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## The Late Phase of Preconditioning Roberto Bolli

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This Review is part of a thematic series on **Preconditioning**, which includes the following articles:

Ischemic Preconditioning in Isolated Cells

The Preconditioning Phenomenon: A Tool for the Scientist or a Clinical Reality?

Myocardial K<sub>ATP</sub> Channels in Preconditioning

The Late Phase of Preconditioning

*Roberto Bolli, Guest Editor*

## The Late Phase of Preconditioning

Roberto Bolli

**Abstract**—Unlike the early phase of preconditioning (PC), which lasts 2 to 3 hours and protects against infarction but not against stunning, the late phase of PC lasts 3 to 4 days and protects against both infarction and stunning, suggesting that it may have greater clinical relevance. It is now clear that late PC is a polygenic phenomenon that requires the simultaneous activation of multiple stress-responsive genes. Chemical signals released by a sublethal ischemic stress (such as NO, reactive oxygen species, and adenosine) trigger a complex cascade of signaling events that includes the activation of protein kinase C, Src protein tyrosine kinases, and nuclear factor  $\kappa$ B and culminates in increased synthesis of inducible NO synthase, cyclooxygenase-2, aldose reductase, Mn superoxide dismutase, and probably other cardioprotective proteins. An analogous sequence of events can be triggered by a variety of stimuli, such as heat stress, exercise, and cytokines. Thus, late PC appears to be a universal response of the heart to stress in general. Importantly, the cardioprotective effects of late PC can be reproduced pharmacologically with clinically relevant agents (eg, NO donors, adenosine receptor agonists, endotoxin derivatives, or opioid receptor agonists), suggesting that this phenomenon might be exploited for therapeutic purposes. The purpose of this review is to summarize current information regarding the pathophysiology and mechanism of late PC. (*Circ Res.* 2000;87:972-983.)

**Key Words:** myocardial ischemia ■ myocardial reperfusion ■ nitric oxide ■ cyclooxygenase  
■ aldose reductase

The heart possesses a remarkable ability to adapt to stress by changing its phenotype in a manner that makes it more resistant to injury. The power of this phenotypic plasticity is perhaps illustrated most eloquently by the phenomenon of ischemic preconditioning (PC), the process whereby a sublethal ischemic stress enhances the tolerance of the myocardium to a subsequent ischemic stress. Originally described as an immediate adaptation of the heart to brief coronary occlusions,<sup>1</sup> ischemic PC was subsequently found to be a biphasic phenomenon, with an early phase of protection that develops within minutes from the initial ischemic insult and lasts 2 to 3 hours and a late (or delayed) phase that becomes apparent 12 to 24 hours later and lasts 3 to 4 days.<sup>2,3</sup> Unlike the early phase, the late phase of ischemic PC protects

not only against myocardial infarction but also against myocardial stunning.<sup>4</sup> Because of this, and because of its sustained duration, considerable interest has recently focused on the late phase and on its clinical relevance. If the mechanism of this adaptive metamorphosis is elucidated, then it should be possible to exploit it to protect the ischemic myocardium in patients.

The recognition that the heart shifts to a preconditioned phenotype upon exposure to stress has undoubtedly been one of the major advances in the field of myocardial ischemia. In the past few years, much has been learned regarding the intricate signaling pathways and genetic changes that underlie this defensive adaptation. The purpose of the present essay is to succinctly summarize current information regarding the

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From the Division of Cardiology, University of Louisville and Jewish Hospital Heart and Lung Institute, Louisville, Ky.

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Correspondence to Roberto Bolli, MD, Division of Cardiology, University of Louisville, Louisville, KY 40292. E-mail [rbolli@louisville.edu](mailto:rbolli@louisville.edu)

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TABLE 1. Classification of Late PC

Stimulus	Protection Against			
	Stunning	Arrhythmias	Infarction	Endothelial Dysfunction
Nonpharmacological PC				
Ischemia	+	?	+	+
Heat stress	?	?	+	?
Rapid pacing	?	+	?	?
Exercise	?	?	+	?
Pharmacological PC				
Endotoxin	?	?	+	?
Cytokines	?	?	+	?
ROS-generating solutions	+	?	?	?
NO donors	+	?	+	?
Adenosine receptor agonists	—	?	+	?
MLA and analogs	+	?	+	?
Opioid agonists	?	?	+	?

pathophysiology and mechanism of late PC. Although this review will focus solely on the heart, it should be noted that late PC has also been observed in other organs (eg, brain, intestine, and liver), suggesting that this is a universal mechanism whereby tissues protect themselves from an impending threat.

### Classification of Late PC

Late PC can be categorized on the basis of the stimulus that elicits this response and of the end point examined (Table 1). Delayed protection against myocardial ischemia/reperfusion injury can be induced by a wide variety of stimuli, which can be broadly classified as nonpharmacological and pharmacological. Besides ischemia, the former include heat stress,<sup>5</sup> rapid ventricular pacing,<sup>6</sup> and exercise.<sup>7</sup> The latter consist of naturally occurring—and often noxious—agents (eg, endotoxin,<sup>8</sup> interleukin-1,<sup>9</sup> tumor necrosis factor- $\alpha$  [TNF- $\alpha$ ],<sup>10</sup> TNF- $\beta$ ,<sup>11</sup> leukemia inhibitory factor,<sup>11</sup> reactive oxygen species [ROS]<sup>12</sup>) and of clinically applicable drugs (NO-releasing agents,<sup>13</sup> adenosine receptor agonists,<sup>14</sup> endotoxin derivatives such as monophosphoryl lipid A [MLA]<sup>15</sup> and its analog RC-552,<sup>16</sup> and opioid receptor agonists<sup>17</sup>). Most of these forms of late PC have been shown to protect against lethal ischemia/reperfusion injury (infarction), and at least some have been demonstrated to protect against reversible postischemic dysfunction (stunning),<sup>18</sup> arrhythmias,<sup>6</sup> and endothelial dysfunction<sup>19</sup> (Table 1). This classification is not purely phenomenological, because recent evidence suggests the existence of mechanistic differences among the various forms of late PC outlined in Table 1 (eg, adenosine and ATP-sensitive potassium [K<sub>ATP</sub>] channels play an obligatory role in ischemia-induced late PC against infarction<sup>14,20–26</sup> but not against stunning<sup>18,24,26,27</sup>). Therefore, to avoid inappropriate generalizations, it is important to define (using the nature of the stimulus and of the protective effect) which specific form of late PC is being examined (eg, NO donor-induced

late PC against stunning; ischemia-induced late PC against infarction, etc) (Table 1).

### Components of the Mechanism of Late PC

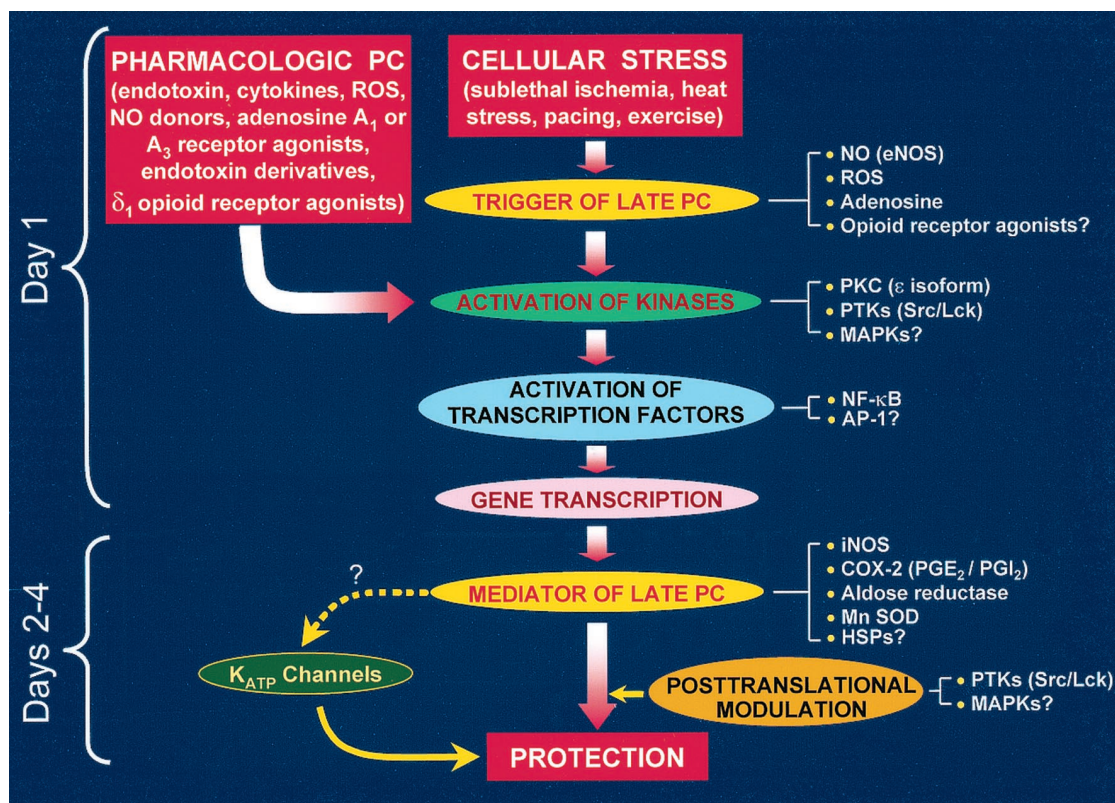
Ischemia-induced late PC is the result of a complex cascade of cellular events that represents an archetypical response of the heart to diverse stressful stimuli (Figure). Conceptually, it is useful to subdivide this cascade into 3 major components: (1) the molecular species that are generated during the first ischemic challenge and are responsible for initiating the adaptation (“triggers” of late PC), (2) the molecular species that are expressed in the heart 24 to 72 hours later and are responsible for conferring protection during the second ischemic challenge (“mediators” of late PC), and (3) the signaling pathways that are activated by the triggers and culminate in the expression of the mediators (Figure). It should be noted that both triggers and signaling pathways operate soon after the PC stimulus; the distinction between them is hierarchical, with the former being upstream of the latter (Figure).

### Triggers (or Initiators) of Late PC

Brief myocardial ischemia and the ensuing reperfusion are associated with major metabolic perturbations that result in the generation of a wide variety of metabolites and ligands. Among these, there is evidence that adenosine, NO, ROS, and perhaps opioid receptor agonists serve as chemical signals that trigger the development of the late phase of ischemic PC (Figure). These substances warn the myocardium that a danger is imminent, essentially acting as a cellular “alarm system” to which the heart responds by switching to a defensive phenotype.

### Adenosine

The concept that adenosine released during the PC stimulus triggers the development of delayed protection was first proposed by Baxter et al<sup>14</sup> and subsequently expanded by the



Schematic representation of the cellular mechanisms underlying late PC. A nonlethal cellular stress (eg, reversible ischemia, heat stress, ventricular pacing, or exercise) causes release of chemical signals (NO, ROS, adenosine, and possibly opioid receptor agonists) that serve as triggers for the development of late PC. These substances activate a complex signal transduction cascade that includes PKC (specifically, the  $\epsilon$  isoform), PTKs (specifically, Src and/or Lck), and probably other as-yet-unknown kinases. A similar activation of PKC and downstream kinases can be elicited pharmacologically by a wide variety of agents, including naturally occurring—and often noxious—substances (eg, endotoxin, interleukin-1, TNF- $\alpha$ , TNF- $\beta$ , leukemia inhibitor factor, or ROS), as well as clinically applicable drugs (NO donors, adenosine A<sub>1</sub> or A<sub>3</sub> receptor agonists, endotoxin derivatives, or  $\delta_1$ -opioid receptor agonists). The recruitment of PKC and distal kinases leads to activation of NF- $\kappa$ B and almost certainly other transcription factors, resulting in increased transcription of multiple cardioprotective genes and synthesis of multiple cardioprotective proteins that serve as co-mediators of protection 2 to 4 days after the PC stimulus. The mediators of late PC identified thus far include iNOS, COX-2, aldose reductase, and Mn SOD. Among the products of COX-2, PGE<sub>2</sub> and/or PGL<sub>2</sub> appear to be the most likely effectors of COX-2-dependent protection. Increased synthesis of HSPs is unlikely to be a mechanism of late PC, although the role of posttranslational modification of preexisting HSPs remains to be determined. In addition, the occurrence of cardioprotection on days 2 to 4 requires the activity of PTKs and possibly p38 MAPKs, potentially because iNOS and other mediators need to undergo posttranslational modulation to confer protection against ischemia. Opening of K<sub>ATP</sub> channels is also essential for the protection against infarction (but not against stunning) to become manifest. The exact interrelationships among iNOS, COX-2, aldose reductase, Mn SOD, and K<sub>ATP</sub> channels are unknown, although recent evidence suggests that COX-2 may be downstream of iNOS (ie, COX-2 is activated by NO).

same group<sup>21,23</sup> and others.<sup>24,28</sup> Unlike NO, however, which is necessary for the development of ischemia-induced delayed protection against both myocardial stunning<sup>29</sup> and myocardial infarction<sup>30</sup> and is also sufficient to trigger both of these forms of adaptation,<sup>13</sup> activation of adenosine receptors is neither necessary nor sufficient to trigger late PC against stunning.<sup>18,24,27</sup> The differential role of adenosine receptors in the genesis of late PC against infarction vis-à-vis late PC against stunning is but one of several pieces of evidence suggesting the existence of important differences in the mechanisms underlying these 2 phenomena (see above).

The identity of the adenosine receptor subtype(s) responsible for triggering late PC is under investigation. Because 2-chloro-N<sup>6</sup>-cyclopentyladenosine (CCPA) is highly selective for A<sub>1</sub> receptors and because CCPA-induced late PC is blocked by selective A<sub>1</sub> receptor antagonists,<sup>28</sup> it seems likely that this agonist elicits delayed cardioprotection by activating

A<sub>1</sub> receptors. Recent studies indicate that delayed protection against infarction can also be triggered by selective activation of adenosine A<sub>3</sub> receptors.<sup>28</sup> Thus, it appears that pharmacological stimulation of either A<sub>1</sub> or A<sub>3</sub> receptors can elicit late PC against infarction. Whether only one or both of these adenosine receptor subtypes contributes to triggering ischemia-induced late PC is unknown, because 8-*p*-sulfophenyl theophylline (the only adenosine receptor antagonist shown to block the development of late PC after an ischemic stress)<sup>14</sup> is not selective for A<sub>1</sub> versus A<sub>3</sub> receptors.

### Nitric Oxide

The first indication that NO triggers late PC was provided by a study in which administration of N<sup>ω</sup>-nitro-L-arginine (L-NA), a nonselective inhibitor of all 3 NO synthase (NOS) isoforms (neuronal [nNOS], endothelial [eNOS], and inducible [iNOS]), before the PC ischemic stimulus was found to



block the development of delayed protection against myocardial stunning,<sup>29</sup> demonstrating that NO generation during the initial PC ischemia is necessary to trigger this cardioprotective phenomenon. A subsequent study demonstrated that NO is also necessary to trigger ischemia-induced late PC against myocardial infarction.<sup>30</sup> Importantly, exposure to exogenous NO is sufficient to reproduce late PC, since pretreatment with NO donors in the absence of ischemia induces a delayed protective effect against both myocardial stunning and infarction that is indistinguishable from that observed during the late phase of ischemic PC.<sup>13,31–33</sup> Administration of nitroglycerin can elicit late PC both by the intravenous<sup>31,33</sup> and the transdermal<sup>33</sup> route; this salubrious effect is not abrogated by the development of nitrate tolerance, indicating that different mechanisms underlie the hemodynamic and PC actions of nitrates.<sup>33</sup> The ability of NO-releasing agents such as nitrates to faithfully mimic the late phase of ischemic PC despite nitrate tolerance supports the possibility of novel clinical applications of these drugs.

Recent studies<sup>34</sup> have provided direct evidence of enhanced biosynthesis of NO in myocardium subjected to brief episodes of ischemia/reperfusion. The source of increased NO formation during the PC ischemia is likely to be eNOS, since the development of late PC is blocked by pretreatment with the nonselective NOS inhibitor L-NA, but not with the relatively selective iNOS inhibitors aminoguanidine and *S*-methylisothiourea.<sup>35</sup> Interestingly, the development of late PC is not affected by pretreatment with the guanylate cyclase inhibitor ODQ<sup>36</sup> but is completely prevented by pretreatment with the antioxidant mercaptopropionyl glycine (MPG).<sup>13,37</sup> NO is known to react rapidly with  $\text{O}_2^-$  to form the peroxy-nitrite anion ( $\text{ONOO}^-$ ), which then protonates and decomposes to generate the hydroxyl radical ( $\text{OH}^\bullet$ ) or some other potent oxidant with similar reactivity.<sup>38,39</sup> Because MPG scavenges both  $\text{ONOO}^-$  and  $\text{OH}^\bullet$ ,<sup>39,40</sup> the ability of MPG to block late PC,<sup>13,37</sup> coupled with the failure of ODQ to do so,<sup>36</sup> suggests that NO triggers this response via formation of  $\text{ONOO}^-$  and/or secondary ROS, rather than via cGMP-dependent pathways.

### Reactive Oxygen Species

The concept that the generation of ROS during the PC ischemia is essential to trigger delayed protection was first proposed by Sun et al.<sup>12</sup> These investigators demonstrated in conscious pigs that the administration of a combination of antioxidants (superoxide dismutase [SOD] plus catalase plus MPG) during the initial ischemic challenge prevented the development of late PC against stunning. Similar findings were obtained in rabbits with MPG alone.<sup>37</sup> MPG has also been found to prevent ischemia-induced late PC against infarction,<sup>41</sup> arrhythmias,<sup>41</sup> and coronary endothelial injury<sup>19</sup> as well as heat stress-induced<sup>42</sup> and exercise-induced<sup>7</sup> late PC against infarction, thus implicating ROS as initiators of these forms of delayed protection as well. Conversely, intracoronary infusion of an ROS-generating solution in rabbits elicits a late-PC response.<sup>43</sup> Taken together, these results<sup>12,19,37,41,42</sup> suggest that sublethal oxidative stress plays a useful role by triggering delayed cardioprotection. Further studies will be necessary to determine the source(s) and the identity of the

ROS responsible for initiating late PC and whether NO and ROS are parts of the same mechanism (ie, whether ROS are derived from the reaction of NO with  $\text{O}_2^-$ , as discussed above) or act in parallel as 2 independent triggers.

### Opioids

Recent data in rats<sup>17</sup> and mice<sup>44</sup> indicate that pharmacological activation of  $\delta_1$ -opioid receptors induces a delayed infarct-sparing effect 24 to 48 hours later. Whether  $\delta_1$ -opioid receptors are also involved in triggering the late phase of ischemia-induced PC is currently unknown.

### Mediators (or Effectors) of Late PC

Ischemic PC causes an increase in the rate of myocardial protein synthesis; if this increase is blocked with cycloheximide, the development of delayed protection is also blocked.<sup>45</sup> Thus, unlike early PC, late PC requires increased synthesis of new proteins, not simply activation of preexisting proteins.<sup>45</sup> The time course of the enhanced tolerance to ischemia, which requires 12 to 24 hours to develop and lasts for 3 to 4 days,<sup>46,47</sup> is also consistent with the synthesis and degradation of cardioprotective proteins. Several proteins have been proposed as possible mediators (or effectors) of the protection afforded by late PC, including NOS, cyclooxygenase-2 (COX-2), aldose reductase, antioxidant enzymes (particularly Mn SOD), and heat stress proteins (HSPs). In addition, considerable evidence implicates  $\text{K}_{\text{ATP}}$  channels as mediators of this defensive phenotype.

### Nitric Oxide Synthase

The first demonstration that the cardioprotective effects of the late phase of ischemic PC are mediated by NOS was provided by 2 studies in conscious rabbits, in which the delayed protection against both myocardial stunning<sup>35</sup> and myocardial infarction<sup>48</sup> was found to be completely abrogated when preconditioned animals were given L-NA 24 hours after ischemic PC, just before the second ischemic challenge. The same effects were observed with the relatively selective iNOS inhibitors aminoguanidine and *S*-methylisothiourea, implicating iNOS as the specific NOS isoform involved in mediating the protective effects of late PC.<sup>35,48</sup> These results were subsequently confirmed by others.<sup>49</sup> Because of the limited selectivity and possible nonspecific effects of iNOS inhibitors, however, conclusive identification of the NOS isoform responsible for enhancing tolerance to ischemia during late PC cannot be attained pharmacologically. Using an in vivo murine model of myocardial infarction, Guo et al<sup>50</sup> were the first to demonstrate that the late phase of ischemic PC is associated with upregulation of myocardial iNOS (whereas eNOS remains unchanged) and that targeted disruption of the *iNOS* gene completely abrogates the delayed infarct-sparing effect, providing unequivocal molecular genetic evidence for an obligatory role of iNOS in the cardioprotection afforded by the late phase of ischemic PC. Immunohistochemical and in situ hybridization studies have identified cardiac myocytes as the specific cell type that expresses iNOS during late PC.<sup>51</sup>

Thus, NO appears to play a dual role in the pathophysiology of the late phase of ischemic PC, acting initially as the trigger<sup>13,29–33</sup> and subsequently as the mediator<sup>35,48–51</sup> of this

adaptive response ("NO hypothesis of late PC").<sup>52</sup> In support of a dual role of NO are also direct measurements of NOS activity, which have shown a biphasic regulation of NOS by ischemic PC, with an increase in calcium-dependent NOS (cNOS [eNOS and/or nNOS]) activity immediately after the PC ischemia followed by an increase in calcium-independent NOS (iNOS) activity (with no change in cNOS activity) 24 hours later.<sup>34</sup> The finding that both ischemic PC and nitroglycerin induce a rapid increase in steady-state levels of iNOS mRNA, which is abolished by administration of L-NA before the PC ischemia<sup>53</sup> (NO-dependent iNOS induction), is also congruent with this concept. Taken together, the studies reviewed above<sup>13,29–35,48–53</sup> support a complex paradigm in which 2 different NOS isoforms are sequentially involved in the pathophysiological cascade of late PC, with eNOS generating the NO that initiates the development of the PC response on day 1 and iNOS then generating the NO that protects against recurrent ischemia on day 2 (reviewed in Reference 52).

The quantitative aspects of the upregulation of iNOS after ischemic PC are noteworthy. In the Guo et al<sup>50</sup> study, the increase in cardiac iNOS protein expression was mild,  $\approx$ 18-fold less than that observed after a lethal dose of lipopolysaccharide. This supports the hypothesis<sup>50</sup> that induction of iNOS after ischemic PC is protective because it is relatively modest, in contrast to other situations (such as inflammation or septic shock) in which iNOS induction is massive and promotes tissue injury. The precise mechanism(s) whereby iNOS-derived NO protects against ischemia remains to be elucidated but appears to involve the activation of guanylate cyclase, given that both the alleviation of stunning and the reduction in infarct size are abrogated by ODQ.<sup>54</sup>

Besides ischemia-induced PC, there is now evidence that other forms of late PC are also iNOS dependent. Guo et al found that the delayed infarct-sparing effects elicited by *S*-nitroso-*N*-acetylpenicillamine and nitroglycerin,<sup>55</sup> by the selective  $\delta_1$ -opioid receptor agonist TAN-670,<sup>44</sup> and by exercise (Y. Guo and R. Bolli, unpublished observations, 2000) were completely abrogated in *iNOS*<sup>-/-</sup> mice, demonstrating that iNOS serves as an obligatory mediator of NO donor-induced,  $\delta_1$ -opioid receptor-induced, and exercise-induced late PC against infarction. Similarly, iNOS activity has been found to be necessary for the delayed infarct size limitation observed after pretreatment with endotoxin derivatives (MLA and RC-552)<sup>16,56,57</sup> and diazoxide.<sup>58</sup> Recent reports on the role of iNOS in CCPA-induced late PC have arrived at conflicting conclusions.<sup>44,59,60</sup>

### Cyclooxygenase-2

An obligatory role of COX-2 in late PC was first shown by Shinmura et al.<sup>61</sup> Using conscious rabbits, this study found that COX-2 protein expression was upregulated 24 hours after ischemic PC, concomitant with an increase in the myocardial levels of prostaglandin (PG) E<sub>2</sub>, 6-keto-PGF<sub>1 $\alpha$</sub>  (the stable metabolite of PGI<sub>2</sub>), and (to a lesser extent) PGF<sub>2 $\alpha$</sub> . Administration of 2 unrelated COX-2-selective inhibitors (NS-398 and celecoxib) 24 hours after ischemic PC abolished the increase in prostanoids and, at the same time, completely blocked the cardioprotective effects of late PC, demonstrating

that COX-2 activity is necessary for this phenomenon to occur.<sup>61</sup> Similar results were subsequently obtained in mice.<sup>62</sup> These observations identify COX-2 as a cardioprotective protein and strongly point to PGE<sub>2</sub> and/or PGI<sub>2</sub> as the likely effectors of COX-2-dependent protection. Induction of COX-2 is generally thought to be detrimental.<sup>63</sup> The finding that COX-2 mediates the antistunning and antiinfarct effects of late PC impels a reassessment of current views regarding this enzyme and supports a more complex paradigm in which COX-2 can play either a beneficial or a deleterious role depending on various factors (eg, the intensity of its induction, the pathophysiological setting, and the ability of specific cell types to metabolize PGH<sub>2</sub> produced by COX-2 into cytoprotective prostanoids).<sup>61</sup>

The recognition that iNOS and COX-2 are co-induced and serve as co-mediators of late PC logically leads to the question of whether there is an interaction between these 2 proteins or they act as independent effectors of cytoprotection. Previous studies have suggested that NO can directly activate COX-2.<sup>64</sup> In keeping with these data, we have recently observed in unpublished studies that inhibition of iNOS blocks the increased COX-2 activity associated with late PC, whereas inhibition of COX-2 has no effect on iNOS activity, suggesting that COX-2 is downstream of iNOS in the preconditioned heart.

### Aldose Reductase

Oxidative stress, osmotic stress, cytokines, and NO are known to upregulate the expression of aldose reductase, an enzyme that catalyzes not only the metabolism of glucose to sorbitol but also the detoxification of ROS-derived lipid aldehydes.<sup>65</sup> Shinmura et al<sup>66</sup> have recently found that the protein expression of aldose reductase is upregulated 24 hours after ischemic PC in conscious rabbits and that inhibition of this enzyme abrogates the infarct-sparing effects observed in untreated animals. Thus, in addition to iNOS and COX-2, aldose reductase is a third necessary mediator of the cardioprotective actions of the late phase of ischemic PC. The mechanism whereby aldose reductase enhances resistance to ischemia/reperfusion remains to be determined but could involve the removal of toxic byproducts of lipid peroxidation such as 4-hydroxy-*trans*-nonenal.<sup>65</sup>

### Antioxidant Enzymes (Mn SOD)

Studies in dogs have shown that ischemic PC induces, 24 hours later, an increase in the protein expression and activity of Mn SOD (while other antioxidant enzymes are unchanged)<sup>67</sup> and that the time course of Mn SOD induction parallels that of protection against lethal ischemia/reperfusion injury.<sup>2</sup> A similar association between the time course of Mn SOD induction and that of delayed cardioprotection has been observed after heat stress,<sup>42</sup> exercise,<sup>7</sup> and administration of CCPA,<sup>68</sup> although in one study<sup>69</sup> heat stress did not augment Mn SOD activity. The increase in Mn SOD activity after heat stress and exercise appears to be caused by the production of ROS, TNF- $\alpha$ , and interleukin-1 $\beta$ .<sup>7,42,70</sup> Importantly, in vivo administration of antisense oligodeoxynucleotides to Mn SOD has been reported to block heat stress-induced,<sup>70</sup> exercise-induced,<sup>7</sup> and CCPA-induced<sup>71</sup> late PC, indicating

that Mn SOD upregulation is essential for these 3 forms of delayed cardioprotection. No such data are available to determine whether Mn SOD is necessary for ischemia-induced late PC in vivo (although evidence supporting this concept has been obtained in isolated neonatal myocytes).<sup>72</sup> An increase in the activity of various antioxidant enzymes (Mn SOD, Cu-Zn SOD, catalase, and/or glutathione peroxidase) has also been reported 24 to 72 hours after pharmacological PC with interleukin-1<sup>73</sup> and endotoxin,<sup>74</sup> concomitant with increased myocardial resistance to ischemia/superfusion injury, but a cause-and-effect relationship remains to be established. Not all studies, however, have found upregulation of antioxidant defenses during late PC. In conscious pigs<sup>75</sup> and rabbits<sup>76</sup> subjected to ischemic PC, no increase in Mn SOD, Cu-Zn SOD, catalase, glutathione peroxidase, or glutathione reductase activity could be detected 24 hours after the PC stimulus, when the delayed cardioprotection was fully manifest. Thus, the role of antioxidant proteins in ischemia-induced late PC is currently unknown.

### Heat Stress Proteins

Although studies in transgenic mice overexpressing HSP70 have shown that this protein confers protection against ischemia/reperfusion injury,<sup>77–79</sup> it remains controversial whether ischemic or pharmacological PC upregulates HSPs in vivo. For example, whereas earlier investigations reported an increase in myocardial HSP70 content 24 hours after ischemic PC,<sup>3,80</sup> subsequent studies found no HSP70 induction in rabbits preconditioned with ischemia,<sup>81</sup> CCPA,<sup>21,82,83</sup> or MLA.<sup>84,85</sup> Furthermore, several studies in rats subjected to whole-body hyperthermia<sup>86–88</sup> or to ischemic PC<sup>89</sup> have shown that the changes in myocardial HSP70 and HSP27 content do not correlate with protection against infarction. In addition, induction of HSP70 by heat stress fails to confer delayed cardioprotection in mice.<sup>69</sup> Recently, another member of the HSP family, HSP27, has been suggested to participate in late PC. Studies in rabbits have shown that CCPA-induced late PC is associated with a redistribution of HSP27 from the membranous to the cytosolic fraction of the homogenate<sup>90</sup> and with increased phosphorylation of this protein, which is abolished by pretreatment with protein kinase C (PKC) or tyrosine kinase inhibitors.<sup>91</sup> Since HSP27 is a substrate for the p38 mitogen-activated protein kinase (MAPK) pathway, which is activated 24 hours after CCPA,<sup>91</sup> the hypothesis has been proposed that posttranslational modulation of HSP27 may play an important role in mediating the delayed cardioprotection afforded by CCPA.<sup>91</sup>

The evidence reviewed above suggests that induction of HSPs is unlikely to account for the cardioprotection afforded by late PC, because the expression of these proteins does not correlate with the presence of the late infarct-sparing effects induced by 4 different stimuli (ischemia, heat stress, adenosine A<sub>1</sub> agonists, and MLA). It appears that increased expression of HSPs might be just a marker of the response of myocytes to stress.

### K<sub>ATP</sub> Channels

Pharmacological studies have provided evidence that opening of K<sub>ATP</sub> channels is necessary for the infarct-sparing effects of

late PC induced by MLA,<sup>20</sup> heat stress,<sup>22</sup> ischemia,<sup>25,26</sup> adenosine A<sub>1</sub><sup>28,83,92</sup> and A<sub>3</sub><sup>28</sup> receptor agonists, and  $\delta_1$ -opioid receptor agonists.<sup>17</sup> The diversity of the PC stimuli that converge on K<sub>ATP</sub> channels suggests that the activity of these channels may be a common distal mechanism of delayed protection against cell death. In contrast to late PC against infarction, however, ischemia-induced late PC against stunning does not appear to require K<sub>ATP</sub> channel activity.<sup>26</sup> The differential role of K<sub>ATP</sub> channels in late PC against stunning versus late PC against infarction provides further evidence that different mechanisms underlie these 2 forms of cardioprotection, as mentioned above.

Major issues that remain to be elucidated are the identity of the K<sub>ATP</sub> channels involved in late PC (ie, sarcolemmal versus mitochondrial) and the mechanism whereby their opening confers protection. Given the limitations of the available pharmacological tools, it seems likely that definitive assessment of the role of mitochondrial versus sarcolemmal K<sub>ATP</sub> channels will require molecular approaches (eg, gene targeting or transgenesis).

### The Signaling Pathway of Late PC

The stimuli listed in Table 1 trigger late PC by activating a complex cascade of signaling events that ultimately results in increased transcription of cardioprotective genes. Among the various families of cellular kinases, there is now convincing evidence that PKC and Src protein tyrosine kinases (PTKs) play an essential role in the development of late PC.

#### Protein Kinase C

The notion that PKC is essential for the genesis of late PC was first proposed by Baxter et al,<sup>93</sup> who found that the delayed infarct-sparing effects of ischemic PC in rabbits were abrogated by pretreatment with the PKC inhibitor chelerythrine. Conversely, administration of the PKC activator dioctanoyl-*sn*-glycerol induced cardioprotection 24 hours later.<sup>94</sup> Subsequent studies have also implicated PKC in the development of CCPA-induced<sup>95</sup> and heat stress-induced<sup>86,88,96</sup> late PC against infarction. Direct evidence that PC stimuli activate PKC in vivo, however, was lacking. Furthermore, no information was available as to which PKC isoform is involved. These issues were addressed in a series of studies in conscious rabbits<sup>97–100</sup> in which it was found that ischemic PC causes selective translocation (and activation<sup>100</sup>) of PKC $\epsilon$  and PKC $\eta$  (2 members of the subfamily of novel PKC isoforms) but does not affect the other 8 isoforms expressed in the rabbit heart (PKC isoforms  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\zeta$ ,  $\iota$ ,  $\lambda$ , and  $\mu$ ) and does not significantly change total PKC activity.<sup>97</sup> Inhibition of PKC $\epsilon$  translocation (and activation<sup>100</sup>) by chelerythrine blocked the development of late PC against myocardial stunning, whereas inhibition of PKC $\eta$  translocation did not, indicating that the translocation of PKC $\epsilon$  (but not that of PKC $\eta$ ) is necessary for late PC to occur.<sup>98</sup> Thus, activation of PKC after ischemic PC is isoform selective and  $\epsilon$  appears to be the specific PKC isotype responsible for the development of delayed protection. The ischemic PC-induced activation of PKC $\epsilon$  is caused by the generation of NO during the initial ischemic stress, because it is blocked by pretreatment with L-NA.<sup>99</sup> Interestingly, administration of NO donors in



the absence of ischemia induces a selective activation of PKC $\epsilon$  quantitatively similar to that induced by ischemic PC<sup>99</sup>; this event is essential for NO donor-induced late PC, since coadministration of chelerythrine blocks both the activation of PKC $\epsilon$  and the delayed protection elicited by the NO donors.<sup>99</sup>

Thus, the recruitment of the  $\epsilon$  isoform of PKC appears to be a critical signaling event in the development of both ischemia-induced and NO donor-induced late PC in rabbits. The recent finding that transgenic expression of constitutively active PKC $\epsilon$  recapitulates both the signaling events and the cardioprotective effects of late PC supports a role of PKC $\epsilon$  in mice as well.<sup>101</sup> The precise mechanism whereby NO activates PKC $\epsilon$  remains to be elucidated. Because MPG blocks NO donor-induced late PC,<sup>13</sup> it seems reasonable to postulate that NO-derived reactive species (ONOO<sup>-</sup> and/or ROS) may activate PKC $\epsilon$  either by direct oxidative modification or via activation of phospholipases.<sup>99</sup>

### Protein Tyrosine Kinases

Since >1000 different PTKs have been identified so far, assessing the contribution of this class of enzymes to late PC represents a gargantuan task. A role of PTKs in the genesis of ischemia-induced late PC was proposed on the basis of studies using genistein,<sup>102</sup> which, however, is a broad inhibitor of most known PTKs and has modest selectivity for these kinases versus PKC and other kinases. Recent studies in conscious rabbits<sup>100,103</sup> have focused on 2 major families of PTKs, the Src PTKs and the epidermal growth factor (EGF) receptor PTKs. It was found that ischemic PC selectively activates 2 members of the Src family of PTKs (Src and Lck) (among the 7 members expressed in the rabbit heart) and that this activation is blocked by chelerythrine, suggesting that these kinases are distal to PKC.<sup>100</sup> Inhibition of Src and Lck activation with lavendustin A (LD-A) completely abrogates the development of late PC against myocardial stunning,<sup>103</sup> indicating that Src and/or Lck signaling plays a causative role in this phenomenon. In contrast, EGF receptor PTKs are not activated by ischemic PC.<sup>100</sup> Thus, Src and/or Lck (but not EGF receptor PTKs) appear to be essential components of the signaling pathway responsible for the development of ischemia-induced late PC and to be downstream targets of PKC phosphorylation in rabbits. Subsequent studies in mice have corroborated these concepts by demonstrating that Lck is a direct substrate of PKC $\epsilon$ <sup>104</sup> and that targeted disruption of the *Lck* gene abolishes ischemia-induced late PC.<sup>105</sup> LD-A has also been found to block CCPA-induced late PC,<sup>95</sup> implicating tyrosine kinases in the genesis of this form of adaptation as well. In contrast, genistein failed to block heat stress-induced late PC.<sup>96</sup>

### Mitogen-Activated Protein Kinases

Another potential downstream target of PKC-dependent signaling during the development of late PC is the MAPK superfamily, which includes 3 major subfamilies: the p44/p42 MAPKs (or extracellular signal-regulated kinases [ERKs]), the p38 MAPKs, and the p46/p54 MAPKs (or c-jun N-terminal kinases [JNKs]). Several studies in isolated hearts

have documented that brief myocardial ischemia/reperfusion is associated with activation of p44/p42 MAPKs, p38 MAPKs, and JNKs,<sup>106,107</sup> but it is unknown whether the ischemic protocols used in those investigations elicit late PC. Recent studies in conscious rabbits have demonstrated that an ischemic PC protocol known to induce delayed cardioprotection activates all of the 3 MAPK subfamilies, although the activation of p38 MAPK is short-lived.<sup>108,109</sup> The activation of p44/p42 MAPKs and JNKs is abolished by chelerythrine, indicating that it is downstream of, and dependent on, activation of PKC.<sup>108,109</sup> Interestingly, selective overexpression of PKC $\epsilon$  in adult rabbit myocytes induces activation of p44/p42 MAPKs and protects against simulated ischemia, an effect that can be abolished by p44/p42 MAPK inhibitors.<sup>108</sup> The critical unresolved issue now is whether the recruitment of MAPKs contributes to the development of late PC or is merely an epiphenomenon. Elucidation of this issue will require studies in *in vivo* models of late PC in which the activity of MAPKs is inhibited either with genetic approaches (eg, gene targeting or transgenesis of dominant-negative mutants) or with pharmacological agents more specific than those (eg, SB203580) currently available.

### Role of Kinases in Mediating Protection

In addition to their role in initiating the development of late PC shortly after the stimulus (day 1), there is mounting evidence that cellular kinases are also necessary for the protection to become manifest 24 hours later (day 2) (Figure). This concept is supported by the recent finding that administration of the PTK inhibitor LD-A before the second ischemic challenge (on day 2) completely abrogates the protective effects of late PC against myocardial stunning and the concomitant increase in iNOS activity.<sup>103</sup> It appears, therefore, that PTKs play a bifunctional role in ischemia-induced late PC, contributing not only to its development shortly after the initial ischemic stress but also to the occurrence of protection 24 hours later. The mechanism whereby PTKs participate in the protection on day 2 remains to be established. Because inhibition of PTKs with LD-A on day 2 abolishes the increase in iNOS activity, it has been suggested that posttranslational modulation of iNOS proteins via tyrosine phosphorylation is necessary to activate this enzyme and to confer tolerance to ischemia/reperfusion injury.<sup>103</sup> In addition, the recent finding that p38 MAPK activity is markedly increased 24 hours after administration of CCPA (concomitant with increased phosphorylation of HSP27) raises the possibility that this subfamily of MAPKs may also be involved in mediating the protective effects of late PC.<sup>91</sup>

### Transcription Factors

The recruitment by the PC stimulus of PKC, Src PTKs, and almost certainly other as-yet-unidentified kinases leads to the activation of transcription factors that govern the expression of the cardioprotective genes responsible for late PC. The first transcription-regulatory element to be identified as an integral component of the late PC response was nuclear factor- $\kappa$ B (NF- $\kappa$ B),<sup>110</sup> which is known to be a major modulator of



TABLE 2. Mechanism of Late PC

Stimulus	Triggers				Signaling Pathway				Mediators						K <sub>ATP</sub> Channels
	Adenosine	NO	ROS	Opioids	PKC	PTKs	MAPKs	NF-κB	iNOS	COX-2	AR	HSPs	Mn SOD		
Nonpharmacological PC															
Ischemia	+	+	+	?	+	+	?	+	++	+	+	?	?	+	
Heat stress	?	?	?	?	+	—	?	?	?	?	?	?	+	+	
Rapid pacing	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
Exercise	?	?	+	?	+	?	?	?	+	?	?	?	+	?	
Pharmacological PC															
Adenosine receptor agonists	+	?	?	?	+	+	?	?	±	?	?	—	+	+	
MLA and analogs	?	?	?	?	?	?	?	?	+	?	?	—	?	+	
NO donors	?	+	+	?	+	+	?	?	++	?	?	?	?	?	
Opioid agonists	?	?	?	+	?	?	?	?	+	?	?	?	?	+	

+ indicates evidence based on pharmacological studies; ++, evidence based on molecular approaches (eg, knockout mice); ±, conflicting evidence; —, studies suggest no role in this form of late PC; and ?, available information insufficient.

AR indicates aldose reductase.

\*Adenosine receptors and K<sub>ATP</sub> channels have been found to participate in late PC against infarction but not against stunning.

iNOS, COX-2, and aldose reductase gene expression. Using conscious rabbits, Xuan et al<sup>110</sup> demonstrated that ischemic PC induces rapid activation of NF- $\kappa$ B and that this event can be mimicked by infusing NO donors in the absence of ischemia. Inhibition of NF- $\kappa$ B with diethyldithiocarbamate completely abrogated the cardioprotective effects observed 24 hours later, indicating that NF- $\kappa$ B plays a critical role in the genesis of late PC.<sup>110</sup> The ischemic PC-induced activation of NF- $\kappa$ B was blocked by pretreatment with L-NA, MPG, chelerythrine, and LD-A (all given at doses previously shown to block late PC), indicating that the cellular mechanism whereby ischemic PC activates NF- $\kappa$ B involves the formation of NO and ROS and the subsequent activation of PKC- and PTK-dependent signaling events.<sup>110</sup> Thus, NF- $\kappa$ B appears to be a common downstream pathway through which multiple signals elicited by ischemic stress (NO, ROS, PKC, and PTKs) act to induce gene expression in the heart.

Subsequent studies have shown that ischemic PC induces both serine and tyrosine phosphorylation of I $\kappa$ B $\alpha$  (the inhibitor of NF- $\kappa$ B) concomitant with PKC-dependent activation of IKK $\alpha$  and IKK $\beta$  (the serine-threonine kinases that phosphorylate I $\kappa$ B $\alpha$ ),<sup>111</sup> suggesting that a dual mechanism accounts for the activation of NF- $\kappa$ B during ischemic PC, one via PKC- and IKK-dependent serine phosphorylation of I $\kappa$ B $\alpha$  and the other via IKK-independent tyrosine phosphorylation of I $\kappa$ B $\alpha$ . The Lck kinase also plays an essential role in the activation of NF- $\kappa$ B induced by ischemia, as this event is absent in *Lck*<sup>-/-</sup> mice.<sup>105</sup> A molecular link between PKC $\epsilon$  (the isoform implicated in the genesis of late PC)<sup>97–100</sup> and NF- $\kappa$ B is further supported by studies in adult cardiac myocytes, in which selective overexpression of PKC $\epsilon$  has been found to induce activation of NF- $\kappa$ B,<sup>112</sup> activation of IKK $\alpha$  and IKK $\beta$ ,<sup>113</sup> and serine and tyrosine phosphorylation of I $\kappa$ B $\alpha$ .<sup>113</sup> Interestingly, PKC $\epsilon$ -induced recruitment of NF- $\kappa$ B in cardiomyocytes is abolished by inhibition of either p44/p42 MAPKs or JNKs.<sup>112</sup>

Recruitment of NF- $\kappa$ B has also been reported in rat hearts subjected to short-term protocols of ischemia/reperfusion in vivo<sup>114</sup> and in vitro,<sup>115</sup> but it is not clear whether these protocols elicit a late PC response in the models used. In addition, activating protein 1 (AP-1) has been found to be activated by brief myocardial ischemia in rats<sup>114</sup> and by overexpression of PKC $\epsilon$  in cardiomyocytes.<sup>112</sup> Whether this transcription factor plays a functional role in the development of the late PC response remains unknown. Given the fact that specific combinations of 2 or more transcription-regulatory proteins are obligatorily required for iNOS gene expression and given the multiplicity of enzymes that co-mediate late PC (eg, COX-2, aldose reductase, and Mn SOD), it seems likely that the upregulation of iNOS, COX-2, and other co-mediators after a PC stimulus involves simultaneous activation of multiple stress-responsive transcription factors acting in an additive or synergistic manner.

### Polygenic Nature of Late PC

As detailed above, there is now solid evidence that, in addition to K<sub>ATP</sub> channel activity,<sup>17,20,22,25,26,28,83,92</sup> at least 3 stress-responsive, inducible proteins (ie, iNOS,<sup>16,34,35,44,48–53,55–59</sup> COX-2,<sup>61,62</sup> and aldose reductase<sup>66</sup>) are required to mediate the protection afforded by the late phase of ischemic PC, and at least 2 proteins (iNOS and Mn SOD) are required to mediate exercise-induced late PC<sup>7</sup> (and possibly adenosine-induced late PC)<sup>44,59,71</sup> (Table 2). Thus, the paradigm of late PC has evolved from the original (and in retrospect naïve) proposal that this adaptation is mediated by one protein to the recognition that the shift of the heart to a defensive phenotype represents a complex response requiring the coordinated activation of multiple genes. This is not dissimilar from other conditions in which the heart changes its phenotype (eg, hypertrophy). As the number of newly identified mediators of late PC increases, so will the number of triggers, transcription-regulatory mechanisms (kinases/transcription factors), and posttranscriptional modulators

involved. Unraveling the complexity of this polygenic phenotypic change will likely be a challenge for years to come.

### Late Versus Early PC

It is useful to recapitulate here some of the fundamental differences between the early and late phases of PC. Both early and late PC limit infarct size, but the infarct-sparing effects of early PC are more robust.<sup>1-3,116</sup> On the other hand, late PC mitigates myocardial stunning, whereas early PC does not.<sup>4</sup> The duration of the protection conferred by the 2 phases (2 to 3 hours<sup>116</sup> versus 72 to 96 hours<sup>21,46,47</sup>) is vastly different, supporting the concept that the late phase may ultimately have greater clinical usefulness. The duration of the early phase cannot be extended by continuous infusion of pharmacological triggers (ie, CCPA)<sup>117</sup> nor by repeated brief ischemic episodes,<sup>118</sup> presumably because of desensitization of adenosine receptors. This problem should not apply to late PC, in which stimuli need to be applied only at 48- to 72-hour intervals to maintain the defensive phenotype. Indeed, Yellon's group has demonstrated that the infarct-sparing effects induced by a single dose of CCPA persist for 72 hours<sup>21</sup> and that repeated administration of CCPA at 48-hour intervals results in the maintenance of continuous protection against infarction for at least 10 days with no evidence of downregulation of A<sub>1</sub> receptor function.<sup>23</sup> These results provide proof of concept that late PC can be exploited pharmacologically to maintain the heart in a chronically preconditioned state without development of tolerance.

### Conclusions

The heart reacts to a sublethal ischemic stress by mobilizing a complex sequence of cellular events that results in a shift from a naïve (nonpreconditioned) to a defensive (preconditioned) phenotype. Although the exact cellular and molecular mechanisms underlying the phenomenon of late PC remain to be deciphered, remarkable progress has been made in the last few years in our understanding of this powerful cardioprotective adaptation. A schematic representation of the mechanism of late PC is presented in the Figure. It is now clear that this is a polygenic process that requires the synthesis of multiple proteins. Specifically, chemical signals released by the ischemic stress (such as NO, ROS, and adenosine) are transduced by a cascade of signaling elements that includes PKC, Src PTKs, and NF- $\kappa$ B to the nucleus where they direct the transcription of iNOS, COX-2, aldose reductase, and probably other cardioprotective genes (Figure). An analogous sequence of events (possibly involving additional genes such as *Mn SOD*) can be triggered by a wide variety of stimuli, including heat stress, exercise, and cytokines. Thus, late PC appears to be a universal response of the heart to stress in general. A sustained cardioprotection similar to that afforded by the late phase of ischemic PC can also be induced pharmacologically with clinically relevant agents, such as NO donors, adenosine A<sub>1</sub> or A<sub>3</sub> receptor agonists, endotoxin derivatives, and  $\delta_1$ -opioid receptor agonists ("PC mimetics"), suggesting that this endogenous adaptive response might be exploited for therapeutic purposes. The extent to which the various forms of nonpharmacological and pharmacological

PC share the same molecular mechanism remains to be established. Some components of the proposed paradigm (eg, PKC, PTKs, iNOS, and Mn SOD) appear to be common to several forms of late PC, but others (eg, adenosine and K<sub>ATP</sub> channels) appear to differ. Deciphering the mechanism of late PC is important not only for our understanding of how the heart adapts to stress but also for its potential clinical implications. The identification of the cellular basis of this phenomenon should provide a conceptual framework for developing novel therapeutic strategies aimed at mimicking the cardioprotective effects of late PC with pharmacological agents (eg, PC-mimetic drugs) or genetic approaches (eg, transfer of cardioprotective genes) that can maintain the heart in a sustained or chronic defensive (preconditioned) state.

### References

1. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation*. 1986; 74:1124-1136.
2. Kuzuya T, Hoshida S, Yamashita N, Fujii H, Oe H, Hori M, Kamada T, Tada M. Delayed effects of sublethal ischemia on the acquisition of tolerance to ischemia. *Circ Res*. 1993;72:1293-1299.
3. Marber MS, Latchman DS, Walker JM, Yellon DM. Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction. *Circulation*. 1993;88: 1264-1272.
4. Bolli R. The early and late phases of preconditioning against myocardial stunning and the essential role of oxyradicals in the late phase: an overview. *Basic Res Cardiol*. 1996;91:57-63.
5. Currie RW, Karmazyn M, Kloc M, Mailer K. Heat-shock response is associated with enhanced postischemic ventricular recovery. *Circ Res*. 1988;63:543-549.
6. Kaszala K, Vegh A, Papp JG, Parratt JR. Time course of the protection against ischaemia and reperfusion-induced ventricular arrhythmias resulting from brief periods of cardiac pacing. *J Mol Cell Cardiol*. 1996;28:2085-2095.
7. Yamashita N, Hoshida S, Otsu K, Asahi M, Kuzuya T, Hori M. Exercise provides direct biphasic cardioprotection via manganese superoxide dismutase activation. *J Exp Med*. 1999;189:1699-1706.
8. Brown JM, Gross MA, Terada LS, Whitman GJR, Banerjee A, White CW, Harken AH, Repine JE. Endotoxin pretreatment increases endogenous myocardial catalase activity and decreases ischemia-reperfusion injury of isolated rat hearts. *Proc Natl Acad Sci U S A*. 1989;86:2516-2520.
9. Brown JM, White CW, Terada LS, Grosso MA, Shanley PF, Mulvin DW, Banerjee A, Whiteman GJ, Harken AH, Repine JE. Interleukin-1 pretreatment decreases ischemia/reperfusion injury. *Proc Natl Acad Sci U S A*. 1990;87:5026-5030.
10. Brown JM, Anderson BO, Repine JE, Shanley PF, White CW, Grosso MA, Banerjee A, Bensard DD, Harken AH. Neutrophils contribute to TNF induced myocardial tolerance to ischaemia. *J Mol Cell Cardiol*. 1992;24:485-495.
11. Nelson SK, Wong GH, McCord JM. Leukemia inhibitory factor and tumor necrosis factor induce manganese superoxide dismutase and protect rabbit hearts from reperfusion injury. *J Mol Cell Cardiol*. 1995; 27:223-229.
12. Sun J-Z, Tang X-L, Park SW, Qiu Y, Turrens JF, Bolli R. Evidence for an essential role of reactive oxygen species in the genesis of late preconditioning against myocardial stunning in conscious pigs. *J Clin Invest*. 1996;97:562-576.
13. Takano H, Tang X-L, Qiu Y, Guo Y, French B, Bolli R. Nitric oxide donors induce late preconditioning against myocardial stunning and infarction in conscious rabbits via an antioxidant-sensitive mechanism. *Circ Res*. 1998;83:73-84.
14. Baxter GF, Marber MS, Patel VC, Yellon DM. Adenosine receptor involvement in a delayed phase of myocardial protection 24 hours after ischemic preconditioning. *Circulation*. 1994;90:2993-3000.

15. Yao Z, Auchampach JA, Pieper GM, Gross GJ. Cardioprotective effects of monophosphoryl lipid A, a novel endotoxin analogue, in the dog. *Cardiovasc Res*. 1993;27:832–838.
16. Xi L, Salloum F, Tekin D, Jarrett NC, Kukreja RC. Glycolipid RC-552 induces delayed preconditioning-like effect via iNOS-dependent pathway in mice. *Am J Physiol*. 1999;277:H2418–H2424.
17. Fryer RM, Hsu AK, Eells JT, Nagase H, Gross GJ. Opioid-induced second window of cardioprotection: potential role of mitochondrial  $K_{ATP}$  channels. *Circ Res*. 1999;84:846–851.
18. Sun J-Z, Tang X-L, Knowlton AA, Park SW, Qiu Y, Bolli R. Late preconditioning against myocardial stunning: an endogenous protective mechanism that confers resistance to postischemic dysfunction 24 hours after brief ischemia in conscious pigs. *J Clin Invest*. 1995;95:388–403.
19. Kaeffer N, Richard V, Thuillel C. Delayed coronary endothelial protection 24 hours after preconditioning: role of free radicals. *Circulation*. 1997;96:2311–2316.
20. Mei DA, Elliott GT, Gross GJ.  $K_{ATP}$  channels mediate late preconditioning against infarction produced by monophosphoryl lipid A. *Am J Physiol*. 1996;271:H2723–H2729.
21. Baxter GF, Yellon DM. Time course of delayed myocardial protection after transient adenosine  $A_1$  receptor activation in the rabbit. *J Cardiovasc Pharmacol*. 1997;29:631–638.
22. Hoag J, Qian Y, Nayeem M, D'Angelo M, Kukreja RC. ATP-sensitive potassium channel mediates delayed ischemic protection by heat stress in rabbit heart. *Am J Physiol*. 1997;273:H2458–H2464.
23. Dana A, Baxter GF, Walker JM, Yellon DM. Prolonging the delayed phase of myocardial protection: repetitive adenosine  $A_1$  receptor activation maintains rabbit myocardium in a preconditioned state. *J Am Coll Cardiol*. 1998;31:1142–1149.
24. Auchampach J, Takano H, Yang Z, Tang X-L, Bolli R. Activation of  $A_3$  adenosine receptors induces late preconditioning against myocardial infarction but not stunning in conscious rabbits. *J Mol Cell Cardiol*. 1999;31:5 A10. Abstract.
25. Bernardo NL, D'Angelo M, Okubo S, Joy A, Kukreja RC. Delayed ischemic preconditioning is mediated by opening of ATP-sensitive potassium channels in the rabbit heart. *Am J Physiol*. 1999;276: H1323–H1330.
26. Takano H, Tang X-L, Bolli R. Differential role of  $K_{ATP}$  channels in late preconditioning against myocardial stunning and infarction in rabbits. *Am J Physiol*. 2000;279:H2350–H2359.
27. Maldonado C, Qiu YM, Tang X-L, Cohen MV, Auchampach J, Bolli R. Role of adenosine receptors in late preconditioning against myocardial stunning in conscious rabbits. *Am J Physiol*. 1997;42:H1324–H1332.
28. Takano H, Bolli R, Tang X-L, Yang Z, Black R, Auchampach J. Activation of  $A_1$  or  $A_3$  receptors produces delayed protection against infarction in conscious rabbits by a mechanism involving  $K_{ATP}$  channels. *Circulation*. 1999;100:I-56. Abstract.
29. Bolli R, Bhatti ZA, Tang X-L, Qiu Y, Zhang Q, Guo Y, Jadoon AK. Evidence that late preconditioning against myocardial stunning in conscious rabbits is triggered by the generation of nitric oxide. *Circ Res*. 1997;81:42–52.
30. Qiu Y, Rizvi A, Tang X-L, Manchikalapudi S, Takano H, Jadoon AK, Wu W-J, Bolli R. Nitric oxide triggers late preconditioning against myocardial infarction in conscious rabbits. *Am J Physiol*. 1997;273: H2931–H2936.
31. Banerjee S, Tang X-L, Qiu Y, Takano H, Manchikalapudi S, Dawn B, Shirk G, Bolli R. Nitroglycerin induces late preconditioning against myocardial stunning via a protein kinase C dependent pathway. *Am J Physiol*. 1999;277:H2488–H2494.
32. Guo Y, Bao W, Tang X-L, Wu W-J, Bolli R. Nitric oxide donors induce late preconditioning against myocardial infarction in mice. *J Mol Cell Cardiol*. 1999;31:A11. Abstract.
33. Hill M, Kodani E, Shinmura K, Tang X-L. Nitroglycerin induces late preconditioning against myocardial infarction despite nitrate tolerance. *Circulation*. 2000;102(suppl II):II-25. Abstract.
34. Xuan Y-T, Tang X-L, Qiu Y, Banerjee S, Takano H, Han H, Bolli R. Biphasic response of cardiac NO synthase to ischemic preconditioning in conscious rabbits. *Am J Physiol*. 2000;279:H2360–H2371.
35. Bolli R, Manchikalapudi S, Tang X-L, Takano H, Qiu Y, Guo Y, Zhang Q, Jadoon AK. The protective effect of late preconditioning against myocardial stunning in conscious rabbits is mediated by nitric oxide synthase: evidence that nitric oxide acts both as a trigger and as a mediator of the late phase of ischemic preconditioning. *Circ Res*. 1997; 81:1094–1107.
36. Kodani E, Tang X-L, Takano H, Shinmura K, Hill M, Bolli R. The role of cyclic guanosine monophosphate (cGMP) in late preconditioning against myocardial stunning in conscious rabbits. *J Mol Cell Cardiol*. 2000;32:A23. Abstract.
37. Tang X-L, Rizvi AN, Qiu Y, Takano H, Zhang Q, Guo Y, Bolli R. Evidence that the hydroxyl radical triggers late preconditioning against myocardial stunning in conscious rabbits. *Circulation*. 1997;96:I-255. Abstract.
38. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci U S A*. 1990;87:1620–1624.
39. Crow JP, Beckman JS. Reactions between nitric oxide, superoxide, and peroxynitrite: footprints of peroxynitrite in vivo. *Adv Pharmacol*. 1995; 34:17–43.
40. Sun JZ, Kaur H, Halliwell B, Li XY, Bolli R. Use of aromatic hydroxylation of phenylalanine to measure production of hydroxyl radicals after myocardial ischemia in vivo: direct evidence for a pathogenetic role of the hydroxyl radical in myocardial stunning. *Circ Res*. 1993;73: 534–549.
41. Yamashita N, Hoshida S, Taniguchi N, Kuzuya T, Hori MA. "Second window of protection" occurs 24 h after ischemic preconditioning in the rat heart. *J Mol Cell Cardiol*. 1998;30:1181–1189.
42. Yamashita N, Hoshida S, Taniguchi N, Kuzuya T, Hori M. Whole-body hyperthermia provides biphasic cardioprotection against ischemia/reperfusion injury in the rat. *Circulation*. 1998;98:1414–1421.
43. Takano H, Tang X-L, Qiu Y, Manchikalapudi S, Wu W-J, French B, Bolli R. Intracoronary administration of oxygen radicals induces late preconditioning against stunning in conscious rabbits. *Circulation*. 1997;96(suppl I):I-256–I-257. Abstract.
44. Guo Y, Bao W, Tang X-L, Wu W-J, Takano H, Bolli R. Pharmacological preconditioning (PC) with adenosine  $A_1$  and opioid  $\delta_1$  receptor agonists is iNOS-dependent. *Circulation*. 2000;102(suppl II):II-121. Abstract.
45. Rizvi A, Tang X-L, Qiu Y, Xuan Y-T, Takano H, Jadoon AK, Bolli R. Increased protein synthesis is necessary for the development of late preconditioning against myocardial stunning in conscious rabbits. *Am J Physiol*. 1999;277:H874–H884.
46. Tang X-L, Qiu Y, Park SW, Sun J-Z, Kalya A, Bolli R. Time course of late preconditioning against myocardial stunning in conscious pigs. *Circ Res*. 1996;79:424–434.
47. Baxter GF, Goma FM, Yellon DM. Characterization of the infarct-limiting effect of delayed preconditioning: timecourse and dose-dependency studies in rabbit myocardium. *Basic Res Cardiol*. 1997;92:159–167.
48. Takano H, Manchikalapudi S, Tang X-L, Qiu Y, Rizvi A, Jadoon A, Zhang Q, Bolli R. Nitric oxide synthase is the mediator of late preconditioning against myocardial infarction in conscious rabbits. *Circulation*. 1998;98:441–449.
49. Imagawa J, Yellon DM, Baxter GF. Pharmacological evidence that inducible nitric oxide synthase is a mediator of delayed preconditioning. *Br J Pharmacol*. 1999;126:701–708.
50. Guo Y, Jones WK, Xuan Y-T, Tang X-L, Bao W, Wu W-J, Han H, Laubach VE, Ping P, Yang Z, Qui Y, Bolli R. The late phase of ischemic preconditioning is abrogated by targeted disruption of the iNOS gene. *Proc Natl Acad Sci U S A*. 1999;96:11507–11512.
51. Wang Y, Zhang S, Guo Y, Bao W, Wu J, Bolli R. Ischemic preconditioning (PC) upregulates inducible nitric oxide synthase (iNOS), but not eNOS, in cardiac myocytes in vivo. *Circulation*. 2000;102(suppl II): II-93. Abstract.
52. Bolli R, Dawn B, Tang X-L, Qiu Y, Ping P, Xuan Y-T, Jones WK, Takano H, Guo Y, Zhang J. The nitric oxide hypothesis of late preconditioning. *Basic Res Cardiol*. 1998;93:325–338.
53. Jones WK, Flaherty MP, Tang X-L, Takano H, Qiu Y, Banerjee S, Smith T, Bolli R. Ischemic preconditioning increases iNOS transcript levels in conscious rabbits via a nitric oxide-dependent mechanism. *J Mol Cell Cardiol*. 1999;31:1469–1481.
54. Kodani E, Tang X-L, Xuan Y-T, Shinmura K, Takano H, Bolli R. Role of cyclic guanosine monophosphate in nitric oxide-dependent late preconditioning in conscious rabbits. *Circulation*. 2000;102(suppl II): II-270. Abstract.
55. Guo Y, Jones WK, Bao W, Tang X-L, Wu WJ, Bolli R. Targeted disruption of iNOS gene abrogates NO donor-induced late preconditioning. *Circulation*. 1999;100:I-562. Abstract.



56. Zhao L, Weber PA, Smith JR, Comerford ML, Elliott GT. Role of inducible nitric oxide synthase in pharmacological "preconditioning" with monophosphoryl lipid A. *J Mol Cell Cardiol.* 1997;29:1567–1576.
57. Xi L, Jarrett NC, Hess ML, Kukreja RC. Essential role of inducible nitric oxide synthase in monophosphoryl lipid A-induced late cardioprotection: evidence from pharmacological inhibition and gene knockout mice. *Circulation.* 1999;99:2157–2163.
58. Ockaili R, Emani VR, Okubo S, Brown M, Krottapalli K, Kukreja RC. Opening of mitochondrial KATP channel induces early and delayed cardioprotective effect: role of nitric oxide. *Am J Physiol.* 1999;277:H2425–H2434.
59. Zhao T, Xi L, Chelliah J, Levasseur JE, Kukreja RC. Inducible nitric oxide synthase mediates delayed myocardial protection induced by activation of adenosine A<sub>1</sub> receptors: evidence from gene-knockout mice. *Circulation.* 2000;102:902–907.
60. Bell RM, Rees DD, Yellon DM. The protection observed during delayed preconditioning of the heart appears independently of iNOS: a study in iNOS knockout mice. *Circulation.* 1999;100:1-242. Abstract.
61. Shinmura K, Tang X-L, Wang Y, Xuan Y-T, Liu S-Q, Takano H, Bhatnagar A, Bolli R. Cyclooxygenase-2 mediates the cardioprotective effects of the late phase of ischemic preconditioning in conscious rabbits. *Proc Natl Acad Sci U S A.* 2000;97:10197–10202.
62. Guo Y, Bao W, Wu W-J, Shinmura K, Tang X-L, Bolli R. Evidence for an essential role of cyclooxygenase-2 as a mediator of the late phase of ischemic preconditioning in mice. *Basic Res Cardiol.* 2000;95:480–485.
63. Vane JR, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. *Annu Rev Pharmacol Toxicol.* 1998;38:97–120.
64. Salvemini D, Misko TP, Masferrer JL, Seibert K, Currie MG, Needleman P. Nitric oxide activates cyclooxygenase enzymes. *Proc Natl Acad Sci U S A.* 1993;90:7240–7244.
65. Srivastava S, Chandra A, Wang L-F, Seifert WE, DaGue BB, Ansari NH, Srivastava SK, Bhatnagar A. Metabolism of the lipid peroxidation product, 4-hydroxy-trans-2-nonenal, in isolated perfused rat heart. *J Biol Chem.* 1998;273:10893–10900.
66. Shinmura K, Liu S-Q, Tang X-L, Kodani E, Xuan Y-T, Bhatnagar A, Bolli R. Aldose reductase is an obligatory mediator of the late phase of ischemic preconditioning. *Circulation.* 2000;102(suppl II):II-120. Abstract.
67. Hoshida S, Kuzuya T, Fuji H, Yamashita N, Oe H, Hori M, Suzuki K, Taniguchi N, Tada M. Sublethal ischemia alters myocardial antioxidant activity in canine heart. *Am J Physiol.* 1993;264:H33–H39.
68. Dana A, Yamashita N, Baxter GF, Yellon DM. Involvement of protein kinases and manganese-superoxide dismutase in adenosine induced delayed preconditioning. *J Mol Cell Cardiol.* 1998;30:A75. Abstract.
69. Xi L, Chelliah J, Nayeem MA, Levasseur JE, Hess ML, Kukreja RC. Whole body heat shock fails to protect mouse heart against ischemia/reperfusion injury: role of 72 kDa heat shock protein and antioxidant enzymes. *J Mol Cell Cardiol.* 1998;30:2213–2227.
70. Yamashita N, Hoshida S, Otsu K, Taniguchi N, Kuzuya T, Hori M. Involvement of cytokines in the mechanism of whole-body hyperthermia-induced cardioprotection. *Circulation.* 2000;102:452–457.
71. Dana A, Jonassen AK, Yamashita N, Yellon DM. Adenosine A<sub>1</sub> receptor activation induces delayed preconditioning in rats mediated by manganese superoxide dismutase. *Circulation.* 2000;101:2841–2848.
72. Yamashita N, Nishida M, Hoshida S, Kuzuya T, Hori M, Taniguchi N, Kamada T, Tada M. Induction of manganese superoxide dismutase in rat cardiac myocytes increases tolerance to hypoxia 24 hours after preconditioning. *J Clin Invest.* 1994;94:2193–2199.
73. Maulik N, Engelman RM, Wei Z, Lu D, Rousou JA, Das DK. Interleukin-1 $\alpha$  preconditioning reduces myocardial ischemia reperfusion injury. *Circulation.* 1993;88:387–394.
74. Maulik N, Watanabe M, Engelman D, Engelman RM, Kagan VE, Kisin E, Tyurin V, Cordis GA, Das DK. Myocardial adaptation to ischemia by oxidative stress induced by endotoxin. *Am J Physiol.* 1995;269:C907–C916.
75. Tang X-L, Qiu Y, Turrens JF, Sun J-Z, Bolli R. Late preconditioning against stunning is not mediated by increased antioxidant defenses in conscious pigs. *Am J Physiol.* 1997;273:H1651–H1657.
76. Tang X-L, Qiu Y, Turrens JF, Bolli R. Ischemic preconditioning does not upregulate antioxidant enzymes in conscious rabbits. *J Mol Cell Cardiol.* 2000;32:A54. Abstract.
77. Plumier JCL, Ross BM, Currie RW, Angelidis CE, Kazlaris H, Kollias G, Pagoulatos GN. Transgenic mice expressing the human heat-shock protein-70 have improved postischemic myocardial recovery. *J Clin Invest.* 1995;95:1854–1860.
78. Marber MS, Mestral R, Chi SH, Sayen MR, Yellon DM, Dillman WH. Overexpression of the rat inducible 70-kD heat stress protein in a transgenic mouse increases the resistance of the heart to ischemic injury. *J Clin Invest.* 1995;95:1446–1456.
79. Radford NB, Fina M, Benjamin IJ, Moreadith RW, Graves KH, Zhao PY, Gavva S, Wiethoff A, Sherry AD, Malloy CR, Williams RS. Cardioprotective effects of 70-kDa heat shock protein in transgenic mice. *Proc Natl Acad Sci U S A.* 1996;93:2339–2342.
80. Knowlton AA, Brecher P, Apstein CS. Rapid expression of heat shock protein in the rabbit after brief cardiac ischemia. *J Clin Invest.* 1991;87:139–147.
81. Baxter GF, Yellon DM. Delayed preconditioning against lethal ischaemic injury. In: Baxter GF, Yellon DM, eds. *Delayed Preconditioning and Adaptive Cardioprotection*. Dordrecht, the Netherlands: Kluwer Academic Publishers; 1998:1–28.
82. Heads RJ, Baxter GF, Latchman DS, Marber MS, Yellon DM. Hsp and cytoskeletal protein expression and localization during delayed preconditioning in the rabbit heart. *J Mol Cell Cardiol.* 1998;30:A20. Abstract.
83. Bernardo NL, Okubo S, Maaieh MM, Wood MA, Kukreja RC. Delayed preconditioning with adenosine is mediated by opening of ATP-sensitive K(+) channels in rabbit heart. *Am J Physiol.* 1999;277:H128–H135.
84. Yoshida KI, Maaieh MM, Shipley JB, Doloresco M, Bernardo NL, Qian YZ, Elliott GT, Kukreja RC. Monophosphoryl lipid A induces pharmacologic preconditioning in rabbit hearts without concomitant expression of 70-kDa heat shock protein. *Mol Cell Biochem.* 1996;159:73–80.
85. Baxter GF, Goodwin RW, Wright MJ, Kerac M, Heads RJ, Yellon DM. Myocardial protection after monophosphoryl lipid A: studies of delayed anti-ischaemic properties in rabbit hearts. *Br J Pharmacol.* 1996;117:1685–1692.
86. Yamashita N, Hoshida S, Nishida M, Igarashi J, Aoki K, Hori M, Kuzuya T, Tada M. Time course of tolerance to ischemia-reperfusion injury and induction of heat shock protein 72 by heat stress in the rat heart. *J Mol Cell Cardiol.* 1997;29:1815–1821.
87. Qian Y-Z, Shipley JB, Levasseur JE, Kukreja RC. Dissociation of heat shock proteins expression with ischemic tolerance by whole body hyperthermia in rat heart. *J Mol Cell Cardiol.* 1998;30:1163–1172.
88. Kukreja RC, Qian YZ, Okubo S, Flaherty EE. Role of protein kinase C and 72 kDa heat shock protein in ischemic tolerance following heat stress in the rat heart. *Mol Cell Biochem.* 1999;195:123–131.
89. Qian Y-Z, Bernardo NL, Nayeem MA, Chelliah J, Kukreja RC. Induction of 72-kDa heat shock protein does not produce second window of ischemic preconditioning in rat heart. *Am J Physiol.* 1999;276:H224–H234.
90. Heads RJ, Baxter GF, Latchman DS, Marber MS, Yellon DM. Hsp and cytoskeletal protein expression and localization during delayed preconditioning in the rabbit heart. *J Mol Cell Cardiol.* 1998;30:A20. Abstract.
91. Dana A, Skarli M, Papakrivopoulou J, Yellon DM. Adenosine A<sub>1</sub> receptor induced delayed preconditioning in rabbits: induction of p38 MAPK activation and Hsp27 phosphorylation via a tyrosine kinase- and protein kinase C-dependent mechanism. *Circ Res.* 2000;86:989–997.
92. Baxter GF, Yellon DM. ATP-sensitive K<sup>+</sup> channels mediate the delayed cardioprotective effect of adenosine A<sub>1</sub> receptor activation. *J Mol Cell Cardiol.* 1999;31:981–989.
93. Baxter GF, Goma FM, Yellon DM. Involvement of protein kinase C in the delayed cytoprotection following sublethal ischaemia in rabbit myocardium. *Br J Pharmacol.* 1995;115:222–224.
94. Baxter GF, Mocanu MM, Yellon DM. Attenuation of myocardial ischaemic injury 24 hours after diacylglycerol treatment in vivo. *J Mol Cell Cardiol.* 1997;29:1967–1975.
95. Dana A, Baxter GF, Yellon DM. Both protein kinase C and protein tyrosine kinase mediate adenosine induced delayed cardioprotection in rabbits. *Circulation.* 1997;96:1-312. Abstract.
96. Joyeux M, Baxter GF, Thomas DL, Ribaut C, Yellon DM. Protein kinase C is involved in resistance to myocardial infarction induced by heat stress. *J Mol Cell Cardiol.* 1997;29:3311–3319.
97. Ping P, Zhang J, Qiu Y, Tang X-L, Manchikalapudi S, Cao X, Bolli R. Ischemic preconditioning induces selective translocation of protein kinase C isoforms  $\epsilon$  and  $\eta$  in the heart of conscious rabbits without subcellular redistribution of total protein kinase C activity. *Circ Res.* 1997;81:404–414.

98. Qiu Y, Ping P, Tang X-L, Manchikalapudi S, Rizvi A, Zung J, Takano H, Wu W-J, Teschner S, Bolli R. Direct evidence that protein kinase C plays an essential role in the development of late preconditioning against myocardial stunning in conscious rabbits and that  $\epsilon$  is the isoform involved. *J Clin Invest*. 1998;101:2182–2198.
99. Ping P, Takano H, Zhang J, Tang X-L, Qiu Y, Li RCX, Banerjee S, Dawn B, Balafonova Z, Bolli R. Isoform-selective activation of protein kinase C by nitric oxide in the heart of conscious rabbits: a signaling mechanism for both nitric oxide-induced and ischemia-induced preconditioning. *Circ Res*. 1999;84:587–604.
100. Ping P, Zhang J, Zheng Y-T, Li RC, Dawn B, Tang X-L, Takano H, Balafonova Z, Bolli R. Demonstration of selective, PKC-dependent activation of Src and Lck tyrosine kinases during ischemic preconditioning in conscious rabbits. *Circ Res*. 1999;85:542–550.
101. Ping P, Zhang J, Zheng Y, Li R, Guo Y, Bao W, Bolli R. Cardiac targeted transgenesis of active PKC $\epsilon$  renders the heart resistant to infarction. *Circulation*. 2000;102(suppl II):II-24. Abstract.
102. Imagawa J, Baxter GF, Yellon DM. Genistein, a tyrosine kinase inhibitor, blocks the “second window of protection” 48 hours after ischemic preconditioning in the rabbit. *J Mol Cell Cardiol*. 1997;29:1885–1893.
103. Dawn B, Xuan Y-T, Qiu Y, Takano H, Tang X-L, Ping P, Banerjee S, Hill M, Bolli R. Bifunctional role of protein tyrosine kinases in late preconditioning against myocardial stunning in conscious rabbits. *Circ Res*. 1999;85:1154–1163.
104. Song C, Zhang J, Gao J, Zheng Y, Cao X, Wead W, Bolli R, Ping P. Lck tyrosine kinase is a direct substrate of PKC $\epsilon$  in cardiac myocytes. *Circulation*. 2000;102(suppl II):II-211. Abstract.
105. Ping P, Guo Y, Zhang J, Li R, Bao W, Wu W, Bolli R. Targeted deletion of Lck tyrosine kinase abrogates the NF- $\kappa$ B activation and the infarct-sparing effect induced by late preconditioning. *Circulation*. 2000;102(suppl II):II-211. Abstract.
106. Sugden PH, Clerk A. “Stress-responsive” mitogen-activated protein kinases (c-Jun N-terminal kinases and p38 mitogen-activated protein kinases) in the myocardium. *Circ Res*. 1998;83:345–352.
107. Ping P, Murphy E. Role of p38 mitogen-activated protein kinases in preconditioning: a detrimental factor or a protective kinase? *Circ Res*. 2000;86:921–922.
108. Ping P, Zhang J, Cao X, Li RCX, Kong D, Tang X-L, Qiu Y, Manchikalapudi S, Auchampach J, Black RG, Bolli R. PKC-dependent activation of p44/p42 MAPKs during myocardial ischemia-reperfusion in conscious rabbits. *Am J Physiol*. 1999;276:H1468–H1481.
109. Ping P, Zhang J, Huang S, Cao X, Tang X-L, Li RC, Zheng Y-T, Qiu Y, Clerk A, Sugden P, Han JH, Bolli R. PKC $\epsilon$ -dependent activation of p46 and p54 JNKs during ischemic preconditioning in conscious rabbits. *Am J Physiol*. 1999;277:H1771–H1785.
110. Xuan Y-T, Tang X-L, Banerjee S, Takano H, Li J-J, Qiu Y, Han H, Li R, Bolli R. Nuclear factor  $\kappa$ B plays an essential role in the genesis of the late phase of ischemic preconditioning in conscious rabbits. *Circ Res*. 1999;84:1095–1109.
111. Zhang J, Bolli R, Tang X-L, Jones K, Ping P. Direct demonstration of both tyrosine and serine phosphorylation of I $\kappa$ B- $\alpha$  during ischemic preconditioning in conscious rabbits. *Circulation*. 1999;100:I-56. Abstract.
112. Li RCX, Ping P, Zhang Z, Wead WB, Cao X, Gao J, Zheng Y, Huang S, Han J-H, Bolli R. PKC $\epsilon$  modulates NF- $\kappa$ B and AP-1 via mitogen activated protein kinases in adult rabbit cardiomyocytes. *Am J Physiol*. 2000;279:H1679–H1689.
113. Li RCX, Zhang J, Zheng Y, Weed W, Gao J, Cao X, Bolli R, Ping P. PKC $\epsilon$  enhances NF- $\kappa$ B activation via modulation of the IKK complex in cardiac myocytes. *Circulation*. 2000;102(suppl II):II-120. Abstract.
114. Chandrasekar B, Freeman GL. Induction of nuclear factor  $\kappa$ B and activation protein 1 in postischemic myocardium. *FEBS Lett*. 1997;401:30–34.
115. Maulik N, Sato M, Price BD, Das DK. An essential role of NF- $\kappa$ B in tyrosine kinase signaling of p38 MAP kinase regulation of myocardial adaptation to ischemia. *FEBS Lett*. 1998;429:365–369.
116. Cohen MV, Baines CP, Downey JM. Ischemic preconditioning: from adenosine receptor to K<sub>ATP</sub> channel. *Annu Rev Physiol*. 2000;62:79–109.
117. Tsuchida A, Thompson R, Olsson RA, Downey JM. The anti-infarct effect of an adenosine A1-selective agonist is diminished after prolonged infusion as is the cardioprotective effect of ischaemic preconditioning in rabbit heart. *J Mol Cell Cardiol*. 1994;26:303–311.
118. Cohen MV, Yang XM, Downey JM. Conscious rabbits become tolerant to multiple episodes of ischemic preconditioning. *Circ Res*. 1994;74:998–1004.