion (21, 22). However, evidence that this interesting mechanism is actually operating in the setting of IPost is currently lacking.

Bradykinin receptor activation

A subsequent study by Penna et al. (23) has implicated the endogenous activation of the GPCR, bradykinin B2, in IPost protection. These authors first demonstrated that two different pharmacological antagonists of the bradykinin B2 receptor abolished IPost protection in perfused rat hearts (23). Interestingly, the authors found that administering bradykinin in early reperfusion only reduced myocardial infarct size if it was perfused in an intermittent manner, in much the same way as IPost is applied using intermittent reperfusion. However, the requirement for intermittent perfusion with bradykinin to confer cardioprotection is unclear, and is in conflict with previous studies reporting cardioprotection with bradykinin perfused continuously at the time of reperfusion (24, 25).

Through the use of various pharmacological inhibitors, the nitric oxide synthase (NOS), cGMP, mitochondrial K_{ATP} (mKATP) channels and reactive oxygen species (ROS) signalling pathway has been reported to mediate bradykinin protection in early reperfusion (23). Another signalling pathway identified in bradykinin-induced cardioprotection is the inhibition of cyclooxygenase enzymes and the generation of prostacyclin PGI₂ (26). This finding has potential clinical implications in that the presence of COX inhibitors may antagonise the protection elicited by IPost in patients presenting with an AMI. Finally, the recent finding that mice lacking the bradykinin B2 receptor were resistant to IPost protection, provides genetic evidence for the obligatory role of endogenous bradykinin B2 receptor activation in the setting of IPost (19). The role for the bradykinin B1 receptor was less clear, as the mice were partially protected by the IPost stimulus (19).

Opioid receptor activation

Recently, Zatta et al. (27) have provided the first experimental evidence to link IPost with the endogenous activation of the opioid GPCR. After demonstrating that the non-specific opioid receptor antagonist, naloxone, was capable of abolishing IPost-protection in the intact rat heart, they investigated the effect of IPost in the presence of pharmacological antagonists of the δ -, κ -, and μ -opioid receptors (27). The data implicated endogenous stimulation of the μ - and possibly the δ -opioid receptors in the setting of IPost (27). In addition, hearts subjected to IPost accumulated higher levels of pro-enkephalin, suggesting perhaps that IPost was capable of increasing endogenous opioid content in the reperfused myocardium (27). A subsequent study has provided further evidence supporting a role for the δ -opioid receptor, and has linked protection initiated by this signalling transduction pathway to nitric oxide, guanylyl cyclase, protein kinase

G and the inhibition of the mitochondrial permeability transition pore (mPTP - see later) (28).

Other receptors

Other G-protein coupled receptors which have been linked to IPost protection using pharmacological inhibitors include preliminary evidence implicating the protease activated receptor 2 (PAR2) (29) and particulate guanylyl cyclase, the natriuretic peptide receptor (30). Experimental evidence exists supporting the pharmacological activation of these receptors as conferring cardioprotection but the mechanism through which these receptors are activated in the setting of IPost, and the downstream signalling pathways which convey the protective effect require further study.

Signal transduction pathways

A number of different signal transduction pathways have been reported to underlie the cardioprotective effect of IPost. These signalling cascades have been comprehensively reviewed in several recent articles (10, 31), and only an overview of the major signalling cascades will be provided here. Among these, the Reperfusion Injury Salvage Kinase (RISK) pathway was the first signalling cascade to be linked to IPost (32, 33), a finding which provided the first line of evidence that IPost was capable of recruiting pro-survival signal transduction cascades.

The reperfusion injury salvage kinase (RISK) pathway

In 1999, our research laboratory first introduced the concept of a pro-survival reperfusion signalling pathway (34), which we subsequently termed the reperfusion injury salvage kinase (RISK) pathway (reviewed in [35, 36]). We and others have demonstrated that the pharmacological activation of pro-survival kinases such as Akt and Erk1/2 (the RISK pathway) at the immediate onset of myocardial reperfusion using a diverse variety of agents, which include growth factors, cytokines, GPCR agonists, natriuretic peptide, adipocytokines, and 'Statins' reduces myocardial infarct size in the region of 40–50% (35, 36). Our laboratory and others have demonstrated that the cardioprotective benefits of IPost are dependent on the activation of Akt and Erk1/2 at the immediate onset of myocardial reperfusion (32, 33). Subsequent studies have confirmed the role for Akt and Erk1/2 in the setting of IPost in both non-diseased animal hearts and diseased ones (37, 38) as well as human atrial muscle (39). Interestingly, obese mice have been reported to be resistant to IPost protection, and this finding was associated with insufficient activation of the RISK pathway in the hearts harvested from obese animals compared to control ones (40). This finding underscores the importance of using relevant experimental animal models capable of simulating disease pathologies present in patients with coronary heart disease (reviewed in [41]).

It is interesting to note that a couple of experimental studies have failed to implicate components of the RISK pathway as mediators of IPost-protection (42, 43), although this may have been due to differences in experimental model, species or the protocol used.

The mechanistic pathways underlying the cardioprotection mediated by the RISK pathway are currently being investigated and experimental studies have implicated the mitochondrial per-

meability transition pore (44, 45), the sarcoplasmic reticulum function (46), and a number of anti-apoptotic signalling pathways as potential effectors of protection. The actual mechanism through which the RISK pathway is actually recruited in the setting of IPost is unresolved, although experimental data suggest that it may be due to activation of cell-surface receptors including the adenosine A2A receptor (20). Other studies have placed its activation downstream of other signalling elements such as sphingosine kinase (47) and the recovery of neutral pH in the first few minutes of myocardial reperfusion (48) – see later sections.

One particular downstream target of the RISK pathway which has been investigated in the setting of IPost is glycogen synthase kinase (GSK-3 β), a protein kinase linked to the regulation of a variety of cellular functions including glycogen metabolism, gene expression, and cellular survival. Experimental studies have demonstrated that the phosphorylation and inhibition of GSK-3 β confers cardioprotective effects through its potential mitochondrial effects which include the inhibition of mPTP opening (49) and the control of mitochondrial adenine nucleotide transport through the outer mitochondrial membrane (50). Two recent studies have examined the role of GSK-3 β as an obligatory mediator of IPost using transgenic mice and have arrived at conflicting conclusions (51, 52). Gomez et al. (51) found that mice containing a mutant form of GSK-3 β (which cannot be phosphorylated and inhibited) were resistant to the myocardial infarct-limiting effects of IPost *in situ*, suggesting that GSK-3 β inactivation is required for IPost. However, an unconventional IPost protocol was used in this study (3x60 s cycles of ischaemia/reperfusion), and one has to speculate whether a more standard IPost protocol (6x10 s cycles of ischaemia/reperfusion) may have elicited cardioprotection in the genetic mutants. In contrast, Nishino et al. (52) have reported that mice with a mutant form of both GSK-3 β and GSK-3 α in which the Akt phosphorylation sites were changed, thereby rendering them to resistant to inactivation, were still amenable to the myocardial infarct-limiting effects of both IPC and IPost suggesting that GSK-3 β and GSK-3 α inactivation is not necessary for cardioprotection in these settings. Therefore, the exact role GSK-3 β plays in the setting of IPost is currently unclear.

Most interest has focused on the Erk1/2 member of the mitogen activated protein kinase family (MAPK). However, two other members of the MAPK family have been examined in the context of IPost, namely JNK and p38MAPK. Their role in cardioprotection in general and in the phenomena of IPC have been much debated in the literature (31, 53). The only study to investigate the role of these kinases suggests that these MAPK's are inhibited in the setting of adult rat cardiomyocytes subjected to simulated IPost (54), suggest that their activation at the onset of myocardial reperfusion is detrimental.

The JAK-STAT pathway

The Janus Kinase (JAK)-Signal transducer and activator of transcription (STAT) pathway conveys extracellular stress signals from interleukin (IL)-6 type cytokine receptors on the plasma membrane to the nucleus where a array of proteins are transcribed relating to a variety of cellular processes including the second window of protection (SWOP), which refers to the car-

dioprotective effect which appears 12–24 hours following the preconditioning stimulus (reviewed in [55]). More recently, the JAK-STAT pathway has been implicated as a mediator of acute cardioprotection in the settings of classical IPC and IPost. Experimental studies have reported that pharmacologically inhibiting the JAK-STAT pathway at the onset of myocardial reperfusion abrogates the infarct-limiting effects of both IPC (56) and IPost (57, 58). However, mice with a cardiac-specific STAT3 deletion were found to still be amenable to the infarct-limiting effects of IPost, providing a suitable IPost protocol was used i.e. IPost using 5x5 s cycles of ischaemia/reperfusion reduced myocardial infarct size but 3x10 s cycles did not (57). Using mice with the same cardiac-restricted STAT-3 deletion, Goodman et al. (58) were able to demonstrate improved LV function using the IPost protocol of 3x10 s cycles of ischaemia/reperfusion, suggesting that STAT-3 may not be an obligatory mediator of IPost. The mechanism underlying the acute form of myocardial protection mediated by the JAK-STAT pathway is unclear but may relate to as yet unidentified mitochondrial effects (55).

IPost studies suggest that during the pre-ischaemic trigger phase of IPC, dual signalling of the JAK-STAT and the PI3K-Akt signalling cascades may be required for cardioprotection (59). However, at the onset of reperfusion and in the context of IPost, the experimental data exploring the interaction between the JAK-STAT pathway and the PI3K-Akt pathway is inconclusive – pharmacological inhibition of STAT3 but not JAK attenuated the phosphorylation of Akt in postconditioned rat hearts, but in postconditioned mice containing the cardiac-specific deletion of STAT3, there was no change in Akt phosphorylation (58). The mechanism responsible for the activation of the JAK-STAT pathway in the setting of IPost is unknown, but it is presumably due to an endogenous cytokine binding to its IL-6-type cytokine receptor in early reperfusion, although this remains to be demonstrated.

Sphingosine kinase

Sphingosine kinase (SPK) is a lipid kinase which generates sphingosine 1 phosphate (S1P), which in turn regulates cell mitosis, apoptosis, cytoskeletal rearrangement, and survival (60). Jin et al. (47) have recently demonstrated an obligatory role for SPhK1 as a mediator of IPost-protection, which is potentially upstream of the RISK pathway. The authors reported that hearts isolated from mice lacking SPhK1, sustained larger myocardial infarcts, were resistant to IPost, and did not demonstrate activation of the Akt and Erk1/2 components of the RISK pathway in response to IPost (47). One can postulate that the S1P formed by SPhK in early reperfusion moves into the extracellular space and activates the S1P-receptor, which then recruits the RISK pathway – whether this mechanism actually operates in the setting in IPost needs to be demonstrated. Interestingly, they also found that the activation of the RISK pathway and generation of S1P was only observed in the infarct-limiting IPost protocol comprising 3x5 s cycles of ischaemia/reperfusion, whereas the non-protective IPost protocols of 3x10 and 3x20 s cycles did not activate the RISK pathway or generate S1P, providing supportive evidence of their mediatory role in IPost (47).

Protein kinase C (PKC)

It is well-established that PKC acts as a critical mediator of protection in the setting of IPC, providing for the ‘memory’ elicited by an IPC-stimulus (reviewed in [61]). Recent studies suggest that PKC may actually link the IPC stimulus to events occurring in the early reperfusion phase (22, 62), where many of the signalling pathways recruited in response to the IPC stimulus prior to ischaemia appear to be re-activated at reperfusion (13, 63). Crucially, IPost has been reported to also be dependent on PKC activation. Penna et al. (64) were the first to demonstrate that the non-specific PKC inhibitor could abolish the infarct-limiting effects of IPost in perfused rat hearts, suggesting that IPost required the activation of PKC to confer cardioprotection. A subsequent study by Zatta et al. (65) have found that IPost-protection could be abolished by pharmacological inhibition of the PKC- ϵ isoform in early reperfusion. The translocation to the mitochondria of the detrimental PKC- δ , was reduced in postconditioned hearts. The mechanism through which IPost activates PKC is unclear. With respect to the cardioprotective effects of PKC, it has been postulated that PKC may sensitise the adenosine A2B receptor on the cell surface (18, 22) and that a special mitochondrial pool of PKC- ϵ confers inhibition of mitochondrial permeability transition pore (mPTP) opening (66) (see later).

The nitric oxide-cGMP-PKG signalling cascade

PKG has emerged as a critical mediator of cardioprotection in both IPC and IPost (reviewed in [67]). Much of the experimental data suggests that in the setting of IPC, it forms the final link in the signalling pathway which begins at the plasma membrane and terminates at the mitochondria (68). In the context of IPost, its myocardial infarct-limiting effects have been demonstrated to be sensitive to pharmacological inhibition of the NO-sGC-cGMP-PKG pathway (15, 69). Activated PKG at the level of the mitochondria is then believed to open the adenosine triphosphate (ATP)-sensitive mitochondrial potassium channel through PKC- ϵ (68). This pathway is presumed to be activated through the Akt component of the RISK pathway via eNOS in response to IPost (32). The downstream target of this pathway is believed to be PKC, resulting in sensitization of the adenosine A2B receptor (22) or the inhibition of mPTP opening (see later) (70, 71).

Hydrogen sulphide

Hydrogen sulphide (H_2S) is a gaseous signalling mediator which has been reported in experimental studies to protect the ischaemic heart, contribute to IPC-protection (72) and activate the RISK pathway (73). Recently, Yong et al. (74) have demonstrated that pharmacologically inhibiting H_2S abrogated the infarct-limiting effects of IPost in perfused rat hearts, suggesting a role for H_2S as an endogenous signalling mediator of IPost. Furthermore, IPost was reported to stimulate the production of H_2S -generating enzymes, although the mechanism for this is unclear, and the administration of exogenous H_2S in early reperfusion was also found to be cardioprotective (74). Importantly, H_2S -induced cardioprotection was reported to be dependent on the activation of the Akt, Erk1/2 and PKC components of the RISK pathway (74).

Calcitonin gene-related peptide

The neurotransmitter calcitonin gene-related peptide (CGRP), which is predominantly released by capsaicin-sensitive sensory nerves, has been linked to IPC protection (75). Li et al. (76) have demonstrated using the perfused rat heart, that pharmacologically inhibiting CGRP, or using capsaicin to deplete CGRP from the sensory nerves, abrogated the infarct-limiting effects of IPost, suggesting that endogenous CGRP acts as a signalling mediator in the setting of IPost. In addition, exogenous CGRP administered at the onset of myocardial reperfusion was able to recapitulate IPost cardioprotection (76). The downstream targets for cardioprotection in this setting are unclear and require further investigation.

Mitochondria as the end-effectors of protection

Many of the signalling pathways conveying the cardioprotective signal of both IPC and IPost appear to converge on the mitochondria, which may come as no great surprise given the critical role this organelle plays in terms of survival and death of the cardiomyocyte. The mechanism through which the signalling cascade actually terminates on the mitochondria is a matter of debate, but the current paradigm proposes that certain protein kinases are able to act at the level of the mitochondria (66), although this is controversial, with the issue being the inability of some investigators to demonstrate the presence of these kinases in mitochondria (77). An alternative mechanism is that these signalling pathways act on other structures within the cell, such as the sarcoplasmic reticulum (46), which then impact on mitochondria. Abdallah et al. (46) have demonstrated that the pharmacological activation of PI3K-Akt and PKG components of the RISK pathway increases the uptake of calcium into the sarcoplasmic reticulum, which would be expected to be beneficial at time of myocardial reperfusion—however, whether this particular mechanism operates in the setting of IPost is unknown.

The mitochondrial permeability transition pore

The mitochondrial permeability transition pore (mPTP) has emerged as a critical target for cardioprotection (reviewed in [78, 79]), a cardioprotective strategy which has been recently applied to the clinical setting of an acute myocardial infarction (80). The irreversible opening of this large non-selective channel in the mitochondrial inner membrane in response to the abrupt reperfusion of ischaemic myocardium causes cardiomyocyte death by uncoupling oxidative phosphorylation. The prevailing conditions of calcium and phosphate overload, oxidative stress, the rapid restoration of neutral pH and ATP depletion present in the first few minutes of myocardial reperfusion are responsible for its opening at this time. Although a regulatory role for mitochondrial cyclophilin D has been demonstrated (81, 82), the molecular identity of the mPTP remains unclear. Experimental studies have excluded the voltage-dependent anion channel (83) and adenine nucleotide translocase (84) from being obligatory components of the mPTP, with a recent study suggesting that the mitochondrial phosphate carrier may play a role (85). Preventing its opening at the onset of myocardial reperfusion using pharmacological mPTP inhibitors (78, 86, 87) or genetically ablating one of its critical components (81, 82, 88), reduces myocardial infarct size by 40–50%, underlining its importance as a target for

cardioprotection. Both IPC (86, 89, 90) and IPost (91) have been reported to prevent mPTP opening at the onset of myocardial reperfusion, although the mechanism underlying this effect is currently unclear. It may involve components of the RISK pathway such as Akt, Erk1/2 or GSK-3 β (44, 45) and/or changes in intracellular pH in the first few moments of myocardial reperfusion (see later) (48, 92).

Argaud et al. (91) found that mitochondria isolated from a perfused rabbit heart which had been subjected to a standard IPost protocol, were more resistant to calcium-induced opening of the mPTP, suggesting that IPost was capable of inhibiting mPTP opening. A subsequent study by the same group, reported that this inhibitory effect on mPTP opening was sensitive to pharmacological PI3K inhibition using either wortmannin or LY294002 at the onset of reperfusion, suggesting that IPost mediated mPTP inhibition through the activation of the PI3K-Akt pathway (45). We have demonstrated that mice lacking cyclophilin-D are resistant to IPost providing confirmatory evidence supporting the role of the mPTP in IPost (88). Further studies suggest that the changes in cellular pH in early reperfusion of postconditioned hearts may also contribute to the inhibition of mPTP opening (92) (see later).

Intracellular pH

During myocardial ischaemia, intracellular pH decreases (pH<7.0) due to the formation of lactic acid, a change which contributes to the sodium and calcium accumulation during this time. In the first few minutes of myocardial reperfusion there is a rapid restoration of neutral pH within the cardiomyocyte, mediated by the wash-out of lactic acid and the actions of the Na⁺-H⁺ exchanger (NHE) and the Na⁺-HCO⁻ co-transporter. Interestingly, IPost (48, 92, 93) and possibly IPC (62) are able to delay the restoration of neutral pH in the early moments of myocardial reperfusion. This transient acidosis in the first couple of minutes of reperfusion may be sufficient to permit the activation of the RISK pathway (48), suppress mPTP opening (92), inhibit cardiomyocyte hypercontracture, and prevent detrimental calcium activation (93), over this critical period of time.

The mechanism through which IPost modifies cellular pH in early reperfusion is currently unclear, but it has been attributed to delayed wash-out of lactate, but another potential explanation could be the inhibition of the NHE by the RISK pathway, which is activated in IPost-treated hearts. A recent study by Avkiran's laboratory (94) has demonstrated that Akt is able to phosphorylate and inhibit the actions of NHE in cardiomyocytes, and so whether this process occurs in postconditioned hearts is an interesting possibility.

The ATP-dependent mitochondrial potassium channel

Previous experimental studies have suggested that the ATP-dependent mitochondrial potassium (mK_{ATP}) channel plays a pivotal role in IPC (95, 96), and recent studies have implicated it as a potential mediator of IPost. However, much of the evidence has been obtained using pharmacological agents to manipulate the mK_{ATP} channel. Studies have demonstrated that the pharmacological inhibition of the mK_{ATP} channel in early reperfusion abolished the infarct-limiting effects of both IPC (62) and IPost (33, 97, 98), but the direct demonstration that either IPC and IPost ac-

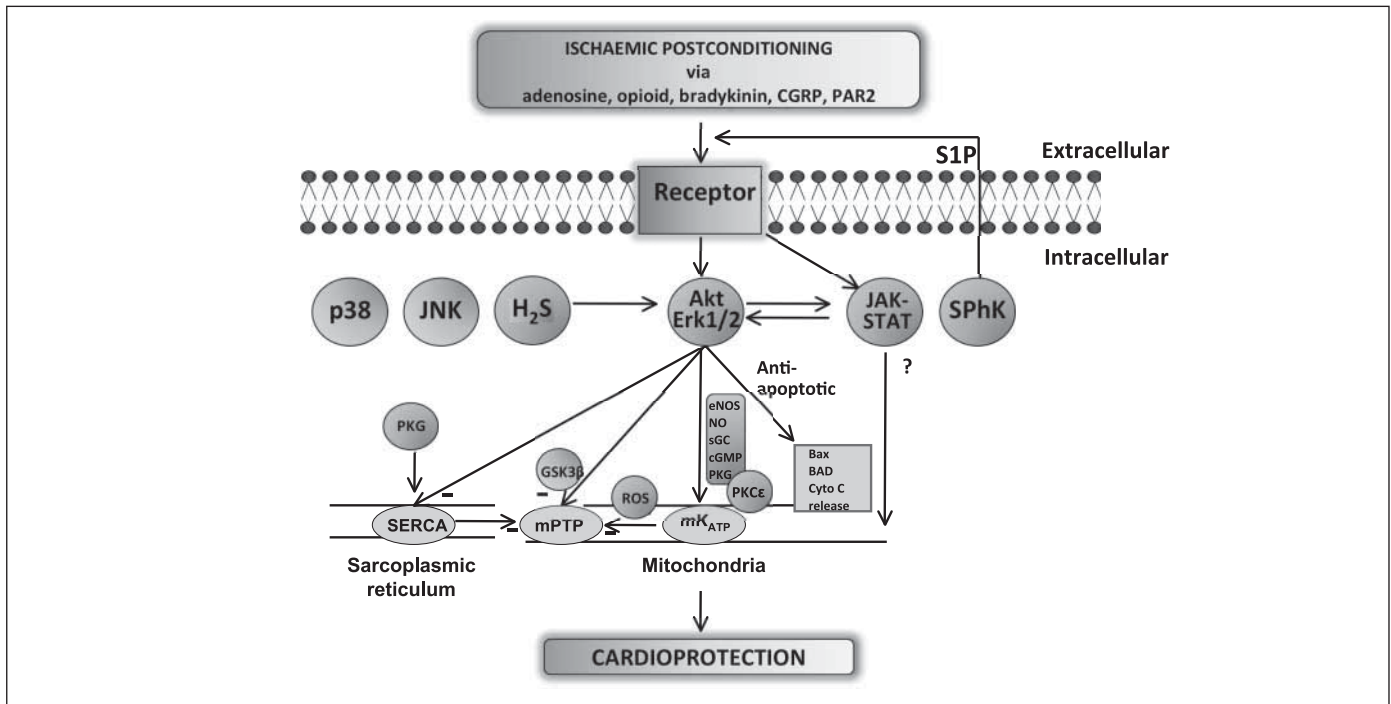


Figure 1: This figure depicts the major signalling pathways recruited at the onset of myocardial reperfusion which mediate the cardioprotective effects of ischaemic postconditioning (IPost). Several different G-protein coupled receptors including the adenosine, bradykinin, opioid, protease activated receptor 2 (PAR2), and sphingosine-1-phosphate (S1P) receptors, the calcitonin-gene related peptide (CGRP) receptor and interleukin (IL)6-type cytokine receptor are believed to initiate the IPost signal in the heart. The activation of the cell-surface receptor activates several signalling cascades including the Akt and Erk1/2 components of the reperfusion injury salvage kinase (RISK) pathway, which then activate downstream targets which terminate at the level of the mitochondria leading to the activation of: (1) GSK-3 β and the subsequent inhibition of the mitochondrial permeability transition pore (mPTP); (2) the activation of protein kinase C (PKC)- ϵ and subsequent opening of the ATP-sensitive mitochondrial potassium (mK_{ATP}) channel, which results in the generation of reactive oxygen

species (ROS), probable hydrogen peroxide, leading to mPTP inhibition. The activation of the Akt, Erk1/2 and PKG components of the RISK pathway has also been reported to activate Sarco/Endoplasmic Reticulum Ca²⁺-ATPase (SERCA), thereby increasing calcium uptake into the sarcoplasmic reticulum via SERCA, potentially preventing mPTP opening. The RISK pathway is also able to recruit several other anti-apoptotic pathways including the phosphorylation and inhibition of the pro-apoptotic factors BAD and BAX, as well as the inhibition of cytochrome C release from the mitochondria and prevention of apoptosis. In addition, IPost is believed to (1) activate sphingosine phosphate kinase (SPhK) which generates sphingosine-1-phosphate (S1P) which moves to the extracellular space and activates the S1P receptor and the downstream RISK pathway; (2) inhibit JNK and p38 MAPK through unknown mechanisms thereby preventing apoptosis; and (3) stimulate the formation of hydrogen sulphide (H₂S) which cardioprotects through the activation of the RISK pathway.

tually induce the opening of this channel in early reperfusion remains to be shown. The question arises as to how IPost results in the opening of the mK_{ATP} channel – whether the mechanism which operates during IPC also occurs in early reperfusion in the setting of IPost needs to be investigated. This mechanism proposes that the signalling pathway initiated by G-protein coupled receptor activation terminates at the level of the mitochondria with the activation of PKG, which then activates the mK_{ATP} channel via mitochondrial PKC- ϵ (66, 68, 71). A recent study by Garland's groups suggests that both IPC and IPost generate a signalosome which conveys the protective signal from the G-protein coupled receptor to the opening of the mK_{ATP} channel which then results in the inhibition of mPTP opening (99).

Signalling ROS

In the context of myocardial ischaemia-reperfusion injury and cardioprotection, ROS have been demonstrated to play dual and opposing roles. The burst of ROS generated in the first few min-

utes of myocardial reperfusion contributes to myocardial injury and cardiomyocyte death, and both IPC (100) and IPost (12) have been reported to reduce the amount of detrimental ROS generated at the onset of myocardial reperfusion. In contrast, ROS also participate as critical signalling mediators in the signal transduction pathway underlying IPC (101). Intriguingly, it appears that ROS signalling in the first few moments of myocardial reperfusion is required for IPost protection (64). Penna et al. (64) were the first to demonstrate that the administration of the non-specific ROS scavenger, N-acetylcysteine (NAC), was able to abrogate the infarct-limiting effect of IPost in perfused rat hearts. Crucially, if NAC was administered after the first three minutes of myocardial reperfusion had elapsed, no effect on IPost was observed, suggesting that ROS signalling during the first couple of minutes of myocardial reperfusion was critical to IPost protection (64). However, the intermittent ROS generation in early myocardial reperfusion failed to confer cardioprotection in isolated perfused rat hearts (23). Subsequent studies using intact

rabbit (102) and murine (103) hearts have confirmed the involvement of a signalling form of ROS in IPost protection.

It is not clear what role signalling ROS may play in early reperfusion in postconditioned hearts, but it is interesting to speculate that the intracellular localization of particular ROS participate in the signalling pathway. It may be that they are required to activate the pro-survival kinases of the RISK pathway, but this remains to be shown. Garlid (71) have proposed that mitochondrial hydrogen peroxide generated in response to PKG activation and the opening of the mitochondrial ATP-dependent potassium channel may lead to mPTP inhibition – whether this process happens in postconditioned hearts remains to be shown directly.

Conclusions

Despite being a form of modified myocardial reperfusion, IPost is actually an active cardioprotective process recruiting a number of intracellular signal transduction pathways which begin with

the generation of autocooids such as adenosine, bradykinin, opioids and others which stimulate their respective plasma membrane receptors on the cardiomyocyte (see Fig. 1 for overview). Several different signalling cascades including the RISK pathway have been implicated and the end-effector of cardioprotection appears to be the mitochondria, and specifically the mitochondrial permeability transition pore. Critically, the identification of the underlying mechanistic pathways opens up the possibility of discovering new pharmacological targets for cardioprotection which can be used to recapitulate the cardioprotection elicited by IPost, thereby obviating the need for an invasive interventional procedure being applied to the heart.

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