Preconditioning and postconditioning: innate cardioprotection from ischemia-reperfusion injury

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Reperfusion is the definitive treatment to salvage ischemic myocardium from infarction. A primary determinant of infarct size is the duration of ischemia. In myocardium that has not been irreversibly injured by ischemia, reperfusion induces additional injury in the area at risk. The heart has potent innate cardioprotective mechanisms against ischemia-reperfusion that reduce infarct size and other presentations of postischemic injury. Ischemic preconditioning (IPC) applied before the prolonged ischemia exerts the most potent protection observed among known strategies. It has been assumed that IPC exerts protection during ischemia. However, recent data suggest that cardioprotection is also exerted during reperfusion. Postconditioning (PoC), defined as brief intermittent cycles of ischemia alternating with reperfusion applied after the ischemic event, has been shown to reduce infarct size, in some cases equivalent to that observed with IPC. Although there are similarities in mechanisms of cardioprotection by these two interventions, there are key differences that go beyond simply exerting these mechanisms before or after ischemia. A significant limitation of IPC has been the inability to apply this maneuver clinically except in situations where ischemic event can be predicted. On the other hand, PoC is applied at the point of service in the hospital (cath-lab for percutaneous coronary intervention, coronary artery bypass grafting, and other cardiac surgery) where and when reperfusion is initiated. Initial clinical studies are in agreement with the success and extent to which PoC reduces infarct size and myocardial injury, even in the presence of multiple comorbidities.

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the myocardium is unsalvageable that is demonstrated by transmural involvement after permanent occlusion. However, it is not known what time boundaries this relates to. The “wavefront” time frame may not be applicable since the time line was developed in ischemic and reperfused myocardium, therefore incorporating both ischemic and reperfusion injuries. Some studies on surgical revascularization suggest significant recovery in patients exhibiting symptoms of evolving infarction well beyond the limit proposed by the wavefront model. The majority of therapeutic approaches for treatment of acute evolving myocardial infarction have been designed to reduce the duration and severity of ischemia; newer generation approaches may target reperfusion injury. Numerous therapies have been advocated to reduce ischemic injury specifically, including drugs that are administered before the ischemic event (i.e., pharmacological preconditioning), hypothermia, and ischemic preconditioning. Indeed, the early studies by Maroko and colleagues (48) introduced the concept of myocardial salvage from ischemic injury. These studies approached the problem by attempting to lower the energy demands of the heart to reduce the overall severity of the energy supply/demand mismatch during ischemia. This approach was not effective in the absence of reperfusion. Ischemic preconditioning, introduced by Murry et al. (49) in 1986, has shown the greatest promise of any cardioprotective strategy and is to a great extent a “gold standard” (21) by which other strategies are compared. It has been thought that preconditioning intervenes during the ischemic period by numerous mechanisms reviewed below. These mechanisms were either triggered by the preconditioning stimulus itself (trigger phase) or mediated by largely molecular mechanisms that were set in motion after the stimulus (mediator phase). However, recent data suggest that preconditioning may also attenuate events that specifically occur during reperfusion (59).

On the other hand, various approaches have been taken to attenuate reperfusion injury, including the systemic or local infusion of adenosine, nitric oxide, oxygen radical scavengers, anti-inflammatory agents, and the filtration of inflammatory cells at the time of reperfusion. Recently, postconditioning, defined as brief intermittent cycles of ischemia alternating with reperfusion applied after the ischemic event (at the onset of reperfusion), has been introduced as a mechanical intervention to attenuate reperfusion injury specifically (87). This review compares and contrasts the cardioprotection phenotype and mechanisms of preconditioning and postconditioning and notes their similarities as well as their differences. The molecular mediators engaged by these two forms of “conditioning” are not discussed, but are reviewed in depth elsewhere (25, 69). Because there are significant clinical implications of these two strategies, their clinical applications are highlighted.

THE PHYSIOLOGICAL END POINTS OF PROTECTION
BY PRECONDITIONING AND POSTCONDITIONING

Infarct size (necrosis and apoptosis). That ischemic preconditioning (IPC) and postconditioning (PoC) reduce infarct size acutely (26, 35, 50, 79, 85, 87) and long term (50) is well established. The total infarct area is likely composed of cardiomyocytes that have died by either necrosis or apoptosis (31; for a review on the effects of preconditioning on apoptosis, see Ref. 92). Both IPC (40) and PoC (60, 75, 80) have been shown to inhibit both forms of cell death. In the rat model, IPC has consistently demonstrated a robust reduction of infarct size (on the order of 50–80% reduction) independent of the animal model used. In contrast, PoC has demonstrated a less-substantial infarct size reduction in smaller animal models (mice, rats, rabbits ~30% reduction) than in larger animal models of coronary artery occlusion (~50% (23)–80% (26) reduction). The reasons underlying this “species or size discrepancy” have not been determined but may relate to species differences in 1) myocardial metabolism and the supply/demand mismatch that defines the severity of ischemia, 2) the complement of endogenous antioxidants expressed in the animal model, 3) the role of inflammatory cells during reperfusion, 4) effectiveness of the PoC algorithm for each species, which is likely multifactorial, but may be related in part to the rate at which reperfusion injury events occur. Relevant to point number 4, for example, Iliodromitis et al. (26) found that the failure of PoC to reduce infarct size reported in the study by Schwartz and Lagragna (57) in the pig model may be related to an insufficient number of reperfusion-occlusion cycles. However, cycles ranging from 30 to 60 s have been used in human studies with similar reductions in infarct size. Furthermore, Halkos et al. (23) found that IPC and PoC exerted similar reduction in infarct size in the canine model, whereas in the rat IPC exerts greater infarct reduction than does PoC. Halkos et al. (23) also showed that the cardioprotection by PoC was not additive to that of IPC in the canine model of left anterior descending coronary artery (LAD) occlusion and reperfusion. However, Yang et al. (77) found that infarct size reduction with IPC and PoC were additive if ischemic times were prolonged in the rabbit model. Whether such additive protection is unmasked with prolonged coronary occlusion in which each strategy is ineffective by itself, or whether this is a species-dependent effect, is not clear and warrants further investigation. The entire question of the optimal algorithm and durations of reperfusion and ischemic segments of the PoC stimulus is open.

Contractile dysfunction. In contrast to the consistent demonstration of infarct size reduction within a given species, the benefits of both IPC and PoC on postischemic contractile function in the absence of infarction (myocardial stunning) or in the presence of infarction is not clear. Kin et al. (34) reported that PoC did not alter global postischemic contractile function using a three-cycle (10 s each) algorithm, but global function (estimated grossly by +dP/dt) was significantly improved using a six-cycle algorithm. However, it must be stated that global function is influenced by a number of factors, paramount of which is infarct size. In a regionally ischemic model in which myocardial stunning was created by 10 min of either LAD or left circumflex coronary artery occlusion in a canine model, PoC did not improve regional wall motion in the area at risk or its time to recovery independent of whether the cycle length was 15, 30, or 60 s (10). The Vinten-Johansen and Zhao laboratory observed a similar lack of protection in stunned myocardium in a canine model of 10-min LAD occlusion and 3 h of reperfusion (Fig. 1). Whether altering the number of cycles or the duration of a fixed number of cycles can improve regional postischemic contractile function has not been answered at this time.

Arrhythmias. IPC has been reported to have antiarrhythmic effects in canine (23, 67) and rat (58) models of coronary artery occlusion-reperfusion. However, it has also been reported that
IPC exacerbates the incidence of arrhythmias. In contrast, three reports support that PoC attenuates postischemic arrhythmias. Halkos et al. (23) showed that PoC reduced the incidence of ventricular arrhythmias by 50% in the canine model, which is vulnerable to arrhythmias at the onset of reperfusion. If the arrhythmias were initiated during the early period of reperfusion, the reocclusion of the previously ischemic coronary artery as part of the PoC algorithm immediately truncated the arrhythmias and consequently restored sinus rhythm. Galagudza et al. (19) and Kloner et al. (36) also reported a reduction in post-ischemic arrhythmias by PoC. The mechanisms by which PoC attenuates arrhythmias at reperfusion may be related to 1) deprivation of oxygen necessary to fuel ion pumps during the occlusion phases of the algorithm, 2) attenuation of calcium transient currents, or 3) attenuation of reactive oxygen species generation, all of which have been implicated in postischemic arrhythmogenesis.

Protection of the coronary vascular endothelium and reduction of the inflammatory response to reperfusion. An inflammatory-like response has been observed to occur at the onset of reperfusion (17, 18, 68). The hallmarks of this response are 1) simultaneous activation of neutrophils (CD11/18 expression) and coronary vascular endothelium (P-selectin expression) and the consequent recruitment of neutrophils to the reperfused coronary vascular endothelium of the area at risk, 2) endothelial dysfunction (decreased endothelium-dependent vasodilation), 3) increased superoxide anion generation by coronary vascular endothelium (50), decreased endogenous nitric oxide generation (42), and 4) release of proinflammatory cytokines into the interstitium and vascular space. These inflammatory-like events have been indirectly correlated with the degree of myocardial necrosis in that attenuation of these events at reperfusion is associated with a decrease in infarct size (2, 41, 64). IPC has been reported to attenuate postischemic coronary vascular endothelial dysfunction in some studies (4, 12) but not in others (4). Endothelial dysfunction assessed by vasodilator responses to acetylcholine has been shown to be preserved by PoC (87). It is not clear which of the physiological effects of PoC are responsible for protection, since the inflammatory-like response is a complex and integrated cascade of events. PoC could reduce superoxide generation that would damage endothelium or limit neutrophil adherence. But at this point, it is difficult to distinguish a cause and effect relation. This is a very controversial area and one that will likely draw more investigative interest in the future. The implication in practice is that the anti-inflammatory response of both IPC and PoC may be amplified by coapplication of various anti-inflammatory and other cardioprotective agents.

Prolonging tissue acidosis. Ischemia causes severe tissue acidosis, which is readily reversed by washout of hydrogen ions and metabolic byproducts at reperfusion. Indeed, this rapid reversal of tissue acidosis may contribute to reperfusion injury. Acidosis prevents opening of the mitochondrial permeability transition pore (mPTP; Ref. 22). In preconditioned hearts, IPC decreases the degree of tissue acidosis achieved during index ischemia, and accelerates the normalization (decrease) in intracellular sodium during the early moments of reperfusion (3). This accelerated normalization of tissue sodium is replicated by repetitive tissue acidosis applied before index ischemia (47). Recently, Cohen and colleagues (9) found that PoC prolonged tissue acidosis during reperfusion, which may attenuate opening of the mPTP. They found that maintaining tissue acidosis by perfusing the heart with hypercapnic buffer in isolated rabbit hearts subjected to 30 min of regional ischemia recapitulated the infarct reduction of PoC, and infarct sparing by PoC was reversed by perfusing with alkalotic buffer during the PoC algorithm. In agreement with this “pH hypothesis,” we found that in the in vivo canine model of ischemia-reperfusion postconditioning significantly prolonged tissue acidosis during the early moments of reperfusion (Fig. 2). How this mechanism of prolonging tissue acidosis and maintaining the mPTP in a closed state interacts with other mechanisms such as triggering by G protein-coupled receptors (GPCR) ligands is not clear.

**RECEPTOR-DEPENDENT MECHANISMS OF PRECONDITIONING AND POSTCONDITIONING**

Stimulation of any G protein-coupled receptor has been shown to trigger preconditioning (84), and a similar involvement of GPCR is being revealed for PoC. These GPCRs include adenosine, bradykinin, norepinephrine, and opioids and may include protease-activated receptor type 2 (PAR2) at least for postconditioning. The involvement of these receptors must satisfy several criteria: 1) the receptors must be present in the target tissue (myocytes, endothelium, inflammatory cells) and active...
during ischemia and/or reperfusion; 2) the stimulating ligand must be endogenously produced and elevated during either ischemia (preconditioning or index) or reperfusion; 3) the endogenous ligand-receptor interaction can be blocked pharmacologically or the protective effects not observed in models in which the specific receptor has been knocked out or the endogenous ligand has been knocked down by siRNA approaches; and 4) the exogenous ligand can mimic the effects of IPC and PoC. Adenosine is released during ischemia and reaches high interstitial and intravascular levels (65, 66). The purine is also rapidly washed out during reperfusion, with high concentrations being detected in coronary perfusate during the early minutes of reflow (32). There are four adenosine receptor subtypes: A1, A2A, A2B, and A3. Blockade of the A1 receptor abolished IPC’s cardioprotection (44), while exogenous application of selective A1-receptor antagonists prior to the index ischemia reduces infarct size (61, 62), which suggests that stimulation of the A1 receptor by adenosine released during the preconditioning stimulus exerts protection before the index ischemia is imposed. Zhao and colleagues (88) found that endogenously released adenosine also exerts cardioprotection during reperfusion and that the A2 receptor, rather than the A1 receptor (90), is involved in modulating infarction during reperfusion. In addition, infusion of adenosine or A2A-selective agonists (CGS-21680) at the onset of reperfusion also reduces infarct size (30, 38). These data suggest that adenosine reduces infarct size when administered at reperfusion by an A2A mechanism. Like IPC, PoC was found to involve the stimulation of adenosine receptors, but receptor stimulation/activation is during reperfusion rather than during ischemia as in IPC. Parenthetically, there is recent data suggesting that adenosine acts during reperfusion after IPC (59), which would make IPC and PoC similar in their mechanisms. Blockade of adenosine receptors at the onset of reperfusion with the non-specific antagonist 8-p-(sulfophenyl)theophylline at reperfusion abrogated the infarct sparing effects of PoC; an A2A-selective blocker as well as an A3-selective blocker also reversed the myocardial salvage by PoC (34), but blockade of the A1 receptor at reperfusion did not abolish PoC cardioprotection (34, 55). These results suggesting a role for the A2A receptor in PoC have been supported by models of A1- and A2A-receptor knockout mice (82). A role for the A2B receptor has been reported by Philipp et al. (55) and Solenkova et al. (59).

Other GPCR have been implicated in the triggering of IPC and PoC. Bradykinin is elevated during and after ischemia. Bradykinin infused in rabbits or mice starting 5 min before reperfusion reduces infarct size (5, 78) and the bradykinin antagonist HOE-140 abrogates IPC (73), suggesting that endogenous bradykinin mediates IPC. In addition, IPC is abrogated in B2-bradykinin receptor knockout mice (81). The role of endogenous bradykinin in the triggering of PoC via activation of B2 receptors has recently been demonstrated (52). Bradykinin is elevated during and after ischemia or before reperfusion (20). The nonselective opioid antagonist naloxone and the peripherally acting antagonist naloxone methiodide abrogate the effects of IPC, suggesting that endogenous opioids, such as the enkephalins, are involved in triggering of IPC. Both κ- and δ-antagonists were found to reverse the infarct reduction by IPC (74). Opioid receptors have also been found to be involved in the triggering of PoC (33) and, like IPC, may involve both the κ- and δ-receptors. Hence the endogenous ligands that trigger IPC and PoC seem to be similar. However, the question of whether these ligands activate their cognate receptors and produce a physiological event at different times (ischemia for IPC, reperfusion for PoC) is open to question, although preliminary data suggest that reperfusion may be playing a greater role in ligand-receptor responses.

It is fortunate that these GPCR ligands exert cardioprotection during both windows of opportunity, although different receptor subtypes may be involved in each phase. Inducing pre- and postconditioning by pharmacological mimetics that operate through activation of GPCRs is of great clinical interest currently. Agents may be administered before cardiac surgery or angioplasty for an additive effect or may be administered just before reperfusion to “pharmacologically postcondition” the heart, assuming that exogenously delivered molecules have the same access to target cells as do endogenously released molecules. A summary of the GPCR ligands and other mechanisms involved in both IPC and PoC is shown in Fig. 3.

**PRECONDITIONING AND POSTCONDITIONING THE HUMAN HEART**

Excised human myocardium demonstrates increased tolerance to ischemia by IPC. Furthermore, studies demonstrated the phenomenon of IPC in the intact human heart in several clinical presentations of ischemia-reperfusion, including preinfarction angina, the warm-up phenomenon, percutaneous coronary intervention (angioplasty), and cardiac surgery (63). In the catheterization laboratory setting, repeated balloon inflations during balloon angioplasty attenuated electrocardiographic changes (ST-segment elevation; Ref. 13), lactate release, and left ventricular segmental dysfunction (28) relative to the first balloon inflation suggestive of a preconditioning-like effect. Laskey (37) reported that repetitive 90-s balloon occlusions during angioplasty reduced ST-segment elevations, increased the rate of ST-segment elevation resolution (a marker of improved viability and perfusion) and increased flow velocity reserve in the infarct-related artery. Whether this alternating ischemia-reperfusion induced by repeated balloon inflations immediately after opening the coronary artery is preconditioning or postconditioning is arguable. Prodromal (preinfarction) angina, defined as anginal pain at rest experienced in the 24 h before the onset of symptoms of myocardial infarction, reduced infarct size by 33% assessed by peak CK-MB release (51). Some of this cardioprotection is likely a preconditioning effect rather than recruitment of collateral vessels.

In cardiac surgery, cardiac motion is electively limited by inducing fibrillation, or complete electromechanical arrest is achieved by either global ischemia (aortic cross-clamping) or chemical cardioplegia. Yellon et al. (83) and Alhulaifi et al. (1) reported that preconditioning the heart by short periods of aortic cross-clamping preceding elective ventricular fibrillation conserved high energy phosphate (i.e., ATP) levels in myocardial biopsy samples, suggestive of a cardioprotective effect. However, a conservation of tissue ATP was not observed in a follow-up study (29). The impact of these observations is limited by the declining use of induced ventricular fibrillation during aorto-coronary artery bypass in favor of some form of chemical cardioplegia and by the hesitation of surgeons to...
repeatedly cross-clamp aortas that may have some degree of atheromatous plaque and/or adherent thrombi that may dislodge and embolize, particularly in the brain, causing stroke. Alternatively, the heart may be preconditioned remotely, for example by inducing transient limb ischemia before ventricular fibrillation or ischemia. Less CK release, greater myocardial ATP levels, and greater dP/dt values (a soft surrogate measure of function in vivo) suggestive of better contractility upon reperfusion were reported after ischemically preconditioning patients undergoing chemical cardioplegia and aortic and mitral valve replacement surgery (45). However, in a study using chemical cardioplegia to arrest the heart during surgery, preconditioning was reported to be associated with increased release of CK-MB compared with nonpreconditioned patients.

Fig. 3. A schematic diagram of triggers, mediators, end effectors, and end points in ischemic preconditioning and postconditioning (PoC). Both cardio-protective maneuvers share similarities in mechanisms, notably a division of participants into the classifications triggers, mediators and effectors. Notable exceptions are 1) obvious application of all three classifications of mechanisms at the onset of reperfusion in PoC; 2) adenosine (Ado) receptor subtype(s) active in IPC is the A1 and/or the A2B, while in PoC the A2A and A1 receptors have been implicated. Other G protein-coupled receptor (GPCR) ligands have been implicated in both IPC and PoC. Mitochondrial K\textsubscript{ATP} channels have been implicated as both triggers and mediators in IPC, whereas in PoC this distinction is not known. Mediators have been assumed to exert protection during index ischemia in IPC, but likely are activated during the early phase of reperfusion in PoC after the initial ischemia-reperfusion sequence. In addition to the inhibiting opening of the mitochondrial permeability transition pore (mPTP), which is common to both maneuvers, PoC also reduces superoxide anion generation (O\textsuperscript{2−}) by cardiomyocytes and endothelium, attenuates calcium (Ca\textsuperscript{2+}), increases preserves endothelial function (increased nitric oxide generation) and attenuates activation and adherence of neutrophils (PMNs). PoC exhibits a broad spectrum of protection in intact myocardium that spans the multiple mechanisms of reperfusion injury. It is still not clear how and if IPC attenuates reperfusion injury specifically.

In summary, preconditioning has been shown to be cardio-protective in patients with coronary occlusive disease, but its clinical application is limited since it is not readily feasible to predict the onset of ischemia in non-surgical settings such as the catheterization laboratory. Whether sequential inflations and deflations of the angioplasty balloon in the catheterization laboratory at the onset of reperfusion are preconditioning or a form of postconditioning in acute PCI is arguable. However, the degree of infarct size reduction after angioplasty is remarkably similar between the studies (~30%), and mechanisms may be similar to those revealed by experimental studies. Efficacy of preconditioning in the surgical arena using chemical cardioplegia is controversial, and surgeons are reticent to literally apply ischemia by cross-clamping the aorta. However, both preconditioning and postconditioning can be applied in off-pump surgery during elective occlusion of the target vessel.
The use of preconditioning mimetics in cardiac surgery is much more appealing. There are relatively few clinical studies on postconditioning, and these have been conducted in small populations of patients with acute myocardial infarction, with little experience in its application in cardiac surgery. Whether cardioprotection exerted by preconditioning or postconditioning is sustained in some experimental models of comorbidities including age, hyperlipidemia (16, 27, 86), diabetes, heart failure, and hypertension, salubrious effects of postconditioning have been reported in patients with multiple comorbidities. Resolving the discrepancies in efficacy of pre- and postconditioning between experimental models and patients with these comorbidities is open to further investigation.

THE FUTURE APPLICATION OF PRECONDITIONING AND POSTCONDITIONING

The strength of IPC and PoC is in their ubiquitous cardioprotection observed in all species tested, including humans, and the protection afforded to other organ systems. A limitation of both conventional IPC and PoC is the reliance on ischemia to induce the stimulus. However, IPC and PoC strategies can be “mimicked” by pharmacological agents given before the index ischemia or at reperfusion, respectively. IPC has the added limitation that it is applied before the ischemic event and therefore can be used only when the event is predictable (unless a chronic preconditioned phenotype can be induced). Whether for pre- or postconditioning, imposing ischemia is not part of the clinical mind-set and, therefore, is performed with great hesitation. Even surgical ischemia is avoided by continuous cardioplegia. In turn, the utility of PoC may be limited in patients in whom the onset of reperfusion is not predictable, for example, in patients with intermittent vasospasm. The utility of IPC and PoC in patients with incomplete occlusive disease has not been investigated. In addition, the duration of ischemia beyond which either IPC or PoC salvage myocardium has not been defined and cannot be predicted from the “wavefront phenomenon” in which unfettered reperfusion has undoubtedly contributed to the extent of infarction. A reduction in reperfusion injury would potentially turn a 4-h infarct into a 1-h infarct and divert the preoccupation of the interventionist from reducing the ischemic time to initiating reperfusion under controlled conditions. The reliance on myocardial ischemia can be overcome by inducing ischemia in other organs and thereby remotely conditioning the heart either before or after the index ischemia. The Holy Grail strategy would be to pharmacologically induce IPC or PoC. Indeed, this has been done for both protocols. The literature is replete with studies in which cardioprotective drugs have been administered either before the index ischemia or at the onset of reperfusion. Some of the drugs demonstrating myocardial salvage when administered at reperfusion include adenosine, nitric oxide, opioids, bradykinin, and erythropoietin, as well as drugs that activate PKC epsilon. In addition, the delivery of PoC mimetics to the target tissue specifically may improve efficacy that is otherwise limited by systemic hemodynamic side effects or other unwanted side effects of the agents. However, the strategy of reperfusion therapeutics to salvage myocardium (as opposed to simply restoring blood flow) has not yet been adopted into daily clinical practice.

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