Postconditioning: a mechanical maneuver that triggers biological and molecular cardioprotective responses to reperfusion

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Abstract Infarct size is determined not only by the duration and severity of ischemia, but also by pathological processes initiated at reperfusion (reperfusion injury). Numerous pharmacological strategies have been reported which administer drugs at or just before the onset of reperfusion, with subsequent salubrious effects, notably a reduction in infarct size. However, few if any of these strategies have become standard of care in the catheterization laboratory setting. Postconditioning, defined as repeated brief cycles of reperfusion interrupted by ischemia (or hypoxia) applied at the onset of reperfusion, was recently introduced as a mechanical strategy to attenuate reperfusion injury. Postconditioning intervenes only during the first few minutes of reperfusion. However, it reduces endothelial activation and dysfunction, the inflammatory response to reperfusion, necrosis, and apoptosis both acutely and long-term. Cardioprotection has been demonstrated by multiple independent laboratories and in multiple species. Postconditioning stimulates G-protein coupled receptors by their cognate endogenously released ligands and surprisingly activates survival kinases that may converge on mitochondrial K_{ATP} channels and the permeability transition pore. Postconditioning has been shown in two clinical studies to reduce infarct size in patients undergoing percutaneous coronary intervention in the catheterization laboratory, and at least five other studies are in some phase of implementation. This significant reduction in infarct size has implications for reduction in heart failure as a consequence of myocardial infarction, but this link has yet to be demonstrated. The salubrious effects of postconditioning are an indirect validation of the experimental and clinical importance of reperfusion injury in the setting of coronary artery occlusion.

Keywords Reperfusion injury · Postconditioning · Myocardial protection · Reperfusion therapeutics

Introduction

Cardiovascular disease is the leading cause of death in both men and women in the United States, accounting for approximately 40% of all deaths in 2000. This percentage is greater than the next leading causes of death combined (cancer, chronic respiratory disease, accidents, diabetes mellitus, and influenza/pneumonia). About 5% of patients with coronary heart disease die of heart failure. The incidence of heart failure as estimated by hospital discharges has increased approximately 145% between 1970 and 2000. Currently, it is estimated that between 2.5 and 3 million people in the United States (representing 1% of the population) has heart failure, with over half a million new cases being diagnosed each year [1–3]. In 1993, the 5 year survival after the diagnosis of heart failure was as low as 25–35% [4], but these mortality statistics have improved with the use of ACE inhibitors and beta blockers. Treatment for heart failure accounts for over 6% of the total health care expenditures.

There are a number of pathological conditions that contribute to the etiology of heart failure. Hypertension is a strong independent risk factor. Myocardial infarction is likewise an independent risk factor for heart failure. In 2005, approximately 1.2 million Americans suffered a

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myocardial infarction, of which 42% died. After myocardial infarction, the surviving myocardium undergoes a complex sequence of remodeling changes that present functioning and hemodynamic advantages in the short-term, but which ultimately cause heart failure in the long-term. In experimental studies, the degree of this deleterious remodeling is proportional to the size of the infarct [5] which causally links the development of heart failure to myocardial infarction. Indeed, permanent or transient coronary artery occlusion is a popular experimental model of heart failure [6]. Accordingly, clinical reports indicate that infarct size estimated by peak plasma creatine kinase activity is an independent predictor of LV remodeling and hence heart failure [7].

In view of the important role that infarct size plays in the etiology of heart failure, limiting infarct size is an important strategy to reduce the incidence and severity of heart failure, in addition to other therapeutic strategies that target the remodeling process (i.e., β-adrenergic inhibitors, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers). It appears likely that the limitation of unfavorable LV remodeling is a physiological consequence of infarct size limitation [8], potentially through a decrease in the untoward remodeling and geometric changes (dilatation) leading to heart failure. Therefore, cardioprotective strategies that reduce infarct size achieve not only short-term benefits, but may have long-term benefits by reducing the human and financial burden of heart failure.

Postconditioning is defined as repeated brief cycles of reperfusion interrupted by ischemia (or acidosis) applied at the onset of reperfusion [9]. In its brief lifetime since 2003 [9], postconditioning has enjoyed a steep trajectory that has taken it from an unlikely bench top experimental ''curiosity'' to a clinically validated strategy that significantly reduced infarct size in patients undergoing percutaneous coronary interventions (PCI) [10–12]. In addition, at the time of this writing, there are five clinical trials either enrolling patients or ready for launch, all outside the United States, and one on ''pharmacological'' postconditioning with adenosine for ST-segment elevation myocardial infarction (STEMI).

Postconditioning is undoubtedly a form of modified reperfusion, and in a broad sense it may be similar to other mechanical interventions that mechanically alter the onset of reperfusion [13], such as gradual or controlled reperfusion [14, 15]. However, it has not been shown that gradual reperfusion and postconditioning exert a similar degree of infarct reduction, or similar vasculoprotection to coronary vascular endothelium, or whether the molecular mechanisms are involved. By its time frame of exerting cardioprotection within the first few minutes of reperfusion [16] postconditioning interrupts events that are occurring within the first few minutes of reperfusion—events that obviously play a causal role in postischemic injury. As Piper and associates [17, 18] and others [14, 19] have stated, the first few minutes of reperfusion are very active indeed in contributing to the pathophysiology of not only myocardial necrosis, but also to endothelial cell dysfunction and apoptosis. A summary of these early events is shown in Table 1. The attenuation of postischemic injury by postconditioning is an indirect and very compelling validation of the existence of reperfusion injury, not only in experimental models, but also in patients undergoing evolving myocardial infarction and clinically destined for angioplasty [10–12].

Protection against acute myocardial infarction

The initial study by Zhao et al. [9] in the canine model of coronary artery occlusion established that postconditioning significantly reduced infarct size. The ability to reduce infarct size has been confirmed by multiple labs and in multiple species, including mouse [20, 21], rat [22–24], rabbit [25–27], canine [9, 28], and pig [29] (but not without some controversy [30]). However, its protection appears to go beyond protecting only myocytes from necrosis; postconditioning also reduces apoptosis [31, 32]. Postconditioning may reduce pro-apoptotic triggers such as the generation of oxygen radicals and accumulation of calcium in mitochondria. However, the vascular endothelium is also spared by postconditioning. It is the opinion of the authors and other investigators that the coronary vascular endothelium is a critical factor in the initiation of the inflammatory response to reperfusion, and as such preservation of

Table 1 Events that occur during the early moments of reperfusion

Generation of reactive oxygen species
    Endothelial cells
    Neutrophils
Surface expression of P-selectin
Endothelial cell dysfunction
    Impaired synthesis and release of nitric oxide
Elaboration of cytokines
    TNFα
    Interleukins-6 and -8
Vascular expression of tissue factor (?)
Realkalinization
    Activation of the sodium/hydrogen (Na^+/H^+) exchanger
Calcium accumulation and dyshomeostasis
    Cytosolic
    Mitochondrial
Hypercontracture
Opening of the mitochondrial permeability transition pore
Activation of survival kinases (PI-3 kinase, ERK1/2)

the coronary vascular endothelium is causally related to a reduction in infarct size, as has been reviewed elsewhere [33–36]. The remainder of the review will discuss the mechanisms by which postconditioning reduces necrosis and apoptosis. The observation that necrosis and infarct size are determinants of heart failure, and apoptosis is an important mechanism leading to heart failure makes postconditioning a potential therapeutic strategy that may have a future impact on the incidence and severity of heart failure secondary to acute myocardial infarction. Indeed, a recent study by Zhu et al. [24] reported that postconditioning reduced infarct size (TTC staining and lactate dehydrogenase activity) and improved function in hearts with chronic (6 weeks) myocardial hypertrophy induced by permanent coronary artery ligation or by one kidney/one clip hypertension. After the chronic condition each heart was subjected to 40 min global ischemia (Langendorff apparatus) and 90 min of reperfusion with or without postconditioning following an algorithm of 10 sec reperfusion and 10 sec complete ischemia repeated for 6 cycles. The infarct-sparing effect of postconditioning was comparable between the non-remodeled (''healthy'') hearts and the two hypertrophy models. However, functional recovery after global ischemia was somewhat less (but still significantly greater than controls) in the one clip/one kidney model than in the infarcted or non-remodeled cohorts.

Mechanical attenuation of reperfusion

A proximal-most mechanism of postconditioning may be the slow reintroduction of oxygen and persistence of tissue acidosis. In elegant experiments performed in regionally ischemic isolated perfused rabbit hearts by Cohen et al. [37], the cardioprotection exerted by postconditioning was replicated by reperfusion with hypercapnic buffer (pH was 6.9) for the first 2 min of reflow. This protection with acidotic perfusate was abrogated by an alkalotic (pH 7.7) buffer, and by the oxygen radical scavenger N-2-mercaptopropionyl glycine. These data suggest that maintaining tissue acidosis for the first 2 min of reperfusion is an important mechanism in postconditioning. Maintaining tissue acidosis may attenuate opening of the mitochondrial permeability transition pore (mPTP), while the gradual reintroduction of oxygen may generate lower levels of oxygen radicals for tissue signaling rather than the large levels associated with tissue injury. More experimentation is needed to confirm this involvement of tissue acidosis by measuring tissue pH and acid products during the short window of postconditioning, and the relationship between tissue pH and the mPTP, and signaling versus cardiodestructive levels of reactive oxygen species. In addition, more research has to determine the relationship between

maintaining tissue acidosis, the gradual reintroduction of oxygen and endogenous ligands that trigger postconditioning (adenosine, bradykinin, opioids). Whether these mechanisms operate independently and some operate in an integrated manner must still be demonstrated.

Inhibition of endothelial dysfunction, neutrophil actions and the inflammatory response to reperfusion

A number of investigators have suggested that some of the early events initiated at reperfusion are similar to an inflammatory-like response [38–42]. There is ample evidence in the experimental literature to support an inflammatory-like response that begins in the very early moments of reperfusion with activation of neutrophils and vascular endothelial cells in the reperfused area, and a subsequent recruitment of neutrophils to first adhere to the endothelium (at first loosely with rolling along the surface, later firm adherence) and then finally migrate into the parenchyma (>4 h after onset of reperfusion). Both neutrophils and the vascular endothelium elaborate pro-inflammatory mediators (TNFα, IL-6, IL-8) and oxidant species that can damage the endothelium in these early minutes [19, 36], and finally damage cardiomyocytes. These same oxidants also neutralize constitutively expressed nitric oxide and form the potent oxidant peroxynitrite. The hallmarks of endothelial dysfunction are: impaired vasorelaxation to acetylcholine, and increased adherence of neutrophils, both related to impaired release of nitric oxide secondary to dysfunction of the endothelial receptors used to stimulate relaxation, a loss of cofactors such as tetrahydrobiopterin, or damage to the eNOS apparatus itself.

Postconditioning has been shown to attenuate endothelial dysfunction, reduce neutrophil adherence to endothelium and their accumulation in the reperfused myocardium, and pro-inflammatory mediators such as TNFα. The importance of neutrophils in reperfusion injury has been disputed by some [43]. Indeed, the persistence of cardioprotection is observed in cell-free model systems which would appear to argue against an active anti-inflammatory component to postconditioning cardioprotection. Preliminary unpublished data from our laboratory (A. Granfeldt Petersen et al.) using neutrophil antiserum to deplete the neutrophil population in an in vivo rodent model of coronary artery occlusion-reperfusion show that both neutrophil depletion and postconditioning separately reduce infarct size to the same extent (~28%), but there was no significant further reduction in infarct size when postconditioning and neutrophil depletion were combined. Whether neutrophils as well as oxidants and pro-inflammatory mediators contribute significantly to the pathogenesis of reperfusion injury, as well as their role in postconditioning, is on on-going and vigorous debate.

G-protein coupled receptors: a possible convergence of triggers of postconditioning

G-protein coupled receptors (GPCR) have recently been implicated in cardioprotection at a very proximal point—the cell membrane. The stimulation of the GPCR for adenosine [44–46], bradykinin [47, 48], and opioids at the time of reperfusion have been associated with a reduction of infarct size. In addition, there may be considerable cross-talk between these receptors, e.g., stimulation of the adenosine receptor by opioid agonists and vice verse [49]. Involvement of three GPCRs in postconditioning is reviewed below. Whether blockade at the G-protein transduction level blocks the effects of these ligands has not been attempted. However, it is interesting that blockade of either adenosine or opioid receptors completely blocks postconditioning, suggesting an all-or-none response, rather than demonstrating a graded or incremental reversal of infarct reduction. In addition, a combination of these GPCR agonists has not been administered to determine if there is an additive or synergistic effect.

Adenosine in postconditioning

Of the four adenosine receptor subtypes (A_1, A_{2A}, A_{2B}, A_3), the A_{2A} and A_3 receptors have been shown to be cardioprotective when activated at reperfusion [46, 50]. Recently, Yang et al. [51] reported that the selective A_{2A} adenosine receptor agonist ATL 146e administered intraperitoneally 2 min before reperfusion reduced infarct size in wild-type mice, but had no effect in global A_{2A} receptor knock-out mice, or chimeric mice in which the A_{2A} receptor was not expressed in bone marrow derived cells. These data support the concept that adenosine acts to attenuate reperfusion injury by an anti-inflammatory mechanism. This study [51] also showed in RAG-1 knock-out mice that inhibition of T lymphocytes may be a mechanism of action, consistent with the notion that T lymphocytes (perhaps CD4+) may generate the cytokines and chemokines that recruit neutrophils to the reperfused myocardium. This anti-inflammatory effect of A_{2A} adenosine receptor stimulation during postconditioning is consistent with the following observations: (1) adenosine levels are elevated during ischemia, and the washout of adenosine is delayed by postconditioning [20]; (2) non-selective inhibition of adenosine receptors [20, 25] and the A_{2A} receptor specifically [20], abrogates the cardioprotection of postconditioning; (3) the reduction of neutrophil adherence, accumulation, and preservation of the vascular endothelium are consistent with the physiological effects of adenosine in models of ischemia-reperfusion. Recently, Philipp et al. [26] from Cohen's and Downey's group have suggested that postconditioning activates the A_{2B} receptor

and PKC rather than the A_{2A} receptor. The purported A_{2B} antagonist MRS 1754 blocked the infarct-sparing effect of postconditioning. Studies have been limited to pharmacological blockade, and lack confirmation in appropriate A_{2B} knock-out or knock-down (siRNA against A_{2B} mRNA) models. Further studies are needed in this area to clarify which receptor subtype(s) are involved in the endogenous adenosine-mediated protection of postconditioning.

Bradykinin

Yang et al. [48] reported that bradykinin administered at reperfusion reduced infarct size by mechanisms that signaled through the reperfusion injury survival kinase pathway and nitric oxide production. Similar results were reported by Bell and Yellon in a murine model of ischemia-reperfusion [47]. There is no formal publication on whether endogenous bradykinin is involved in postconditioning.

Opioids in postconditioning

There are three opioid receptor subtypes, the μ, δ, and κ. The δ and κ receptors are expressed in cardiomyocytes and coupled to Gi. The myocardium is able to synthesize the three major endogenous opioid peptides (enkephalins, endorphins, and dynorphins). The release of opioid peptides and subsequent stimulation of κ and δ receptors has been implicated in the cardioprotection of preconditioning [52]. Recent evidence has suggested that exogenous opioids administered at reperfusion are cardioprotective [53, 54]. Accordingly, the non-selective opioid morphine, or the δ opioid receptor agonists BW373U86 and fentanyl isothiocyanate administered at reperfusion, reduce infarct size comparable to that observed with a pretreatment administration in the rat model of coronary artery occlusion and reperfusion [53]. However, κ opioid receptor stimulation at reperfusion was not cardioprotective. The mechanism of this opioid-induced infarct salvage was suggested to be activation of the RISK pathway components PI-3 kinase, mammalian target of rapamycin (mTOR), p70s6 kinase, and phosphorylation and subsequent inactivation of GSK-3β. Hence activation of opioid receptors at reperfusion is cardioprotective; a cardioprotective role for *endogenous* opioids is also suggested by the antagonist data.

Since opioid precursors and peptides may be synthesized and released in myocardium during ischemia/reperfusion, the hypothesis may be put forward that opioids mediate in part the infarct sparing effect of postconditioning. Kin et al. [55] demonstrated the involvement of endogenous opioid peptides in postconditioning. In the rat model of 30 min coronary artery occlusion followed by 3 h of reperfusion, postconditioning cardioprotection was completely abrogated by the non-subtype selective antagonist

naloxone and its peripherally restricted quaternary derivative naloxone methiodide administered 5 min before reperfusion. Blockade of either the δ receptor (naltrindole hydrochloride) or the κ receptor (nor-binaltorphimine dihydrochloride) abrogated postconditioning as well. Whether μ receptor stimulation was involved was not clear. Hence, in contrast to preconditioning, in which only the δ receptor is suspected of being involved, both the κ and δ opioid receptors may be involved in postconditioning. Further studies by Zatta et al. (unpublished) show that postconditioning increases precursor proenkephalin levels in area at risk myocardium, and achieves a normalization of active enkephalin levels without effecting methionine enkaphalin or methionine-enkephalin-arginine-phenylalanin, in contrast to preconditioning which preserves active enkephalin metabolites. The increased enkephalin pool may allow an increased stimulation of opioid receptors, or a sustained release over an undetermined period of reperfusion.

Signaling in postconditioning cardioprotection

PI3-K and ERK ½

Surprisingly, the very short period of intervention that defines postconditioning activates a complex and growing array of molecular pathways. This network of pathways has been reviewed comprehensively by Hausenloy and Yellon [56]. The reperfusion injury survival kinases (RISK) [56, 57] PI3 kinase and MEK-ERK1/2 are distal targets of GPCR activation, so activation of these kinase pathways would be consistent with the involvement of GPCR ligands. Both pathways are stimulated at early reperfusion, placing this timing within the temporal frame of postconditioning. The PI3 kinase/Akt pathway likely is proximal to endothelial nitric oxide synthase [56], the activation of which has been found to be important to cardioprotection by postconditioning [27], and involves two targets that have been shown to be pivotal to postconditioning cardioprotection—the mitochondrial K_{ATP} channel [27, 58] and the mitochondrial permeability transition pore m(PTP) [59]. The mPTP is a potential ''switch'' not only of cell survival or death, but of pursuit along the necrotic or apoptotic pathways. Inhibition of the PI3 kinase pathway has, in most studies, abrogated infarct reduction by postconditioning [23, 25]. Likewise, the MEK-ERK1/2 pathway has also been reported to be an important pathway engaged by postconditioning [27, 60]. Interestingly, both preconditioning and postconditioning may have these two pathways in common. The involvement of these two pathways is entirely consistent with the importance of GPCR stimulation by endogenous ligands such as adenosine and opioids.

Protein kinase C (PKC)

PKC is a key signaling pathway in cardioprotection by preconditioning [61]. GPCR ligands such as adenosine and opioid agonists stimulate phospholipase C and the generation of inositol triphosphate and diacyglycerol, the latter of which stimulates PKC. In rodents, PKCε and δ are the predominant isoforms involved in preconditioning [62] and postconditioning, whereas PKCα is important in cardioprotection by preconditioning in pigs [63]. PKCδ may be involved in the pathogenesis of myocardial injury after ischemia-reperfusion [64, 65]. This isoform translocates to mitochondria within the first 5 min of reperfusion, which is within the time frame of the postconditioning effect. PKCδ is associated with pathophysiological events including increased superoxide anion generation, mitochondrial dysfunction and the release of cytochrome c and downstream pro-apoptotic factors [66, 67]. On the other hand, PKCε is associated with cardioprotection [68, 69] potentially by inhibiting the mitochondrial permeability transition pore [70]. Zatta et al. [71] recently reported that the infarct sparing effect of postconditioning in a rat model was abrogated by the non-selective PKC antagonist chelerythrine administered 5 min before the onset of reperfusion. Similar results were also reported by Penna et al. [72]. In addition, Zatta et al. [71] also showed that infarct size reduction by postconditioning was reversed by the PKCε specific inhibitor KIE1-1. Inhibition of PKCδ with rottlerin given 5 min before reperfusion by itself reduced infarct size, but did not alter (i.e., further reduce) the infarct size reduction achieved with postconditioning (Fig. 1). Postconditioning increased total cell homogenate levels of phosphorylated PKCε relative to the decreased levels observed after ischemia-reperfusion, suggesting a translocation site other than the mitochondrion, while phosphorylated levels and mitochondrial translocation of PKCδ were reduced. These data suggest that postconditioning cardioprotection is dependent on PKC signaling, and the mechanical maneuver increases the cardioprotective component of endogenous PKC signaling (PKCε) while simultaneously decreasing the cardio-destructive component (PKCδ). Philipp et al. [26] have also reported that cardioprotection by postconditioning is dependent on PKC signaling since the non-selective PKC inhibitor chelerythrine given 5 min before reperfusion aborted the infarct sparing effect of postconditioning in an in vivo rabbit model of coronary artery occlusion and postconditioning (4 cycles of 30 sec reperfusion/ischemia). A signaling role for PKC is consistent with the involvement of GPCR activators of PKC, specifically endogenous adenosine [20] and opioids.

Postconditioning and reactive oxygen species (ROS)

Reactive oxygen species include the superoxide anion and its products (H_2O_2 and $\bullet OH$), and nitric oxide and its
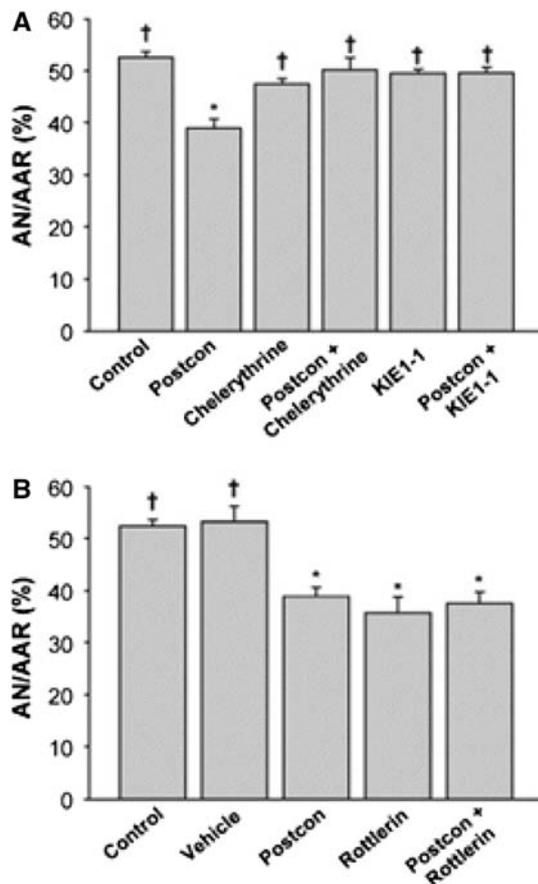
Fig. 1 The effects of PKC inhibitors on infarct size (area of necrosis/area at risk mass) reduction by postconditioning (postcon) in the rat model of 30 min coronary artery occlusion and 3 h of reperfusion. (**A**) Effects of the non-selective PKC inhibitor chelerythrine (5 mg/kg) administered intravenously 5 min before reperfusion or the PKCε inhibitor KIE1-1 (3.8 mg/kg). (**B**) The effects of the PKCδ inhibitor rottlerin (0.3 mg/kg body weight) alone or given before postconditioning. Values are mean ± standard errors of the mean. $*P < 0.05$ versus control; $†P < 0.05$ versus postconditioning group (From Zatta et al. [71] with permission)

products (especially ONOO-). Under normal conditions oxidants are generated during the reduction of oxygen to water in mitochondria. Oxidants are part of cellular homeostasis, signaling, differentiation, mitosis, and immune responses. On the other hand, ROS are also an integral response to stresses such as ischemia-reperfusion. Initially described as the ''oxygen paradox'' by Hearse et al. [73] a robust generation of ROS has been shown to occur at reperfusion in myocardium [74, 75] and in isolated mitochondria [76]. In addition, the direct administration of ROS in concentrations approximating those observed at reperfusion causes injury to myocardium [77]. ROS are elaborated by cardiomyocytes, coronary vascular endothelium and inflammatory cells such as neutrophils. ROS cause injury by oxidation of lipid peroxidation of membranes, protein denaturation, and by causing breaks in genomic DNA. Hence, ROS play a dual role: that of signaling molecule and injury mechanism. This dual role of ROS may be based on several factors related to (1) the concentration achieved locally; (2) conversion of less reactive species into more potent species; (3) the environment favoring release of iron to fuel Fenton chemistry; and (4) the activation or shift of key ROS-generating enzymes. For example, the normally active xanthine dehydrogenase shifts to xanthine oxidase under ischemic conditions. In addition, cytokines and thrombin released at reperfusion can activate endothelial NADPH oxidase.

Preconditioning is mediated, in part, by elaboration of superoxide anions, possibly from mitochondria via signaling cascades that involve GPCR, PKC and K_{ATP} channels. Conversely, postconditioning may lead directly or indirectly to a reduction in ROS, which may be a factor differentiating the two cardioprotective strategies. Sun et al. [78] demonstrated that hypoxic postconditioning—the in vitro simulation of ''ischemic'' postconditioning—in cultured neonatal rat cardiomyocytes reduced ROS generation (superoxide anions and H_2O_2), which was associated also with attenuated cytosolic and mitochondrial calcium loading, and reduced cell death. In in vivo models of coronary artery occlusion-reperfusion, postconditioning was associated with less dihydroethidium-positive signal from oxidant-generating cells in the ischemic-reperfused myocardium [16, 28]. These observations are consistent with the reduction in lipid peroxidation products reported by several investigators in in vivo models of coronary artery occlusion and reperfusion [9, 16, 28]. In addition, postconditioning attenuated superoxide generation from the coronary vascular endothelium in the reperfused area after either 3 h or 24 h of reperfusion [79]. This reduction in endothelial ROS generation may be, in part, responsible for the preservation of endothelial function of epicardial coronary arteries observed in in vivo postconditioned hearts [9]. These data would suggest that postconditioning reduces oxidant-induced injury. However, Penna et al. [72] demonstrated in isolated rat hearts that the scavenger N-acetyl cysteine (NAC) given at reperfusion abrogated the infarct reduction by postconditioning. In addition, delaying administration of NAC until after the postconditioning algorithm was applied did not abolish the maneuver's protection. These data suggest that the mechanisms of postconditioning cardioprotection may involve both preservation of the ROS-related signaling pathways and a reduction in oxidant-induced injury. Further research is necessary to clarify this very interesting duality. Postconditioning may also preserve antioxidant reserves in previously ischemic myocardium. Serviddio et al. [76] reported that postconditioning attenuated the oxidation of glutathione (GSH), the major intracellular antioxidant system. Hence, a reduction in the ROS burden may not only

prevent direct tissue injury, but may preserve endogenous antioxidant levels in the myocardium.

The mitochondrial transition pore

The mitochondrial permeability transition pore (mPTP) is a voltage-dependent pore localized in the inner mitochondrial membrane. In the normally closed position, the mPTP impedes the movement of small molecules <1500 Da and water into the mitochondrial matrix. Opening of this pore allows transfer of small molecules into the matrix exacerbated by the osmotic properties of the matrix proteins. The inner membrane is capable of sustaining increases in matrix volume because of the convolutions of the cristae. However, the outer membrane is more vulnerable to such swelling, and will rupture, with a resulting collapse of the transmembrane potential, loss of ATP synthetic capacity, and release of pro-apoptotic factors such as cytochrome c. Key pathophysiological features of reperfusion conspire to open the mPTP at the onset of reperfusion, with the resultant accumulation of calcium and oxidants, and alkalinization of intracellular pH (acidosis impedes mPTP opening). Accordingly, opening of the mPTP has been shown to occur at reperfusion [80], notably within the first few minutes [81], which again places this event within the postconditioning window. Inhibition of mPTP opening with cyclosporine A, sanghliferin A or NIM811 reduces cell death and infarct size. After in vivo postconditioning, Argaud et al. [59] demonstrated that mitochondria isolated from the ischemic-reperfused myocardium were resilient to Ca^{2+}-induced mPTP opening similar to that achieved by the mPTP opening inhibitor NIM811. These mitochondrial responses were associated with better preserved morphology. There are still questions whether the harvested mitochondria represented a more robust population in the postconditioning group versus the control group, and the comparison to NIM811 is indirect evidence only that attenuation of mPTP is causally linked to cardioprotection. However, these data are suggestive and present a good spring board for more definitive studies on the causal relationship of mPTP status and cardioprotection with postconditioning. A subsequent study demonstrated that attenuation of mPTP was associated with phosphorylation of Akt [82]. However, whether protection from mPTP opening by postconditioning is localized to cardiomyocytes or other cells involved in reperfusion injury is not known.

Postconditioning in hearts with co-morbidities

A preliminary report by Donato et al. [83] showed that postconditioning reduced infarct size and improved functional recovery following 30 min of global ischemia (isolated perfused preparation) and 30 min of reperfusion with or without postconditioning (2 cycles of 30 sec reperfusion/30 sec ischemia). A full-length publication has not appeared from these authors. In contrast, Iliodromitis et al. [84] reported that postconditioning did not reduce infarct size in a rabbit model of chronic (6 weeks) hypercholesterolemia with atherosclerosis, whereas the cardioprotection of preconditioning persisted. As discussed above, postconditioning cardioprotection was shown by Zhu et al. [24] to persist in two rodent models of myocardial remodeling (myocardial infarction, hypertension). Whether postconditioning efficacy is lost in diabetes or older hearts is not known as yet. Therefore, there are some experimental data that suggest postconditioning may be of limited value in models with isolated co-morbidities. However, these experimental data are inconsistent with the salubrious effects of postconditioning in patients undergoing percutaneous coronary intervention (PCI) in the catheterization laboratory. In the study by Staat et al. [10] the patients undergoing postconditioning had demographics similar to those treated by standard PCI, and consistent with the normal population of patients undergoing such procedures: mean age was 58 ± 4 years, 38% had high blood pressure, 57% were smokers, 80% had dyslipidemia, and 20% had diabetes. These patients were therefore ''models'' of multiple risk factors. In the study by Darling et al. [11] which also reported reduction in infarct size in patients treated by postconditioning (multiple balloon inflations), there were similar co-morbidities in the postconditioned group as in the standard PCI group. Therefore, although experimental models may identify limitations of one cardioprotective strategy or another, the translation of these results to the clinical scenario has to be done with appropriate caution and validation.

Concluding remarks

The concept that reperfusion is essential for myocardial salvage is abundantly justified experimentally and clinically. However, the concepts that (1) reperfusion is a component of the ''wavefront'' pattern of injury and (2) reperfusion as an opportunity to apply cardioprotective therapeutics to reduce postischemic injury and infarct size has enjoyed limited appreciation for some decades [85, 86], and has not been given due attention until very recently [13, 17, 18, 40]. Although hundreds of studies have investigated cardioprotective strategies applied at reperfusion, few if any have been adopted into clinical practice [87]. Postconditioning has highlighted the importance of the first few minutes of reperfusion in determining infarct size even 24 h or more after reperfusion [12, 59, 79]. In

addition, postconditioning has been used in limited but growing numbers of clinical trials after only 3 short years since its introduction. It is hoped that this and other strategies aimed at attenuating post-ischemic injury by applying a cardioprotective strategy at reperfusion will be tested in clinical studies, and if appropriate, be adopted into the armamentarium of reperfusion therapeutics for patients undergoing PCI.

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