Reperfusion Injury Salvage Kinase and Survivor Activating Factor Enhancement Prosurvival Signaling Pathways in Ischemic Postconditioning: Two Sides of the Same Coin

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Abstract

The discovery of ischemic postconditioning (IPost) has rejuvenated the field of cardioprotection. As an interventional strategy to be applied at the onset of myocardial reperfusion, the transition of IPost from a bench-side curiosity to potential clinical therapy has been impressively rapid. Its existence also confirms the existence of lethal myocardial reperfusion injury in man, suggesting that 40%–50% of the final reperfused myocardial infarct may actually be due to myocardial reperfusion injury. Intensive analysis of the signal transduction pathways underlying IPost has identified similarities with the signaling pathways underlying its preischemic counterpart, ischemic preconditioning. In this article, the reperfusion injury salvage kinase pathway and the more recently described survivor activating factor enhancement pathway, two apparently distinct signaling pathways that actually interact to convey the IPost stimulus from the cell surface to the mitochondria, where many of the prosurvival and death signals appear to converge. The elucidation of the reperfusion signaling pathways underlying IPost may result in the identification of novel pharmacological targets for cardioprotection. *Antioxid. Redox Signal.* 14, 893–907.

Introduction

THE RECENT DISCOVERY in 2003 of ischemic post-conditioning (IPost), an interventional strategy for limiting myocardial infarct size when applied at reperfusion (137), has revitalized the field of cardioprotection. However, the actual term IPost was first coined in 1996 to describe ventricular premature beat-driven intermittent reperfusion protecting against reperfusion-induced ventricular fibrillation (93). And of course many years before this, the concept of modifying the process of myocardial reperfusion as a strategy for infarct-size limitation had been described in experimental studies dating back to the 1980s (99). The term IPost has highlighted the importance of intervening at the onset of myocardial reperfusion to protect the ischemic heart, a clinically more relevant time point for intervention in patients presenting with an acute myocardial infarction. As such, its clinical application has been rapid for both ST-elevation myocardial infarction patients undergoing primary percutaneous coronary intervention (PCI) (120, 125) and for patients undergoing on-pump cardiac surgery (80). Its transition to the clinical setting has proven the existence of lethal myocardial

reperfusion injury in man, and these early clinical studies suggest that 40%–50% of the final reperfused infarct may be attributable to myocardial reperfusion injury (134). Of course, previous attempts to intervene at the onset of reperfusion using pharmacological agents as a strategy for protecting against myocardial reperfusion injury has been extensively investigated, and in terms of translation of the treatment strategy into clinical therapy, this has been hugely disappointing (11, 66, 79, 134). However, initial progress has been made with more novel approaches for preventing myocardial reperfusion injury, such as cyclosporine A (105) as a mitochondrial permeability transit pore inhibitor and atrial natriuretic peptide (65).

In common with its preischemic counterpart, ischemic preconditioning (IPC), intensive investigation of the signaling pathways underlying IPost have identified a number of different signal transduction pathways conveying the cardioprotective signal from the sarcolemma to the mitochondria, some of which overlap with IPC. This article reviews two of these reperfusion signaling pathways, the reperfusion injury salvage kinase (RISK) pathway and the more recently described survivor activating factor enhancement (SAFE)

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pathway, two apparently distinct signal transduction pathways that may actually interact to convey IPost cardioprotection.

The RISK Pathway: Origins, Applications, and Controversies

The actual term RISK pathway was first coined by our research group in 2002, in a study investigating the signal transduction pathway underlying the infarct-limiting effects of urocortin administered at reperfusion (110). In that initial study, the mitogen extraregulated kinase 1/2 (MEK1/2)–extraregulated kinase 1/2 (Erk1/2) prosurvival kinase pathway was implicated as the mediator of urocortin-induced cardioprotection and the first member of the RISK pathway (110). Crucially, the administration of urocortin at the onset of myocardial reperfusion reduced infarct size and increased Erk1/2 activation over and above that achieved by reperfusion alone—importantly, the pharmacological inhibition of urocortin-induced Erk1/2 activation abolished the infarct-limiting effects of urocortin (110).

However, the concept of a cardioprotective antiapoptotic reperfusion pathway was actually first proposed in the late 1990s with the discovery that a variety of growth factors were capable of limiting myocardial infarct size when administered at the immediate onset of myocardial reperfusion (133). However, it must be appreciated that although these growth factors were first investigated for their potential antiapoptotic effects, it has in fact been their dramatic effects on necrosis and reductions in myocardial infarct size, which has been their most important contribution. Of course, the idea of protecting the heart by intervening at the time of reperfusion had been previously explored using a variety of approaches, some of which have been less successful, including antioxidant therapy, calcium channel blockers, anti-inflammatory agents, and so forth, with mixed results (134). However, initial results from more novel approaches such as cyclosporine A (105) and atrial natriuretic peptide (65) have been more promising. The novel aspect, therefore, was the possibility of recruiting prosurvival signaling pathways to protect the heart against lethal myocardial reperfusion injury. In this review article, the focus will be on the involvement of the RISK pathway in IPost signaling. For a more comprehensive account of the RISK pathway, the reader is directed to several recent reviews (17, 45, 48).

Reperfusion Signaling in IPost: The RISK Pathway

In the original description of IPost in 2003 (137), the infarct-limiting effects were attributed to the prevention of myocardial reperfusion injury-induced calcium overload, less oxidative stress, preserved endothelial function, attenuated apoptotic cell death, less myocardial inflammation, and edema. However, the first study to implicate an actual signal transduction pathway as a mediator of IPost was by our research group in 2004 (127). In that study, we demonstrated that six 10-s cycles of reperfusion and ischemia applied at the immediate onset of myocardial reperfusion phosphorylated Akt, endothelial nitric oxide synthase (eNOS), and p70S6K to a greater extent than uninterrupted reperfusion in the isolated perfused rat heart (Fig. 1). Crucially, the pharmacological inhibition of phosphatidyl inositol 3 kinase (PI3K) during the
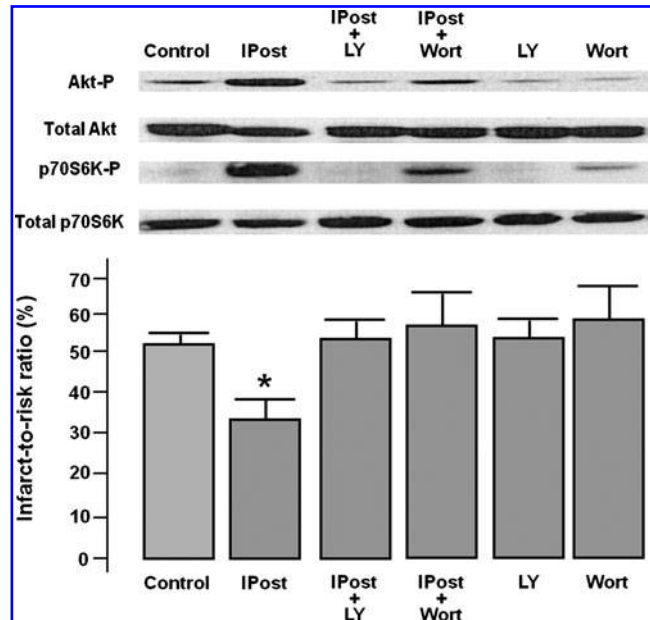
FIG. 1. This graph shows the infarct-to-risk ratios (%) and relative densitometries of Akt and p70S6K phosphorylation during myocardial reperfusion in controls hearts and in hearts treated with ischemic postconditioning (IPost) in the presence or absence of the phosphatidyl inositol 3 kinase inhibitors LY294002 or Wortmannin ($n > 6$ per group; $*p < 0.05$). This figure was adapted from ref. (127).

IPost protocol using either LY294002 or Wortmannin abolished the infarct-limiting effects of IPost and abrogated the phosphorylation of Akt, eNOS, and p70S6K (Fig. 1). Interestingly, the recruitment of the RISK pathway is shared with the phenomenon of IPC (43), suggesting that the RISK may be a common pathway for cardioprotection, which can be activated either prior to ischemia or at the onset of myocardial reperfusion (44, 47).

The first study to implicate the MEK1/2-Erk1/2 component of the RISK pathway was that by Yang *et al.* (132), who demonstrated that pharmacological inhibition of MEK/12 during IPost abrogated cardioprotetion in the isolated rabbit heart. A number of experimental studies have subsequently confirmed the involvement of the RISK pathway in IPost signaling and these are briefly summarized in Table 1. There are several other protein kinases that have been implicated in IPost signaling, which could also be considered components of the RISK pathway, such as protein kinase C (PKC), protein kinase G, p38 mitogen-activated protein kinase (p38MAPK), and Jun N-terminal kinase MAPK.

The mechanism responsible for activating components of the RISK pathway in IPost is not clear, but has been attributed to cell-surface receptor binding of autacoids such as adenosine.

Upstream Activators of the RISK Pathway

The actual mechanism through which components of the RISK pathway are activated at the onset of myocardial reperfusion are not clear. Most evidence supports the activation of cell-surface receptors during the IPost stimulus, which recruit components of the RISK pathway. It is clear from the

literature that recruiting components of the RISK pathway at the onset of myocardial reperfusion to confer cardioprotection can be initiated *via* the pharmacological activation of a variety of cell-surface receptors including G-protein coupled receptors, cytokine receptors, tyrosine kinase receptors, and serine threonine receptors (48, 49). The realization that the signaling pathways underlying IPost included components of the RISK pathway resulted in a number of different pharmacological postconditioning agents being investigated, including inhalational anesthetics, which have been extensively studied as pharmacological postconditioning agents capable of recruiting the RISK pathway and its downstream components (17). Interestingly, some of these receptors that convey cardioprotection when activated by pharmacological agents appear to be involved in IPost cardioprotection. Two experimental studies have demonstrated directly the involvement of cell-surface receptors in the activation of the RISK pathway. Morrison *et al.* (90) reported the failure of IPost to activate the RISK pathway in mice lacking the adenosine A2A receptor, suggesting that, in this case, the A2A receptor may be responsible for the endogenous activation of the RISK pathway in the postconditioned heart. There has been some disagreement on the adenosine receptor subtype involved in IPost signaling. Other experimental studies have implicated the adenosine A2B receptor and the A3 receptor as potential triggers of cardioprotection. However, why IPost should be selective for a specific adenosine receptor subtype is not clear, given that at the onset of myocardial reperfusion the myocardium would be awash with adenosine. A more recent study has linked the binding to the sphingosine kinase-1 receptor as a potential upstream activator of the RISK pathway (56). Mice lacking the sphingosine kinase-1 receptor were found to be resistant to IPost and did not phosphorylate Akt and Erk1/2 in response to a standard IPost stimulus. It is probable that many of the other cell-surface receptors implicated as triggers of IPost, such as the bradykinin (104) and opioid (136) receptors, also activate the RISK pathway but this remains to be demonstrated directly. IPost has also been reported to protect the heart by delaying the restoration of physiological pH (20, 31), which then prevents the activation of calpain (55). The actual interplay between these beneficial effects of IPost against myocardial reperfusion injury and the activation of the RISK pathway at this time is unclear, although preliminary evidence suggests that delaying pH restoration may facilitate RISK pathway activation through an unknown mechanism (31).

Downstream Effectors of the RISK Pathway

There are a number of downstream effectors of the RISK pathway that could be responsible for the cardioprotection elicited by IPost. Many of these terminate on the mitochondria, an organelle that occupies an essential role in cardiomyocyte survival and death signaling.

The mitochondrial permeability transition pore

The opening of the mitochondrial permeability transition pore (mPTP) at the onset of myocardial reperfusion is a critical determinant of lethal myocardial reperfusion injury, such that pharmacologically inhibiting its opening at this time can reduce myocardial infarct size by 40%–50% in both the labora-

tory (3, 40, 41) and clinical setting (105). Although the actual identity of the pore-forming units of the mPTP is unknown, several studies have demonstrated mitochondrial cyclophilin-D to be a major regulatory component of the mPTP, such that mice lacking cyclophilin-D appear resistant to mPTP opening and sustain greatly reduced myocardial infarct sizes (5, 77, 94). Studies from our laboratory and others have demonstrated that mPTP inhibition at the time of reperfusion is the end-effector in both IPC (41) and IPost (4). Experiments are ongoing to determine the mechanism through which mPTP is inhibited in these settings, but potential mechanisms include the prosurvival kinase pathways [RISK (12, 22, 59) and SAFE] and less oxidative stress (19). We and others have demonstrated that a variety of pharmacological agents capable of limiting myocardial infarct size when administered at the onset of myocardial reperfusion do this through the activation of the RISK pathway and the subsequent inhibition of mPTP (8, 22, 59, 78). The actual mechanism through which the Akt and Erk1/2 components of the RISK pathway mediate mPTP inhibition is unclear, although potential explanations include (i) the generation of nitric oxide by eNOS, a downstream target of the RISK pathway, can inhibit mPTP opening (62); (ii) Akt may modulate mitochondrial morphology, thereby rendering mitochondria more resistant to mPTP opening (42, 101); (iii) Akt may modulate intracellular calcium handling by increasing sarcoplasmic reticulum calcium uptake and thus may prevent mPTP opening (1); and (iv) glycogen synthase kinase 3 beta (GSK3β), a downstream target of both Akt and Erk1/2, may act as a point of convergence for a variety of prosurvival signaling pathways resulting in mPTP inhibition (59, 60).

Antiapoptotic signaling pathways

The possibility of recruiting antiapoptotic signaling pathways had been one of the original reasons for proposing the RISK pathway as a prosurvival signaling pathway, particularly given the close association of apoptotic cell death with the reperfusion phase (133). Interestingly, although a large number of potential antiapoptotic pathways exist downstream of the RISK pathway, relatively few have actually been investigated in the context of cardioprotection, yet alone IPost. These antiapoptotic mechanisms include the phosphorylation and inhibition of proapoptotic proteins such as Bcl2-associated death factor (57) and Bcl2-associated X protein, the activation of antiapoptotic proteins such as PIM-1 kinase (13), the effect of which is preservation of mitochondrial integrity, and a favorable increase in the antiapoptotic proteins such as B-cell lymphoma 2 and B-cell lymphoma 2 extra large. There are a number of other potential antiapoptotic mechanistic pathways recruited by the RISK pathway—these are reviewed elsewhere (46, 48).

Modification of the RISK Pathway in Comorbidities

Many of the comorbidities that exist with ischemic heart disease, such as diabetes, hypertension, age, hyperlipidemia, and so on, may impact on the cardioprotection elicited by IPost (26). The actual interplay between these confounding factors and IPost cardioprotection need to be taken into account when designing appropriate experimental animal studies. The effect of these comorbidities is particularly important with respect to signal transduction, as conditions such

TABLE 1. MAJOR EXPERIMENTAL STUDIES INVESTIGATING THE REPERFUSION INJURY SALVAGE KINASE PATHWAY IN IPOST SIGNALING

Study	Model	IPost protocol	RISK pathway	New insight
Tsang et al. 2004 (127)	Isolated rat heart	Six RI cycles of 10 s/10 s	↓IS linked to Akt phosphorylation	First study to link IPost to the PI3K-Akt component of RISK pathway. Downstream eNOS and p70S6K implicated.
Yang et al. 2004 (132)	Isolated rabbit heart	Three RI cycles of 30 s/30 s	↓IS blocked by MEK1/2 inhibitor	First study to link IPost with MEK1/2-Erk1/2 component of the RISK pathway.
Chiari et al. 2005 (17)	In vivo rabbit heart	Three RI cycles of 20 s/20 s	↓IS linked to Akt phosphorylation	First study to link IPost isoflurane postconditioning with RISK pathway.
Darling et al. 2005 (21)	Isolated rabbit heart	Four RI cycles of 30 s/30 s	↓IS linked to Erk but not Akt phosphorylation	First study to link IPost to Erk1/2 component of RISK pathway. However, first study to not implicate Akt.
Schwartz and Granha 2006 (112)	In vivo porcine heart	Three RI cycles of 30 s/30 s	No ↓IS. Akt and Erk1/2 phosphorylation	First study to report dissociation of IPost with RISK pathway activation.
Zhu et al. 2006 (138)	Isolated post-MI and hypertension remodeled rat hearts	Six RI cycles of 10 s/10 s	↓IS linked to Akt but not Erk1/2 phosphorylation	First study to link IPost to Akt activation in post-MI and hypertension-remodeled myocardium. Downstream (eNOS, p70S6K, GSK3β) implicated.
Manintveld et al. 2007 (83)	In vivo rat heart	Three RI cycles of 30 s/30 s	↓IS linked to Akt phosphorylation	First study to demonstrate IPost increasing IS following 15-min coronary artery occlusion.
Fujita et al. 2007 (31)	In vivo canine heart	Four RI cycles of 60 s/60 s	↓IS associated with Akt phosphorylation	First study to implicate transient acidosis in IPost.
Sivaraman et al. 2007 (113)	Isolated human atrial trabeculae	Four RI cycles of 60 s/60 s	Improved functional recovery blocked by kinase inhibitors	First study to implicate RISK pathway in human heart tissue.
Morrison et al. 2007 (90)	Isolated murine hearts lacking adenosine A2A receptor.	Six RI cycles of 10 s/10 s	Improved functional recovery and less Trop-T release linked to Akt/Erk1/2 phosphorylation	First study to implicate adenosine A2A receptor upstream of RISK pathway in IPost.

Study	Model	Protocol	Effect	Comments
Jin et al. 2008(56)	Isolated murine hearts lacking sphingosine kinase-1 receptor.	Three RI cycles of 5 s/5 s	↓IS linked to Akt/Erk1/2 phosphorylation	First study to implicate sphingosine kinase-1 receptor upstream of RISK pathway in IPost.
Bouhidel et al. 2008 (14)	In vivo murine murine heart.	Six RI cycles of 10 s/10 s	↓IS linked to Akt/Erk1/2 phosphorylation	First time that adenosine monophosphate-activated protein kinase activation and downregulation of phosphatase on tensin homolog, MAP kinase phosphatase-3, and protein phosphatase 2C implicated in IPost signaling. Obese mice not protected by IPost (Akt not phosphorylated).
Wagner et al. 2008 (129)	Isolated rat heart	Three RI cycles of 30 s/30 s	↓IS linked to ERK1/2 and GSK3β phosphorylation	No cardioprotection or kinase activation in the presence of the metabolic syndrome.
Oikawa et al. 2008 (98)	In vivo rat heart	Six RI cycles of 10 s/10 s	↓IS linked to Akt/Erk1/2 phosphorylation	First study to demonstrate coronary stenosis in left anterior descending blocking IPost and Akt/Erk1/2 phosphorylation.
Skyschally et al. 2009 (115)	In vivo porcine heart	Six RI cycles of 20 s/20 s	↓IS but no additional Akt or Erk1/2 phosphorylation	Failure to find RISK pathway activation in IPost.
Murozono et al. 2009 (91)	Isolated rat heart	Five RI cycles of 10 s/10 s	↓IS linked to Akt phosphorylation	First study to show heat shock and IPost acting in synergistic manner.
Tamareille et al. 2009 (123)	Isolated rat heart	Three RI cycles of 30 s/30 s	↓IS linked to GSK3β phosphorylation; protection blocked by PI3K/MEK1/2 kinase inhibitors	First study to show erythropoietin more effective than IPost.
Honisch et al. 2010 (51)	In vivo rat heart	Three RI cycles of 30 s/30 s	↓IS linked to Akt and GSK3β phosphorylation	

Erk, extraregulated kinase; eNOS, endothelial nitric oxide synthase; GSK3β, glycogen synthase kinase 3 beta; IS, infarct size; MEK, mitogen extraregulated kinase; PI3K, phosphatidyl inositol 3 kinase; RI, reperfusion ischemia; RISK, reperfusion injury salvage kinase.

as diabetes and age may downregulate certain signaling pathways. For example, downregulation of the Erk1/2-GSK3β signaling pathway rendered the hearts excised from the metabolic syndrome rat resistant to the infarct-limiting effects of IPost (129). A similar loss of IPost cardioprotection was observed in relation to downregulation of PI3K-Akt and MEK1/2-Erk1/2 signaling in the presence of a coronary stenosis (98) and obesity (14). This effect of comorbidities on cardioprotection has been also observed in pharmacological postconditioning using inhaled anesthetics. In the presence of hyperglycemia, the *in vivo* rabbit heart was resistant to the infarct-limiting effects of isoflurane postconditioning and Akt-eNOS activation was absent (107).

Controversial Issues Surrounding RISK Pathway Signaling in IPost

The involvement of the RISK pathway in IPost signaling has sparked several controversial issues. The first of these was by Darling *et al*. (21), who failed to implicate the PI3K-Akt component of the RISK pathway in the *ex vivo* postconditioned rabbit heart. However, other studies have provided evidence for the role of the PI3K-Akt pathway in IPost signaling in the intact rabbit heart (17) (Table 1).

In the porcine heart, the disparate findings concerning the RISK pathway have been even more interesting. First, Schwartz and Granha (112) reported dissociation between RISK pathway activation and the infarct-limiting effects of IPost in the *in vivo* porcine heart. Using three 30-s cycles of reperfusion and ischemia, these authors were able to demonstrate significant Akt and Erk1/2 phosphorylation in the absence of cardioprotection. One could argue that although the IPost stimulus may have been adequate to activate the RISK pathway it was not strong enough to elicit infarct limitation. Curiously, a subsequent study by Skyschally *et al*. (115) reported almost the opposite findings—these authors managed to demonstrate infarct limitation with IPost (six 20-s cycles of reperfusion and ischemia), but in the absence of RISK pathway activation. Of note, inhalational anesthetics, which are known to activate components of the RISK pathway, were used in this study, which may explain why no difference in RISK pathway activation was observed in IPost-treated hearts. The failure of kinase inhibitors to block IPost cardioprotection may be due to the fact that they were administered *via* the intracoronary route as opposed to being given peripherally prior to the onset of myocardial reperfusion.

A recent study has questioned the role of GSK3β, a downstream component of the RISK pathway, as a target for IPost. A wealth of data, much of it pharmacological, suggests that the phosphorylation and inhibition of GSK3β constitutes a common mechanism of cardioprotection that underlies IPost (59). However, Nishino *et al*. (97) reported that mice containing a form of GSK3β resistant to phosphorylation and inhibition could still be cardioprotected by IPost. This finding contrasts with that of Gomez *et al*. (32), who reported that mice expressing a different form of GSK3β, which was also resistant to phosphorylation and inhibition, were resistant to IPost.

The controversial issues surrounding RISK pathway activation in IPost signaling have been used to argue for the existence of RISK-independent pathways underlying IPost. One of these alternative signaling pathways is the SAFE pathway, which is the next topic of this review article.

Reperfusion Signaling in IPost: The SAFE Pathway

In addition to the inconsistencies, noted in the previous section, surrounding RISK signaling in IPost, one particular postconditioning mimetic does not appear to limit myocardial infarct size at the time of reperfusion through the RISK pathway. Tumor necrosis factor (TNF) can mimic IPost in isolated murine hearts in the absence of Akt activation, and its protective effect is not affected by the PI3K inhibitor, Wortmannin (69). Taken together, these data support the existence of an alternative prosurvival signal transduction pathway for protecting the ischemic myocardium against lethal myocardial reperfusion injury. In this respect, Lecour has recently described this novel prosurvival pathway, which involves the activation of TNF and the transcription factor, signal transducer and activator of transcription 3 (STAT3), as the SAFE pathway (70, 71). The SAFE pathway was first discovered in the setting of IPC, but its role in IPost has been only recently confirmed.

TNF Is Part of the SAFE Pathway

TNF and the heart

TNF is a proinflammatory cytokine discovered in macrophages as a cytotoxic factor that can induce necrosis in certain murine tumors (16). First produced as a 212-amino-acid-long type II transmembrane protein, it is cleaved by TNF-converting enzyme (also called ADAM 17) (9) to release the homotrimeric soluble TNF (56 kDa). Expressed in all nucleated cell types of the myocardium, including cardiomyocytes (84), TNF exerts its major effects after binding onto its cell-surface receptors, TNF receptor 1 (TNFR1 or p55) and TNF receptor 2 (TNFR2 or p75). Both receptors are present in the heart (126), and when activated, they form homotrimers, thereby facilitating signal transduction (81). The existence of a mitochondrial binding protein to allow receptor-independent delivery of TNF from the cell surface to the mitochondria has been also proposed (15).

The two TNFRs differ in their signal pathways and are differentially expressed and regulated in the human heart (2). The concentration of both receptors is increased in coronary artery disease (108) and the signaling pathways coupled to TNFR1 include both apoptotic and protective signaling, whereas the signaling pathways coupled to TNFR2, although poorly studied, seem to convey protective signaling only (Fig. 2). Apoptosis is induced by the adaptor protein, Fas-associated death domain protein, which recruits and activates the apoptosis initiator caspase-8 (19). The protective effects mediated by the receptors may occur *via* the activation of nuclear factor kappa-B, Jun N-terminal kinase (81). TNFR binding also leads to diacylglycerol formation *via* phospholipase C (111), which in turn activates the sphingolipid signaling pathway and PKC (111). Another prosurvival factor activated in response to TNFRs binding is the Janus kinase (JAK)/STAT3 pathway (described later).

TNF and myocardial infarction

Although low physiological levels of TNF do not seem to alter the function of the heart (85), an increase in TNF, as observed in myocardial infarction (24, 50, 109) or heart failure (76), exerts a negative inotropic effect and leads to contractile dysfunction in a concentration-dependent manner (114, 135).
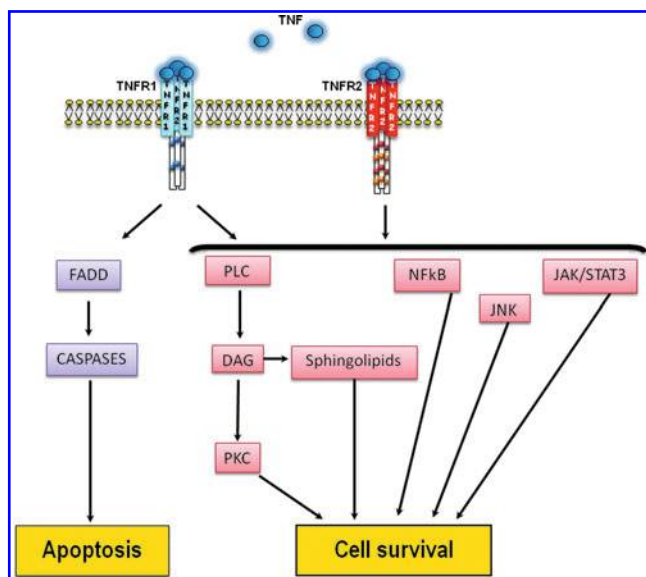
FIG. 2. Activation of TNF receptors (TNFRs) leads to either apoptosis or cell survival. DAG, diacylglycerol; FADD, Fas-associated death domain; JAK/STAT3, Janus kinase/signal transducer and activator of transcription 3; JNK, Jun N-terminal kinase; PKC, protein kinase C; PLC, phospholipase C. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

This effect is most likely due to an alteration in intracellular calcium homeostasis (135) and the formation of free radicals (25). However, animal studies exploring the exact role of TNF in ischemia-reperfusion injury (IRI) have led to conflicting results. Mice lacking TNF are protected from IRI (82) and the presence of TNF antibodies limits the infarct size in animal studies (6, 38), therefore supporting the concept of a deleterious effect of TNF in myocardial infarction. In contrast, exogenous TNF protects cardiomyocytes against hypoxic injury (95). Similarly, knockdown of both TNFRs in mice subjected to coronary artery ligation results in an increase in infarct size, whereas the infarct size in TNFR1 knockout mice was reduced or unchanged (27, 69, 88). Apart from the concentration of

TNF, the type of receptor activated in the setting of IRI is another critical factor that will influence the deleterious or beneficial effect of TNF.

TNF in pre- and post-postconditioning

The requirement of TNF release for both classic and delayed IPC to confer protection is now strongly supported in the literature. In rats, mice, or rabbits, classic IPC is associated with an increased level of preischemic TNF (7, 117), but a decreased level of endogenous TNF released during IRI (7, 63). TNF antibodies can reduce the infarct-sparing effect of delayed IPC in a rat model (131) or preconditioning with microembolization in a pig model (114). Similarly, TNF knockout mice fail to be preconditioned (54, 67, 117) and exogenous TNF given as a preconditioning mimetic can reduce the infarct size to a similar extent to IPC both *in vivo* or *in vitro* (72–74, 124). Interestingly, the protection with exogenous TNF can only be achieved with a washout phase prior to the sustained ischemia, therefore suggesting that the time when TNF is produced is another criteria, together with the concentration and the type of receptors activated, to consider for the deleterious *versus* protective effect of TNF (70, 73).

Similar to IPC, IPost reduces levels of plasma TNF during the reperfusion phase in anesthetized rats subjected to coronary artery ligation (64). In a Langendorff system, TNFR1 knockout mice hearts but not TNF or TNFR2 knockout mice hearts are protected with an IPost stimulus (69) (Fig. 3). Similarly, the addition of TNFR2 antibodies abolishes the infarct-sparing effect of IPost, whereas TNFR1 antibodies have no effect (69). All together, these data strongly support the requirement of TNF release in a dose- and time-dependent manner in both IPC and IPost and the protective effect mediated by TNF is acting *via* its binding to the TNFR2.

JAK/STAT3 Is Part of the SAFE Pathway

JAK/STAT3 pathway in the heart

Traditionally believed to act as a transcription factor after activation and translocation of STAT3 to the nucleus, the JAK/STAT3 pathway plays a critical role in the myocardial response of the heart in various physiopathological
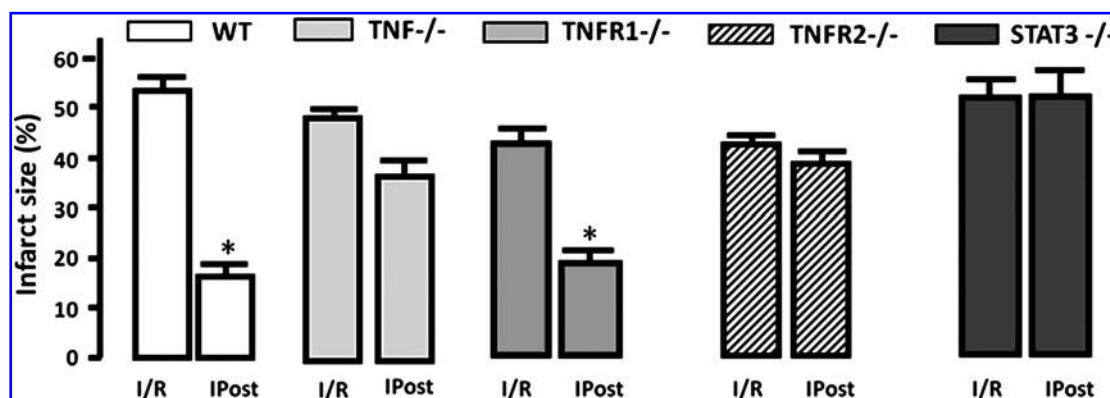
FIG. 3. Activation of the SAFE pathway in IPost. This graph shows that TNF, TNR receptor 2 knockout mice, and cardiac-specific STAT3-deficient mice but not TNFR 1 knockout animals failed to be protected by IPost in the isolated heart model ($n > 6$ per group; *$p < 0.05$). This figure was adapted from ref. (69).

Table 2. MAJOR EXPERIMENTAL STUDIES INVESTIGATING THE SURVIVOR ACTIVATING FACTOR ENHANCEMENT PATHWAY IN IPost SIGNALING

Study	Model	IPost protocol	SAFE pathway	New insight
Suleman et al. 2006 (121)	Isolated rat heart	Four RI cycles of 10 s/10 s	↓IS linked to STAT3 phosphorylation	First study to link IPost to STAT3.
Boengler et al. 2008 (10)	In vivo mouse heart	Three RI cycles of 10 s/10 s	Loss of cardioprotection in aged STAT3 knockout mice	First study to link the effect of IPost in aged animal to STAT3 activation.
Goodman et al. 2008 (34)	Isolated murine heart	Three RI cycles of 10 s/10 s	↓IS linked to Akt and STAT3 phosphorylation	First study to link RISK and SAFE in IPost. However, mice lacking cardiac STAT3 still amenable to IPost.
Fuglesteg et al. 2008 (29)	Isolated mouse cardiomyocyte	Insulin at onset of reperfusion	Loss of cardioprotection in STAT3 knockout mice	First study to link insulin to STAT3 in cardioprotection.
Somers et al. 2009 (119)	Isolated mouse heart	Sphingosine-1 phosphate given at the onset of reperfusion	Loss of cardioprotection in TNF or STAT3 knockout mice	First study to link sphingosine-1 phosphate IPost to STAT3 and TNF.
Lacerda et al. 2009 (69)	Isolated mouse heart	Six RI cycles of 10 s/10 s	Loss of cardioprotection in STAT-3, TNF or TNFR2 knockout mice	First study to link IPost to TNF and TNFR2.

MI, myocardial infarction; SAFE, survivor activating factor enhancement; STAT3, signal transducer and activator of transcription 3; TNFR, tumor necrosis factor receptor.

conditions such as myocardial infarction, myocarditis, or hypertrophy. The recent discovery that activated STAT3 can also translocate to the mitochondria suggests a control of STAT3 in the energy production of the cells (130).

JAK2 proteins are a family of tyrosine kinases constitutively associated with the cytoplasmic domain of cytokine and growth factor receptors. Upon activation of the receptors, JAK proteins phosphorylate and create a docking site for a STAT3 protein, which in turn is activated by phosphorylation. Tyrosine phosphorylation of STAT3 enables STAT3 to dimerize and translocate to the nucleus. Serine phosphorylation of STAT3 is required for its translocation to the mitochondria, where it interacts with GRIM-19–containing complexes I and II to regulate the electron transport chain (92).

JAK/STAT3 and myocardial infarction

Following coronary artery ligation *in vivo*, phosphorylation of STAT3 occurs, which is maintained for up to 24 h (96). Reperfusion after 25 min of ligation further increases its activation (87). Although acute ischemia followed by up to 2 h of reperfusion did not affect the infarct size of cardiomyocyte-specific STAT3-depleted mouse hearts compared with their littermate controls (10, 69, 116), a 10-fold cardiac overexpression of STAT3 in mice reduced the infarct size following IRI (102, 103). In contrast, the addition of the JAK tyrosine kinase inhibitor, AG490, in an isolated rat heart undergoing IRI protected the heart (53, 86).

JAK/STAT3 in pre- and postconditioning

The role of STAT3 in early preconditioning was first suggested in an isolated rat heart model whereby STAT3 phosphorylation was increased following the preconditioning stimulus, and the addition of AG490, the JAK/STAT3 inhibitor, during the preconditioning stimulus ablated the protection against infarction (39). Further, hearts from mice in which STAT3 was ablated by MLC2v promoter–driven activation of the Cre-recombinase failed to be protected by either an IPC stimulus or a pharmacological preconditioning stimulus such as TNF and diazoxide (116). Additionally, the presence of AG490 at the onset of reperfusion also abolished the protective effect of IPC, thereby suggesting that STAT3 phosphorylation is also required during the reperfusion period and not limited to its activation during the preconditioning stimulus (74, 122). Interestingly, the coronary effluent from a preconditioned heart activates the JAK/STAT3 pathway in a donor heart, conferring cardioprotection by limiting apoptosis (52).

In 2006, the role of STAT3 in IPost was also suggested in an isolated rat heart model whereby the addition of the JAK/STAT3 inhibitor AG490 at the onset of reperfusion abolished the cardioprotective effect of IPost (121) (Table 2). Both ischemic and TNF postconditioning with TNF failed to confer an infarct-sparing effect in isolated STAT3-deficient murine hearts subjected to IRI (10, 69). Further, the absence of protection observed in TNF or TNFR 2 knockout mice was associated with the absence of STAT3 phosphorylation (69). Similarly, postconditioning the heart with sphingosine-1 phosphate (S1P) is associated with an increase in STAT3 phosphorylation and its cardioprotective effect is abolished in STAT3-deficient mice (119). In both IPC and IPost, activation of STAT3 occurs within minutes and its cardioprotective effect can be seen very rapidly, therefore suggesting that its effect is

mediated by its translocation to the mitochondria (10, 130) rather than its translocation to the nucleus where it will regulate the transcription of various genes.

Upstream Targets of the SAFE Pathway

The upstream activators of the SAFE pathway have been poorly studied. Nevertheless, many pharmacological agents capable of mimicking IPC or IPost may confer their cardioprotective effect *via* the SAFE pathway. Although not always demonstrated in the heart, angiotensin II (28, 100), bradykinin (58, 106), adrenoreceptors (33), leptin (37), opioids, and cannabinoids (18, 89) can activate both TNF and STAT-3. Similarly, insulin given at the time of reperfusion confers cardioprotection *via* the activation of the JAK/STAT-3 pathway (29), and preliminary data from the Hatter Institute in Cape Town demonstrate that insulin, given at the onset of reperfusion in isolated TNF knockout mice hearts, fails to protect the heart against IRI. Interestingly, adenosine can mimic IPC in wild-type mice and its protective effect is unaffected in TNF knockout mice (117) or in cardiomyocyte-specific STAT3-deficient mice (116). These data strongly suggest that the cardioprotective effect of adenosine is independent of the SAFE pathway. Another postconditioning mimetic is S1P (128), also known as a downstream target of TNFRs (Fig. 2) (73). Surprisingly, exogenous S1P requires the activation of the SAFE pathway as it fails to induce cardioprotection in both TNF knockout mice and cardiomyocyte-specific STAT3-deficient mice (61, 119), therefore suggesting a complex regulatory cross-talk mechanism between TNF and S1P.

Dowstream Targets of the SAFE Pathway

All these data clearly indicate that the activation of TNF, TNFR2, and STAT3, major components of the SAFE pathway, are required for IPost to confer protection, but very little is known about the signaling pathways activated downstream of STAT3. TNF-induced protection requires the activation of PKC, the mitochondrial ATP-dependent potassium channel, and NFκ-B (73, 118). In addition, pharmacological preconditioning with TNF is associated with an increase in mitochondrial free radicals and an addition of free radical scavengers such as mercaptoprpionyl glycine abolished its cardioprotective effect (68, 72). In contrast, neither p38MAPK, Akt, Erk, nor GSK3β seems to be involved in TNF-induced cardioprotection, therefore suggesting that they do not act as the downstream target of the SAFE pathway (69, 74, 124). STAT3 mediates cardioprotection *via* the phosphorylation and inactivation of the proapoptotic factors Bcl2-associated death factor (23, 74) and Bcl2-associated X protein (52). The mPTP is described as an end-effector of IPost and the RISK pathway; our initial data suggest that the SAFE pathway may also target the mPTP (unpublished data, 2010).

Modification of the SAFE Pathway in Comorbidities

Interestingly, IPost signaling through the SAFE pathway may also be influenced by comorbidities. Boengler *et al.* (10) noted that the infarct-limiting effects of IPost were lost in aged mice, and this inability to postcondition the aged mice was related to inadequate activation of the JAK-STAT3 pathway, a component of the SAFE pathway. Whether other

FIG. 4. Reperfusion injury salvage kinase (RISK) and SAFE pathway signaling in IPost. Activation of cell-surface receptors in response to an IPost stimulus recruits both the RISK and survivor activating factor enhancement (SAFE) pathways. These signal transduction pathways then terminate in the mitochondria and nucleus and activate antiapoptotic pathways. eNOS, endothelial nitric oxide synthase; Erk, extra-regulated kinase; GSK3β, glycogen synthase kinase 3 beta; Mek, mitogen extra-regulated kinase; mPTP, mitochondrial permeability transition pore; NO, nitric oxide; PI3K, phosphatidyl inositol 3 kinase; PKCε, protein kinase C epsilon; PKG, protein kinase G; ROS, reactive oxygen species; SPK, sphingosine kinase; TNF, tumor necrosis factor; TNFR2, tumor necrosis factor receptor 2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).
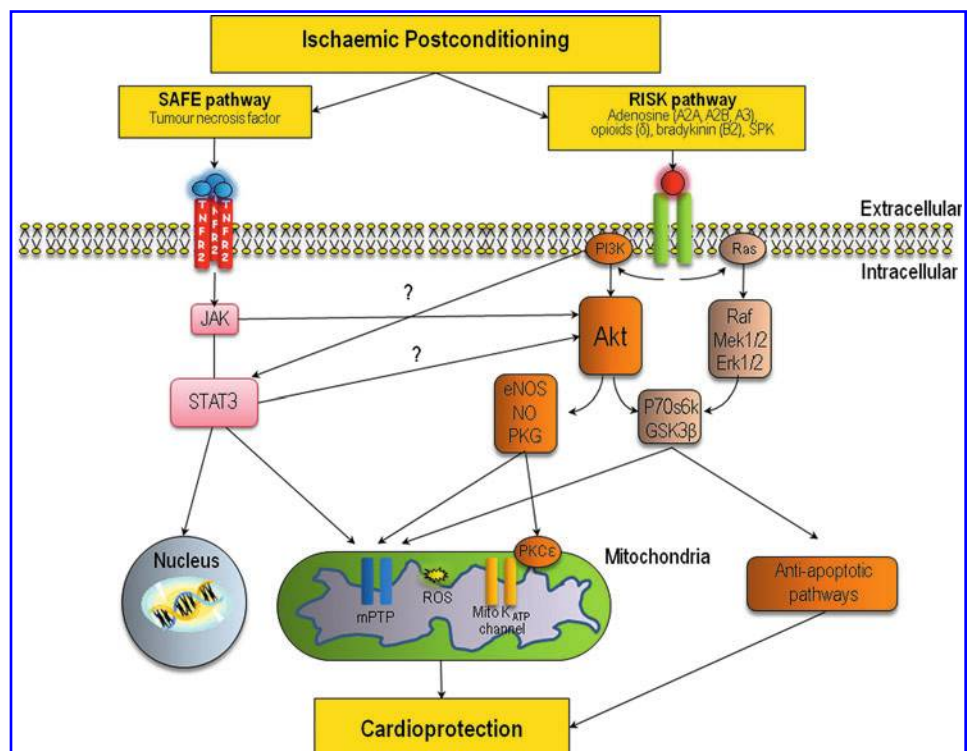
comorbidities are able to impact on signaling through the SAFE pathway remains to be determined.

Two Sides of the Same Coin: Interplay Between the SAFE and RISK Pathways

It is clear that IPost can protect the ischemic heart from myocardial reperfusion injury through the activation of either the RISK or SAFE pathway (Fig. 4). Interestingly, the pharmacological inhibition of either pathway appears to abrogate IPost cardioprotection completely, which suggests that either there is an interaction between the two pathways, or the resolution of our animal models cannot distinguish between partial and complete inhibition of cardioprotection. Studies are ongoing to investigate the actual interplay between the RISK and SAFE pathways. All things considered, it appears that the mitochondria and specifically the mPTP may act as a point of convergence for the two IPost signaling pathways. The study by Goodman *et al.* (34) suggested the possibility of an interaction between the RISK and SAFE pathways in IPost signaling. These authors found that pharmacological inhibition of STAT3 with Stattic (a specific STAT3 inhibitor) abrogated both the IPost-induced phosphorylation of STAT3 and Akt, suggesting that Akt may be downstream of STAT3. Interestingly, the pharmacological inhibition of PI3K blocked the IPost-induced phosphorylation of Akt but not STAT3, suggesting that STAT3 is not downstream of PI3K-Akt. In the setting of morphine-induced postconditioning, Gross *et al.* (36) found that morphine-induced Akt phosphorylation could be blocked by AG-490, a JAK2 inhibitor, and that morphine-induced STAT3 phosphorylation could be blocked by Wortmannin, a PI3K inhibitor, suggesting an interaction between the two pathways.

Conclusions

The elucidation of the signal transduction pathways underlying IPost has identified a number of signaling pathways, which include the RISK and the SAFE pathways. The identification of the major components of these two pathways has identified several novel pharmacological targets for protecting the ischemic heart at the time of myocardial reperfusion. Further work is needed to elucidate the relationship between these two reperfusion signaling pathways.

References

1. Abdallah Y, Gkatzoflia A, Gligorievski D, Kasseckert S, Euler G, Schluter KD, Schafer M, Piper HM, and Schafer C. Insulin protects cardiomyocytes against reoxygenation-induced hypercontracture by a survival pathway targeting SR Ca2+ storage. *Cardiovasc Res* 70: 346–353, 2006.

2. Al Lamki RS, Brookes AP, Wang J, Reid MJ, Parameshwar J, Goddard MJ, Tellides G, Wan T, Min W, Pober JS, and Bradley JR. TNF receptors differentially signal and are differentially expressed and regulated in the human heart. *Am J Transplant* 9: 2679–2696, 2009.

3. Argaud L, Gateau-Roesch O, Muntean D, Chalabreysse L, Loufouat J, Robert D, and Ovize M. Specific inhibition of the mitochondrial permeability transition prevents lethal reperfusion injury. *J Mol Cell Cardiol* 38: 367–374, 2005.

4. Argaud L, Gateau-Roesch O, Raisky O, Loufouat J, Robert D, and Ovize M. Postconditioning inhibits mitochondrial permeability transition. *Circulation* 111: 194–197, 2005.

5. Baines CP, Kaiser RA, Purcell NH, Blair NS, Osinska H, Hambleton MA, Brunskill EW, Sayen MR, Gottlieb RA, Dorn GW, Robbins J, and Molkentin JD. Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature* 434: 658–662, 2005.

6. Belosjorow S, Bolle I, Duschin A, Heusch G, and Schulz R. TNF-alpha antibodies are as effective as ischemic preconditioning in reducing infarct size in rabbits. *Am J Physiol Heart Circ Physiol* 284: H927–H930, 2003.

7. Belosjorow S, Schulz R, Dorge H, Schade FU, and Heusch G. Endotoxin and ischemic preconditioning: TNF-alpha concentration and myocardial infarct development in rabbits. *Am J Physiol* 277: H2470–H2475, 1999.

8. Bhamra GS, Hausenloy DJ, Davidson SM, Carr RD, Paiva M, Wynne AM, Mocanu MM, and Yellon DM. Metformin protects the ischemic heart by the Akt-mediated inhibition of mitochondrial permeability transition pore opening. *Basic Res Cardiol* 103: 274–284, 2008.

9. Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL, Reddy P, Srinivasan S, Nelson N, Boiani N, Schooley KA, Gerhart M, Davis R, Fitzner JN, Johnson RS, Paxton RJ, March CJ, and Cerretti DP. A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. *Nature* 385: 729–733, 1997.

10. Boengler K, Buechert A, Heinen Y, Roeskes C, Hilfiker-Kleiner D, Heusch G, and Schulz R. Cardioprotection by ischemic postconditioning is lost in aged and STAT3-deficient mice. *Circ Res* 102: 131–135, 2008.

11. Bolli R, Becker L, Gross G, Mentzer R, Jr., Balshaw D, and Lathrop DA. Myocardial protection at a crossroads: the need for translation into clinical therapy. *Circ Res* 95: 125–134, 2004.

12. Bopassa JC, Ferrera R, Gateau-Roesch O, Couture-Lepetit E, and Ovize M. PI 3-kinase regulates the mitochondrial transition pore in controlled reperfusion and postconditioning. *Cardiovasc Res* 69: 178–185, 2006.

13. Borillo GA, Mason M, Quijada P, Volkers M, Cottage C, McGregor M, Din S, Fischer K, Gude N, Avitable D, Barlow S, Gustafsson AB, Glembotski C, Gottlieb RA, Brown JH, and Sussman MA. Pim-1 kinase protects mitochondrial integrity in cardiomyocytes. *Circ Res* 106: 1265–1274, 2010.

14. Bouhidel O, Pons S, Souktani R, Zini R, Berdeaux A, and Ghaleh B. Myocardial ischemic postconditioning against ischemia-reperfusion is impaired in ob/ob mice. *Am J Physiol Heart Circ Physiol* 295: H1580–H1586, 2008.

15. Busquets S, Aranda X, Ribas-Carbo M, Azcon-Bieto J, Lopez-Soriano FJ, and Argiles JM. Tumour necrosis factor-alpha uncouples respiration in isolated rat mitochondria. *Cytokine* 22: 1–4, 2003.

16. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, and Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci U S A* 72: 3666–3670, 1975.

17. Chiari PC, Bienengraeber MW, Pagel PS, Krolikowski JG, Kersten JR, and Warltier DC. Isoflurane protects against myocardial infarction during early reperfusion by activation of phosphatidylinositol-3-kinase signal transduction: evidence for anesthetic-induced postconditioning in rabbits. *Anesthesiology* 102: 102–109, 2005.

18. Cianchi F, Papucci L, Schiavone N, Lulli M, Magnelli L, Vinci MC, Messerini L, Manera C, Ronconi E, Romagnani P, Donnini M, Perigli G, Trallori G, Tanganelli E, Capaccioli S, and Masini E. Cannabinoid receptor activation induces apoptosis through tumor necrosis factor alpha-mediated ceramide *de novo* synthesis in colon cancer cells. *Clin Cancer Res* 14: 7691–7700, 2008.

19. Clarke SJ, Khaliulin I, Das M, Parker JE, Heesom KJ, and Halestrap AP. Inhibition of mitochondrial permeability transition pore opening by ischemic preconditioning is probably mediated by reduction of oxidative stress rather than mitochondrial protein phosphorylation. *Circ Res* 102: 1082–1090, 2008.

20. Cohen MV, Yang XM, and Downey JM. The pH hypothesis of postconditioning: staccato reperfusion reintroduces oxygen and perpetuates myocardial acidosis. *Circulation* 115: 1895–1903, 2007.

21. Darling CE, Jiang R, Maynard M, Whittaker P, Vinten-Johansen J, and Przyklenk K. Postconditioning via stuttering reperfusion limits myocardial infarct size in rabbit hearts: role of ERK1/2. *Am J Physiol Heart Circ Physiol* 289: H1618–H1626, 2005.

22. Davidson SM, Hausenloy D, Duchen MR, and Yellon DM. Signalling via the reperfusion injury signalling kinase (RISK) pathway links closure of the mitochondrial permeability transition pore to cardioprotection. *Int J Biochem Cell Biol* 38: 414–419, 2006.

23. Deuchar GA, Opie LH, and Lecour S. TNFalpha is required to confer protection in an *in vivo* model of classical ischaemic preconditioning. *Life Sci* 80: 1686–1691, 2007.

24. Dorge H, Schulz R, Belosjorow S, Post H, van de SA, Konietzka I, Frede S, Hartung T, Vinten-Johansen J, Youker KA, Entman ML, Erbel R, and Heusch G. Coronary microembolization: the role of TNF-alpha in contractile dysfunction. *J Mol Cell Cardiol* 34: 51–62, 2002.

25. Ferdinandy P, Danial H, Ambrus I, Rothery RA, and Schulz R. Peroxynitrite is a major contributor to cytokine-induced myocardial contractile failure. *Circ Res* 87: 241–247, 2000.

26. Ferdinandy P, Schulz R, and Baxter GF. Interaction of cardiovascular risk factors with myocardial ischemia/reperfusion injury, preconditioning, and postconditioning. *Pharmacol Rev* 59: 418–458, 2007.

27. Flaherty MP, Guo Y, Tiwari S, Rezazadeh A, Hunt G, Sanganalmath SK, Tang XL, Bolli R, and Dawn B. The role of TNF-alpha receptors p55 and p75 in acute myocardial ischemia/reperfusion injury and late preconditioning. *J Mol Cell Cardiol* 45: 735–741, 2008.

28. Frolkis I, Gurevitch J, Yuhas Y, Iaina A, Wollman Y, Chernichovski T, Paz Y, Matsa M, Pevni D, Kramer A, Shapira I, and Mohr R. Interaction between paracrine tumor necrosis factor-alpha and paracrine angiotensin II during myocardial ischemia. *J Am Coll Cardiol* 37: 316–322, 2001.

29. Fuglesteg BN, Suleman N, Tiron C, Kanhema T, Lacerda L, Andreasen TV, Sack MN, Jonassen AK, Mjos OD, Opie LH, and Lecour S. Signal transducer and activator of transcription 3 is involved in the cardioprotective signalling pathway activated by insulin therapy at reperfusion. *Basic Res Cardiol* 103: 444–453, 2008.

30. This reference has been deleted.

31. Fujita M, Asanuma H, Hirata A, Wakeno M, Takahama H, Sasaki H, Kim J, Takashima S, Tsukamoto O, Minamino T, Shinozaki Y, Tomoike H, Hori M, and Kitakaze M. Prolonged transient acidosis during early reperfusion contributes to the cardioprotective effects of postconditioning. *Am J Physiol Heart Circ Physiol* 292: H2004–H2008, 2007.

32. Gomez L, Paillard M, Thibault H, Derumeaux G, and Ovize M. Inhibition of GSK3beta by postconditioning is required to prevent opening of the mitochondrial permeability transition pore during reperfusion. *Circulation* 117: 2761–2768, 2008.

33. Gong KZ, Zhang H, Du JH, and Zhang YY. Crosstalk between signaling pathways of adrenoreceptors and signal transducers and activators of transcription 3 (STAT3) in heart. *Acta Pharmacol Sin* 28: 153–165, 2007.

34. Goodman MD, Koch SE, Fuller-Bicer GA, and Butler KL. Regulating RISK: a role for JAK-STAT signaling in postconditioning? *Am J Physiol Heart Circ Physiol* 295: H1649–H1656, 2008.

35. Gordon JM, Dusting GJ, Woodman OL, and Ritchie RH. Cardioprotective action of CRF peptide urocortin against simulated ischemia in adult rat cardiomyocytes. *Am J Physiol Heart Circ Physiol* 284: H330–H336, 2003.

36. Gross ER, Hsu AK, and Gross GJ. The JAK/STAT pathway is essential for opioid-induced cardioprotection: JAK2 as a mediator of STAT3, Akt, and GSK-3 beta. *Am J Physiol Heart Circ Physiol* 291: H827–H834, 2006.

37. Guo Z, Jiang H, Xu X, Duan W, and Mattson MP. Leptin-mediated cell survival signaling in hippocampal neurons mediated by JAK STAT3 and mitochondrial stabilization. *J Biol Chem* 283: 1754–1763, 2008.

38. Gurevitch J, Frolkis I, Yuhas Y, Lifschitz-Mercer B, Berger E, Paz Y, Matsa M, Kramer A, and Mohr R. Anti-tumor necrosis factor-alpha improves myocardial recovery after ischemia and reperfusion. *J Am Coll Cardiol* 30: 1554–1561, 1997.

39. Hattori R, Maulik N, Otani H, Zhu L, Cordis G, Engelman RM, Siddiqui MA, and Das DK. Role of STAT3 in ischemic preconditioning. *J Mol Cell Cardiol* 33: 1929–1936, 2001.

40. Hausenloy DJ, Duchen MR, and Yellon DM. Inhibiting mitochondrial permeability transition pore opening at reperfusion protects against ischaemia-reperfusion injury. *Cardiovasc Res* 60: 617–625, 2003.

41. Hausenloy DJ, Maddock HL, Baxter GF, and Yellon DM. Inhibiting mitochondrial permeability transition pore opening: a new paradigm for myocardial preconditioning? *Cardiovasc Res* 55: 534–543, 2002.

42. Hausenloy DJ and Scorrano L. Targeting cell death. *Clin Pharmacol Ther* 82: 370–373, 2007.

43. Hausenloy DJ, Tsang A, Mocanu M, and Yellon DM. Ischemic preconditioning protects by activating pro-survival kinases at reperfusion. *Am J Physiol Heart Circ Physiol* 288: H971–H976, 2005.

44. Hausenloy DJ, Tsang A, and Yellon DM. The reperfusion injury salvage kinase pathway: a common target for both ischemic preconditioning and postconditioning. *Trends Cardiovasc Med* 15: 69–75, 2005.

45. Hausenloy DJ and Yellon DM. New directions for protecting the heart against ischaemia-reperfusion injury: targeting the reperfusion injury salvage kinase (RISK)-pathway. *Cardiovasc Res* 61: 448–460, 2004.

46. Hausenloy DJ and Yellon DM. Survival kinases in ischemic preconditioning and postconditioning. *Cardiovasc Res* 70: 240–253, 2006.

47. Hausenloy DJ and Yellon DM. Preconditioning and post-conditioning: united at reperfusion. *Pharmacol Ther* 116: 173–191, 2007.

48. Hausenloy DJ and Yellon DM. Reperfusion injury salvage kinase signalling: taking a RISK for cardioprotection. *Heart Fail Rev* 12: 217–234, 2007.

49. Hausenloy DJ and Yellon DM. Cardioprotective growth factors. *Cardiovasc Res* 83: 179–194, 2009.

50. Herskowitz A, Choi S, Ansari AA, and Wesselingh S. Cytokine mRNA expression in postischemic/reperfused myocardium. *Am J Pathol* 146: 419–428, 1995.

51. Honisch A, Theuring N, Ebner B, Wagner C, Strasser RH, and Weinbrenner C. Postconditioning with levosimendan reduces the infarct size involving the PI3K pathway and KATP-channel activation but is independent of PDE-III inhibition. *Basic Res Cardiol* 105: 155–167, 2010.

52. Huffman LC, Koch SE, and Butler KL. Coronary effluent from a preconditioned heart activates the JAK-STAT pathway and induces cardioprotection in a donor heart. *Am J Physiol Heart Circ Physiol* 294: H257–H262, 2008.

53. Hwang YC, Shaw S, Kaneko M, Redd H, Marrero MB, and Ramasamy R. Aldose reductase pathway mediates JAK-STAT signaling: a novel axis in myocardial ischemic injury. *FASEB J* 19: 795–797, 2005.

54. Ichikawa Y, Miura T, Nakano A, Miki T, Nakamura Y, Tsuchihashi K, and Shimamoto K. The role of ADAM protease in the tyrosine kinase-mediated trigger mechanism of ischemic preconditioning. *Cardiovasc Res* 62: 167–175, 2004.

55. Inserte J, Barba I, Hernando V, and Garcia-Dorado D. Delayed recovery of intracellular acidosis during reperfusion prevents calpain activation and determines protection in postconditioned myocardium. *Cardiovasc Res* 81: 116–122, 2009.

56. Jin ZQ, Karliner JS, and Vessey DA. Ischaemic postconditioning protects isolated mouse hearts against ischaemia/reperfusion injury via sphingosine kinase isoform-1 activation. *Cardiovasc Res* 79: 134–140, 2008.

57. Jonassen AK, Sack MN, Mjos OD, and Yellon DM. Myocardial protection by insulin at reperfusion requires early administration and is mediated via Akt and p70s6 kinase cell-survival signaling. *Circ Res* 89: 1191–1198, 2001.

58. Ju H, Venema VJ, Liang H, Harris MB, Zou R, and Venema RC. Bradykinin activates the Janus-activated kinase/signal transducers and activators of transcription (JAK/STAT) pathway in vascular endothelial cells: localization of JAK/STAT signalling proteins in plasmalemmal caveolae. *Biochem J* 351: 257–264, 2000.

59. Juhaszova M, Zorov DB, Kim SH, Pepe S, Fu Q, Fishbein KW, Ziman BD, Wang S, Ytrehus K, Antos CL, Olson EN, and Sollott SJ. Glycogen synthase kinase-3beta mediates convergence of protection signaling to inhibit the mitochondrial permeability transition pore. *J Clin Invest* 113: 1535–1549, 2004.

60. Juhaszova M, Zorov DB, Yaniv Y, Nuss HB, Wang S, and Sollott SJ. Role of glycogen synthase kinase-3beta in cardioprotection. *Circ Res* 104: 1240–1252, 2009.

61. Kelly R, King J, and Lecour S. Sphingosine 1 phosphate-induced cardioprotection is mediated by STAT-3. *J Mol Cell Cardiol* 44: 740, 2008 [Abstract].

62. Kim JS, Ohshima S, Pediaditakis P, and Lemasters JJ. Nitric oxide: a signaling molecule against mitochondrial perme-ability transition- and pH-dependent cell death after reperfusion. *Free Radic Biol Med* 37: 1943–1950, 2004.

63. Kimura H, Shintani-Ishida K, Nakajima M, Liu S, Matsumoto K, and Yoshida K. Ischemic preconditioning or p38 MAP kinase inhibition attenuates myocardial TNF alpha production and mitochondria damage in brief myocardial ischemia. *Life Sci* 78: 1901–1910, 2006.

64. Kin H, Wang NP, Mykytenko J, Reeves J, Deneve J, Jiang R, Zatta AJ, Guyton RA, Vinten-Johansen J, and Zhao ZQ. Inhibition of myocardial apoptosis by postconditioning is associated with attenuation of oxidative stress-mediated nuclear factor-kappa B translocation and TNF alpha release. *Shock* 29: 761–768, 2008.

65. Kitakaze M, Asakura M, Kim J, Shintani Y, Asanuma H, Hamasaki T, Seguchi O, Myoishi M, Minamino T, Ohara T, Nagai Y, Nanto S, Watanabe K, Fukuzawa S, Hirayama A, Nakamura N, Kimura K, Fujii K, Ishihara M, Saito Y, Tomoike H, and Kitamura S. Human atrial natriuretic peptide and nicorandil as adjuncts to reperfusion treatment for acute myocardial infarction (J-WIND): two randomised trials. *Lancet* 370: 1483–1493, 2007.

66. Kloner RA and Rezkalla SH. Cardiac protection during acute myocardial infarction: where do we stand in 2004? *J Am Coll Cardiol* 44: 276–286, 2004.

67. Labruto F, Yang J, Vaage J, and Valen G. Role of tumor necrosis factor alpha and its receptor I in preconditioning by hyperoxia. *Basic Res Cardiol* 100: 198–207, 2005.

68. Lacerda L, Smith RM, Opie L, and Lecour S. TNFalpha-induced cytoprotection requires the production of free radicals within mitochondria in C2C12 myotubes. *Life Sci* 79: 2194–2201, 2006.

69. Lacerda L, Somers S, Opie LH, and Lecour S. Ischaemic postconditioning protects against reperfusion injury via the SAFE pathway. *Cardiovasc Res* 84: 201–208, 2009.

70. Lecour S. Activation of the protective survivor activating factor enhancement (SAFE) pathway against reperfusion injury: does it go beyond the RISK pathway? *J Mol Cell Cardiol* 47: 32–40, 2009.

71. Lecour S. Multiple protective pathways against reperfusion injury: a SAFE path without Aktion? *J Mol Cell Cardiol* 46: 607–609, 2009.

72. Lecour S, Rochette L, and Opie L. Free radicals trigger TNF alpha-induced cardioprotection. *Cardiovasc Res* 65: 239–243, 2005.

73. Lecour S, Smith RM, Woodward B, Opie LH, Rochette L, and Sack MN. Identification of a novel role for sphingolipid signaling in TNF alpha and ischemic preconditioning mediated cardioprotection. *J Mol Cell Cardiol* 34: 509–518, 2002.

74. Lecour S, Suleman N, Deuchar GA, Somers S, Lacerda L, Huisamen B, and Opie LH. Pharmacological preconditioning with tumor necrosis factor-alpha activates signal transducer and activator of transcription-3 at reperfusion without involving classic prosurvival kinases (Akt and extracellular signal-regulated kinase). *Circulation* 112: 3911–3918, 2005.

75. This reference has been deleted.

76. Levine B, Kalman J, Mayer L, Fillit HM, and Packer M. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. *N Engl J Med* 323: 236–241, 1990.

77. Lim SY, Davidson SM, Hausenloy DJ, and Yellon DM. Preconditioning and postconditioning: the essential role of

the mitochondrial permeability transition pore. *Cardiovasc Res* 75: 530–535, 2007.

78. Lim SY, Davidson SM, Paramanathan AJ, Smith CC, Yellon DM, and Hausenloy DJ. The novel adipocytokine visfatin exerts direct cardioprotective effects. *J Cell Mol Med* 12: 1395–1403, 2008.

79. Ludman, A, Yellon DM, and Hausenloy DJ. Cardiac preconditioning for ischaemia: lost in translation. *Disease Models Mech* 3: 35–38, 2010.

80. Luo W, Li B, Chen R, Huang R, and Lin G. Effect of ischemic postconditioning in adult valve replacement. *Eur J Cardiothorac Surg* 33: 203–208, 2008.

81. MacEwan DJ. TNF ligands and receptors—a matter of life and death. *Br J Pharmacol* 135: 855–875, 2002.

82. Maekawa N, Wada H, Kanda T, Niwa T, Yamada Y, Saito K, Fujiwara H, Sekikawa K, and Seishima M. Improved myocardial ischemia/reperfusion injury in mice lacking tumor necrosis factor-alpha. *J Am Coll Cardiol* 39: 1229–1235, 2002.

83. Manintveld OC, Te Lintel HM, van den Bos EJ, Suurenbroek GM, Dekkers DH, Verdouw PD, Lamers JM, and Duncker DJ. Cardiac effects of postconditioning depend critically on the duration of index ischemia. *Am J Physiol Heart Circ Physiol* 292: H1551–H1560, 2007.

84. Mann DL. Stress-activated cytokines and the heart: from adaptation to maladaptation. *Annu Rev Physiol* 65: 81–101, 2003.

85. Marino MW, Dunn A, Grail D, Inglese M, Noguchi Y, Richards E, Jungbluth A, Wada H, Moore M, Williamson B, Basu S, and Old LJ. Characterization of tumor necrosis factor-deficient mice. *Proc Natl Acad Sci U S A* 94: 8093–8098, 1997.

86. Mascareno E, El Shafei M, Maulik N, Sato M, Guo Y, Das DK, and Siddiqui MA. JAK/STAT signaling is associated with cardiac dysfunction during ischemia and reperfusion. *Circulation* 104: 325–329, 2001.

87. McCormick J, Barry SP, Sivarajah A, Stefanutti G, Townsend PA, Lawrence KM, Eaton S, Knight RA, Thiemermann C, Latchman DS, and Stephanou A. Free radical scavenging inhibits STAT phosphorylation following *in vivo* ischemia/reperfusion injury. *FASEB J* 20: 2115–2117, 2006.

88. Monden Y, Kubota T, Inoue T, Tsutsumi T, Kawano S, Ide T, Tsutsui H, and Sunagawa K. Tumor necrosis factor-alpha is toxic via receptor 1 and protective via receptor 2 in a murine model of myocardial infarction. *Am J Physiol Heart Circ Physiol* 293: H743–H753, 2007.

89. Montecucco F, Lenglet S, Braunersreuther V, Burger F, Pelli G, Bertolotto M, Mach F, and Steffens S. CB(2) cannabinoid receptor activation is cardioprotective in a mouse model of ischemia/reperfusion. *J Mol Cell Cardiol* 46: 612–620, 2009.

90. Morrison RR, Tan XL, Ledent C, Mustafa SJ, and Hofmann PA. Targeted deletion of A2A adenosine receptors attenuates the protective effects of myocardial postconditioning. *Am J Physiol Heart Circ Physiol* 293: H2523–H2529, 2007.

91. Murozono Y, Takahashi N, Shinohara T, Ooie T, Teshima Y, Hara M, Saikawa T, and Yoshimatsu H. Hyperthermia-induced cardioprotection is potentiated by ischemic postconditioning in rats. *Exp Biol Med (Maywood)* 234: 573–581, 2009.

92. Myers MG, Jr. Cell biology. Moonlighting in mitochondria. *Science* 323: 723–724, 2009.

93. Na HS, Kim YI, Yoon YW, Han HC, Nahm SH, and Hong SK. Ventricular premature beat-driven intermittent restoration of coronary blood flow reduces the incidence of reperfusion-induced ventricular fibrillation in a cat model of regional ischemia. *Am Heart J* 132: 78–83, 1996.

94. Nakagawa T, Shimizu S, Watanabe T, Yamaguchi O, Otsu K, Yamagata H, Inohara H, Kubo T, and Tsujimoto Y. Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. *Nature* 434: 652–658, 2005.

95. Nakano M, Knowlton AA, Dibbs Z, and Mann DL. Tumor necrosis factor-alpha confers resistance to hypoxic injury in the adult mammalian cardiac myocyte. *Circulation* 97: 1392–1400, 1998.

96. Negoro S, Kunisada K, Tone E, Funamoto M, Oh H, Kishimoto T, and Yamauchi-Takihara K. Activation of JAK/STAT pathway transduces cytoprotective signal in rat acute myocardial infarction. *Cardiovasc Res* 47: 797–805, 2000.

97. Nishino Y, Webb IG, Davidson SM, Ahmed AI, Clark JE, Jacquet S, Shah AM, Miura T, Yellon DM, Avkiran M, and Marber MS. Glycogen synthase kinase-3 inactivation is not required for ischemic preconditioning or postconditioning in the mouse. *Circ Res* 103: 307–314, 2008.

98. Oikawa M, Yaoita H, Watanabe K, and Maruyama Y. Attenuation of cardioprotective effect by postconditioning in coronary stenosed rat heart and its restoration by carvedilol. *Circ J* 72: 2081–2086, 2008.

99. Okamoto F, Allen BS, Buckberg GD, Bugyi H, and Leaf J. Reperfusion conditions: importance of ensuring gentle versus sudden reperfusion during relief of coronary occlusion. *J Thorac Cardiovasc Surg* 92: 613–620, 1986.

100. Omura T, Yoshiyama M, Ishikura F, Kobayashi H, Takeuchi K, Beppu S, and Yoshikawa J. Myocardial ischemia activates the JAK-STAT pathway through angiotensin II signaling in *in vivo* myocardium of rats. *J Mol Cell Cardiol* 33: 307–316, 2001.

101. Ong SB, Subrayan S, Shiang YL, Yellon DM, Davidson SM, and Hausenloy DJ. Inhibiting mitochondrial fission to protect the heart from ischemia-reperfusion injury. *Circulation* 121: 2012–2022, 2010.

102. Oshima Y, Fujio Y, Nakanishi T, Itoh N, Yamamoto Y, Negoro S, Tanaka K, Kishimoto T, Kawase I, and Azuma J. STAT3 mediates cardioprotection against ischemia/reperfusion injury through metallothionein induction in the heart. *Cardiovasc Res* 65: 428–435, 2005.

103. Osugi T, Oshima Y, Fujio Y, Funamoto M, Yamashita A, Negoro S, Kunisada K, Izumi M, Nakaoka Y, Hirota H, Okabe M, Yamauchi-Takihara K, Kawase I, and Kishimoto T. Cardiac-specific activation of signal transducer and activator of transcription 3 promotes vascular formation in the heart. *J Biol Chem* 277: 6676–6681, 2002.

104. Penna C, Mancardi D, Tullio F, and Pagliaro P. Postconditioning and intermittent bradykinin induced cardioprotection require cyclooxygenase activation and prostacyclin release during reperfusion. *Basic Res Cardiol* 103: 368–377, 2008.

105. Piot C, Croisille P, Staat P, Thibault H, Rioufol G, Mewton N, Elbelghiti R, Cung TT, Bonnefoy E, Angoulvant D, Macia C, Raczka F, Sportouch C, Gahide G, Finet G, Andre-Fouet X, Revel D, Kirkorian G, Monassier JP, Derumeaux G, and Ovize M. Effect of cyclosporine on reperfusion injury in acute myocardial infarction. *N Engl J Med* 359: 473–481, 2008.

106. Qin LJ, Gu YT, Zhang H, and Xue YX. Bradykinin-induced blood-tumor barrier opening is mediated by tumor necrosis factor-alpha. *Neurosci Lett* 450: 172–175, 2009.

107. Raphael J, Gozal Y, Navot N, and Zuo Z. Hyperglycemia inhibits anesthetic-induced postconditioning in the rabbit heart via modulation of phosphatidylinositol-3-kinase/Akt and endothelial nitric oxide synthase signaling. *J Cardiovasc Pharmacol* 55: 348–357, 2010.

108. Safranow K, Dziedziejko V, Rzeuski R, Czyzycka E, Wojtarowicz A, Binczak-Kuleta A, Jakubowska K, Olszewska M, Ciechanowicz A, Kornacewicz-Jach Z, Machalinski B, Pawlik A, and Chlubek D. Plasma concentrations of TNF-alpha and its soluble receptors sTNFR1 and sTNFR2 in patients with coronary artery disease. *Tissue Antigens* 74: 386–392, 2009.

109. Samnegard A, Hulthe J, Silveira A, Ericsson CG, Hamsten A, and Eriksson P. Gender specific associations between matrix metalloproteinases and inflammatory markers in post myocardial infarction patients. *Atherosclerosis* 202: 550–556, 2009.

110. Schulman D, Latchman DS, and Yellon DM. Urocortin protects the heart from reperfusion injury via upregulation of p42/p44 MAPK signaling pathway. *Am J Physiol Heart Circ Physiol* 283: H1481–H1488, 2002.

111. Schutze S, Potthoff K, Machleidt T, Berkovic D, Wiegmann K, and Kronke M. TNF activates NF-kappa B by phosphatidylcholine-specific phospholipase C-induced "acidic" sphingomyelin breakdown. *Cell* 71: 765–776, 1992.

112. Schwartz LM and Lagranha CJ. Ischemic postconditioning during reperfusion activates Akt and ERK without protecting against lethal myocardial ischemia-reperfusion injury in pigs. *Am J Physiol Heart Circ Physiol* 290: H1011–H1018, 2006.

113. Sivaraman V, Mudalgiri NR, Di Salvo C, Kolvekar S, Hayward M, Yap J, Keogh B, Hausenloy DJ, and Yellon DM. Postconditioning protects human atrial muscle through the activation of the RISK pathway. *Basic Res Cardiol* 102: 453–459, 2007.

114. Skyschally A, Gres P, Hoffmann S, Haude M, Erbel R, Schulz R, and Heusch G. Bidirectional role of tumor necrosis factor-alpha in coronary microembolization: progressive contractile dysfunction versus delayed protection against infarction. *Circ Res* 100: 140–146, 2007.

115. Skyschally A, van Caster P, Boengler K, Gres P, Musiolik J, Schilawa D, Schulz R, and Heusch G. Ischemic postconditioning in pigs: no causal role for RISK activation. *Circ Res* 104: 15–18, 2009.

116. Smith RM, Suleman N, Lacerda L, Opie LH, Akira S, Chien KR, and Sack MN. Genetic depletion of cardiac myocyte STAT-3 abolishes classical preconditioning. *Cardiovasc Res* 63: 611–616, 2004.

117. Smith RM, Suleman N, McCarthy J, and Sack MN. Classic ischemic but not pharmacologic preconditioning is abrogated following genetic ablation of the TNFalpha gene. *Cardiovasc Res* 55: 553–560, 2002.

118. Somers S, Lacerda L, Opie L, and Lecour S. NFkB triggers TNF induced cardioprotection in C2C12. *J Mol Cell Cardiol* 38: 1040, 2005 [Abstract].

119. Somers S, Opie L, and Lecour S. Sphingosine-1-phosphate (S1P) can mimic ischaemic postconditioning via activation of the STAT-3 pathway. *Eur Heart J* 30: 729, 2009 [Abstract].

120. Staat P, Rioufol G, Piot C, Cottin Y, Cung TT, L'Huillier I, Aupetit JF, Bonnefoy E, Finet G, Andre-Fouet X, and Ovize M. Postconditioning the human heart. *Circulation* 112: 2143–2148, 2005.

121. Suleman N, Opie L, and Lecour S. Ischemic postconditioning confers cardioprotection via phosporylation of STAT-3. *J Mol Cell Cardiol* 40: 977, 2006 [Abstract].

122. Suleman N, Somers S, Smith R, Opie LH, and Lecour SC. Dual activation of STAT-3 and Akt is required during the trigger phase of ischaemic preconditioning. *Cardiovasc Res* 79: 127–133, 2008.

123. Tamareille S, Ghaboura N, Treguer F, Khachman D, Croue A, Henrion D, Furber A, and Prunier F. Myocardial reperfusion injury management: erythropoietin compared with postconditioning. *Am J Physiol Heart Circ Physiol* 297: H2035–H2043, 2009.

124. Tanno M, Gorog DA, Bellahcene M, Cao X, Quinlan RA, and Marber MS. Tumor necrosis factor-induced protection of the murine heart is independent of p38-MAPK activation. *J Mol Cell Cardiol* 35: 1523–1527, 2003.

125. Thibault H, Piot C, Staat P, Bontemps L, Sportouch C, Rioufol G, Cung TT, Bonnefoy E, Angoulvant D, Aupetit JF, Finet G, Andre-Fouet X, Macia JC, Raczka F, Rossi R, Itti R, Kirkorian G, Derumeaux G, and Ovize M. Long-term benefit of postconditioning. *Circulation* 117: 1037–1044, 2008.

126. Torre-Amione G, Kapadia S, Lee J, Bies RD, Lebovitz R, and Mann DL. Expression and functional significance of tumor necrosis factor receptors in human myocardium. *Circulation* 92: 1487–1493, 1995.

127. Tsang A, Hausenloy DJ, Mocanu MM, and Yellon DM. Postconditioning: a form of "modified reperfusion" protects the myocardium by activating the phosphatidylinositol 3-kinase-Akt pathway. *Circ Res* 95: 230–232, 2004.

128. Vessey DA, Li L, Kelley M, Zhang J, and Karliner JS. Sphingosine can pre- and post-condition heart and utilizes a different mechanism from sphingosine 1-phosphate. *J Biochem Mol Toxicol* 22: 113–118, 2008.

129. Wagner C, Kloeting I, Strasser RH, and Weinbrenner C. Cardioprotection by postconditioning is lost in WOKW rats with metabolic syndrome: role of glycogen synthase kinase 3beta. *J Cardiovasc Pharmacol* 52: 430–437, 2008.

130. Wegrzyn J, Potla R, Chwae YJ, Sepuri NB, Zhang Q, Koeck T, Derecka M, Szczepanek K, Szelag M, Gornicka A, Moh A, Moghaddas S, Chen Q, Bobbili S, Cichy J, Dulak J, Baker DP, Wolfman A, Stuehr D, Hassan MO, Fu XY, Avadhani N, Drake JI, Fawcett P, Lesnefsky EJ, and Larner AC. Function of mitochondrial Stat3 in cellular respiration. *Science* 323: 793–797, 2009.

131. Yamashita N, Hoshida S, Otsu K, Taniguchi N, Kuzuya T, and Hori M. Involvement of cytokines in the mechanism of whole-body hyperthermia-induced cardioprotection. *Circulation* 102: 452–457, 2000.

132. Yang XM, Proctor JB, Cui L, Krieg T, Downey JM, and Cohen MV. Multiple, brief coronary occlusions during early reperfusion protect rabbit hearts by targeting cell signaling pathways. *J Am Coll Cardiol* 44: 1103–1110, 2004.

133. Yellon DM and Baxter GF. Reperfusion injury revisited: is there a role for growth factor signaling in limiting lethal reperfusion injury? *Trends Cardiovasc Med* 9: 245–249, 1999.

134. Yellon DM and Hausenloy DJ. Myocardial reperfusion injury. *N Engl J Med* 357: 1121–1135, 2007.

135. Yokoyama T, Vaca L, Rossen RD, Durante W, Hazarika P, and Mann DL. Cellular basis for the negative inotropic effects of tumor necrosis factor-alpha in the adult mammalian heart. *J Clin Invest* 92: 2303–2312, 1993.

136. Zatta AJ, Kin H, Yoshishige D, Jiang R, Wang N, Reeves JG, Mykytenko J, Guyton RA, Zhao ZQ, Caffrey JL, and Vinten-Johansen J. Evidence that cardioprotection by postconditioning involves preservation of myocardial opioid content and selective opioid receptor activation. *Am J Physiol Heart Circ Physiol* 294: H1444–H1451, 2008.

137. Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton RA, and Vinten-Johansen J. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol* 285: H579–H588, 2003.

138. Zhu M, Feng J, Lucchinetti E, Fischer G, Xu L, Pedrazzini T, Schaub MC, and Zaugg M. Ischemic postconditioning protects remodeled myocardium via the PI3K-PKB/Akt reperfusion injury salvage kinase pathway. *Cardiovasc Res* 72: 152–162, 2006.

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Abbreviations Used

DAG = diacylglycerol
Erk = extraregulated kinase
eNOS = endothelial nitric oxide synthase
FADD = Fas-associated death domain
GSK3β = glycogen synthase kinase 3 beta
IPC = ischemic preconditioning
IPost = ischemic postconditioning
IRI = ischemia-reperfusion injury
IS = infarct size
JAK = Janus kinase
JNK = Jun N-terminal kinase
MAPK = mitogen-activated protein kinase
MEK = mitogen extraregulated kinase
MI = myocardial infarction
mPTP = mitochondrial permeability transition pore
NO = nitric oxide
PI3K = phosphatidyl inositol 3 kinase
PKC = protein kinase C
PKCε = protein kinase C epsilon
PKG = protein kinase G
PLC = phospholipase C
RI = reperfusion ischemia
RISK = reperfusion injury salvage kinase
ROS = reactive oxygen species
SAFE = survivor activating factor enhancement
S1P = sphingosine-1 phosphate
SPK-1 = sphingosine kinase-1
STAT3 = signal transducer and activator of transcription 3
TNFR = tumor necrosis factor receptor

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1. C. Zhou, Y. Yao, Z. Zheng, J. Gong, W. Wang, S. Hu, L. Li. 2012. Stenting technique, gender, and age are associated with cardioprotection by ischaemic postconditioning in primary coronary intervention: a systematic review of 10 randomized trials. *European Heart Journal* . [CrossRef]

2. Antonio Rodríguez-Sinovas, Jose A. Sánchez, Celia Fernandez-Sanz, Marisol Ruiz-Meana, David Garcia-Dorado. 2012. Connexin and pannexin as modulators of myocardial injury. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1818**:8, 1962-1970. [CrossRef]

3. Claudia Penna , Maria-Giulia Perrelli , Pasquale Pagliaro . Mitochondrial Pathways, Permeability Transition Pore, and Redox Signaling in Cardioprotection: Therapeutic Implications. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]

4. Robert M. Bell, Derek M. Yellon. 2012. Conditioning the whole heart—not just the cardiomyocyte. *Journal of Molecular and Cellular Cardiology* **53**:1, 24-32. [CrossRef]

5. Jingyuan Li, Soban Umar, Andrea Iorga, Ji-Youn Youn, Yibin Wang, Vera Regitz-Zagrosek, Hua Cai, Mansoureh Eghbali. 2012. Cardiac vulnerability to ischemia/reperfusion injury drastically increases in late pregnancy. *Basic Research in Cardiology* **107**:4. . [CrossRef]

6. Claudia Penna, Teresa Pasqua, Maria-Giulia Perrelli, Pasquale Pagliaro, Maria Carmela Cerra, Tommaso Angelone. 2012. Postconditioning with glucagon like peptide-2 reduces ischemia/reperfusion injury in isolated rat hearts: role of survival kinases and mitochondrial KATP channels. *Basic Research in Cardiology* **107**:4. . [CrossRef]

7. O. Morel, T. Perret, N. Delarche, J.-N. Labeque, B. Jouve, M. Elbaz, C. Piot, M. Ovize. 2012. Pharmacological approaches to reperfusion therapy. *Cardiovascular Research* . [CrossRef]

8. W. Shi, J. Vinten-Johansen. 2012. Endogenous cardioprotection by ischaemic postconditioning and remote conditioning. *Cardiovascular Research* . [CrossRef]

9. D. J. Hausenloy, E. K. Iliodromitis, I. Andreadou, A. Papalois, G. Gritsopoulos, M. Anastasiou-Nana, D. T. Kremastinos, D. M. Yellon. 2011. Investigating the Signal Transduction Pathways Underlying Remote Ischemic Conditioning in the Porcine Heart. *Cardiovascular Drugs and Therapy* . [CrossRef]

10. Sophie Tamareille, Victor Mateus, Nehmat Ghaboura, Julien Jeanneteau, Anne Croué, Daniel Henrion, Alain Furber, Fabrice Prunier. 2011. RISK and SAFE signaling pathway interactions in remote limb ischemic perconditioning in combination with local ischemic postconditioning. *Basic Research in Cardiology* . [CrossRef]

11. Yoshitaka Kawaraguchi, David M. Roth, Hemal H. Patel. 2011. From Local to Global. *Anesthesiology* 1. [CrossRef]

12. Pasquale Pagliaro , Francesca Moro , Francesca Tullio , Maria-Giulia Perrelli , Claudia Penna . 2011. Cardioprotective Pathways During Reperfusion: Focus on Redox Signaling and Other Modalities of Cell Signaling. *Antioxidants & Redox Signaling* **14**:5, 833-850. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]

13. Kerstin Boengler , Gerd Heusch , Rainer Schulz . 2011. Mitochondria in Postconditioning. *Antioxidants & Redox Signaling* **14**:5, 863-880. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]

14. Pasquale Pagliaro , Claudia Penna . 2011. Cardiac Postconditioning. *Antioxidants & Redox Signaling* **14**:5, 777-779. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]

15. T. T. Nielsen, N. B. Stottrup, B. Lofgren, H. E. Botker. 2011. Metabolic fingerprint of ischaemic cardioprotection: importance of the malate-aspartate shuttle. *Cardiovascular Research* . [CrossRef]

16. Sze Wong, Po Chiu, Hoi Leung, Limin Zhou, Zhong Zuo, Philip Y Lam, Kam Ko. 2011. Myocardial post-conditioning with Danshen-Gegen decoction protects against isoproterenol-induced myocardial injury via a PKC#/mKATP-mediated pathway in rats. *Chinese Medicine* **6**:1, 7. [CrossRef]

17. Arjun Iyer, Gayathri Kumarasinghe, Mark Hicks, Alasdair Watson, Ling Gao, Aoife Doyle, Anne Keogh, Eugene Kotlyar, Christopher Hayward, Kumud Dhital, Emily Granger, Paul Jansz, Roger Pye, Phillip Spratt, Peter Simon Macdonald. 2011. Primary Graft Failure after Heart Transplantation. *Journal of Transplantation* **2011**, 1-9. [CrossRef]

18. Ann-Dorit Andersen, Bo Hjorth Bentzen, Henrik Salling, Henrik Klingberg, Morten Kanneworff, Morten Grunnet, Stine F. Pedersen. 2011. The Cardioprotective Effect of Brief Acidic Reperfusion after Ischemia in Perfused Rat Hearts is not Mimicked by Inhibition of the Na^+/H^+ Exchanger NHE1. *Cellular Physiology and Biochemistry* **28**:1, 13-24. [CrossRef]