Remote ischaemic preconditioning: underlying mechanisms and clinical application

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REMOTE ISCHAEMIC PRECONDITIONING (RIPC) represents a strategy for harnessing the body’s endogenous protective capabilities against the injury incurred by ischaemia and reperfusion. It describes the intriguing phenomenon in which transient non-lethal ischaemia and reperfusion of one organ or tissue confers resistance to a subsequent episode of lethal ischaemia reperfusion injury in a remote organ or tissue. In its original conception, it described intramyocardial protection, which could be relayed from the myocardium served by one coronary artery to another. It soon became apparent that myocardial infarct size could be dramatically reduced by applying brief ischaemia and reperfusion to an organ or tissue remote from the heart before the onset of myocardial infarction. The concept of remote organ protection has now been extended beyond that of solely protecting the heart to providing a general form of inter-organ protection against ischaemia-reperfusion injury. This article reviews the history and evolution of the phenomenon that is RIPC, the potential mechanistic pathways underlying its cardioprotective effect, and its emerging application in the clinical setting.

**KEYWORDS**
Ischaemia; Reperfusion; Infarction; Preconditioning

1. Introduction

New treatment strategies are required to reduce myocardial injury and improve clinical outcomes in patients with coronary heart disease, the leading cause of death worldwide. Following an acute myocardial infarction (AMI), timely myocardial reperfusion using either thrombolytic therapy or primary percutaneous coronary intervention (PPCI) to restore blood flow in the infarct-related coronary artery remains the most effective intervention for limiting myocardial infarct size, preserving left ventricular (LV) ejection fraction, preventing LV remodelling, and improving clinical outcomes.

In the experimental setting, myocardial infarct size can be dramatically reduced by subjecting the heart to one or more episodes of non-lethal myocardial ischaemia and reperfusion prior to the sustained coronary artery occlusion, an endogenous cardioprotective phenomenon termed IPC (ischaemic preconditioning). Its clinical application, however, is restricted to the elective settings of cardiac surgery, in which the timing of the ischaemic insult can be readily anticipated. Patients presenting with an AMI require a treatment strategy, which can be implemented at the time of myocardial reperfusion. In this regard, the ability to reduce myocardial infarct size in the setting of PPCI by interrupting myocardial reperfusion with short-lived episodes of coronary re-occlusion, a phenomenon termed ischaemic postconditioning (IPost), has renewed interest in the reperfusion phase as a target for cardioprotection. However, both IPC and IPost necessitate an invasive treatment being applied directly to the myocardium in order to achieve cardioprotection, which in some clinical settings can be impractical and harmful.

An alternative more amenable strategy is to apply the cardioprotective stimulus to an organ or tissue remote from the heart, an approach encapsulated by the phenomenon of remote ischaemic preconditioning (RIPC), an idea which was first conceived by Przyklenk et al. in 1993. These authors made the intriguing discovery that inducing brief episodes of ischaemia and reperfusion in the circumflex coronary artery territory had the capacity to reduce the subsequent size of the myocardial infarct, arising from the occlusion of the left anterior descending coronary artery. This form of intramyocardial protection was later extended to non-cardiac organs, with the report that myocardial infarct size could actually be reduced in the animal heart by inducing brief ischaemia and reperfusion in either the kidney or the small intestine immediately prior to the sustained coronary artery occlusion (reviewed in and further). Furthermore, the newly conceived idea of applying a remote preconditioning stimulus after the onset of the myocardial infarction but prior to reperfusion, a concept termed remote ischaemic postconditioning (RIPost), offers the...
possibility of applying this cardioprotective strategy to patients presenting with an AMI. The concept of RIPC has now been extended to different organs and tissues such that it has emerged as a true strategy of inter-organ protection against the detrimental effects of acute ischaemia-reperfusion injury (Figure 1).

2. Brief limb ischaemia and reperfusion as a remote preconditioning stimulus

These early experimental studies had depended on using a non-cardiac organ as the remote preconditioning stimulus, which of course requires an invasive operative procedure to apply the preconditioning ischaemia. However, for the clinical application of this cardioprotective strategy a less invasive method for applying the remote preconditioning ischaemia was required. Initial progress was made in this direction by Birnbaum et al., in 1997, with the critical observation that briefly restricting blood flow to skeletal muscle of the lower limb and pacing the gastrocnemius leg muscle prior to an acute coronary artery occlusion was able to reduce the subsequent sustained myocardial infarct size by 65% in the rabbit heart, a phenomenon which was termed ‘IPC at a distance’. A less invasive method of inducing hind-limb ischaemia as a remote preconditioning stimulus was introduced by Oxman et al., who demonstrated that applying a tourniquet to the hind-limb to induce 10 min of limb ischaemia had the ability to reduce reperfusion arrhythmias in a rat heart following a sustained ischaemic insult.

As a non-invasive RIPC stimulus, brief limb ischaemia can also be achieved in human volunteers by applying an inflated blood pressure cuff to the upper or lower limb. MacAllister and co-workers, have pioneered the use of this approach in human volunteers and more recently in patients with stable coronary heart disease (see Figure 2 for a summary). In the original study, transient ischaemia and reperfusion of the arm was induced by inflating a blood pressure cuff placed on the upper arm to 200 mmHg for 5 min and deflating the cuff for 5 min; a cycle which was repeated three times. This RIPC stimulus was able to attenuate the endothelial dysfunction in the contralateral arm arising from a 20 min episode of sustained cuff inflation. The same experimental model has been used to reproduce the concept of delayed RIPC, in which the preconditioning upper limb ischaemia confers an improvement in endothelial function in the contralateral arm 24–48 h later. Most recently, using this model, the phenomenon of RIPC has been demonstrated in healthy human volunteers and patients with stable coronary heart disease.

3. Potential mechanisms underlying remote preconditioning

The actual mechanism through which an episode of brief ischaemia and reperfusion in an organ or tissue exerts protection against a subsequent sustained insult of ischaemia-reperfusion injury in a remote organ or tissue is currently unclear. Studies suggest that some of the underlying mechanistic pathways and signal transduction cascades
activated within remotely preconditioned cardiomyocytes may be similar to those recruited in the setting of IPC and postconditioning.\textsuperscript{14} However, the mechanistic pathway linking the remote organ or tissue to the heart is currently unclear although several mechanisms have been proposed and are reviewed here (Figure 2). It is important to appreciate that these mechanistic pathways may interact with each other and are therefore not mutually exclusive.

3.1 Evidence for a potential humoral factor in remote preconditioning

The finding that a period of reperfusion of the remote preconditioning organ was required in addition to the brief ischaemia, suggested that the reperfusion period may be needed to ‘washout’ a substance or humoral factor generated by the preconditioning ischaemia, which was then transported to the heart (Table 1).\textsuperscript{4,15} This hypothesis was consolidated by a study reporting that blood taken from a remote preconditioned rabbit which had been subjected to simultaneous IPC of the heart and kidney, could reduce a subsequent untreated rabbit,\textsuperscript{16} suggesting the transfer of one or more humoral cardioprotective factors. The same authors went onto demonstrate that coronary effluent from an isolated rabbit heart treated with a standard IPC protocol, could reduce myocardial infarct size by 69%\textsuperscript{17} and improve recovery of LV function\textsuperscript{18} when used to perfuse an untreated isolated rabbit heart. The investigators demonstrated no difference in concentrations of either adenosine or noradrenaline in the coronary effluent from preconditioned hearts vs. control hearts, suggesting that neither of these were the humoral cardioprotective factor.\textsuperscript{17}

Convincing evidence in support of a humoral mechanism for RIPC was provided in an elegant experimental study by Konstantinov \textit{et al.}\textsuperscript{19} Remote limb preconditioning of a pig that had received a donor heart was able to reduce myocardial infarct size in the denervated donor heart, providing strong evidence that a humoral mediator was responsible for RIPC protection, although an afferent sensory nerve pathway from the limb cannot be excluded.\textsuperscript{19} A similar type of study was conducted by Kristiansen \textit{et al.}\textsuperscript{20} who

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<td>Intestinal ischaemia 15’ I 10’ R</td>
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<td>Neural pathway first implicated</td>
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<td>Schoemaker and Heijningen\textsuperscript{24}</td>
<td>Rat</td>
<td>Intestinal ischaemia 15’ I 10’ R</td>
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<td>Ding \textit{et al.}\textsuperscript{36}</td>
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<td>I/R%; 34%</td>
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<td>Liem \textit{et al.}\textsuperscript{37}</td>
<td>Rat</td>
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<td>Adenosine receptor binding implicated as neural mediator</td>
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<td>Dong \textit{et al.}\textsuperscript{38}</td>
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<td>McClanahan \textit{et al.}\textsuperscript{4}</td>
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<td>Oxman \textit{et al.}\textsuperscript{10}</td>
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</tr>
<tr>
<td>Pell \textit{et al.}\textsuperscript{23}</td>
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<td>I/R%; 45% Preserved ATP levels, less pH</td>
<td>Adenosine receptor binding and K\textsubscript{ATP} channel opening first implicated</td>
</tr>
<tr>
<td>Takaoka \textit{et al.}\textsuperscript{35}</td>
<td>Rabbit</td>
<td>Renal ischaemia 10’ I 20’ R</td>
<td>I/R%; 53% Preserved ATP levels, less pH</td>
<td>Adenosine receptor binding implicated</td>
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<td>Rabbit</td>
<td>Myocardial and renal ischaemia of donor animal 5’ I 10’ R (\times 5)</td>
<td>I/R%; 77% in acceptor animal</td>
<td>Humoral factor transferred in blood</td>
</tr>
<tr>
<td>Dickson \textit{et al.}\textsuperscript{17}</td>
<td>Rabbit</td>
<td>Myocardial ischaemia of donor heart 5’ I 10’ R (\times 3)</td>
<td>I/R%; 69% in acceptor heart</td>
<td>Humoral factor transferred in blood</td>
</tr>
<tr>
<td>Dickson \textit{et al.}\textsuperscript{18}</td>
<td>Rabbit</td>
<td>Myocardial ischaemia of donor heart 5’ I 10’ R (\times 3)</td>
<td>Improved recovery of LVF in acceptor heart</td>
<td>Humoral factor transferred in coronary effluent. Not adenosine or noradrenaline</td>
</tr>
<tr>
<td>Xiao \textit{et al.}\textsuperscript{41}</td>
<td>Rat</td>
<td>Intestinal ischaemia 4’ I 4’ R (\times 3)</td>
<td>I/R%; 50%</td>
<td>CGRP and nitric oxide implicated</td>
</tr>
</tbody>
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\textit{Continued}
demonstrated that hearts excised from a rat that had been remote limb preconditioned experienced a smaller infarct size, when subjected to a sustained episode of myocardial ischaemia and reperfusion on a Langendorff-apparatus.

3.1.1 Attempting to identify the humoral mediator of remote ischaemic preconditioning

Experimental studies have attempted to identify the humoral factor, which conveys the preconditioning signal from the remote organ to the heart (Table 1). Using a proteomic method, Lang et al.\(^2\) failed to identify a specific protein $>8 \text{kDa}$ in rats subjected to a remote renal preconditioning stimulus. Serejo et al.\(^3\) collected the effluent from preconditioned rat hearts and identified temperature-sensitive hydrophobic substances with molecular weights $>3.5 \text{kDa}$, which conferred cardioprotection through the activation of protein kinase C (PKC). However, the actual identity of the humoral mediator remains unknown.

Other studies have investigated whether endogenous substances such as adenosine,$^{23}$ bradykinin,$^{24}$ opioids,$^{25}$ CGRP (calcitonin gene-related peptide),$^{26}$ and endocannabinoids$^{27}$ are released from the remote organ during the preconditioning ischaemia and are carried to the heart in the blood stream where they then activate intracellular pathways of cardioprotection. Alternatively, the endogenous mediator may activate afferent neural pathways within the remote preconditioned organ to confer cardioprotection, as is the case with adenosine, bradykinin and CGRP (discussed later). A recent experimental study has implicated the transcription factor hypoxia-inducible factor (HIF)-1$\alpha$ as a potential mediator of RIPC-induced cardioprotection.$^{28}$

3.1.2 Opioids

The involvement of opioid signalling in RIPC was first reported by Patel et al.$^{29}$ in 2002 (Table 1). They demonstrated that the non-specific opioid receptor antagonist, naloxone, was capable of abolishing the myocardial infarct-limiting effects conferred by remote intestinal preconditioning in the rat.$^{25}$ Dickson et al.$^{29}$ implicated opioid receptor binding in the protective effect of coronary effluent from preconditioned rats hearts on ischaemic intestinal tissue. Subsequent experimental studies have implicated the $\delta$-opioid receptor in remote limb preconditioning of skeletal muscle in the pig$^{30}$ and in remote infra-renal aortic preconditioning of the rat heart.$^{31}$ However, a recent study failed to find evidence for the involvement of the $\delta$-opioid receptor and instead implicated the $\kappa$-opioid receptor in RIPC using femoral artery occlusion as the

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|}
\hline
Study & Infarction model & RIPC stimulus & Endpoint & Mechanistic insight \\
\hline
\hline
Wolfrum et al.$^{39}$ & Rat & Intestinal ischaemia $15^\circ$ I $15^\circ$ R & I/R$\%$ 48% & Bradykinin receptor (B2) binding and PKC-$\varepsilon$ activation implicated \\
\hline
Patel et al.$^{25}$ & Rat & Intestinal ischaemia $15^\circ$ I $10^\circ$ R & I/R$\%$ 44% & Opioid receptor binding first implicated \\
Weinbrenner et al.$^{48}$ & Rat & Infra-renal aortic occlusion $15^\circ$ I $10^\circ$ R & I/R$\%$ 71% & PKC activation implicated \\
Tokuno et al.$^{32}$ & Mouse & Permanent bilateral internal carotid occlusion & I/R$\%$ 12% 24 h later & iNOS activation implicated \\
Weinbrenner et al.$^{31}$ & Rat & Infra-renal aortic occlusion $15^\circ$ I $10^\circ$ R & I/R$\%$ 63% & Reactive oxygen species first implicated. \\
Singh and Chopra$^{34}$ & Rat & Renal ischaemia $5^\circ$ I $10^\circ$ R ($\times 4$) & I/R$\%$ 78% & Opioid $\delta$ receptor implicated \\
Konstantinov et al.$^{19}$ & Pig & Hindlimb ischaemia (tourniquet) $5^\circ$ I $5^\circ$ R ($\times 4$) & I/R$\%$ 57% in denervated donor heart & angiotensin I receptor binding first implicated \\
Kristiansen et al.$^{20}$ & Rat & Hindlimb ischaemia (tourniquet) $5^\circ$ I $5^\circ$ R ($\times 4$) & I/R$\%$ 56% in explanted perfused heart & Strong evidence against neural pathway. \\
Wolfrum et al.$^{42}$ & Rat & Intestinal ischaemia $15^\circ$ I $15^\circ$ R & I/R$\%$ 57% & Strong evidence against neural pathway. \\
Lang et al.$^{21}$ & Rat & Renal ischaemia $10^\circ$ I $20^\circ$ R & I/R$\%$ 35% & K$_{ATP}$ channel implicated \\
Zhang et al.$^{32}$ & Rat & Femoral artery occlusion $5^\circ$ I $5^\circ$ R ($\times 3$) & I/R$\%$ 63% & CGRP and PKC-$\varepsilon$ activation implicated. Attempt to identify humoral mediator using proteomics \\
Hajrasouliha et al.$^{27}$ & Rat & Intestinal ischaemia $15^\circ$ I $15^\circ$ R & I/R$\%$ 49% & $\kappa$-opioid receptor instead of $\delta$-opioid receptor implicated. mPTP inhibition also suggested \\
Heidbreder et al.$^{49}$ & Rat & Intestinal ischaemia $15^\circ$ I $15^\circ$ R & I/R$\%$ 50% & CB2 cannabinoid receptor binding but not CB1 implicated \\
Kant et al.$^{28}$ & Rat & Renal ischaemia $15^\circ$ I $5^\circ$ I $5^\circ$ R ($\times 4$) & I/R$\%$ 55% & JNK, p38 and Erk1/2 MAPK activation in remote organ implicated \\
Peralta et al.$^{45}$ & Rat & Hepatic ischaemia $10^\circ$ I $10^\circ$ R ($\times 1$) & Inflammatory response & Suppressed inflammatory response \\
Konstantinov et al.$^{44}$ & Mouse & Femoral artery ischaemia $5^\circ$ I $5^\circ$ R ($\times 6$) & Systemic response & Modification of gene expression \\
\hline
\end{tabular}
\caption{Continued}
\end{table}

\footnotesize
1, Ischaemia; R, Reperfusion; I/R%, myocardial infarct size expressed as a percentage at risk volume.
preconditioning stimulus to reduce myocardial infarct size in rat hearts.\textsuperscript{32} It has been proposed that endogenous opioids generated by the preconditioning stimulus in the remote organ enter the blood stream where they act directly on the myocardium to confer cardioprotection (Figure 2),\textsuperscript{25} although further studies are required to both investigate this proposal and delineate the individual contributions of the different receptor subtypes to RIPC.

3.1.3 Endocannabinoids

Previous studies have implicated binding at the CB2 endocannabinoid receptor of the endogenous cannabinoid system in protection from myocardial ischaemia reperfusion injury.\textsuperscript{33} A recent experimental study has implicated endogenous activation of the CB2 receptor in the myocardial infarct-limiting effects of remote intestinal preconditioning, using a pharmacological CB2 antagonist to abolish RIPC protection.\textsuperscript{27} The authors proposed that endocannabinoids generated by the intestinal ischaemia may enter the blood stream and activate CB2 receptors on the myocardium, but of course further studies are required to test this hypothesis (Figure 2).

3.1.4 Other receptor ligands

It has been reported that the reduction in myocardial infarct size elicited by remote renal preconditioning in the rat can be abolished by losartan, an Angiotensin I receptor blocker,\textsuperscript{34} but the subsequent mechanism of protection remains to be explored. Whether noradrenaline acts as a mediator of cardioprotection in the setting of RIPC is not conclusive, with conflicting studies.\textsuperscript{10,17}

3.2 Evidence for a potential neural pathway in remote preconditioning

One of the early studies of RIPC first provided potential evidence that a neural pathway may underlie the cardioprotection elicited by remote preconditioning of a non-cardiac organ. Gho et al.\textsuperscript{15} demonstrated that the reduction in myocardial infarct size induced by brief ischaemia and reperfusion of the anterior mesenteric artery could be reversed in the presence of the ganglion blocker, hexamethonium. The hypothesis for a neural pathway was further developed with the proposition that endogenous substances such as adenosine,\textsuperscript{23} bradykinin,\textsuperscript{24} CGRP,\textsuperscript{26} released by the remote preconditioned organ, stimulated afferent nerve fibres, which then relay to efferent nerve fibres terminating on the myocardium to confer cardioprotection.

3.2.1 Adenosine

In 1998, our laboratory was the first to implicate adenosine as a potential mediatory factor underlying cardioprotection in the setting of RIPC, demonstrating that the administration of the non-specific adenosine receptor antagonist, 8-sulphophenyltheophylline (8-SPT), prior to the RIPC protocol could abolish the reduction in myocardial infarct size induced by a remote preconditioning stimulus in the rabbit kidney.\textsuperscript{23} In a subsequent study by Takao et al.,\textsuperscript{35} it was demonstrated that 8-SPT administered after the renal RIPC stimulus also had the ability to block cardioprotection suggesting that myocardial adenosine receptor binding was required for cardioprotection, a finding which was supported by their finding of elevated plasma levels of adenosine in blood sampled from the carotid artery of rabbits subjected to RIPC compared with those treated with IPC alone.\textsuperscript{35}

Ding et al.\textsuperscript{36} later demonstrated that renal nerve section abolished the cardioprotective effect induced by a preconditioning renal ischaemia stimulus providing strong supportive evidence of a neural pathway. They then reported that during the renal preconditioning stimulus, renal afferent nerve discharge was increased and that this enhanced neural activity could be abrogated by 8-SPT.\textsuperscript{36} Further confirmatory evidence implicating adenosine in a neural pathway of cardioprotection was provided by Liem et al.\textsuperscript{37} who after confirming that the prior administration of hexamethonium or 8-SPT abolished the myocardial infarct size reduction induced by brief mesenteric ischaemia and reperfusion, demonstrated that the local administration of adenosine into the mesenteric vascular bed also conferred cardioprotection in a manner which was sensitive to hexamethonium.\textsuperscript{37} These findings suggested that brief episodes of ischaemia of the small intestine might generate adenosine, which would then activate mesenteric afferent sensory nerves. However, the investigators went on to report that 8-SPT administered after the remote preconditioning stimulus was also able to inhibit cardioprotection, suggesting that adenosine receptor binding in the heart may also be required for protection.\textsuperscript{37}

A subsequent experimental study has provided confirmatory evidence of an adenosine-mediated neural pathway using limb ischaemia as the preconditioning stimulus.\textsuperscript{38} Dong et al.\textsuperscript{38} demonstrated that the myocardial infarct-limiting effect of remote hindlimb preconditioning was abolished by dissecting the femoral nerve, suggesting that an intact neural pathway is required for the sensory afferent neural signalling from the preconditioning limb. Injection of intra-femoral arterial adenosine was also found to reduce myocardial infarct size.\textsuperscript{38}

3.2.2 Bradykinin

Schoemaker and van Heijningen\textsuperscript{24} demonstrated that the reduction in myocardial infarct size elicited by brief mesenteric artery occlusion and reperfusion could be abolished by prior administration of HOE140, a specific bradykinin B2 receptor antagonist (Table 1). Interestingly, they went on to find that intra-mesenteric arterial administration of bradykinin was also able to confer cardioprotection in a manner, which was sensitive to ganglion blockade by hexamethonium.\textsuperscript{24} The authors suggested that bradykinin generated during the remote preconditioning intestinal ischaemia, may stimulate mesenteric afferent sensory nerves, which then mediate the cardioprotective effect.\textsuperscript{24}

These findings were confirmed in a subsequent study by Wolfrum et al.\textsuperscript{39} who also observed that the activation of myocardial PKC-ɛ by brief intestinal ischaemia was blocked by HOE-140 and hexamethonium, suggesting that PKC-ɛ was positioned downstream of bradykinin and the neural pathway.

3.2.3 Calcitonin gene-related peptide

Several experimental studies have implicated CGRP, a neurotransmitter released from capsaicin-sensitive sensory nerves, as a potential mediator of both IPC\textsuperscript{40} and RIPC. (Table 1).\textsuperscript{26,41} These can be summarized as follows: remote intestinal preconditioning generates nitric oxide (NO) which stimulates capsaicin-sensitive sensory nerves in
the intestinal vasculature, releasing CGRP into the bloodstream (where levels have reported to be increased by RIPC), which is then carried to the heart where it activates myocardial PKC-β.30,42

3.3 Evidence for a systemic response in remote preconditioning

Several experimental studies have examined the effect of remote preconditioning of an organ or tissue on the myocardial gene transcription profile,33,44 and the inflammatory response,45 and have discovered that the inflammatory response is suppressed and a favourable profile of gene transcription appears to be activated that is both anti-inflammatory and anti-apoptotic (Figure 2 and Tables 1 and 2). The relevance of such a response to the cardioprotective effect elicited by RIPC is currently unclear and requires further investigation.

3.4 Myocardial mechanisms of cardioprotection in remote preconditioning

Once the cardioprotective signal has been conveyed from the remote preconditioning organ to the heart, intracellular signal transduction mechanisms are recruited within cardiomyocytes, which are similar to those that participate in IPC46 and postconditioning.47 These include the ligand binding to G-protein cell surface coupled receptors such as adenosine,23 bradykinin,24 opioids,25 angiotensin,34 and endocannabinoids.27 The binding to these cell surface receptors appears to then activate intracellular kinases such as PKC-ε,39 and other signalling components such as reactive oxygen species,46 NO (discussed later) and the mitochondrial K<sub>ATP</sub> channel (Figure 2).23 Whether RIPC also activates pro-survival kinases of the reperfusion injury salvage kinase (RISK) pathway and results in the inhibition of the mitochondrial permeability transition pore (mPTP), as in IPC and postconditioning, remains to be determined.14 In this regard, an interesting recent study suggests that the activation of the mitogen-activated protein kinases (MAPKs) JNK, p38 and Erk1/2 within the remote organ may contribute to RIPC-induced cardioprotection.49 Its authors reported that small intestinal remote preconditioning-activated MAPKs within the intestinal tissue but not the myocardium and reduced myocardial infarct size, and that pharmacologically inhibiting the activation of these kinase abolished RIPC. However, it remains to be determined whether Akt and these MAPKs would have been activated within the myocardium at the onset of myocardial reperfusion as part of the RISK pathway.

3.4.1 K<sub>ATP</sub> channel

Both the myocardial sarcolemmal and the mitochondrial K<sub>ATP</sub> channels have been implicated as critical triggers or mediators in the cardioprotective phenomenon of IPC.46 The current paradigm proposes that ligand-receptor

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<td>No change in CK. Reduced LDH</td>
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<td>Kharbanda et al.11</td>
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<td>Upper limb ischaemia 5' 1 5' R (× 3)</td>
<td>Improved endothelial function</td>
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<td>Inflammatory gene expression</td>
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<td>Lower limb ischaemia 5' 1 5' R (× 3)</td>
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<td>Upper limb endothelial function</td>
<td>16</td>
<td>Upper limb ischaemia 5' 1 5' R (× 3)</td>
<td>Improved endothelial function</td>
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<td>Cheung et al. 200655</td>
<td>Paediatric cardiac surgery</td>
<td>37</td>
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<td>Reduced troponin-T release (↓ 43%) Less inotropic requirement and airway resistance.</td>
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<td>Ali et al.57</td>
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<td>82</td>
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</tr>
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<td>Loukogeorgakis et al.13</td>
<td>Upper limb endothelial function</td>
<td>25</td>
<td>Upper limb ischaemia 5' 1 5' R (× 3)</td>
<td>RIPC has a systemic response</td>
</tr>
<tr>
<td>Zhou et al.59</td>
<td>Coronary flow velocity in elective PCI</td>
<td>18</td>
<td>Upper limb ischaemia 5' 1 5' R (× 3)</td>
<td>Augmented coronary flow velocity</td>
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I, Ischaemia; R, Reperfusion.
binding at the cell surface activates signal transduction pathways, which terminate at the mitochondria and result in opening of the mitochondrial K$_{\text{ATP}}$ channels. This leads to the generation of mitochondrial reactive oxygen species, which then mediates cardioprotection by either activating pro-survival kinases or inhibiting the opening of the mPTP (discussed later). Whether this signalling paradigm applies to the setting of RIPC is currently unknown although several studies have linked the opening of these channels to RIPC using pharmacological antagonists of the K$_{\text{ATP}}$ channels such as glibenclamide and 5-hydroxydecanoate. Whether the K$_{\text{ATP}}$ channel exerts its effects in the remotely preconditioned organ or tissue vs. the heart is currently unknown and needs further investigation.

### 3.4.2 Nitric oxide
NO plays a critical role as a mediator of cardioprotection in the setting of both classical and delayed IPC. The results concerning the involvement of NO in RIPC have been investigated using pharmacological inhibitors of NO synthase and have produced conflicting results. However, Tokuno et al. have implicated iNOS activation as a trigger for delayed RIPC of the heart using cerebral ischaemia as the preconditioning stimulus. They demonstrated that the bilateral occlusion of the internal carotid arteries to induce permanent cerebral ischaemia could reduce myocardial infarct size in murine hearts 24 h later, and that this cardioprotective effect was absent in iNOS mice. The actual role NO plays in the mechanistic pathway underlying RIPC remains to be determined, although some experimental studies have linked it to the activation of CGRP in the remote organ.

### 3.4.3 Protein kinase C
It is well-established that PKC plays a critical role as a mediator of the preconditioning signal in the setting of IPC with the PKC-$\epsilon$ isoform being the major cardioprotective isoform. Experimental studies have linked PKC activation to the phenomenon of RIPC by demonstrating that cardioprotection can be abolished by the non-specific PKC blocker, chelerythrine. Subsequent studies have gone on to demonstrate myocardial PKC-$\epsilon$ activation in response to binding at the bradykinin B2 receptor and in response to the mediator CGRP in hearts protected by RIPC.

### 3.4.4 Mitochondrial permeability transition pore
The mPTP is a non-specific high-conductance channel of the inner mitochondrial membrane, whose opening in the first few minutes of myocardial reperfusion mediates cell death by uncoupling oxidative phosphorylation leading to ATP depletion and by inducing mitochondrial swelling. Preventing its opening at the time of myocardial reperfusion exerts powerful cardioprotection, a mechanism which is believed to underpin the endogenous cardioprotective phenomena of IPC and postconditioning. A recent experimental study has indirectly implicated the mPTP in RIPC cardioprotection by demonstrating that remote limb preconditioning can reduce myocardial infarct size in a rat heart in a manner which is sensitive to a $\kappa$-opioid receptor blocker. Furthermore, in cardiomyocytes, the $\kappa$-opioid agonist was demonstrated to induce mPTP opening. However, more direct evidence is required to implicate mPTP inhibition in the heart as a major cardioprotective mechanism in the setting of RIPC.

### 3.4.5 Reactive oxygen species
Oxidative stress appears to play a dual role in the setting of acute myocardial ischaemia-reperfusion injury. Its detrimental role is as a mediator of lethal reperfusion injury. However, its beneficial signalling role is believed to mediate the cardioprotective effects elicited by both IPC and postconditioning. A study by Weinbrenner et al. suggest a possible beneficial signalling role for reactive oxygen species in the setting of RIPC. They discovered that a free radical scavenger was able to abolish the protection elicited by RIPC. Whether the free radicals are generated in the preconditioned organ or tissue or in the myocardium is currently unclear and requires further examination.

### 4. Clinical application of remote ischaemic preconditioning
The clinical application of RIPC requires an intervention which can be instituted prior to the index myocardial ischaemic event, restricting its implementation to the clinical settings in which the index ischaemic event can be reliably anticipated, such as in the setting of surgery (Table 2 for a summary of clinical studies). The first attempt to apply the concept of RIPC in the clinical setting was a study comprising only eight patients, in which remote limb preconditioning failed to affect CK-MB in elective patients undergoing cardiac surgery. However, this study was underpowered; it only measured CK-MB 5 min after declamping the aorta; cuff inflation to 300 mmHg was used; and finally it used an inadequate RIPC protocol comprising two cycles of 3 min upper limb ischaemia followed by 2 min reperfusion.

The first successful application of RIPC in the clinical setting was by Cheung et al. who reported that a standard RIPC stimulus using four 5 min cycles of lower limb ischaemia was able to reduce myocardial injury, improve airway resistance, and decrease inotrope score in 17 children undergoing corrective cardiac surgery for congenital heart disease. Recently, we went on to demonstrate that RIPC using three-5 min cycles of upper limb ischaemia was capable of reducing myocardial injury (as indicated by a 43% reduction in serum troponin-I released over 72 h) in adult patients undergoing elective CABG surgery.

Most recently, RIPC using limb ischaemia has also been reported to be beneficial in the setting of elective surgery for repair of an abdominal aortic aneurysm (AAA) surgery, an operation which is associated with significant myocardial and renal injury. Ali et al. demonstrated that invasive lower limb ischaemia using two 10 min episodes of iliac artery occlusion was capable of reducing myocardial injury (as indicated by a 27% reduction in serum troponin-I released over the peri-operative period) and preserving renal function during elective AAA surgical repair. Interestingly, in the setting of low-risk uncomplicated single vessel PCI, RIPC using bilateral upper limb ischaemia was reported to actually increase CK-MB and troponin-I over the first 24 h. The reason for this discrepancy is not clear, but may be attributed to the use of bilateral arm ischaemia as the RIPC stimulus and the fact that in this clinical setting no actual acute ischaemia-reperfusion injury is sustained. However, in another study using the same clinical setting a marginal increase in coronary flow velocity was noted in
patients subjected to RIP prior to elective PCI. The significance of these findings are not clear.

5. From remote ischaemic preconditioning to postconditioning

The introduction of IPost in 2003 by Zhao et al. has provided a cardioprotective intervention which can be applied after the onset of myocardial ischaemia and at the time of myocardial reperfusion, opening up its application to the setting of an AMI treated before revascularization by either thrombolytic therapy or PCI. In addition, RIPost using brief limb ischaemia and reperfusion may offer a novel strategy for reducing cerebral infarct size and improving functional recovery in patients presenting with an acute ischaemic stroke.

7. Conclusion

RIPC represents an effective strategy for limiting myocardial infarction that has been successfully applied to the protection of other non-cardiac organs against ischaemia-reperfusion injury. The ability to use transient limb ischaemia as the remote preconditioning stimulus has facilitated its translation from the 'bench-side' to the 'bedside' for the benefit of children and adults undergoing elective cardiac surgery and adults undergoing surgery for repair of an AAA. Further clinical evaluation is required to determine whether RIP can improve clinical outcomes in these patient groups, which may result in a change in clinical practice. Crucially, this intervention is both non-invasive and virtually cost-free facilitating its implementation into other surgical settings. Furthermore, it has the potential to reduce myocardial injury and improve clinical outcomes in patients presenting with an AMI or a cerebrovascular accident.

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