Protecting the heart from ischemia/reperfusion injury: an update on remote ischemic preconditioning and postconditioning

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Purpose of review
The most effective strategy for reducing acute myocardial ischemic injury is timely and effective reperfusion. However, myocardial reperfusion can induce further cardiomyocyte death (reperfusion injury). Interventions that protect the heart from ischemia/reperfusion injury, reducing infarct size, can involve remote ischemic preconditioning and postconditioning. These interventions have a promising potential clinical application, and have been the focus of recent research. In this review, we provide an update of remote ischemic preconditioning and postconditioning mechanisms.

Recent findings
Remote ischemic preconditioning cardioprotection can occur via a humoral pathway and/or a neural pathway. These two pathways have been described as mechanistically different, but it has been suggested that they could be interdependent. However, remote ischemic postconditioning mainly involves the humoral pathway. In this review, we will discuss the different pathways and mechanisms involved in remote ischemic preconditioning and postconditioning.

Summary
Remote ischemic preconditioning and postconditioning is possible to perform in a clinical setting by intermittent ischemia of an upper or lower limb. Furthermore, clinical trials using this procedure in the context of predictable ischemia-reperfusion have produced promising results, and other studies to define the potential clinical use of these strategies are ongoing.

Keywords
myocardial infarction, remote postconditioning, remote preconditioning

INTRODUCTION

Ischemic heart disease is the leading cause of death worldwide [1]. Therefore, novel therapeutic strategies are required to protect the heart against ischemia/reperfusion injury, preserve myocardial function, prevent heart failure, and improve clinical outcomes in patients with ischemic heart disease.

Myocardial ischemic conditioning refers to an intervention that protects the heart from ischemia/reperfusion injury. This conditioning may be provided before (preconditioning), during (perconditioning) or after the prolonged ischemic insult (postconditioning). In this review, we will only focus on the description of the remote ischemic preconditioning and postconditioning (rIPC and rPostC) mechanisms.

DEFINITION OF REMOTE ISCHEMIC PRECONDITIONING AND POST-CONDITIONING

Przyklenk *et al.* [2] demonstrated 24 years ago – that brief cycles of ischemia/reperfusion of the circumflex coronary artery protected remote virgin myocardium from the left anterior descending coronary artery occlusion. The phenomenon of rIPC has been described in different organs and tissues, emerging as a strategy of inter-organ protection against the effects of acute ischemia/reperfusion injury.

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KEY POINTS

- rIPC cardioprotection is critically dependent on afferent innervation of the remote organ and intact parasympathetic activity.
- rIPC induces a cardiac Akt and eNOS phosphorylation, the opening of K^+_{ATP} channels and the release of H_2O_2 by the mitochondria. All these phenomena occur prior to the myocardial ischemia, that could act as 'triggers of remote ischemic preconditioning.
- rPostC stimulus applied during the reperfusion period appears to recruit a completely different mechanism of rIPC, which is likely to be humoral.

Interestingly, in 2005, Kerendi *et al.* [3] demonstrated that brief ischemia/reperfusion applied to a distant organ at the onset of myocardial reperfusion (rPostC) reduces myocardial infarct size. As rPostC is applied during the onset of reperfusion, it has made more of an impact in clinical practice.

STIMULUS OF REMOTE ISCHEMIC PRECONDITIONING AND POSTCONDITIONING

The preconditioning stimulus starts in the remote organ or tissue to reach the target organ (heart, brain, kidney, etc.) by different pathways. To this end, Dong *et al.* [4] showed that femoral nerve transection performed before transient limb ischemia abolished rIPC cardioprotection in a rat model. They also showed that intrafemoral arterial adenosine administration mimicked the effects of rIPC, which was only partially abolished by prior treatment with an adenosine A_1 receptor blocker. Similarly, in another study, intramesenteric arterial adenosine administration induced cardioprotection, and this beneficial effect was blocked by either pretreatment with hexamethonium or by an adenosine blocker [5].

In the same way, systemic pretreatment with nitric oxide synthase blockers (*N*(ω)-nitro-L-arginine methyl ester, L-NAME) may abrogate rIPC cardioprotection [6▪]. It is well known that nitric oxide donors infused into the heart can increase intracellular nitric oxide bioavailability, which is important to achieve the preconditioning effect [7]. However, local nitric oxide release and exogenous nitric oxide donors may be neuro-inhibitory to renal sensory nerve stimulation [8], which in turn may contribute to the preconditioning stimulus resulting from renal ischemia. Thus, Steensrud *et al.* [9] have shown that intrafemoral arterial adenosine administration leads to the release of a dialyzable cardioprotective factor into the bloodstream. Furthermore, they also demonstrated that the effect of adenosine and rIPC by transient limb ischemia is completely abolished by prior femoral nerve section and pretreatment with an nitric oxide donor, but is unaffected by pretreatment with an nitric oxide synthase blocker.

Nevertheless, it is difficult to analyze the components of this stimulus. Thus, adenosine and nitric oxide could act as critical triggers as well as mediators in remote preconditioning cardioprotection. The involvement of adenosine receptors in the rPostC mechanism has been described by Kerendi *et al.* [3], who suggested that the source of adenosine was the reperfused kidney. However, whether adenosine was able to activate adenosinergic receptors directly in the heart or elsewhere was not clarified by the authors. Adenosine may also act indirectly by stimulating the release of other substances that are cardioprotective, for example, kinins and prostacyclins.

COMMUNICATION PATHWAYS

Humoral hypothesis

The earliest studies of rIPC hypothesized that the cardioprotection elicited by IPC could be transferred via blood transfusion to a nonpreconditioned animal. The existence of a circulating cardioprotective blood factor was demonstrated in a model of heart porcine transplantation [10], where hind limb preconditioning in a recipient animal provided significant cardioprotection to the subsequently transplanted and denervated donor heart. Furthermore, Takaoka *et al.* [11] reported that plasma adenosine concentrations in the carotid artery were elevated following renal ischemia and reperfusion in a rabbit model of remote preconditioning by renal artery occlusion, suggesting that the kidney is a potential source of circulating adenosine. A sufficient adenosine concentration in the arterial blood to cause hypotension was reported by Pell *et al.* [12] in a model of remote preconditioning. They noted a transient (~30 s) reduction in diastolic arterial blood pressure whenever the renal occlusion was relieved, that was abolished by the general adenosine receptor blocker 8-(*p*-sulfophenyl) theophylline. These data suggest that adenosine was present in vasodilator concentrations in the arterial system despite the transit time through the general circulation.

Similarly, Konstantinov *et al.* [13] demonstrated that rIPC of the recipient animal decreased ischemia/reperfusion injury in the donor heart following orthotopic heart transplantation, via a K^+_{ATP} channel. This study suggested that a circulating factor

persists after the rIPC stimulus is applied, and excludes an afferent neurogenic mechanism of cardioprotection. The identity of the factor remains unclear, and some authors have suggested different candidates for the blood-borne cardioprotective factors of rIPC, for example, stromal cell-derived factor-1α (SDF-1α) [14], exosomes [15], nitrite [16], microRNA-144 [17], hypoxia-inducible factor 1α (HIF-1α) [18] and apolipoprotein α-I [19]. However, these studies have failed to demonstrate that the cardioprotective factor was actually responsible for the beneficial effect.

As mentioned above, Kerendi *et al.* [3] described that rPostC reduced infarct size through the activation of adenosine receptors. These authors suggested that the release of adenosine from the reperfused kidney is responsible for cardioprotection. However, the source of adenosine was not definitively identified. In addition, the authors did not determine the type of adenosine receptors involved in the cardioprotection.

Neural hypothesis

The involvement of a neurogenic pathway in remote cardioprotection has been demonstrated by different authors [20,21]. To this end, it has been suggested that the sensory arm of the neural pathway leading from the remote organ or tissue may be recruited. Different experimental studies have demonstrated that activation of sensitive fibers by topical capsaicin or nociceptive stimuli can mimic the rIPC cardioprotection [22]. However, the neural components of the pathway downstream of this sensory afferent neuron, via a remote organ or tissue, remain unclear. Lambert *et al.* [23▪] demonstrated that forearm ischemia/reperfusion in humans is associated with sympathetic nervous activation, and that rIPC attenuates and delays sympathetic activation observed during the ischemic period. rIPC-induced sympathetic attenuation during ischemia was associated with improved reactive hyperemia, an index of endothelial function, and prevention of oxidative stress formation.

The participation of the autonomic nervous system in the rIPC mechanism was demonstrated by Gourine's group, who showed that rIPC activates a neural pathway, that signals to the heart through the vagus nerves [21]. In accordance with the findings of Gourine's group, we showed that rIPC activates a neural afferent path, that involves the femoral and sciatic nerves and the spinal cord. The cardioprotective signal was shown to reach the heart through the vagus nerve (efferent pathway), and acetylcholine activated the preconditioning (IPC) phenomenon by acting on muscarinic receptors [24].

In agreement with these results, Mastitskaya *et al.* [25] demonstrated that rIPC activates a group of preganglionic parasympathetic neurons, in the dorsal vagal motor nucleus (DVMN). In addition, whenever DVMN was genetically silenced, the rIPC cardioprotection was abolished, indicating that this group of preganglionic parasympathetic neurons is necessary to transfer the protective signal from the conditioned limb to the heart.

It is therefore clear that the vagus nerve is the efferent pathway of rIPC. However, and as Pickard *et al.* [26▪] mentioned, determining which branch of the vagus nerve is responsible for carrying the cardioprotection signal to the heart will facilitate complete understanding of the rIPC mechanism. To this end, we performed a set of experiments in which we selectively sectioned the right and left vagal nerves, and we also performed a bilateral vagotomy (Fig. 1, data not published). Interestingly, only the right vagus section abolished the protective effect of preconditioning on infarct size, showing that only the right vagal myocardial innervation is necessary to provide preconditioning to the heart. Moreover, it is unknown whether rIPC applied to an upper (arm) versus a lower (thigh) extremity would alter the efficacy of rIPC due to considerable differences in vascularization and innervation. Concerning this, Dezfulian *et al.* [27▪▪] showed that the physiological (reactive hyperemia) and biochemical (plasma nitrite concentration) effects or rIPC were not significantly different whenever IPC was applied to the arm versus the thigh in healthy individuals. Future studies should expand on this pathway, considering

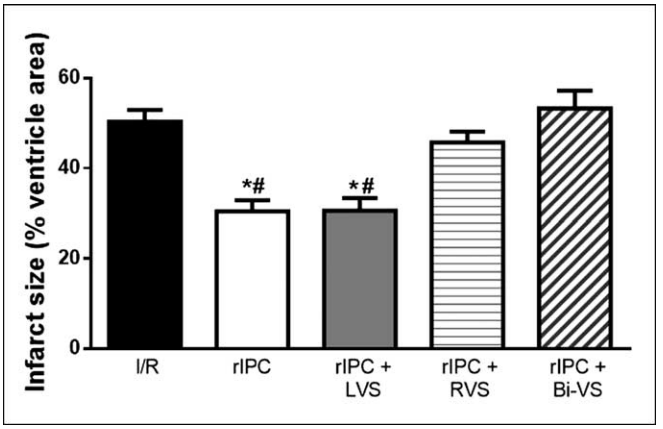

FIGURE 1. Infarct size, expressed as a percentage of the left ventricular area. rIPC significantly reduced the infarct size, the effect of which was abolished by bilateral and right vagal section. Bi-VS, bilateral vagal section; LVS, left vagal section; rIPC, remote ischemic preconditioning; RVS, right vagal section (I/R, $n=8$; rIPC, $n=10$; rIPC + LVS, $n=7$; rIPC + RVS, $n=7$; rIPC + Bi-VS, $n=7$). $^{*}P<0.05$ versus I/R; $^{\#}P<0.05$ versus rIPC + RVS and rIPC + Bi-VS.

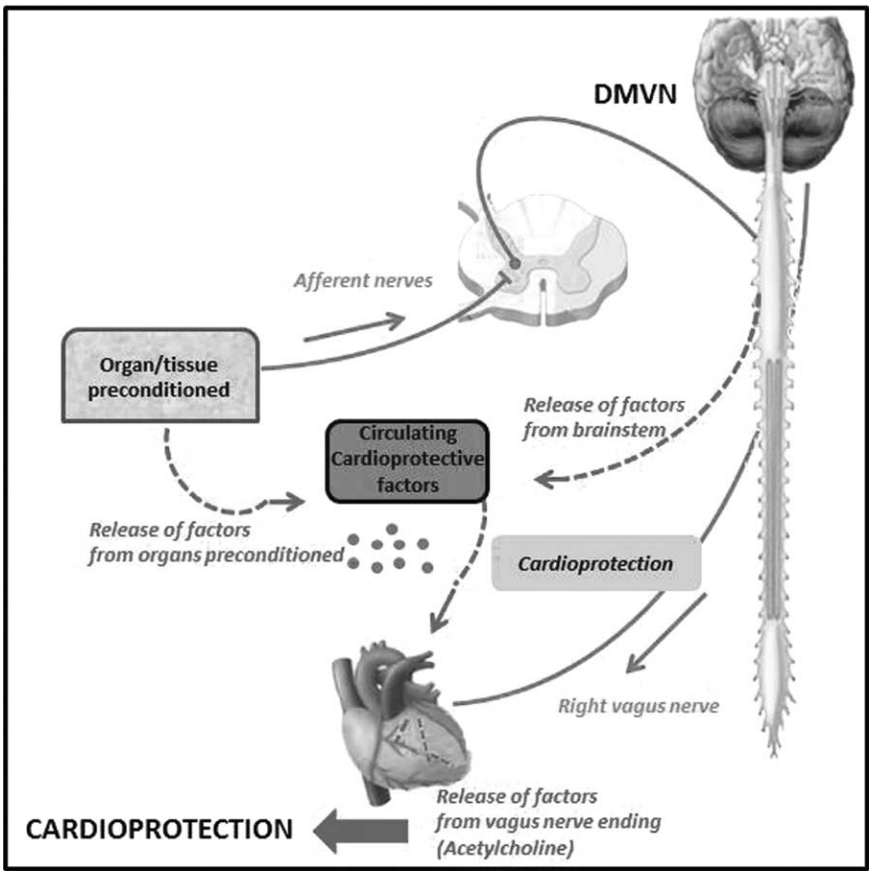

FIGURE 2. The link between the neural pathway (green solid lines) and the humoral pathway (broken red lines) in the mechanism of rIPC. Cycles of brief ischemia/reperfusion induced the local release of factors, which then activated local sensory afferent neurons. A study showing the participation of the neurons in the dorsal motor vagal nucleus (DMVN) in the rIPC mechanism, providing parasympathetic innervation of the left ventricle. The potential sites of cardioprotective factor(s) release include (1) from the conditioned limb itself, (2) from the central nervous system, (3) from pre/postganglionic parasympathetic nerve endings within the heart (broken green lines); and (4) from a nonconditioned remote organ/tissue receiving parasympathetic innervation.

differences not only between the arm versus the thigh but also between the right and the left arm.

The possible involvement of a neural pathway in the mechanism of rPostC was studied by Basalay *et al.* [21], who demonstrated that the neural pathway of cardioprotection is only effective when activated before myocardial ischemia (rIPC). Thus, the cardioprotection obtained by an rPostC stimulus applied during the reperfusion period appears to recruit a completely different mechanism, which is likely to be humoral.

Currently, it is believed that transmission of the rIPC signal to the target organ is multifactorial, requiring a combination of humoral, neuronal, and systemic mechanisms, and may be model-dependent. Indeed, the release of humoral factors in response to rIPC is dependent on sensory innervation to the preconditioned limb [26▪]. However, the downstream target of sensory nerve activation leading to the release of the humoral factor is unclear (Fig. 2). Mastitskaya *et al.* [28▪] demonstrated that vagal innervation to the gut is essential for the communication of rIPC, possibly via the release of a blood-borne factor. We do not fully agree with these data as cervical, but not sub-diaphragmatic vagotomy abrogated rIPC, showing that direct cardiac vagal innervation is crucial for rIPC communication [6▪,24].

Conversely, the existence of a cardiac intrinsic neural network that processes sensory information and modulates efferent autonomic outputs from intrinsic cardiac ganglia is well known. This neural network is also affected by ischemia/reperfusion damage and the subsequent remodeling postmyocardial infarction, worsening ventricular function [29,30]. Therefore, it is important to study the role of these intracardiac neurons in the mechanism of rIPC.

MYOCARDIAL MECHANISMS OF REMOTE PRECONDITIONING AND POSTCONDITIONING

Once the cardioprotective signal has been conveyed from the remote preconditioning organ to the heart, intracellular signal transduction mechanisms are recruited within cardiomyocytes [31].

In a recent study [6▪], we evaluated the signaling pathway that is activated at the heart level, after the rIPC stimulus, but before myocardial ischemia. The cervical vagal section (CVS), performed before the rIPC protocol, completely abolished the beneficial effects of rIPC. However, subdiaphragmatic vagal section (sub-VS) did not modify the rIPC effect, thus demonstrating that denervation of organs – other than the heart – do not contribute to the loss of rIPC. As activation of muscarinic receptors can increase nitric oxide synthesis, we studied the possible

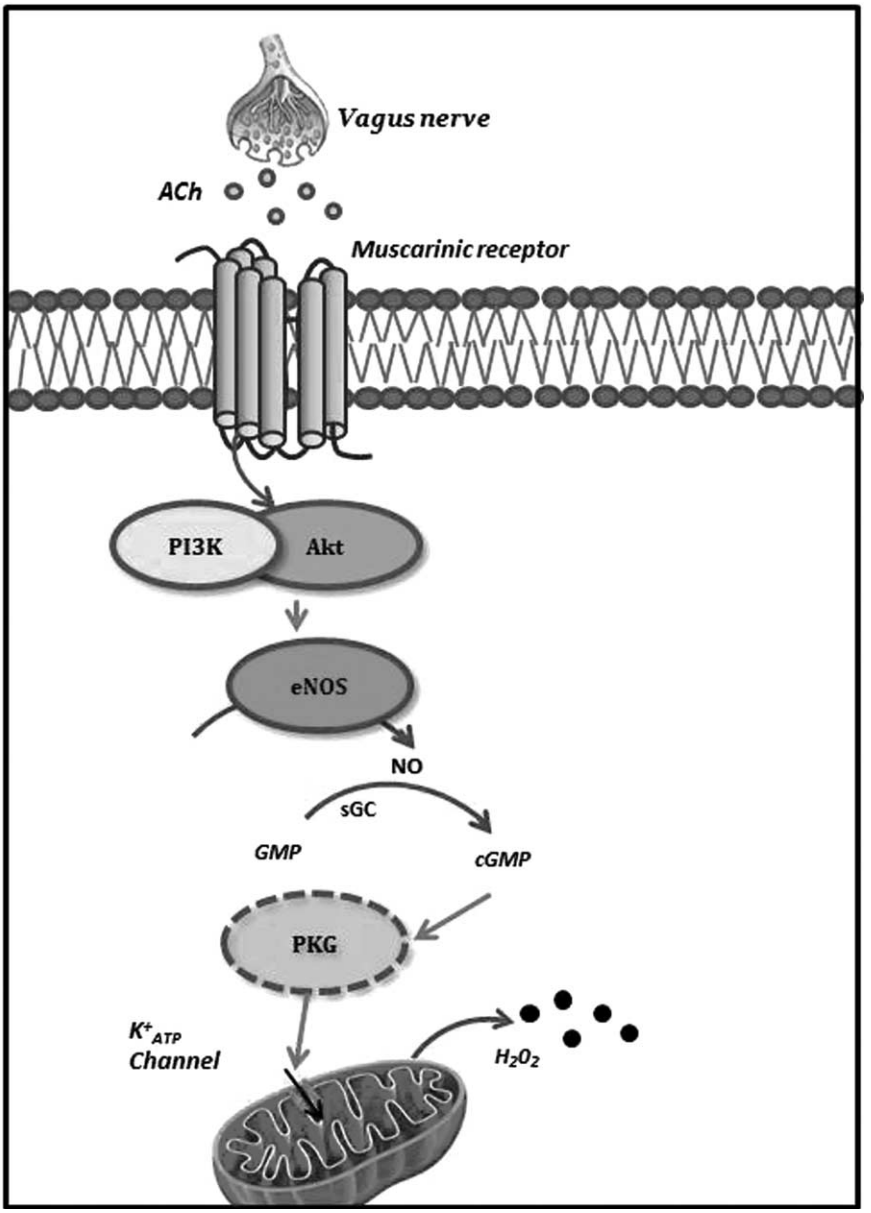

FIGURE 3. Schematic illustration of the intracellular pathways activated by remote ischemic preconditioning before myocardial ischemia. Acetylcholine, released from cardiac vagal nerve endings, activates muscarinic receptors located in the cardiomyocyte plasma membrane, inducing the phosphorylation of Akt and eNOS enzymes. Subsequently, activation of soluble guanylate cyclase and protein kinase G could lead to opening of mK^+_{ATP} channels and an increase in H_2O_2 mitochondrial production. Thus, H_2O_2 could act as a second messenger of the rIPC protective signal. Ach, acetylcholine; eNOS, endothelial nitric oxide synthase; mK^+_{ATP}, mitochondrial K^+_{ATP} channels; NO, nitric oxide; PKG, protein kinase G; sGC, soluble guanylate cyclase.

involvement of nitric oxide in the observed infarct size reduction caused by rIPC. In this regard, administration of L-NAME, during the rIPC protocol, completely abolished the protective effect of rIPC, indicating a central role of nitric oxide in rIPC activation. Given that nitric oxide can induce the opening of mK^+_{ATP} channels, we administered 5-HD before the rIPC protocol. The mK^+_{ATP} channels blocker completely abolished the effect of rIPC, thus demonstrating the involvement of mK^+_{ATP} channels in rIPC [6▪].

In addition, we studied the role of Akt and endothelial nitric oxide synthase (eNOS) phosphorylation in the rIPC mechanism. rIPC induced a significant increase in the phosphorylation of cardiac Akt and eNOS, which was abolished by CVS, in hearts that had not yet been subjected to ischemia/reperfusion. Taken together, these results clearly indicate the involvement of the Akt-eNOS pathway in the heart – as a trigger of the rIPC mechanism – prior to the ischemia/reperfusion cardiac insult.

On the other hand, mitochondrial reactive oxygen species (ROS) production plays a relevant role in IPC [32], but ROS production was not addressed in rIPC. We measured H_2O_2 mitochondrial production in rIPC hearts; there was a significant increase in H_2O_2 release as a result of rIPC, and this effect was attenuated by CVS, L-NAME, and blockade of the mK^+_{ATP} channels by 5-HD. Clearly, rIPC induced activation of Akt and eNOS phosphorylation, mK^+_{ATP} channel opening, and mitochondrial H_2O_2 production in the heart before the index myocardial ischemia [6▪]. In addition, the protective effect of rIPC was abolished by CVS, but not by sub-VS, reinforcing the hypothesis of a parasympathetic vagal pathway (Fig. 3).

Gedik *et al.* [33▪▪] showed that the transfer of rIPC pig plasma attenuated ischemia/reperfusion-induced mitochondrial ROS production after improved adenosine diphosphate (ADP)-stimulated complex I respiration, indicating that mitochondria are involved in reducing ROS formation, supporting the notion that mitochondria are a myocardial target organelle of the protection provided by rIPC.

Less is known about the activation mechanism of rPostC. Peng *et al.* [34] demonstrated that rPostC attenuated delayed neuronal death and spatial learning and memory deficits induced by cerebral ischemia/reperfusion. This neuroprotective effect was abolished by the administration of L-NAME and LY-294002 (a PI3K/Akt antagonist). In agreement with this study, Breivik *et al.* [35] demonstrated that the coronary effluent of preconditioned hearts contains humoral factor(s), and significantly reduced infarct size whenever administered during early ischemic reperfusion in recipient rat hearts, mimetic of rPostC. Moreover, these data suggest that this cardioprotection is partly mediated by PI3K/Akt signaling during ischemic reperfusion.

Furthermore, experimental evidence concerning the involvement of ROS in the mechanism of myocardial rPostC is scarce. However, rPostC produces neuroprotection against cerebral ischemia/reperfusion injury via mobilizing the endogenous adaptive mechanisms of the brain. Thus, rPostC has been demonstrated to inhibit oxidative stress by increasing the activity of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase in different cerebral ischemia models, including focal cerebral ischemia and global brain ischemia in adult rats [36].

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Conflicts of interest

There are no conflicts of interest.

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