Review

Survival kinases in ischemic preconditioning and postconditioning

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Abstract

Despite nearly twenty years of research into the field of ischemic preconditioning, the actual mechanism of protection remains unclear. However, much progress has been made in elucidating the signal transduction pathways that convey the extracellular signal initiated by the preconditioning stimulus to the intracellular targets of cardioprotection, with many of these pathways involving the activation of a diverse array of survival protein kinase cascades. The powerful protective benefits of ischemic preconditioning have not yet been realised in the clinical arena, not least because of the prerequisite for any preconditioning intervention to be applied prior to the onset of index ischemia, which in the case of an acute myocardial infarction is difficult to institute. In this regard, the newly described phenomenon of ischemic postconditioning, which comprises a cardioprotective intervention that can be applied at the time of myocardial reperfusion, offers a far more attractive and amenable approach to myocardial protection. Interestingly, certain survival protein kinase cascades recruited at the time of myocardial reperfusion appear to be shared by both ischemic preconditioning and postconditioning, thereby offering a potentially common target of cardioprotection. The often disputed roles these different protein kinases play in mediating the cardioprotective effects of ischemic preconditioning and postconditioning are reviewed in this article, and include protein kinases C, G, and A, members of the MAPK family (Erk1/2, p38, JNK and BMK1), the PI3K–Akt cascade, and the JAK-STAT pathway.

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1. Introduction

Nearly twenty years after its initial description, the phenomenon of ischemic preconditioning, whereby episodes of intermittent sublethal ischemia and reperfusion confer resistance against a subsequent lethal episode of myocardial ischemia [1], continues to occupy those in the research field investigating its apparent complex mechanism of protection. However, huge progress has been made in the understanding of the signal transduction pathways which convey the extracellular signal, initiated by the preconditioning stimulus, to the intracellular targets of cardioprotection, with many of these pathways involving the activation of a diverse array of survival protein kinases [2].

The practical application of ischemic preconditioning (IPC) in the clinical arena of myocardial ischemia–reperfusion has not materialized, not least because of the prerequisite for any preconditioning intervention to be applied prior to the onset of index ischemia, which in the case of an acute myocardial infarction, is difficult to predict. However, IPC may offer potential benefits in different settings of clinical myocardial ischemia–reperfusion injury, such as at time of cardiac bypass surgery, in which the episode of myocardial ischemia can be predicted (reviewed in Ref. [3]). Intervening at time of myocardial reperfusion, following the onset of an acute myocardial infarction, offers a more attractive and amenable approach to cardioprotection. In this respect, the newly described phenomenon of ischemic postconditioning, in which the application of intermittent episodes of myocardial ischemia and reperfusion at end of the index ischemic period confer a cardioprotective effect on a par to IPC [4], provides one such intervention.
Interestingly, the mechanism of postconditioning-induced protection also comprises signal transduction pathways that involve protein kinases. Somewhat surprisingly, these two cardioprotective phenomena appear to recruit a common signalling pathway at the time of myocardial reperfusion, offering a potentially common target for cardioprotection.

This article reviews the protein kinases that have been implicated in ischemic preconditioning and postconditioning and then describes the protein kinase signaling pathways that appear to be shared by these two diverse cardioprotective phenomena.

2. Protein kinases and ischemic preconditioning

2.1. PKC and ischemic preconditioning

Studies have identified 11 isoforms of protein kinase C (PKC), classified as follows: (1) classical or conventional PKC (α, β1, β2I and γ) which are activated by calcium and lipids (such as diacylglycerol, phosphatidylserine); (2) novel PKC (δ, ε, η, θ, μ) which are activated by lipids but not calcium, and (3) atypical PKC (λ, i, ξ) which are not activated by either lipids or calcium (reviewed in Ref. [2]).

In 1994, Armstrong et al. [5] were the first to implicate PKC as a potential mediator of IPC-induced protection, demonstrating that the specific PKC inhibitor, calphostin, abrogated the protective effect of preconditioning. Subsequent studies from the laboratory of Ytrehus et al. [6] demonstrated indirectly that IPC induced the translocation of PKC from the cytosol to the membrane fraction in isolated rabbit hearts. In addition, the pharmacological activation of PKC also mimicked IPC-induced protection [6]. Our laboratory [7] went on to implicate PKC as a potential mediator of delayed IPC.

2.1.1. Controversial role of PKC in ischemic preconditioning

In contrast, several studies have failed to demonstrate a role for PKC as a mediator of IPC-induced protection [8]. Indeed, some studies have suggested that PKC activation may actually be detrimental, as pharmacologically inhibiting PKC activation was shown to be cardioprotective [9].

2.1.2. Isoform-specific roles of PKC in ischemic preconditioning

Preconditioning studies have implicated PKC-α, PKC-δ, and PKC-ε in the rat heart [10] and PKC-ε and PKC-η in the rabbit heart [11,12].

Interestingly, Inagaki et al. [13] have demonstrated opposing roles for PKC-δ and PKC-ε in cardioprotection, with activation of the former being detrimental and activation of the latter being protective.

2.1.3. Potential upstream activators of PKC in ischemic preconditioning

2.1.3.1. PKC as critical mediator of IPC. Studies have demonstrated PKC to be a point of convergence for a range of preconditioning mimetics that signal through the GPCR including adenosine [14], bradykinin [15], and so on. These GPCR ligands activate PKC via stimulation of phospholipase C, which breaks down membrane lipids to diacylglycerol, which contributes to PKC activation.

2.1.3.2. The signal transduction pathway implicated in IPC. In the current paradigm of signal transduction in IPC, signaling through the PI3K–Akt–eNOS–cGMP–PKG pathway [16,17], leads to the opening of the ATP-dependent mitochondrial potassium (mKATP) channel [18], which is believed in turn to activate survival kinases through the generation of reactive oxygen species (ROS) (see Fig. 1) [19,20]. In support of this paradigm, studies have demonstrated the PI3K–Akt pathway [21], nitric oxide [22], the mKATP channel [23], and ROS [24] to be upstream of PKC activation in the preconditioned heart.

2.1.4. Potential downstream targets of PKC in ischemic preconditioning

2.1.4.1. Ecto-5’-nucleotidase-adenosine as a downstream target of PKC in IPC. Using the intact dog heart, Kitakaze et al. [25] reported that IPC protects the myocardium by phosphorylating and activating ecto-5’-nucleotidase, an enzyme which generates adenosine during myocardial ischemia, in a PKC-α-dependent manner.

2.1.4.2. Reduced intracellular acidification. Studies have demonstrated that the IPC-induced reduction in intracellular acidosis during ischemia (which reduces intracellular Na+ and subsequent Ca2+ loading during ischemia), may be dependent on PKC activation, through the activation of the sodium–hydrogen exchanger [26].

2.1.4.3. The KATP channel as a downstream target of PKC in IPC. Studies have demonstrated that PKC activates sarcolemmal KATP channels [27], and the mKATP channel [28], although the actual role the mKATP channel plays in IPC has been recently contested [29].

2.1.4.4. Other survival kinases as potential downstream targets of PKC in IPC. Studies have demonstrated the activation of other survival kinases such as p38 MAPK [30], JNK MAPK [31], Erk1/2 MAPK [32], tyrosine kinases such as Src and Lck [33] downstream of PKC in the setting of IPC.

2.1.4.5. Reduced apoptotic cell death. Studies have demonstrated that IPC reduces apoptotic cell death an
effect which appears to be dependent on the PKC-ε isoform [34].

2.1.4.6. Reduced oxidative stress at reperfusion. Vanden Hoek et al. [35] reported that hypoxic preconditioning reduced the oxidant stress generated at time of reoxygenation and this anti-oxidant effect may be mediated by PKC.

2.1.4.7. Activation of transcription factors and mediators of delayed preconditioning. In delayed IPC of rabbit myocardium, Bolli’s laboratory have demonstrated that PKC-ε is responsible for activating nuclear transcription factors, such as NF-κB and AP-1 [36], and mediators such as COX-2 [37] and aldose reductase [38].

2.1.4.8. Mitochondria and the mitochondrial permeability transition pore as a downstream target of PKC in IPC. Studies have demonstrated that PKC-ε forms signaling modules with p38, JNK and Erk1/2 at the mitochondria [39]. A recent study suggests that PKC-ε may actually convey the preconditioning signal from PKG in the cytosol to the mKATP channel in the inner mitochondrial membrane [18]. The opening of the mitochondrial permeability transition pore (mPTP) on reperfusion of ischemic myocardium is a critical mediator of cell death [40], and inhibiting
its opening appears fundamental to IPC-induced protection [41,42]. Korge et al. [43] have demonstrated that PKC activation protected rabbit cardiac mitochondria by inhibiting mPTP opening, and another study suggests that PKC-ε can associate with components of the mPTP and inhibit its opening [44], suggesting that PKC-ε may mediate the inhibition of mPTP opening induced by IPC.

2.1.4.9. Gap junctions as a downstream target of PKC in IPC. IPC has been demonstrated to protect the myocardium by reducing gap junction communication through the phosphorylation of Cx43 [45], and studies suggest that the activation of PKC may contribute to connexin-43 phosphorylation [45].

2.2. PKG and ischemic preconditioning

Protein kinase G (PKG) is a cGMP-dependent serine/threonine protein kinase that was first implicated as a potential mediator of IPC in studies demonstrating that cGMP levels were increased in preconditioned hearts [46].

2.2.1. Possible upstream activators of PKG in ischemic preconditioning

Extensive work by Downey’s laboratory [47,48] has elucidated the components of the signal transduction pathway recruited by IPC during the preconditioning protocol (see Fig. 1), with PKG occupying the penultimate step before the mKATP channel [18].

2.2.2. Possible downstream targets of PKG in ischemic preconditioning

2.2.2.1. The KATP channel as a downstream target of PKG in IPC. Studies have suggested that PKG can modulate the sarcolemmal KATP in rabbit myocytes [49], and that PKG mediates the opening of the mKATP channel possibly via PKC-ε, although the actual mechanism remains to be demonstrated [18,47,48,50].

2.2.2.3. Other potential downstream targets of PKG as effectors of protection

Takuma et al. [51], have demonstrated using rat brain mitochondria that cGMP and PKG are able to inhibit mPTP opening. We postulate therefore that PKG activation may mediate the IPC-induced inhibition of mPTP opening that occurs at time of myocardial reperfusion.

Studies suggest that the activation of PKG can reduce gap junction communication [52], although whether the IPC-induced activation of PKG can decrease gap junction conductance remains to be shown directly.

2.3. PKA and ischemic preconditioning

Studies have reported the activation of PKA during the preconditioning protocol and its contribution to IPC-induced protection [53]. Interestingly, in common with p38 MAPK (see Section 2.7.3), there may exist a dual role for PKA in preconditioning, with its activation during ischemia being detrimental and its activation during the preconditioning phase being protective [54].

2.3.1. Potential upstream activators and downstream effectors of PKA in IPC

The activation of PKA during the preconditioning protocol appears to correlate with the generation of ischemia-induced cAMP [55].

Inserte et al. [56] have suggested that IPC protects the myocyte against calpain-mediated sarcolemmal disruption at time of myocardial reperfusion, and that this action may be mediated by PKA.

A study using the intact dog heart has reported that transient activation of PKA during the preconditioning protocol protects the myocardium by inhibiting the small GTPase Rho and its downstream Rho kinase [57], but the subsequent downstream effectors of cardioprotection are unclear.

2.4. Tyrosine kinase and ischemic postconditioning

Tyrosine kinases can be divided into: (1) receptor tyrosine kinases which may act as preconditioning triggers by activating PKC and (2) cytosolic receptor tyrosine kinases which may act as preconditioning mediators by acting downstream or in parallel with PKC.

Maulik et al. [30] demonstrated that genistein, the tyrosine kinase antagonist, could block IPC-induced protection in rat hearts. Studies in swine hearts have suggested that tyrosine kinase may act in parallel to PKC, indicating the presence of redundant parallel pathways in porcine myocardium [58]. Imagawa et al. [59] were the first to demonstrate the role of tyrosine kinase as a potential mediator of myocardial protection induced by delayed IPC.

Studies by Downey’s group [60,61] suggest that Src tyrosine kinase in conjunction with the epidermal growth factor receptor may be required to activate the PI3K–Akt pathway, implicated in IPC-induced protection (see Fig. 1).

The mechanism by which the receptor tyrosine kinases induce cardioprotection is unclear but it may relate to their activation of the mitogen-activated protein kinases.

2.5. The mitogen-activated protein kinase family

The MAPK family comprises 4 major serine/threonine protein kinase cascades displaying the same conserved three-tier module of a MAPK kinase kinase, a MAPK kinase and MAPK [62]. They comprise the 42 and 44-kDa extracellular signal-regulated kinase (Erk1/2), p38, the c-Jun NHP2 terminal kinase (JNK), and the big MAP kinase 1 (BMK1 or Erk5), and they convey extracellular signals to their intracellular targets through the activation of various
intracellular signalling pathways, and control a diverse array of cellular processes [62].

2.6. Erk1/2 and ischemic preconditioning

The Erk MAPK was first described in 1990 as a serine/threonine protein kinase that was tyrosine phosphorylated by various extracellular signals including insulin and NGF [63]. The role of Erk1/2 as a potential mediator of protection in the setting of IPC has been controversial with the majority of studies supporting its role in IPC, although several studies have failed to demonstrate a role for Erk1/2 in IPC (see Tables 1a and b in the on-line supplement).

2.6.1. Upstream activators of Erk1/2 in IPC

2.6.1.1. PKC-ε as an upstream activator of Erk1/2 in IPC. Studies have demonstrated PKC to be an upstream activator of Erk1/2 in the setting of IPC [32].

2.6.1.2. Reactive oxygen species as an upstream activator of Erk1/2 in IPC. Studies have demonstrated that the ROS generated in response to a preconditioning stimulus, is able to activate Erk1/2 (see Fig. 1) [20].

2.6.2. Downstream targets of Erk1/2 in IPC

2.6.2.1. Subcellular localisation and transcription factors. Studies have demonstrated that in response to a preconditioning stimulus Erk1/2 redistributes to the nucleus [32], the intercalated discs [64], the cytosol [65] and the mitochondria [39]. At the nucleus, Erk1/2 has been demonstrated to activate NF-κB [32], and AP-1 [32], transcription factors implicated in delayed IPC.

2.6.2.2. Anti-apoptotic effects of Erk1/2 in IPC. The reduction in apoptotic myocyte death induced by myocardial ischemia–reperfusion injury has been demonstrated to contribute to the protection mediated by IPC [66]. Although Erk1/2 activation has not been examined directly in this setting, its activation would be expected to reduce cell death through several different anti-apoptotic mechanisms (reviewed in Ref. [67]).

2.6.2.3. Mitochondria and the mitochondrial permeability transition pore. In murine myocytes over-expressing PKC-ε, Erk1/2 has been demonstrated to form signalling modules with PKC-ε at the level of mitochondria [39], and PKC-ε has been demonstrated to associate with components of the mPTP and inhibit its opening [44]. Through p90RSK, Erk1/2 can phosphorylate and inhibit GSK-3β [68], the consequence of which is to inhibit mPTP opening in different settings of cardioprotection [69]. Therefore, whether Erk1/2 activation in preconditioned hearts mediates protection by inhibiting mPTP opening needs further investigation.

2.6.2.4. Gap junctions as a downstream target of Erk1/2 in IPC. Interestingly, Gao et al. [70] have demonstrated translocation of Erk1/2 to intercalated discs, the location of gap junctions. Whether Erk1/2 activation reduces gap junction communication as a cardioprotective mechanism in IPC is currently unknown.

2.6.2.5. Hypoxia-inducing factor-1α as a downstream target of Erk1/2 in IPC. Hypoxia-inducing factor-1α (HIF-1α) is activated in response to hypoxia and regulates genes concerned with cellular survival against hypoxia, and has been implicated in mediating the protection induced by IPC [71]. Studies suggest that HIF-1α may be a potential downstream target of Erk1/2 in the setting of preconditioning [72].

2.6.3. Biphasic activation of Erk1/2 in IPC

Recent studies suggest that IPC actually induces two phases of Erk1/2 activation, the first occurring immediately following the IPC stimulus with the second taking place at time of myocardial reperfusion [65,73]. Interestingly, inhibiting the first phase of Erk1/2 activation has been demonstrated to abolish the phase of activation at the time of reperfusion [64], suggesting that the first phase of Erk1/2 activation may be required to execute the second phase of Erk1/2 activation, perhaps through the translocation of Erk1/2 to its demonstrated site of action.

2.7. p38 MAPK and ischemic preconditioning

The p38 MAPK is believed to comprise 4 main isoforms, p38α, p38β p38γ and p38δ [62]. The role of p38 MAPK in the setting of cardioprotection has been surrounded by controversy and has been the topic of several reviews [74,75].

The studies investigating the role of p38 MAPK in cardioprotection have attracted much controversy, and can be divided into the following categories (see Tables 2a–c of the on-line supplement):

1. Those studies demonstrating that preconditioning activates p38 MAPK during ischemia and that inhibiting its activity during ischemia abrogates protection.
2. Studies finding that preconditioning protects by attenuating the ischemia-induced activation of p38 MAPK and that pharmacologically inhibiting p38 MAPK during ischemia–reperfusion confers cardioprotection in non-preconditioned hearts.
3. Studies demonstrating that IPC transiently activates p38 MAPK during the preconditioning phase, but reduces the activation that occurs during ischemia.

2.7.1. p38 MAPK as a mediator of cardioprotection in IPC

Weinbrenner et al. [76] were the first to demonstrate the activation of p38 MAPK during ischemia in precondi-
tioned isolated rabbit hearts. Subsequent studies have confirmed the role of p38 MAPK as a potential mediator of IPC-induced protection (see Table 2a of the on-line supplement).

2.7.1.1. Potential upstream activators of p38 MAPK in preconditioning. In the setting of IPC, studies have demonstrated that both tyrosine kinase and PKC are upstream of p38 MAPK [76] in the signaling pathway. Whether p38 MAPK is activated by the ROS produced in response to mKATP channel opening (see Fig. 1) has not been shown directly.

2.7.1.2. Potential downstream targets of p38 MAPK in IPC.

2.7.1.2.1. MAPKAP kinase 2, HSP 27 and the cytoskeleton. An important substrate of p38 MAPK is the MAPK-activating protein kinase (MAPKAPK) 2, a kinase that has been demonstrated to phosphorylate the small heat shock protein (HSP) 27 [77], which has been demonstrated to stabilise the actin cytoskeleton. Studies have found that hypoxic preconditioning resulted in a p38 MAPK-dependent translocation of the HSP27 from the cytosol to the cytoskeleton [78].

Another small heat shock protein αB crystallin has been demonstrated to confer protection against ischemia–reperfusion injury, and studies have reported its phosphorylation and translocation in response to IPC to be dependent on p38 MAPK [79].

2.7.1.2.2. Mitochondria and mitochondrial permeability transition pore. Baines et al. [39] have reported that in murine hearts over-expressing PKC-ε, p38 MAPK forms signalling modules with PKC-ε at the level of the mitochondria. Further studies are required to elucidate the role p38 MAPK may play at the level of mitochondria in the setting of cardioprotection.

2.7.1.2.3. Gap junctions as a downstream target of p38 MAPK in IPC. Using intact pig hearts, Schulz et al. [45] demonstrated co-localisation of p38 MAPK with connexin-43, and increased activity of p38β MAPK in preconditioned hearts, suggesting that IPC may protect by activating connexin-43 may act to reduce gap junction permeability.

2.7.1.2.4. The subcellular redistribution of p38 MAPK in response to IPC. Recent studies suggest that in response to a preconditioning stimulus p38 MAPK translocates to the nucleus, myofilaments and sarcolemma [64].

2.7.2. p38 MAPK as a detrimental factor in ischemia–reperfusion

Several studies have reported that IPC reduces p38 activity during ischemia and pharmacologically inhibiting p38 MAPK activation, during ischemia–reperfusion is cardioprotective, suggesting that p38 MAPK activation during ischemia is detrimental for cellular survival (see Tables 2b and c of the on-line supplement).

2.7.3. Reconciling the dichotomous role of p38 MAPK in cardioprotection

2.7.3.1. The phase of p38 MAPK activation in IPC.

Studies suggest that IPC can activate [76] or reduce [80] p38 MAPK during the sustained ischemic episode. The generally accepted view is that IPC transiently activates p38 MAPK during the preconditioning phase [31], and reduces the p38 MAPK activation that occurs during the sustained ischemic phase [80].

A recent study suggests that IPC activates p38 MAPK during the post-ischemic reperfusion phase, as well as immediately following IPC [64], indicating biphasic activation of p38 MAPK in response to IPC, as has been observed with both Akt and Erk1/2 (see Section 2.6.3) [73].

2.7.3.2. The importance of the p38 MAPK isoform. Studies have suggested that the p38α isoform may mediate cell death and the p38β isoform may contribute to cell survival [81]. In line with this, studies have suggested that it is the p38α isoform which is increased during ischemia [31,82], and that hypoxic preconditioning protects myocytes by reducing the activation of this isoform during hypoxia [82]. Conversely, Schulz et al. [45] found p38β MAPK activity to be increased in preconditioned swine hearts.

2.7.3.3. The use of pharmacological inhibitors. Many of the studies investigating a role for p38 MAPK in cardioprotection have relied heavily on the use of pharmacological inhibitors of p38 MAPK such as SB203580, which can have non-specific actions such as the inhibition of JNK at higher concentrations [83]. The use of transgenic knockouts of the different p38 MAPK isoforms should help elucidate the role of this kinase in IPC [82].

2.8. JNK and ischemic preconditioning

JNK was the next member of the MAPK family to be revealed. Discovered in 1991, it was found to differ from Erk1/2 in that it appeared to be activated by cellular stress such as heat, osmotic shock, UV light, endotoxins and cytokines, hence the alternative name stress-activated protein kinase (SAPK) [84].

The role of JNK in the setting of IPC has been controversial with studies, suggesting both a protective and detrimental aspect to JNK activation.

2.8.1. JNK activation as a potential mediator in IPC

Studies have demonstrated JNK activation in response to a preconditioning stimulus [85] and that JNK mediates the protective effect of IPC (see Table 3a of the on-line supplement) [86]. Other reports have found JNK activation in response to a preconditioning stimulus but have failed to
find it contributing to protection [87] (see Table 3a of the on-line supplement). Yet other studies have failed to find activation of JNK in the setting of IPC [88] (see Table 3b of the on-line supplement).

### 2.8.1.1. JNK activation as a potential detrimental kinase in IPC

Using a rat model of cerebral ischemia, Gu et al. [89] found that IPC appeared to inhibit the JNK activation which occurred during the index ischemic phase, suggesting a detrimental role for JNK activation in the setting of IPC (see Table 3b of the on-line supplement).

Unfortunately, a recently published study examining the effect of JNK1/2 transgenic knockouts has only served to complicate matters further [90]. In this study transgenic JNK knockout mice were found to be protected in vivo against myocardial ischemia–reperfusion injury. However, transgenic mice with over-active MKK7, the MAPK kinase upstream of JNK1/2 also paradoxically displayed resistance to myocardial ischemia–reperfusion injury [90].

### 2.8.2. Upstream activators of JNK in ischemic preconditioning

#### 2.8.2.1. Reactive oxygen species as an activator of JNK in IPC

Studies have suggested that reactive oxygen species (ROS) may mediate the reperfusion-induced activation of JNK [91]. Whether JNK is activated by ROS generated in response to a preconditioning stimulus is unknown and remains to be demonstrated.

#### 2.8.2.2. PKC-ε as an activator of JNK

Ping et al. [31] demonstrated using rabbit myocytes that hypoxic preconditioning-induced activation of JNK is dependent on PKC-ε.

### 2.8.3. Downstream targets

#### 2.8.3.1. Transcription factors

Li et al. [92] reported that PKC-ε overexpression activated the transcription factors NF-κB and AP-1 (important mediators of delayed preconditioning) in a JNK-dependent manner.

#### 2.8.3.2. Mitochondria

Baines et al. [39] reported that over-expressing active PKC-ε in murine myocytes, resulted in the formation of signalling modules comprising JNK along with Erk1/2 and p38 MAPK in the mitochondrial fraction, although the activity of JNK was not actually increased.

### 2.9. BMK1 and ischemic preconditioning

Recently a new member of the MAPK family has been described, called the big MAP kinase 1 (BMK1 or Erk5), with studies demonstrating that BMK1 activity is increased in preconditioned hearts [93]. Potential protective mechanisms include reduced gap junction permeability [94] and the phosphorylation and inhibition of the pro-apoptotic factor, BAD [95].

### 2.10. PI3K, Akt and ischemic preconditioning

#### 2.10.1. Upstream activators of Akt in ischemic preconditioning

Tyrosine kinase is a known upstream activator of PI3K–Akt [96], and studies have demonstrated Src tyrosine kinase [60] to be upstream of PI3K–Akt activation in the signalling pathway of IPC (see Fig. 1). Interestingly, Wang et al. [98] have demonstrated that diazoxide was able to activate Akt, suggesting that as well as being upstream of the mKATP channel, Akt may also be activated downstream of the mKATP channel, perhaps by the ROS that is generated on opening of the channel.

#### 2.10.2. Downstream targets of Akt in ischemic preconditioning

##### 2.10.2.1. Akt as a mediator of the preconditioning signal

In their study implicating Akt as a mediator of IPC-induced protection, Tong et al. [21] demonstrated IPC-induced Akt phosphorylation to be upstream of both PKC-ε and NO production. This finding has been confirmed by studies from Downey and co-workers demonstrating both eNOS and PKC-ε to be downstream of Akt [16–18] (see Fig. 1).

##### 2.10.2.2. Akt as an effector of preconditioning-induced protection

Many of the downstream targets phosphorylated by Akt activate various anti-apoptotic pathways (reviewed in Ref. [67]). Recent studies have suggested that hypoxic preconditioning inhibits apoptosis in rat myocytes through the activation of Akt [99]. Studies also suggest that Akt may protect the myocardium against ischemia–reperfusion injury by acting at the level of the mitochondria, and inhibiting mPTP opening. A recent study by Juhaszova et al. [69] have demonstrated that a diverse array of cardioprotective agents, including preconditioning mimetics protect...
myocytes by inhibiting mPTP opening, with GSK-3β, a downstream target of Akt, being fundamental to this effect on the mPTP. A recent study by our laboratory has demonstrated that over-expressing Akt protects cells against oxidative stress by inhibiting mPTP opening [100].

2.10.3. Biphasic activation of Akt in IPC

In common with Erk1/2 activation in IPC (see Section 2.6.3), studies suggest that there may also be two phases of Akt activation in response to a preconditioning stimulus [73]. Importantly, studies have demonstrated the second phase of Akt activation which occurs at reperfusion is required for IPC-induced protection [73,101], and that PI3K–Akt activation may be required for up to 50 min of reperfusion [101].

2.11. JAK-STAT and ischemic preconditioning

The Janus kinase (JAK)-signal transducers and activators of transcription (STAT) pathway is a stress-responsive cascade that conveys signals from the cell membrane to the nucleus, where gene expression is modulated (reviewed in Ref. [102]).

In the context of delayed preconditioning, the activation of the JAK-STAT (STAT1 and STAT3) pathway results in transcriptional upregulation of iNOS [103] and COX-2 [104], known distal mediators/effectors of protection. A recent study suggests that a PKC-ε–Raf1–MEK1/2–Erk1/2 signaling module activates COX-2 through the JAK-STAT pathway [37].

2.12. Sphingokinase and ischemic preconditioning

Recent studies have implicated sphingosine-1-phosphate, a lipid-signaling molecule generated by sphingokinase (SK), as a potential mediator of IPC-induced protection [105]. Interestingly, the activation of SK in this setting has been demonstrated to be dependent on PKC-ε activation [105].

2.13. Overview of the survival kinases in ischemic preconditioning

In Fig. 1, we present a simplified hypothetical scheme providing an overview of the important signal transduction pathways recruited in the setting of IPC. The intermittent episodes of myocardial ischemia–reperfusion which comprise an ischemic preconditioning stimulus generate adenosine and bradykinin which activate their GPCR, activating in turn a signal transduction pathway comprising a tyrosine kinase, PI3K, Akt, eNOS, guanylate cyclase, PKG, and PKC-ε, finally terminating at the inner mitochondrial membrane, inducing the opening of the mitochondrial K_{ATP} channel [18]. The opening of this mitochondrial channel is believed to generate reactive oxygen species [19] which are released into the cytosol where they activate other survival kinases implicated in IPC including p38 MAPK, JNK MAPK [91], Erk1/2 MAPK [20], BMK1, PKC [24], tyrosine kinase and Akt. The potential downstream effectors of protection are reviewed in the previous sections (Fig. 1). Many of these survival kinases activate nuclear transcription factors and mediators of delayed IPC. Other less extensively investigated signal transduction pathways implicated in IPC include the JAK-STAT pathway which has been mainly implicated in delayed preconditioning through its activation of nuclear transcription factors, and the cAMP–PKA pathway the consequences of which are less clear (Fig. 1).

3. Protein kinases and ischemic postconditioning

When the concept of ischemic postconditioning was first described in 2003 by Vinten-Johansen’s laboratory [4], the mechanism of protection was initially attributed to the intervention reducing the detrimental effects of lethal reperfusion injury such as reduced oxidative stress, reduced mitochondrial calcium accumulation, improved endothelial function and reduced inflammation. However, subsequent studies suggest that protection is mediated through the recruitment of signal transduction pathways as is the case with IPC [106].

3.1. PI3K–Akt and ischemic postconditioning

Recent studies suggest that ischemic postconditioning protects the myocardium by activating the PI3K–Akt pathway at time of myocardial reperfusion in the isolated rat heart [106,107], the isolated rabbit heart [108] and the intact rabbit heart [109], although a subsequent study in the isolated rabbit heart has failed to demonstrate a role for this kinase cascade in ischemic postconditioning [110].

3.1.1. Upstream activators of Akt in ischemic postconditioning

The mechanism through which ischemic postconditioning activates Akt at time of myocardial reperfusion is currently unclear, although the retention of endogenous adenosine and the stimulation of the A2a adenosine receptor may be partly responsible [108,111], although this remains to be demonstrated directly.

3.1.1.1. Downstream targets of Akt in ischemic postconditioning

The mechanism through which Akt confers protection in the postconditioned heart is currently unclear, although the mechanisms cited in the section on Akt and IPC may be relevant (Section 2.10.2). Of note, studies have demonstrated the activation of p70S6K and eNOS downstream of Akt in postconditioned hearts [106]. In addition, the inhibition of mPTP opening at time of myocardial reperfusion is a potential mechanism [107], especially as Argaud et al. [112] have demonstrated the inhibition of mPTP opening in postconditioned isolated rat hearts.
3.2. Erk1/2 and ischemic postconditioning

Recent studies suggest that ischemic postconditioning protects the myocardium by also activating the MEK1/2–Erk1/2 pathway at time of myocardial reperfusion in the isolated rabbit heart [110,113].

3.2.1. Upstream activators of Erk1/2 in ischemic postconditioning

As with Akt, the mechanism through which Erk1/2 is activated in postconditioned hearts is currently unknown, although adenosine, PKC or ROS could all be potential candidates.

3.2.2. Downstream targets of Erk1/2 in ischemic postconditioning

The mechanism through which Erk1/2 activation mediates protection in postconditioned hearts is unknown and warrants further investigation, although the mechanisms cited in Section 2.6.2 may be relevant.

3.3. PKG and ischemic postconditioning

Preliminary work by Burley and Baxter’s laboratory [115] appears to implicate PKG as a potential mediator of protection in postconditioned hearts. Using the isolated perfused rat heart, the reduction in infarct size observed in postconditioned heart was abrogated by a PKG inhibitor [115].

3.3.1. Upstream activators of PKG in postconditioning

PKG is activated by the nitric oxide (NO)–cGMP pathway and previous studies have implicated NO as a potential mediator of protection in postconditioned hearts [113].

3.3.2. Downstream targets of PKG in postconditioning

PKG has been demonstrated, possibly via PKC-ε, to open the mKATP channel in preconditioned hearts although this is believed to occur pre-ischemically [18], and interestingly, the purported mKATP channel blocker, 5-hydroxydecanoate, has been demonstrated to block protection in postconditioned hearts [113]. Alternatively PKG activation may mediate mPTP inhibition in postconditioned hearts [51].

3.4. PKC and ischemic postconditioning

A recently study by Zatta and colleagues [114] has implicated the survival kinase PKC-ε as a potential mediator of postconditioning-induced protection in intact rat hearts. Further work will be required to ascertain the upstream activators and downstream targets of PKC-ε in this context,

![Fig. 2. Overview of survival kinases in ischemic postconditioning. A simplified hypothetical scheme providing an overview of the important signal transduction pathways recruited in the setting of ischemic postconditioning. Generation of adenosine in postconditioning activates the survival kinases Akt, Erk1/2 and PKG. The actual role of tyrosine kinase (TK) as a mediator of postconditioning-induced protection has not actually been demonstrated. GPCR, G protein coupled receptor; GC, guanylate cyclase. K_ATP, mitochondrial ATP-dependent potassium channel; NO, nitric oxide.](https://academic.oup.com/cardiovascres/article-abstract/70/2/240/283226)
although the candidates referred to in Section 2.1 may also apply here.

3.5. Overview of the survival kinases in ischemic postconditioning

The field of ischemic postconditioning is still in its infancy, and so far only four protein kinases have been examined as potential mediators of postconditioning-induced protection, and these are depicted in Fig. 2.

Further studies are required to determine whether other survival kinases activated at time of myocardial reperfusion such as, JNK MAPK and p38 MAPK, contribute to the protection observed in the postconditioned heart.

4. Protein kinase cascades shared by ischemic preconditioning and postconditioning

Interestingly, it appears that there exists protein kinase cascades which are activated at the time of myocardial reperfusion by both ischemic preconditioning and postconditioning [116,117]. These include the PI3K–Akt and the MEK1/2–Erk1/2 pathways, the activation of which at time of myocardial reperfusion has been demonstrated to contribute to the protection induced by ischemic preconditioning [73,101] and postconditioning [3,106,108,113].

The important implications of this finding are that the pharmacological activation of these survival kinase cascades may allow one to harness the powerful cardioprotective benefits of ischemic preconditioning and postconditioning without the need for applying such treatment protocols, which in the clinical settings of myocardial reperfusion injury may be difficult, although a recent study has demonstrated the effective application of an ischemic postconditioning protocol at time of coronary angioplasty for an acute myocardial infarction [118].

5. Controversy surrounding the role of protein kinases in cardioprotection

A recurring theme encountered throughout the course of this article is that the role of protein kinases as potential mediators of protection in the settings of ischemic preconditioning and postconditioning has attracted great controversy irrespective of the protein kinase involved [2,8,74,75]. The reasons for this are manifold and include: (1) the use of different species, varying animal models of cardioprotection, and inconsistent preconditioning and postconditioning stimuli; (2) the phase of kinase activation implicated; (3) the reliance on using pharmacological inhibitors of kinase activity; (4) the reliance on measuring kinase activity, when subcellular redistribution may be more accurate; (5) the presence of different protein kinase isoforms which in some situations may have opposing actions on cell survival, and (6) the fact that many of these protein kinases play fundamental roles in cellular growth, cell cycling and cell survival means that availability of transgenic knockouts of these kinases is a problem.

6. Conclusions

Intensive investigation of the signal transduction mechanisms recruited in the setting of ischemic preconditioning and more recently ischemic postconditioning have implicated a diverse array of survival protein kinase cascades, including protein kinase C, G and A, the MAPK family, the PI3K–Akt cascades and the JAK-STAT pathway. Because of the complex nature and interaction of these kinases, this field of research has often been surrounded in controversy.

Interestingly, ischemic preconditioning has been found to recruit survival kinase cascades at the time of myocardial reperfusion, suggesting that the influence of IPC encompasses the myocardial reperfusion phase. Importantly, some of these kinase pathways activated at time of myocardial reperfusion appear to be shared with ischemic postconditioning, thereby offering a common target of cardioprotection. Therefore the pharmacological activation of the survival kinase cascades PI3K–Akt and MEK1/2–Erk1/2 at the time of myocardial reperfusion (reviewed in Ref. [67]) may be able to offer the powerful cardioprotective potential conferred by ischemic preconditioning and postconditioning.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cardiores.2006.01.017.

References


Imagawa J, Baxter GF, Yellon DM. Genistein, a tyrosine kinase

Lochner A, Genade S, Tromp E, Podzuweit T, Moolman JA.

Makaula S, Lochner A, Genade S, Sack MN, Awan MM, Opie LH.

Takuma K, Phuagphong P, Lee E, Mori K, Baba A, Matsuda T. Anti-

Han J, Kim N, Kim E, Ho WK, Earm YE. Modulation of ATP-

Krieg T, Philipp S, Cui L, Dostmann WR, Downey JM, Cohen

Iliodromitis EK, Papadopoulos CC, Markianos M, Paraskevaidis IA,

Baines CP, Song CX, Zheng YT, Wang GW, Zhang J, Wang OL,


[100] Yang XM, Philipp S, Downey JM, Cohen MV. Postconditioning’s protection is not dependent on circulating blood factors or cells but involves adenosine receptors and requires PI3-kinase and guanylyl cyclase activation. Basic Res Cardiol 2005;100:57–63.


[114] Yang XM, Philipp S, Downey JM, Cohen MV. Postconditioning’s protection is not dependent on circulating blood factors or cells but involves adenosine receptors and requires PI3-kinase and guanylyl cyclase activation. Basic Res Cardiol 2005;100:57–63.


[116] Downey JM, Cohen MV. We think we see a pattern emerging here. Circulation 2005;111:120–1.
