Abstract

Reperfusion has the potential to introduce additional injury that is not evident at the end of ischaemia per se, i.e. reperfusion injury. Reperfusion injury is expressed as endothelial and microvascular dysfunction, impaired blood flow, metabolic dysfunction, cellular necrosis, and apoptosis. There is an impressive array of mechanisms contributing to reperfusion injury. Postconditioning, defined as brief periods of reperfusion alternating with re-occlusion applied during the very early minutes of reperfusion, mechanically alters the hydrodynamics of early reperfusion. However, postconditioning also stimulates endogenous mechanisms that attenuate the multiple manifestations of reperfusion injury listed above. These mechanisms include ligands, such as adenosine and opioids, that act as proximal triggers to stimulate molecular pathways involving mediators such as protein kinase C, mitochondrial ATP-sensitive potassium channels, and survival kinases. Postconditioning may also inhibit deleterious pathways such as p38 and JNK mitogen-activated protein (MAP) kinases and attenuate the damage to endothelial cells and cardiomyocytes from oxidants, cytokines, proteases, and inflammatory cells. Postconditioning has been shown to inhibit the mitochondrial permeability transition pore. Hence, postconditioning marshals a variety of endogenous mechanisms that operate at numerous levels and target a broad range of pathological mechanisms. Two clinical studies in patients with acute myocardial infarction have demonstrated that postconditioning was effective in reducing infarct size. Postconditioning indirectly supports the concept of reperfusion injury in animal models of ischaemia–reperfusion and in patients, and exerts cardioprotection that is equivalent to that of ischaemic preconditioning.

Keywords: Postconditioning; Preconditioning; Reperfusion injury; Myocardial infarction; Endothelial dysfunction

1. Introduction

Rapidly initiating reperfusion is the most effective treatment to reduce infarct size resulting from myocardial ischaemia. However, reperfusion has the potential to introduce additional lethal injury that is not evident at the end of ischaemia (reperfusion injury). The pathogenesis of reperfusion injury has been reviewed elsewhere in this focused issue, and in other reviews [1–8]. Even a cursory glance at these reviews indicates that reperfusion injury is a complex process involving numerous mechanisms exerted in the intracellular and extracellular environments. Perhaps predicted by its diverse and numerous causes, reperfusion injury is expressed physiologically in equally diverse ways including 1) endothelial and vascular dysfunction and the sequelae of impaired blood flow, 2) metabolic dysfunction, 3) contractile dysfunction, 4) dysrhythmias, 5) cellular necrosis and 6) apoptosis. One may surmise that effective treatment of myocardial reperfusion injury would necessitate a broader approach rather than a “magic bullet” aimed at only one of these mechanisms. The mechanical interventions of ischaemic preconditioning and “postconditioning” represent interventions with multiple and interacting components marshaled against myocardial reperfusion injury by endogenous cardioprotective mechanisms.

2. Cardioprotection with postconditioning: physiological outcomes

Postconditioning is defined as rapid intermittent interruptions of blood flow in the early phase of reperfusion, as
depicted in Fig. 1. The duration of these alternating periods of reperfusion and ischaemia has evolved over time, but in general the cycles are measured in seconds, and are empirically shorter in smaller species (i.e. 10–15s in rats and mice) and longer in larger species (30s in canines and rabbits) and human (60s) [9]. Although the initial study used the term “ischaemic” postconditioning, the “ischaemic” has since been omitted since 1) it is not apparent whether the brief periods of re-occlusion, the intervening periods of reperfusion, or their combination, provide the stimulus for cardioprotection.

2.1. Infarct size reduction

The first study to demonstrate cardioprotective effects with postconditioning was performed in a canine model of 1h coronary occlusion and 3h of reperfusion [10]. As shown in Fig. 2, the postconditioning algorithm was 30s of reperfusion followed by 30s of coronary occlusion, which were repeated for three cycles at the onset of reperfusion. Relative to the Control group, postconditioning significantly reduced infarct size. Surprisingly, the infarct reduction was comparable to that observed in a group treated with ischaemic preconditioning (Fig. 3), which is considered the “gold standard” of cardioprotection [11].

Subsequent studies from our laboratory confirmed the infarct size reduction and other physiological outcomes observed with postconditioning in a similar canine model, [12] and in rat [13] and murine [14] models of ischaemia–reperfusion. Importantly, Kin et al. [13] demonstrated that the period during which postconditioning is applied is critical to cardioprotection; if the alternating reperfusion–re-occlusion algorithm was applied 1min after the onset of reperfusion, the infarct sparing effect was not observed. The loss of cardioprotection after delaying postconditioning was also demonstrated by Yang et al. [15] and Philipp et al. [16]. As will be reviewed below and elsewhere in this focused issue, there are many cellular and molecular events involved in the pathogenesis of myocardial infarction occurring during the early moments of reperfusion that are modulated by postconditioning.

To determine whether protection by postconditioning was additive to that of preconditioning, the protocols of preconditioning and postconditioning were combined in the acute canine model [12]. Relative to either intervention alone,
the combination of postconditioning and preconditioning did not demonstrate additive protection, whether assessed by infarct size, superoxide anion production by post-ischaemic myocardium, or post-ischaemic endothelial function. Similar results were obtained by Tsang et al. [17] in isolated perfused rat hearts. However, Yang et al. [15] demonstrated in an in vivo rabbit model of 45 min occlusion and 3 h of reperfusion that the combination of preconditioning and postconditioning reduced infarct size significantly more than either maneuver alone. Species differences may underlie the inconsistent observations. Alternatively, an additive effect may be unmasked after longer periods of occlusion. That is, longer occlusions may expose an additive “threshold” in the same way that postconditioning reduces the threshold for protection with inhalational anaesthetics (see below) [18].

One question that arises is whether the cardioprotection of such a brief maneuver persists for longer periods, or whether the protection is simply a delay in its pathogenesis. This is a critical question because postconditioning may attenuate events involved in early reperfusion injury, but not those involved in later events (6–24h). Indeed, the reduction of myocardial infarct size has been shown to persist for 24 and up to 72h [19,20]. Therefore, the infarct sparing effect of postconditioning appears to represent a long-term reduction rather than a delay in the inevitable injury.

2.2. Reduction in endothelial activation, dysfunction, neutrophil adherence

The coronary vascular endothelium is damaged by ischaemia–reperfusion; this damage is expressed as 1) a reduction in vasodilator responses to the nitric oxide synthase stimulator acetylcholine which has been shown to be linked with a reduction in basal nitric oxide generation [21], 2) a proclivity to neutrophils to adhere to coronary vascular endothelium secondary to the loss of basal nitric oxide generation [22], 3) increased surface expression of P-selectin [23,24], and 4) increased superoxide anion generation. Zhao et al. [10] showed that post-ischaemic coronary artery endothelial dysfunction assessed by the vasodilator responses to incremental concentrations of acetylcholine were improved by postconditioning. In addition, postconditioning decreased the surface expression of P-selectin, the adhesion of neutrophils (PMN) to post-ischaemic coronary artery vascular endothelium, and accumulation of neutrophils in the area at risk (myeloperoxidase activity). In agreement with the decrease in endothelial injury (superoxide anion generation) by postconditioning, Halkos et al. [12] reported that less dihydroethidium fluorescence was apparent in the perivascular area within the area at risk of postconditioned hearts. These data may suggest that postconditioning attenuates activation and dysfunction of the vascular endothelium. However, whether the reduction in cell-mediated pro-inflammatory state is causally related to other physiological outcomes such as necrosis, microvascular injury, or apoptosis, or is rather a pedestrian response to less injury is not certain. A cause–effect role of attenuated neutrophil and endothelial cell activation has been reported, [25] but whether there is a cause–effect relationship in the cardioprotection by postconditioning remains a controversial interpretation since postconditioning also exerts cardioprotection in neutrophil-free systems [26–28].

2.3. Reduction of apoptosis

In addition to necrosis, cardiomyocytes also undergo apoptosis, which is genetically programmed cell death, and phenotypically distinct from necrosis. Some studies suggest that apoptosis is triggered by reperfusion after transient coronary artery occlusion [29,30]. There are few studies that have investigated the effects of postconditioning on apoptosis. Zhao et al. [31] have reported in abstract form that “hypoxic postconditioning” of isolated neonatal cardiomyocytes following a prolonged period of hypoxia reduces apoptosis as detected by TUNEL assay and the presence of DNA ladders caused by endonuclease activity. The reduction in apoptosis may involve inhibition of caspases-3 and -9, and maintenance of the Bcl-2/Bax ratio favouring an anti-apoptotic status.

2.4. Section summary

Taken together, the results summarised above suggest that postconditioning exerts potent cardioprotection when it is applied at the onset of reperfusion, but cardioprotection is lost if the maneuver is delayed even briefly. Cardioprotection with postconditioning has now been confirmed by other investigators from different laboratories [12,13,15–18,27,32–34]. Hence, postconditioning as a reperfusion therapy attenuates multiple manifestations of post-ischaemic injury in multiple cell types in the myocardium. There are no data to date testing whether postconditioning attenuates myocardial stunning in models of regional ischaemia–reperfusion, which may be an area of future research.

3. Mechanisms involved in postconditioning

In the preconditioning literature, the concept of triggers, mediators and end effectors has been used to describe the mechanisms involved in its cardioprotective effects as summarised in Table 1. Some studies indeed suggest that a similar lexicon can be used to describe cardioprotection with postconditioning by showing that similar pathways and signals may be involved. For example, Penna et. [35] reported in a recent study that reactive oxygen species may be acting as a trigger of protection in the very early phase of postconditioning. Classical ligand triggers have also been reported to be involved in postconditioning [36,37]. Furthermore, K\textsubscript{ATP} channels and protein kinase C pathways may be evoked after the postconditioning “trigger”, and therefore may be acting as mediators. However, if similar pathways
and molecules are involved in preconditioning and postconditioning, they evidently have very different roles by virtue of the timing and the compartment in which their effects are exerted. In addition, it is not clear whether the reduction of some molecule from cytotoxic levels to cytoprotective levels, as postulated for reactive oxygen species, is a mechanism of cardioprotection, in which case it is difficult to categorize these molecular modifications as simply triggers or mediators. A comparison of triggers, mediators and end effectors in pre- and postconditioning appears in Table 1.

3.1. Triggers that stimulate cardioprotection in postconditioning

3.1.1. Generation of reactive oxygen species (ROS)

As reviewed elsewhere, [4,6,38] ROS have been thought to be an important contributor to reperfusion injury. ROS oxidize proteins and membrane lipids, and activate redox-sensitive signaling cascades. Upon the onset of reperfusion there is a “respiratory burst” lasting several minutes that originates from a number of cellular sources including endothelial cells, [39] cardiomyocytes and activated neutrophils [25,40]. This oxidative “burst” is followed by a moderately but persistently elevated production of superoxide anions. However, ROS also contribute to intracellular signaling and endogenous cardioprotection, and therefore may have a dual role in the setting of postconditioning.

Zhao et al. [10] and Kin et al. [13] have observed elevated superoxide radical generation in post-ischaemic myocardium and vascular endothelial cells after as long as 3 h of reperfusion, but whether postconditioning attenuates specifically the early burst of ROS is not known at this time.

The generation of ROS in post-ischaemic myocardium was associated with increased plasma levels of malondialdehyde, a presumptive marker of lipid peroxidation secondary
to oxidant generation. Furthermore, postconditioning significantly reduced superoxide anion (O$_{2}^-$) generation in vivo in post-ischaemic myocardium after 1[10] and 3[13] h of reperfusion, which was associated with a reduction in plasma lipid peroxidation products (malondialdehyde activity). In neonatal cardiomyocytes in vitro[41] “hypoxic postconditioning” reduced superoxide anion generation detected by lucigenin-enhanced chemiluminescence, cytochrome c reduction, and dihydroethidium fluorescence. This in vitro reduction in ROS with postconditioning was associated with decreased lactate dehydrogenase activity (a presumptive marker of morphological injury, necrosis) in the culture medium. In addition, Servidio et al. [33] reported that a brief period of hypoxic reperfusion, a variant of postconditioning without the alternating periods of normoxia, decreased peroxide production during the reoxygenation phase following hypoxia. A reduction of ROS may be achieved by increasing the endogenous anti-oxidant defense potential of the myocardium, for example by preserving reduced glutathione levels [33]. Again, whether the reduction in ROS represents an active mechanism of postconditioning or a muted response to less tissue injury remains unknown at this time.

In opposition to having cytotoxic effects, ROS have also been associated with cardioprotection signaling in ischaemic preconditioning. The recent results reported by Penna et al. [35] in which the ROS scavenger N-acetyl-cysteine (NAC) given after a postconditioning period (5 cycles of 10s alternating ischaemia and reperfusion in isolated rat hearts) did not block postconditioning, whereas NAC application just before postconditioning or only during the postconditioning period blocked the infarct size reduction of postconditioning, suggests that ROS may be involved as a signal in triggering cardioprotection. The fact that the ex vivo model excluded some significant sources of ROS, notably neutrophils and activated endothelium, may have limited the generation of ROS to lower levels than observed in vivo. The quandary of whether ROS reduction or generation is the operative mechanism in postconditioning has not been resolved. It is possible that both events are taking place, consistent with the prevailing data, and that the cytotoxic “burst” is attenuated and prevented from overwhelming the cytoprotective signaling function of ROS.

3.1.2. Induction of pro-inflammatory cytokines

Zhao et al. [10] and Halkos et al. [12] suggest that one mechanism by which postconditioning reduced reperfusion injury was by inhibiting the inflammatory response to reperfusion. However, this concept is contrasted by the observation that cardioprotection with postconditioning has been reproduced in in vitro cell culture systems[31,41], and in isolated buffer-perfused heart preparations[26,27] thereby suggesting that there is a component of protection that is independent of inflammatory cells. Although preliminary reports suggest that the pro-inflammatory cytokines tissue necrosis factor alpha (TNFα) and interleukin-6 (IL-6) are reduced by postconditioning [31], a functional role for cytokines and indeed inflammatory cells has yet to be demonstrated. In addition, while cytokines are known to recruit neutrophils to ischaemic-reperfused myocardium[42], it is not known whether there is a cause and effect link between reduced cytokines or inflammatory cells and the cardioprotection exerted by postconditioning.

3.1.3. Expression of tissue factor

Tissue factor has recently been suspected of participating in ischaemia–reperfusion injury[43,44] by thrombin-induced activation of the vascular endothelium. Thrombin stimulates surface expression of P-selectin on the vascular endothelium; P-selectin surface expression on endothelial cells is the first step in the recruitment of neutrophils to ischaemic-reperfused myocardium. In addition, thrombin may directly contribute to cell death, possibly by mechanisms associated with calcium overload[45]. In a preliminary report by Jiang et al. [46] using a closed-chest porcine model of 75 min LAD occlusion (angioplasty balloon catheter) and 3 h of reperfusion, postconditioning achieved by the 30s reperfusion and 30s re-occlusion significantly reduced infarct size. Tissue factor protein expression and thrombin activity in the area at risk myocardium were reduced in postconditioned hearts compared to abruptly reperfused controls. In addition, P-selectin immunoreactivity in vascular endothelium was markedly reduced in postconditioned hearts, consistent with previous observations in open-chest models[10]. Hence, downregulation of the tissue factor-thrombin pathway and associated inhibition of local inflammatory responses to ischaemia–reperfusion may be a newly identified mechanism of cardioprotection by postconditioning.

3.1.4. The role of endogenous adenosine

The cardioprotective role of adenosine in ischaemia and reperfusion via receptor-mediated mechanisms has been reviewed by Gross and Gross in this focused issue (pages 212–221), and will not be discussed in detail here. Kin et al. [14] hypothesised that postconditioning delayed the washout of endogenously released adenosine, and that adenosine receptor activation was involved in postconditioning’s cardioprotection. In a murine model of global ischaemia–reperfusion, Kin et al. [14] showed that postconditioning indeed delayed the washout of endogenously released adenosine and other purine intermediates during the early minutes of reperfusion [14]. The infarct size reduction of postconditioning was blocked by the non-selective adenosine receptor antagonist, 8-$\mu$-sulfophenyl theophylline, and by A$_{2A}$ and A$_{3}$ selective antagonists given before post-conditioning, but not by A$_{1}$ receptor antagonist. From previous studies, activation of the A$_{2A}$ and A$_{3}$ receptors has been associated with infarct size reduction. Activation of these receptor subtypes has been associated with attenuation of endothelial activation, and neutrophil activation and adherence[47–50], which are consistent with the in vivo changes observed after postconditioning. Involvement of...
endogenous adenosine in protection by postconditioning was further demonstrated in studies using an in situ rabbit model of ischaemia and reperfusion [16].

Adenosine has also been implicated in the cardioprotection exerted by “remote” postconditioning [51]. In this concept, coronary artery reperfusion after “index” myocardial ischaemia was preceded by a 5 min occlusion of one renal artery. The renal artery occlusion was released 1 min before release of the coronary artery occlusion. Infarct size was significantly reduced by this remote postconditioning. The infarct sparing effect was abrogated by the non-subtype selective adenosine receptor blocker 8-β-sulphophenyl theophylline (8-SPT) given only 5 min before release of the coronary artery occlusion. Permanent renal artery occlusion did not reduce myocardial infarct size. However, a delay in the renal artery occlusion–reperfusion by 1 min did not reduce infarct size. Hence, there may be a soluble factor such as adenosine released upon renal reperfusion that is transmitted directly through the circulation or via neural pathways that can protect the reperfused myocardium from reperfusion injury.

3.1.5. The role of endogenous opioids

Opioids have been reported to be involved in triggering preconditioning protection [52,53] as discussed by Gross and Gross in this focused issue (pages 212–221). Kin et al. [37] recently reported in abstract form that inhibition of opioid receptors with the non-selective opioid receptor antagonist naloxone or the peripherally retained opioid receptor antagonist naloxone methidehine, both administered 5 min before reperfusion in the absence or presence of postconditioning, reversed the infarct sparing effect of postconditioning in the in vivo rat model. Furthermore, inhibition of specifically the κ receptor subtype or the δ receptor subtype reversed the infarct sparing effects of postconditioning. It is not known, however, whether postconditioning augments the release of endogenous opioids themselves or enhances receptor binding characteristics. The activation of protein kinase C and opening of mitochondrial KATP channels after postconditioning (see below) would be consistent with the involvement of endogenous opioids.

3.1.6. The role of endogenous nitric oxide (NO)

Nitric oxide (NO) interacts in a number of biochemical reactions relevant to events occurring during reperfusion [54]. It reacts with and neutralizes superoxide anions at diffusion-limited rates, but a product of this bi-radical quenching is the potentially cytotoxic oxidant peroxynitrite. The temporal coincidence of the “respiratory burst” of superoxide anions with endogenous production of NO by coronary vascular endothelium during early reperfusion [55] may generate significant local concentrations of peroxynitrite [55]. While normal endothelium releases NO, the generation of NO by the coronary vascular endothelium is impaired after reperfusion [56,57]. The endothelial injury observed soon after onset of reflow may be, in part, related to impaired endogenous production of NO. On the other hand, NO attenuates neutrophil events, and has been shown to reduce infarct size and post-ischaemic coronary vascular endothelial injury when given at reperfusion [58,59]. Therefore, like ROS, NO may have a dual role in ischaemia–reperfusion.

As reviewed in this focused issue by Cohen, Yang and Downey (pages 231–239), NO also acts as an intracellular signaling molecule implicated in the cardioprotective effects of preconditioning. Yang et al. [15] were the first to show that NO is involved in the cardioprotection of postconditioning. In the in situ rabbit heart model of coronary artery occlusion–reperfusion, the nitric oxide synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) infused just before the onset of reperfusion reversed the infarct sparing effects of postconditioning. The involvement of NO via the cGMP pathway was reported by Pagliaro et al. [32] and again by Yang et al. [27]. NO could ostensibly be functioning in any of the levels (signaling, anti-inflammatory) discussed above, and its role in ischaemia–reperfusion may involve all levels of activity. Exactly which of these functions postconditioning harnesses has yet to be determined. However, the report by Pagliaro et al. [32] strongly suggest that an anti-inflammatory (i.e. inhibition of neutrophils) mechanism is not the sole mechanism of NO-mediated protection, and that signaling through the KATP channel activation and inhibition of mitochondrial permeability transition pore (mPTP) opening may be likely actions of NO after postconditioning. However, the involvement of NO in KATP activation and closure of the mPTP has yet to be determined.

3.2. Mediators involved in postconditioning

3.2.1. Activation of intracellular protein kinase C

Protein kinase C (PKC) is a key enzyme in the signal transduction of preconditioning as discussed by Inagaki, Churchill and Mochly–Rosen in this focused issue (pages 222–230). In contrast to preconditioning, little has been reported on the role of PKC and its isoforms in postconditioning. Zatta et al. report in this focused issue (pages 315–324) that the PKC inhibitor chelerythrine administered 5 min before reperfusion in the in vivo rat model of ischaemia–reperfusion blocks the infarct reduction of postconditioning. In addition, blockade of PKCε reversed the infarct-sparing effects of postconditioning. Western blot analysis of PKCε and PKCδ shows that postconditioning increases the cardioprotective [60] isoform (PKCε) while the PKCδ isoform, shown to be deleterious in ischaemia–reperfusion, [61] was decreased. These results are confirmed by Penna et al. [35] who showed that chelerythrine administered either just before or after the postconditioning algorithm abrogated the infarct reduction of postconditioning.

3.2.2. Activation of survival and death kinases

The role of survival kinases in ischaemia–reperfusion has been reviewed in detail by Hausenloy and Yellon in this
focused issue (pages 240–253) and will not be repeated here. The involvement of survival and death kinases in postconditioning is currently being investigated by several groups. In isolated buffer-perfused rat [17] and rabbit hearts, [27] the reduction in infarct size with postconditioning was associated with activation of the pro-survival PI3K-Akt pathway [16,27]. Tsang et al. [17] reported that administration of the PI-3-K inhibitors LY294002 or wortmannin during the first 15 min of reperfusion in isolated perfused rat hearts eliminated postconditioning-enhanced expression of phospho-Akt levels and activation of the downstream targets e-NOS and p70s6K in area at risk myocardium, demonstrating a cardioprotective role for the PI-3-K-Akt pathway in postconditioning. A study by Yang et al. [27] in isolated perfused rabbit hearts confirmed the involvement of the PI-3-K pathway. However, in the in situ rabbit heart model of regional ischaemia–reperfusion, Yang et al. [15] reported that activation of the mitogen-activated protein/extracellular signal regulating kinase MEK/ERK 1/2 pathway was also involved in infarct size reduction with postconditioning since infarct size reduction with postconditioning was abrogated by the MEK 1/2 inhibitor PD98059. On the other hand, Darling et al. [26] using isolated perfused rabbit hearts with regional ischaemia–reperfusion reported no attenuation of the infarct sparing effect of postconditioning with a PI3-kinase inhibitors (LY294002). However, they observed complete abrogation of infarct sparing with the ERK1/2 antagonist PD98059 administered just before reperfusion and postconditioning. These data were consistent with increased p-ERK and no change in p-Akt in the subepicardial (assumed non-infarcted) area at risk myocardium compared with full reperfusion controls [26]. The reasons underlying these seemingly disparate observations are not clear, but may be related to differences in methodologies and sampling sites (transmural [17] versus subepicardial [26] samples). Nevertheless, the involvement of ERK1/2 in postconditioning, but not in preconditioning [15] may be a feature that distinguishes the two cardioprotective maneuvers.

Evidence from in vitro and in vivo models has shown that death kinases such as the p38 and the c-jun amino-terminal kinases (JNK-1 and 2)/stress-activated protein kinase (SAPK) linked to myocardial injury after ischaemia and reperfusion are also activated in response to stimuli present at reperfusion such as inflammatory cytokines and oxidants [62–64]. There is little information on the modulation of death kinases in postconditioning. In a preliminary report by Zhao et al. [31] using isolated neonatal rat cardiomyocytes, intermittent reoxygenation and hypoxia (“hypoxic postconditioning”) inhibited the expression of p-38 and JNK mitogen-activated protein kinases. This reduction in death kinases was associated with reduced TNFα generation in the culture medium, but a functional link was not demonstrated. Taken together, the above data provide evidence that modulation of death and survival kinases during the early moments of reperfusion may be involved in the cardioprotection of postconditioning. But how postconditioning alters the stimulators of the kinases, or whether the balance of these death-survival kinases is important in protection remain to be clarified.

3.2.3. Reduction in intracellular Ca$^{2+}$ overload

It is well-known that accumulation of intracellular calcium (Ca$^{2+}$) is lethal to cardiomyocytes [65,66]. The consequence of excessive accumulation of intracellular Ca$^{2+}$ during the early reperfusion phase leads to numerous secondary effects, including stimulation of contractile “rigor” [67,68], mitochondrial dysfunction, over-stimulation of (Ca$^{2+}$)-dependent enzymes, and opening of the mitochondrial permeability transition pore [69]. In neonatal rat cardiomyocytes subjected to 3 h hypoxia and 6 h of reoxygenation, “hypoxic postconditioning” with alternating exposure to 3 cycles of 5 min hypoxic and normoxic conditions preceding reoxygenation reduced intracellular and mitochondrial Ca$^{2+}$ loading compared to non-postconditioned cardiomyocytes. This was associated with a reduction in cardiomyocyte death assessed by propidium iodide and lactate dehydrogenase [41]. However, the signaling pathways and physiological consequences of this lower intracellular Ca$^{2+}$ by postconditioning are not known at present, especially in vivo.

3.2.4. Opening of K$_{ATP}$ channels

K$_{ATP}$ channels, particularly the mitochondrial K$_{ATP}$ channels, are reported to be involved in cardioprotection, [70–73] especially preconditioning [74,75]. The possible link between K$_{ATP}$ channel activation and the mitochondrial permeability transition pore suggests that mitochondrial K$_{ATP}$ channels may be considered more of a mediator than its previous role as an end effector. This position as mediator is reflected in Table 1. Yang et al. [15] in an in situ rabbit model of coronary artery occlusion and reperfusion reported that infarct size reduction by postconditioning was dependent on opening of K$_{ATP}$ channels [15]. The non-selective K$_{ATP}$ channel inhibitor, glibenclamide or the selective mitochondrial (mito)-K$_{ATP}$ channel inhibitor, 5-HD, abrogated the infarct sparing effect of postconditioning [15]. In a preliminary study from our laboratory [19] using a canine model of 60 min of canine coronary artery occlusion and 24 h of reperfusion, the infarct reduction by postconditioning was blocked by 5-HD, but not by HMR1908, both administered 5 min before the onset of reperfusion, suggesting that protection involves specific activation of the mito-K$_{ATP}$ channels.

3.3. Effect of postconditioning on end-effectors involved in reperfusion injury

3.3.1. Inhibition of the mitochondrial permeability transition pore (mPTP)

The “end effectors” involved in preconditioning cardioprotection are reviewed in this focused issue by Garcia-Dorado et al. (pages 274–285). Opening of the mitochon-
Mitochondrial permeability transition pore (mPTP) is considered a key event in cell death after ischaemia–reperfusion [76,77]. The mPTP remains in a largely closed state during ischaemia, but then increases its open probability during early reperfusion [78], possibly triggered by the increase in oxidant generation and intracellular calcium accumulation that occurs coincidentally during early reperfusion. The mitochondrial transition pore as a target in postconditioning is described by Gateau-Roesch, Argaud and Ovize in this focused issue as well (pages 264–273). Argaud et al. [20] reported that postconditioning reduced calcium-induced opening of the mPTP in mitochondria isolated from the area at risk myocardium. Postconditioning was also associated with a reduction in infarct size after both acute and long-term (72h) reperfusion. Bopassa et al. [79] demonstrated in isolated perfused rat hearts that maintenance of mPTP closure was associated with PI3-K activation, which is consistent with the activation of survival kinase pathways described above, but the functional involvement of these pathways and regulation of the mPTP in vivo is not yet clear.

4. Pharmacological intervention: can the cardioprotective effects of postconditioning be mimicked or enhanced?

One goal in applying reperfusion therapeutics is to pharmacologically mimic postconditioning. In addition, no one drug or maneuver can target all mechanisms of reperfusion injury, but combination therapy integrating pharmacological agents and mechanical maneuvers such as postconditioning may provide a broader spectrum approach to attenuate reperfusion injury. In the broader sense, studies in which pharmacological agents have been transiently applied at the onset of reperfusion may have, in fact, pharmacologically induced a “postconditioned state” [80–82], with the important caveat that the pulsatile hydrodynamic conditions of postconditioning were not reproduced by drug intervention. Inhalational anaesthetics such as isoflurane [83] and sevoflurane [84] given just before the onset of reperfusion reduce infarct size in a postconditioning-like manner, termed “anaesthetic postconditioning”. Isoflurane administered only at the onset of reperfusion decreased infarct size in association with attenuation of mPTP opening and through a glycogen synthase kinase 3β (GSK3β) mechanism [85]. The infarct size reduction of sevoflurane was shown by Obal et al. [84] to be mediated, in part, by activation of K_ATP channels. Recently, Chiari et al. [18] reported in in vivo rabbit hearts subjected to 30min of coronary artery occlusion and 3h of reperfusion that the administration of the inhalational anaesthetic isoflurane at 1.0 minimal alveolar concentration (MAC) in short (10 or 20s) cycles (mimicking the pulsatile nature of postconditioning) reduced infarct size to levels achieved by the effective 20s cycles of mechanical postconditioning. Infarct reduction by either isoflurane or mechanical postconditioning was abrogated by wortmannin, suggesting a PI-3-kinase-Akt related mechanism. Protection was not observed with either a 10-s algorithm of mechanical postconditioning or 0.5 MAC isoflurane. However, if the two ineffective treatments were combined, infarct reduction was again observed, suggesting that isoflurane could “lower the threshold” of cardioprotection by postconditioning [18]. Wortmannin blocked the infarct size reduction of the isoflurane-postconditioning combination. In a preliminary study [86], we found that administration of sodium/hydrogen exchanger inhibitor, cariporide at a dose of 5mg/kg at the onset of reperfusion in sequence with postconditioning in the in vivo rat model reduced infarct size relative to each procedure alone. Therefore, postconditioning may be enhanced by pharmacological agents, or may reduce the effective dose of a cardioprotective drug.

5. Postconditioning the human heart

The ultimate validation and utility of a cardioprotective therapy is its application to humans presenting with coronary artery disease and concomitant risk factors, differing presenting ages, genders and various levels of general health. Two studies have recently reported that conventional postconditioning is an effective treatment in a select patient population with coronary artery disease. In a study by Laskey [87], 17 patients undergoing percutaneous coronary intervention were ultimately enrolled to receive standard of care angioplasty involving 90s of uninterrupted balloon inflation without further treatment (n=7) or repeated balloon inflation (“conditioning”, n=10) of 90s’ duration applied 3–5min after the angioplasty inflation. “Conditioning” after angioplasty reduced the magnitude of ST-segment elevation compared to Controls, and accelerated the rate at which ST-segment elevation normalized after reperfusion. Furthermore, blood flow velocity reserve was significantly improved in “conditioned” hearts. Staat et al. [9] reported a multi-center randomised clinical trial of 37 patients with total coronary artery occlusion undergoing angioplasty/stenting. Patients that achieved a TIMI flow grade of 2–3 at completion of the angioplasty/stent procedure were randomised to receive either standard of care treatment thereafter (no further mechanical intervention) or postconditioning with 4 cycles of 1-min re-inflation followed by 1min deflation of the angioplasty balloon. Infarct size (area under the creatine kinase curve) was significantly less, and the coronary blood flow achieved was greater in the postconditioned patients. There were no adverse events in the postconditioned patients. Together, these two studies [9,87] suggest that postconditioning represents a safe and efficient cardioprotective intervention for treatment of reperfusion injury in patients with ischaemic heart disease. These data must be reproduced by other clinical trials, and in broader patient populations,
postconditioning may involve parallel, up-stream or downstream effects on inflammatory responses, protein kinases, K_ATP channels and the mitochondrial permeability transition pore (mPTP). Preservation of endogenous factors such as adenosine, nitric oxide and opioids by postconditioning may trigger receptor-mediated downstream mechanisms. Inhibition of the generation of reactive oxygen species (free radicals), release of cytokines and inhibition of other pro-inflammatory stimuli such as tissue factor by postconditioning may reduce inflammatory and oxidant responses that may or may not involve neutrophil–endothelial cell interactions. Dual (stimulatory and inhibitory) effects of postconditioning on protein kinases cause activation of PI-3K/Akt, ERK1/2, and inhibition of p38 and JNK, that may or may not involve neutrophil–endothelial cell interactions. Inhibition of the mechanical event of postconditioning engages conventional endogenous “triggers” of protection such as adenosine and opioids that not only transduce their cardioprotective effects via signal transduction pathways (PKC), but also elicit protection from signaling molecules (NO). Finally, by preserving other anatomical and cellular structures (e.g. endothelium), postconditioning may enhance the myocardium’s endogenous cardioprotective mechanisms. Hence, the pluripotent mechanisms of postconditioning more appropriately address the multiple mechanisms involved in reperfusion injury.

Future studies are needed to determine whether postconditioning reverses other aspects of post-ischaemic injury such as contractile dysfunction in “stunned” myocardium. In addition, pharmacological agents may be applied to mimic or enhance cardioprotection in a combinational strategic approach to reducing post-ischaemic injury. Furthermore, catheters and devices will be developed to facilitate the application of postconditioning in patients, and thus extend the armamentarium of reperfusion therapeutics beyond the standard of care and facilitated PCI [90] available today. Lastly, conventional or pharmacological postconditioning may be applied directly to other organs, indirectly via other “remote” organs (remote postconditioning) and in other procedures such as organ transplantation.

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