A critical review of mechanisms regulating remote preconditioning-induced brain protection

Robert Meller and Roger P. Simon

Translational Stroke Program, Neuroscience Institute, Morehouse School of Medicine, Atlanta, Georgia; and Grady Memorial Hospital, Atlanta, Georgia

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Meller R, Simon RP. A critical review of mechanisms regulating remote preconditioning-induced brain protection. J Appl Physiol 119: 1135–1142, 2015. First published May 7, 2015; doi:10.1152/japplphysiol.00169.2015.—Remote preconditioning (rPC) is the phenomenon whereby brief organ ischemia evokes an endogenous response such that a different (remote) organ is protected against subsequent, normally injurious ischemia. Experiments show rPC to be effective at evoking cardioprotection against ischemic heart injury and, more recently, neuroprotection against brain ischemia. Such is the enthusiasm for rPC that human studies have been initiated. Clinical trials suggest rPC to be safe (phase II trial) and effective in reducing stroke incidence in a population with high stroke risk. However, despite the potential of rPC, there is a large gap in knowledge regarding the effector mechanisms of rPC and how it might be orchestrated to improve outcome after stroke. Here we provide a critical review of mechanisms that are directly attributable to rPC-induced neuroprotection in preclinical trials of rPC.

BECAUSE OF ITS HIGH METABOLIC ACTIVITY, the brain is vulnerable to injury by hypoxic and ischemic conditions. Hypoxia (reduced oxygen) and anoxia (no oxygen) are pathological components of ischemia (lack of blood flow supplying oxygen and glucose). In response to high-altitude hypoxic conditions, cerebral edema may induce brain ischemia as well. Tolerance to brain ischemia has been studied with a primary focus on stroke neuroprotection. Hypoxia tolerance has focused on understanding evolutionarily conserved adaptive species, such as deep sea turtles, hibernating mammals, underground adapted mammals (mole rats), and drought/anoxia-resistant fish (21, 22, 57, 74). While the temporal initiation of cell damage due to hypoxia, compared with ischemia, may differ, similar pathophysiologically cell death mechanisms are shared by these insults (reviewed in Refs. 5 and 43, respectively), namely, the induction and activation of programmed cell death–associated cascades, oxidative stress, and excitotoxicity. Therefore a better understanding of endogenous neuroprotection, be it evolutionarily adapted or transiently induced, may provide valuable insight for brain protection when hypoxic or ischemic conditions are encountered. In this review we focus on acquired or induced brain protection, as described by the ischemic tolerance phenomenon.

The phenomenon of ischemic tolerance in the brain has been observed with multiple paradigms, in multiple species, and by multiple research groups (19, 23). Ischemic tolerance is induced by exposing the brain to a prior ischemic stress (preconditioning), but at a level that does not induce injury. The brain then becomes protected against subsequent normally injurious durations of ischemia. Ischemic tolerance in brain is divided into two temporal phases, with different mechanisms of action. Rapid ischemic tolerance occurs 30-60 min after the preconditioning event and is mediated by intracellular signaling cascades and posttranslational protein modification (45). A second phase or time window of protection occurs 24–72 h after the preconditioning event and is referred to as delayed tolerance. While the events occurring in cells that lead to the rapid tolerant state may activate mechanisms of delayed neuroprotection, there are considerable differences between the two protected states. Specifically, delayed ischemic tolerance requires new protein synthesis after the preconditioning event, whereas rapid tolerance does not (6, 46). Genes whose expression changes after preconditioning subsequently change the transcriptome and proteomic response in brain from that of harmful ischemia to that of tolerance (71, 72).

While the neuroprotection observed by ischemic tolerance is remarkable, the clinical utility of this observation is tempered by the requirement to induce mild brain ischemia (preconditioning) prior to an ischemic event. Clinically such events are difficult to predict. The brain is very sensitive to ischemic injury; hence it is a substantial challenge to administer a preconditioning ischemic stress directly to the brain. For this reason, the idea that preconditioning using limb ischemia (remote preconditioning, rPC) results in significant brain pro-
The protective potential of rPC was first described in a model of myocardial injury in 1993 (60). The first study showing a reduction in brain infarction/injury following rPC in a stroke model was reported by Dave et al. in 2006 (16). Bilateral hindlimb ischemia was induced with a tourniquet, and neuroprotection was assessed in a model of global ischemia in rats (asphyxial cardiac arrest). They found that protection was observed when ischemia was induced 48 h after rPC (as defined by number of normal neurons in the hippocampus assessed 72 h later). This initial model was followed up by the observation that unilateral hindlimb ischemia (femoral artery occlusion) resulted in protection in a focal model of ischemia in rats (transient middle cerebral artery occlusion with a microfilament) (62). Notably, protection was observed within both rapid and delayed tolerance time windows (1 h and 48 h after preconditioning, respectively). One unexpected result was the observation that rPC also has an “intermediate” tolerance time window of 12 h (62). However, in neither of these studies were the molecular mechanisms of tolerance induction addressed.

Since these studies, rPC-induced neuroprotection has been described in multiple species. Methods to induce rPC include bilateral hindlimb ischemia with tourniquets and unilateral hindlimb ischemia with a tourniquet or direct femoral artery occlusion. Based on the initial cardiac ischemia work, studies have used a series of 5- to 10-min limb ischemic events, typically three or four, interspersed with equivalent reperfusion durations. Tolerance has been reported in both rapid (0–1 h after rPC) and delayed (>24 h) temporal windows as well as treatment after the ischemic event [postconditioning (Refs. 55 and 76)]. Interestingly, the temporal extinction of rPC has not been determined (6), nor has the effect of repetitive administration of rPC in rodents been investigated (75, 86).

**Molecular Determinants of Remote Preconditioning**

The molecular determinants of rPC have not been investigated in detail. Indeed, many reviews have taken the assumption that a mechanism of direct focal brain ischemia-induced preconditioning may be relevant for rPC. However, some studies have directly questioned mechanisms of rPC-induced neuroprotection. Here we review some of the key findings.

**Adenosine receptor activation.** Adenosine A_1_ receptor (A1R) activation has been reported as a key mechanism of both rapid and delayed ischemic tolerance following direct preconditioning of neurons or brain (50, 52, 64, 82). The A1R antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 1 mg/kg ip) blocks rPC-induced neuroprotection in rats (32). This study utilized a rapid model of tolerance, whereby animals underwent three cycles of 5-min right hindlimb ischemia and 5-min reperfusion. Animals were allowed to recover for 1 h and then subjected to an occlusion of the ipsilateral (right) middle cerebral artery for 2 h. The ability of an A1R agonist to induce/mimic tolerance was also investigated, by administering 2-chloro-N(6)-cyclopentyladenosine (CCCP; 1 mg/kg ip) 24 h in advance of the 2-h middle cerebral artery occlusion. The protective effect of rPC and its antagonism by DPCPX was determined by diffusion weighted imaging (DWI) of the animals prior to vital dye staining [triphenyltetrazolium chloride (TTC)] of the brains (32).
The study also investigated markers of stress and inflammation (32). Superoxide dismutase (SOD) and manganese SOD (MnSOD) activity were enhanced in brain after rPC + middle cerebral artery occlusion compared with animals subjected to middle cerebral artery occlusion only. Copper SOD (CuSOD) activity was unchanged, and nitrite levels were reduced. All of these changes were blocked by the adenosine A1R antagonist DPCPX. In contrast, elevations in tumor necrosis factor alpha (TNF-α) levels following middle cerebral artery occlusion were reduced after rPC; this effect was only partially blocked by DPCPX. It was not clear whether the reduction in inflammatory markers was due to rPC-mediated suppression of inflammation or a muted inflammatory response to less brain injury.

**Hypoxia-induced factor 1α.** The transcription regulatory protein hypoxia-induced factor 1α (HIF-1α) has been extensively studied as a response to low-oxygen conditions and thus ischemic preconditioning in brain (10, 66). Its role in brain neuroprotection following rPC has not been established to date; however, since it is widely acknowledged to play a role in ischemic tolerance in brain, we include it in our review. The role of HIF-1α in cardiac preconditioning has been investigated in two studies. It was shown that raised plasma levels of the anti-inflammatory cytokine interleukin-10 (IL-10) and myocardial protection were reduced in mice heterozygous for HIF-1α (11). Increased expression of IL-10 mRNA levels in cultured myocytes after hypoxia was blocked by an inhibitor of HIF-1α (acrilavine) (11).

In a separate study, HIF-1α expression increased in the hindlimb subjected to rPC, and cardioprotection following rPC was also observed in HIF-1α heterozygous mice (single-allele knockout) (36). The difference between the two results may be explained by the fact that the first group used a delayed tolerance model (24 h between rPC and myocardial ischemia) and the second used a rapid tolerance model (5 min between last limb ischemia and myocardial ischemia). A role of a transcription factor in mediating rapid ischemic tolerance is not expected, given that the effect does not require de novo protein synthesis (46).

**Inflammatory factors.** A number of studies suggest that rPC reduces inflammation in organs subjected to rPC followed by harmful ischemia. In the study by Wei et al. (80) in which rPC (3 × 15-min left hindlimb femoral artery ligation) was blocked by hexamethonium and capsaicin, rPC also prevented blood-brain barrier dysfunction (permeability and breakdown) following harmful stroke [common carotid artery ligation (30 min) and permanent occlusion of left middle cerebral artery by cauterization] and a reduction of inflammation-associated signaling molecules (galectin-9/TIM3). However, the effect of rPC alone on inflammatory cascade components was not assessed in this study.

Evidence that rPC regulates inflammatory gene expression in blood is supported by the study of Konstantinov et al. (40), who used microarray analysis of gene expression in blood collected from humans prior to and 15 and 24 h after rPC protocol (3 × 5-min forearm ischemia). Pathway analysis of the gene expression profiles revealed a large number of genes associated with pathways regulating Toll-like receptor signaling, TNF-α receptor signaling, and TNF synthesis. Interpretation of these pathways suggests that rPC affects TNF-α-mediated signaling. However, in a study by Hu et al. (32) a reduction in TNF-α levels was observed after middle cerebral artery occlusion in rPC-pretreated rats. While TNF-α is an inflammatory, cell death-promoting agent, it should be noted that TNF-α can precondition brain, and neurons in culture, and plays an important role in the preconditioning effects of Toll-receptor ligands (24, 58, 59, 65, 73).

Proteomic analysis has been performed on plasma after rPC (30). Rats were subjected to 5-min limb ischemia followed by 5-min or 10-min reperfusion. Seven proteins were shown to be regulated after rPC procedures [apolipoprotein C-III, haptoglobin alpha chain, transthyretin (monomeric), hemoglobin beta chain, transthyretin (dimeric), apolipoprotein A-IV, and fibrinogen beta chain].

**Mammalian target of rapamycin.** Mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase associated with cell growth and cell survival signaling. It is activated by AKT, another protein kinase strongly associated with antiapoptotic cell signaling. A role for mTOR in rPC-induced hippocampal protection following ischemia is indicated given that rapamycin (an inhibitor; 5 mg/kg ip) blocks rPC-induced protection (84). rPC was accomplished by occluding the renal artery [3 cycles of 5 min, with 5-min reperfusion 24 h prior to global brain ischemia (bilateral occlusion of the common carotid arteries)]. In addition, the improvement in behavioral function (latency time in passive avoidance tests) in animals subjected to rPC prior to harmful ischemia compared with nonpreconditioned animals subjected to harmful ischemia was blocked by rapamycin. Western blot analyses showed an increase in mTOR phosphorylation, but not total protein levels, following rPC, whereas SOD levels were not changed. Protein levels were measured 24 h after rPC or global ischemia in the animals, suggesting that at the time of protection mTOR phosphorylation was increased.

**ATP-activated potassium channels.** ATP-activated potassium channels (K_{ATP}) channels have been shown to mediate rapid and delayed ischemic tolerance (4, 31, 33, 54, 63). While no studies have shown a role in rPC, they have been studied in remote postconditioning, whereby remote limb ischemia is applied after the harmful ischemic event (76). Delayed remote postconditioning administered 3 and 6 h after anterior cerebral artery and middle cerebral artery occlusion in rats resulted in a reduction in infarct volume compared with non-postconditioned animals. This was paralleled with an improvement in neurological deficits in the animals. Diazoxide (5 mg/kg iv) administered after harmful ischemia mimicked the protection, and the effects of remote postconditioning at 6 h were blocked by the K_{ATP} channel blocker 5-HD (20 mg/kg iv). When diazoxide and remote postconditioning were administered there was no additive effect on protection. Hence together these data support a role for K_{ATP} channels being regulated in animals subjected to ischemia and then remote postconditioning. Whether K_{ATP} channels regulate preconditioning is not yet clear.

**Nitric oxide and survival kinase signaling.** Two studies show that nitric oxide plays a role in remote preconditioning- and postconditioning-induced brain neuroprotection, using a global ischemia model (53, 85). Rats were subjected to global brain ischemia by permanent blockage of the vertebral arteries and transient ligation of the common carotid arteries (8 min). rPC was performed by transient ligation of both femoral arteries (3 cycles of 10-min occlusion and then release) (85).
After rPC, cell death in the ischemia-sensitive CA1 region of the hippocampus was reduced compared with non-postconditioned animals. Protection was reduced when the nitric oxide synthase inhibitor nitro-L-arginine methyl ester (L-NAME; 5 mg/kg ip) was administered to the animals prior to the preconditioning.

Postconditioning was performed by transient repeated ligation of the femoral arteries, which also produced neuroprotection against global ischemia (53). In addition to L-NAME blocking the protective effect of postconditioning, protection was reduced after administration of the AKT inhibitor LY294002. The authors show an increase in the endothelial nitric oxide synthase enzyme in the hippocampus in postconditioned animals and a concurrent increase in AKT phosphorylation.

A protective effect of remote postconditioning has been described in a focal ischemia model, whereby bilateral femoral artery occlusion-induced remote postconditioning prevented brain injury after 100-min middle cerebral artery occlusion (55). The protective effect was also blocked by administration of the nitric oxide synthase inhibitor L-NAME. In addition, the extracellular signal-regulated protein kinase (ERK) or mitogen-activated protein kinase (MAPK) inhibitor U0126 also blocked remote postconditioning-induced neuroprotection. However, in this study no effect of remote postconditioning on phosphorylated AKT levels was observed.

These studies are notable as they describe a consistent role for nitric oxide after pre- and postconditioning. Indeed, nitric oxide-induced ERK/MAPK activation has been described previously for focal ischemia-induced preconditioning (25), and survival kinases have been shown to play a role in direct ischemic preconditioning-induced ischemic tolerance (46, 47, 66, 81). Inhibition of AKT, but not inhibition of ERK with U0126, has been shown to partially block neuroprotection following direct focal ischemia-induced postconditioning (56). Again these studies suggest that while some similarities may exist between remote and direct brain ischemic preconditioning and postconditioning, differences may also be observed.

**Summary of Molecular Mechanistic Studies**

These reviewed studies represent what limited mechanistic studies have been performed on rPC-induced brain protection. Like focal brain ischemic preconditioning, a role for both adenosine and nitric oxide as a mediator seems strong. The source of the adenosine is not yet clear, but both a humoral and a central nervous mechanism would be feasible. Adenosine agonists are protective when applied directly to neurons (52). The direct release of adenosine in brain or blood following rPC has not been shown, nor is it clear whether the release is essential for either a rapid tolerance effect or a delayed tolerance effect. Other studies suggest that rPC affects immune cell responses, either by reducing their activation following harmful ischemia or via paracrine/endocrine effects. Clearly, brain protection against ischemia reduces the degree of inflammatory response in brain after harmful stroke; however, cause and consequence still need to be clearly defined.

**Physiological Mechanisms of Remote Preconditioning: Humoral or Neural Mechanism?**

A review of the literature shows evidence for both humoral and neural mechanisms regulating rPC (29, 37). However, many studies have been performed in models of ischemic cardioprotection, whereas fewer have been performed in stroke models.

The strongest support for a humoral agent regulating rPC comes from multiple studies of cardioprotection. Unilateral femoral vein occlusion promotes rPC-induced cardioprotection (42). Venous blood collected from remote preconditioned rabbits reduced the volume of heart infarction when transfused to a non-preconditioned rabbit subjected to cardiac ischemia (18). However, the time window of transfer was short (minutes) and more in line with a potential rapid tolerance mechanism. In a similar study it was reported that blood collected from humans subjected to limb rPC also resulted in cardioprotection of an isolated perfused rat heart (34), and that the protective factor may be a small molecule (15 kDa). An alternative source for the humoral agent could be endothelium-derived microparticles. Microparticles isolated from animals subjected to rPC (3 × 5 min of unilateral femoral artery occlusion) reduce infarct volume when administered to animals after 2-h middle cerebral artery occlusion-induced brain ischemia (69). However, remote postconditioning was more effective than the transfusion of particles at producing neuroprotection (69). Recent studies suggest that a microRNA (miR-144) may mediate cardioprotection following rPC, but the effect of miR-144 has not been studied in brain (41).

In a study by Lim et al. (42), supporting the hypothesis that a humoral factor regulates rPC-induced tolerance, it was also shown that resecting both the femoral and sciatic nerves prevents cardioprotection following limb ischemia (induced by femoral artery occlusion), thereby supporting a neural mechanism as well. Additional studies report rPC-induced cardioprotection to be inhibited by vagus nerve transection, spinal cord resection, and paralytic agents in rabbit models (20). Spinal mechanisms involving opioid receptors have also been implicated in cardioprotection induced by rPC (61). Therefore there is strong evidence that, in addition to humoral factors regulating tolerance, neural mechanisms may regulate cardioprotection following rPC as well.

There is direct evidence that neural mechanisms regulate rPC-induced tolerance to brain ischemia. Occlusion of the abdominal aorta to induce hindlimb ischemia can induce protection against 2-h middle cerebral artery occlusion in rats (44). The temporal window was reported to be narrow, as protection was only observed 24 h after rPC, but not at 48 or 72 h after preconditioning. If a neural mechanism regulated rPC, then blocking the spinal input of sensory afferents would be expected to block protection following rPC. Blockade of spinal processing of sensory inputs with the pharmacological ganglionic blocker hexamethonium reduced the protection afforded by rPC (44). A separate study showed that hexamethonium and capsaicin block rPC-induced stroke protection in a rapid ischemic tolerance model (stroke was modeled 15 min after the final rPC event) (80). In a study similar in design to the above-mentioned Lim et al. study, it was reported that severing the sciatic nerve blocks rPC-induced ischemic tolerance in brain (83).
So which one is it: humoral or neural? Studies show protection of heart by both a neural and a humoral mediator of rPC (reviewed in Ref. 29). Blood gene expression has been shown to be affected by rPC procedures in humans (40). However, evidence of a humoral factor regulating brain neuroprotection would be further strengthened by a study using a parabiosis model (whereby two animals’ circulatory systems are surgically joined). If rPC mediates protection via a humoral factor, this may enable the development of a small-molecule mimetic or a blood test for responders (37).

For brain protection, there is currently weaker evidence of a humoral factor and more evidence of a neural event. Studies show that blocking neural inputs to the CNS blocks rPC-induced brain protection (44, 80, 83). If a neural pathway is involved, alternative methods of stimulating this response may be found. However, to date no key brain structure(s) has shown to mediate the neurogenic aspect of rPC.

Given current understanding, it is not surprising that the safest conclusion is that potentially both mechanisms play a role in rPC. Clearly, these questions need addressing, and further investigations in this area would help elucidate the key mechanisms of brain protection. It is not yet clear whether the events of humoral- and neural-mediated rPC-induced protection are sequential or parallel. It is not yet clear whether mechanisms of heart and brain protection following preconditioning are similar; indeed, differences between heart and brain have been observed in direct ischemic preconditioning studies (77). Furthermore, are the molecular and physiological mechanisms of acute or rapid and delayed ischemic tolerance in brain after rPC different, as has been reported for direct brain preconditioning (46)?

Is Remote Preconditioning Ready for Clinical Trials?

Clinical investigations of rPC for brain ischemia are already in progress (26, 37, 38, 49), yet potential uses of rPC may include other situations when brain ischemia or hypoxia are observed. For example, a recent trial of rPC for altitude adaption was reported (9), and rPC has been investigated for exercise training regimens (34). Yet questions remain as to whether rPC mechanisms have been sufficiently elucidated for such trials (39). Has rPC been replicated and understood enough to have proceeded to clinical trials (79)? If not, what more should we be doing to improve the preclinical data for rPC?

The standard to which we compare the preclinical data is the Stroke Treatment Academic Industry Roundtable (STAIR) guidelines, which were published to improve the evidence of preclinical stroke therapeutics prior to embarking on clinical trials. The failure of previous neuroprotective therapies resulted in the STAIR criteria and other more rigorous requests of preclinical data prior to the embarkation on clinical trials (2, 67, 79). A review of the STAIR recommendations suggests that preclinical stroke research determine 1) dose-response curves; 2) therapeutic time windows in “good” animal models; 3) outcomes from blinded, physiologically controlled studies; 4) both histology and functional outcomes; 5) responses in gyrencephalic species; and 6) treatment effects in both transient and permanent occlusion stroke models (79).

Earlier studies established the dose-response nature of rPC and reported their model development. For example, in one of the earlier studies three cycles of ipsilateral hindlimb ischemia preconditioning, but not two cycles of rPC, resulted in a protected brain 2 days later (62). Interestingly, in the same study two cycles of rPC induced protection in a rapid model of tolerance. Similarly, the global ischemia study of Dave et al. (16) investigated 15- and 30-min rPC (bilateral hindlimb) prior to global ischemia (asphyxial cardiac arrest). Interestingly, in this model both durations of rPC induced protection. Dose-response relationships have also been described in remote postconditioning studies (55). The pressure required for a limb cuff to achieve hypoxic conditions in the upper and lower limbs was recently described. This study showed that upper limbs require lower pressure to induce hypoxic conditions compared with lower limbs (determined by blood gas analysis and blood flow measurement). The most consistent effect was observed at 180 mmHg in both upper and lower limbs (70).


This observation is important for translational studies and suggests that ischemia induced by inflating a pressure cuff follows a dose-response relationship. Hence, experimental studies have established a dose-response-like relationship with respect to the number of cycles of rPC and protection.

The therapeutic time window of rPC-induced protection appears to be common between studies. Most report two time windows, as seen for focal ischemia-induced preconditioning studies, a rapid window of protection (15–60 min after rPC) (16, 32, 62, 80) and a delayed window (24–72 h after rPC) (44, 62, 84). What is not clear from such studies is when the time window of protection closes (extinction).

The monitoring of physiological variables and randomization varies between studies. Typical of most small-animal experiments, animal temperature was typically monitored throughout surgeries (16, 44, 62, 80, 84). Some also measured blood gasses and pH (16). Larger-animal studies typically show more extensive details of monitoring (35). Many studies explicitly report randomizing animals to treatment groups, for example, but not limited to, References 44, 62, 80, and 84.

The response to function outcome has been described in multiple studies. Brain infarction is correlated to the loss of function (7, 8). Most studies of brain protection include histological assessment, using either a vital dye (TTC) (62, 80) or traditional cytology stains such as hematoxylin and eosin stain (16) or cresyl violet staining of sections (80). Both remote preconditioning and postconditioning studies have included behavioral and neurological assessment of brain function (53, 55, 76, 80). These data suggest that rPC reduces brain functional deficits following ischemic injury.

rPC has been described in gyrencephalic species. One of the lasting criticisms against the use of rodents for stroke research is that they have simple brain structures (lissencephalic), whereas human brains have convolutions (gyrencephalic). Hence information is usually requested to prove protection in a gyrencephalic species prior to patient trials (2). Brain ischemia induced by circulatory arrest (global ischemia) is reduced in pigs when treated with rPC prior to the induction circulatory arrest. Pigs have gyrencephalic brains, hence the criterion is met. However, care should be noted against such a rigid stance. It was recently shown that neuroprotective agents that produce protection against experimental stroke in lissencephalic species (a postsynaptic density protein 95 blocking peptide) (1) was also equally effective in gyrencephalic species (13–15).
rPC appears to provide protection in multiple organs, but also in multiple forms of ischemic injury models. Examples of transient and focal brain ischemia protection are reported (62, 80) as well as global ischemia induced by vessel occlusion (84) and circulatory arrest (16, 35).

A review of each factor shows rPC to have shown efficacy in many of the criteria laid out by the STAIR guidelines, although perhaps not all together in a single study. Yet while the preclinical data are strong, we do not have a unified mechanism of how rPC [or preconditioning for that matter (3, 48)] evokes tolerance. Hopefully such studies are in progress, as these will no doubt reveal novel therapeutic approaches to induce protection.

Conclusions and Future Perspectives

As has been noted in reviewing the preclinical studies, a variety of approaches to induce limb ischemia have been utilized: bilateral and unilateral ligations, hindlimb and forelimb occlusions. It is unclear whether leg vs. arm occlusion is equipotent (70); a clear case for a biomarker to assess protection potential could be made here (37). Clearly, a systematic study to identify the best paradigm is needed.

Biomarkers may assist in identifying those who would benefit from rPC and those who become tolerant once they receive rPC (37). Other studies suggest that preconditioning may be reduced in its effectiveness either as we age or when other comorbidities occur (12, 17, 51). Clearly identifying patients in whom rPC is less effective is critical. An alternative view is that small details regarding the rPC procedure may affect its efficacy. However, since no central molecular mechanism has been identified as a key regulator of rPC-induced ischemic tolerance, we may be missing a critical focus.

There is much guarded optimism for the application of rPC as a neuroprotective strategy. The simplicity of the approach is perhaps the most provocative feature of this process: rPC is not invasive and could be administered at home by a patient. It is not yet clear what the mechanism(s) of protection is; however, multiple studies show the procedure to induce remarkable neuroprotection. As efficacy is proven in situations of ischemia, and trials are on the way, it is clear that other potential opportunities of rPC to have utility are waiting. Recent studies of athlete performance and altitude adaptation also open the potential for rPC to move outside the clinic (9, 34). Clearly, the adaption to altitude and the adaption to hypoxia have many applications, not limited to high-altitude recreation. It is of note, however, that a common issue of extreme high-altitude exposure, high-altitude cerebral edema, can increase the occurrence of brain ischemia (27, 28, 68). Therefore, rPC may have beneficial effects not only for performance but also in protecting against extreme altitude. Hopefully as studies shed light on such processes, this may increase the use of this approach as well as identify potentially novel uses of this approach.

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