

# Preconditioning: what it is and how it works

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*Preconditioning, which was first described in 1986, demonstrates that preservation of ischemic myocardium is at least theoretically possible. An appreciation of the putative biochemical pathway of preconditioning would allow the clinician to precondition the heart pharmacologically. During ischemia, numerous agents are released by the myocardium, including adenosine, catecholamines, angiotensin II, bradykinin, and endothelin. All of these agents can contribute to preconditioning, but adenosine, acting through its A<sub>1</sub> receptor, is central to the preconditioning phenomenon. Current evidence suggests that adenosine and its receptor elicit protection through the activation of protein kinase C (PKC). Many agonists of PKC-coupled receptors are released by the ischemic myocardium, and exogenous administration of any one of these is capable of eliciting protection. The ATP-sensitive potassium (K<sup>+</sup><sub>ATP</sub>) channel remains the most likely candidate for the ultimate preconditioning end-effector. Human hearts can be preconditioned, and angioplasty has emerged as a powerful tool for testing preconditioning-mimetic agents in man. Preconditioning's early protective phase is followed by a delayed phase of protection, the "second window of protection," which might be more amenable to prophylactic treatment of high-risk patients. As our knowledge of preconditioning's mechanism grows, more and more strategies for protecting the ischemic myocardium are sure to emerge.*

An elusive goal of cardiology has been the identification of interventions that can limit the amount of myocardial necrosis caused by a coronary occlusive event. A major complication of acute myocardial infarction is loss of ventricular mass. Since the heart cannot regenerate myocardium, patients with myocardial infarction are left with a permanent deficit in pumping ability. For decades, clinicians and scientists have sought either pharmacologic or mechanical interventions that might spare ischemic myocardium. A substantive advance followed on the heels of the understanding of the pathobiology of myocardial infarction. When it became apparent that formation of a thrombus was the final step in occlusion of a coronary artery preceding myocardial infarction, efforts were directed at developing strategies for removing the offending thrombus. Thrombolysis with tissue-type plasminogen activator (tPA) and streptokinase has successfully addressed this problem by dissolving clots, thus producing reperfusion of the ischemic segments. Large clinical trials have clearly demonstrated that this strategy leads to successful reperfusion of acutely occluded coronary arteries in at least 75% of cases, but more importantly, that it results in salvage of myocardium with better residual myocardial function and better long-term prognosis.

In current practice, thrombolysis can rarely be instituted early enough to prevent substantial tissue loss. As a result, an adjunct intervention has been sought that would preserve viability until reperfusion could be instituted. This search has been complicated by the fact that the actual sequence of events that occur within an ischemic tissue and lead to its death is still poorly understood. Thus, it has not been possible to design such an intervention based on proven pharmacological principles, but rather we have had to rely primarily on random testing. As a result, there have been many false starts and failures. For example, both  $\beta$ -blockers and calcium channel

antagonists, two families of agents used widely in the treatment of ischemic cardiac syndromes, were among the first agents tested. However, after extensive examination, both proved to be largely ineffective in salvaging jeopardized myocardium. More recently, ischemic preconditioning has emerged as the model for such an intervention. If the mechanism of preconditioning can be understood and duplicated pharmacologically, then infarct size and the associated incidence of congestive heart failure in patients experiencing acute coronary thrombosis should be substantially reduced.

Ischemic preconditioning was first described in 1986 by Murry et al.<sup>1</sup> Their initial observation was so improbable and so defied conventional wisdom that it was followed by several years of doubt and inactivity. These investigators noted that the size of an infarct resulting from a 40-minute occlusion of a branch of a coronary artery of a dog heart could be markedly reduced if they first "preconditioned" the heart with a sublethal ischemic insult. In their protocol, this "insult" consisted of four cycles of 5 minutes of coronary occlusion with each occlusion followed by 5 minutes of

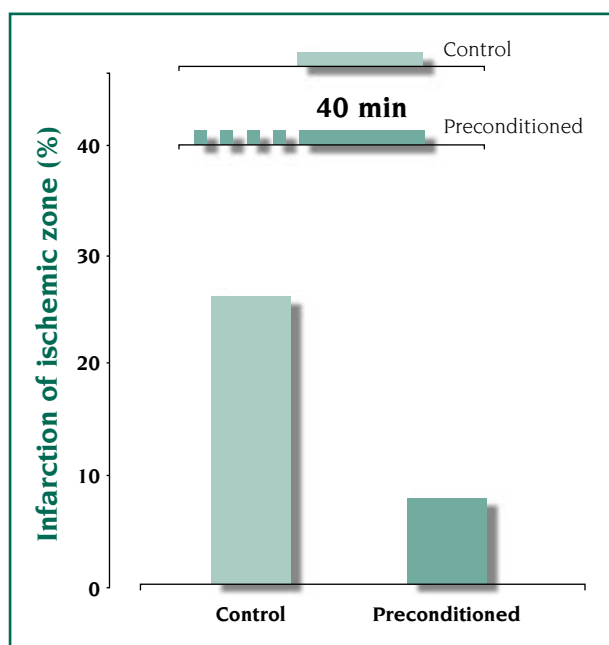
reperfusion. They found that the heart adapted itself within minutes to become resistant to ischemia-induced infarction (*Figure 1*). In other words, more ischemia was better! This phenomenon, now known as "classic" or "early" ischemic preconditioning, has been documented in all species tested to date,<sup>2</sup> including dog, rat, rabbit, and pig, as well as in human isolated cardiomyocytes<sup>3</sup> and atrial muscle.<sup>4</sup> It is obviously not possible to directly test whether in vivo human hearts can be preconditioned against infarction, but there is mounting circumstantial evidence for preconditioning's presence in man during both percutaneous transluminal coronary angioplasty and coronary revascularization surgery as will be discussed in detail later.

Despite a rather slow beginning, current activity can now only be described as frenzied as many investigators search for the mechanism of this unique protection. It is hoped that the forthcoming answers will enable the community to develop strategies that can be used clinically. While brief ischemic episodes might be used effectively to precondition the heart in the cardiac catheterization laboratory or the operating theater, ischemic preconditioning would be of no practical use in the setting of acute myocardial infarction. An appreciation of the putative biochemical pathway of this preconditioning phenomenon, however, should reveal sites where timely pharmacological intervention would allow the clinician to harness the power of ischemic preconditioning.

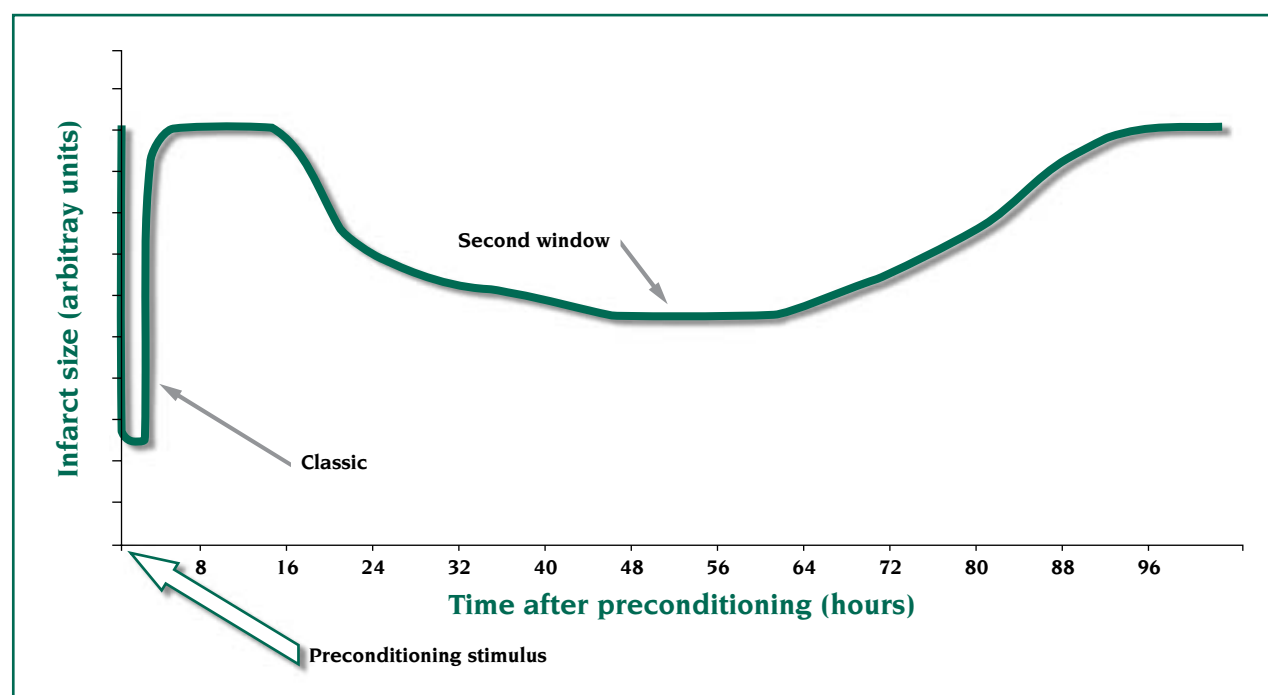
## PRECONDITIONING'S NATURAL HISTORY

In the rabbit and dog, a single 5-minute period of ischemia followed by 5 or 10 minutes of reperfusion is sufficient to put the heart into the preconditioned state.<sup>2</sup> Multiple cycles of ischemia offer no more protection against infarction than a single cycle, indicating either an all-or-none, or, more likely, a saturating type of kinetics. Two 2-minute occlusions do not elicit protection in the rabbit, while a single 5-minute occlusion does, suggesting that in the rabbit heart a relatively sharp threshold for protection exists somewhere between 2 and 5 minutes of ischemia. In man, however, there is evidence that as little as 90 seconds of coronary occlusion occurring during the course of routine angioplasty may be sufficient to precondition the heart.

The window of protection from preconditioning is quite short. Reports vary, but the protection wears off in



**Figure 1.** In this pioneering study by Murry et al.,<sup>1</sup> infarct size as a percentage of the ischemic zone was measured in dogs after either a 40-minute coronary occlusion (control) or a 40-minute occlusion preceded by four cycles of 5-minute occlusion/5-minute reperfusion (preconditioned) (see key in upper part of Figure). The repetitive episodes of brief ischemia prior to the more prolonged ischemic insult resulted in 75% salvage of myocardium otherwise earmarked for infarction ( $P < 0.001$ ). This early observation clearly documented the power of ischemic preconditioning.



**Figure 2.** Schematic representation of the timing and effectiveness of the classic and second window of preconditioning. Whereas myocardial protection related to the former is seen for only several hours after the preconditioning stimulus, protection of the second window appears between 16 to 24 hours and persists for nearly 72 hours. However, the protective effect on ischemic myocardium is more marked for classic preconditioning.

about 1 hour in most anesthetized in situ or isolated heart models.<sup>2</sup> In conscious rabbits, protection may last as long as 4 hours.<sup>5</sup> Interestingly, a second window of protection reappears 24 hours after preconditioning and may persist for as long as 2 to 3 days.<sup>6</sup> This reappearance of a protective state is undoubtedly related to a different mechanism than is early or “classic” preconditioning. Nonetheless, presence of this late phase broadens the potential for possible clinical applications. This second window of preconditioning also protects the myocardium against infarction, but, in our hands at least, the anti-infarct effect of the second window is less potent than that of classic preconditioning.<sup>7</sup> *Figure 2* illustrates the two windows of preconditioning.

Preconditioning does not protect against all aspects of ischemia/reperfusion injury. Preconditioning reportedly attenuates both ischemia- and reperfusion-induced arrhythmias in dog<sup>8</sup> and rat,<sup>9</sup> and perhaps the conscious rabbit model.<sup>10</sup> However, we and others have been unable to observe an antiarrhythmic effect in open-chest pig or rabbit (personal observation). A second window of protection against arrhythmias has also been reported for canine hearts.<sup>8</sup> Classic preconditioning seems to offer little protection against stunned myocardium.<sup>11</sup> However, the second window of

protection is associated with a strong antistunning effect in the rabbit heart.<sup>12</sup> Classic preconditioning has a clear beneficial effect on the recovery of mechanical function after global ischemia in the isolated rat heart. However, in other models, including the dog and the isolated rabbit heart (personal observation), such protection has not been very reproducible. Preconditioning's effect on postischemic function of human atrial strips has shown better reproducibility.<sup>4</sup> It is the authors' opinion that the preconditioning-induced improvement in postischemic ventricular function in the rat and human atrial models is the result of a reduction in myocyte necrosis rather than any effect on stunning. Perhaps the reason why clear protection is not seen in rabbit or canine myocardium is that stunning contributes more to the deficit in postischemic function in these species.

#### MODELS FOR THE ANTI-INFARCT EFFECT OF PRECONDITIONING

Currently, we use several different models to measure preconditioning's anti-infarct effect. In the first, the whole heart is exposed to a period of regional ischemia and reperfusion. As in Murry et al's original report,<sup>1</sup> the heart may be in situ, in which case it is

innervated and perfused with blood, or in the case of the rat and rabbit, may be removed from the host and perfused with a buffered salt solution. Infarction during regional ischemia in the buffer-perfused rabbit heart has been found to be very similar to that in the in situ heart. The isolated heart has obvious advantages when it is to be treated pharmacologically, since the dose and schedule of any agent can be precisely controlled in the perfusate and adverse hemodynamic systemic effects are minimized. On the other hand, the in situ model more closely mimics the clinical situation.

A third model involves isolated cardiomyocytes. There are two categories. In the first, the cells are incubated in hypoxic buffer that may or may not include metabolic blockers, and the rate of cell death is measured by either counting cells stained with vital dyes (cells with an intact sarcolemma will not stain) or quantitating the amounts of cytosolic enzymes, such as lactate dehydrogenase, leaking into the medium.

Armstrong and Ganote<sup>13</sup> have introduced the pellet model, which is slightly different. In this variation of the model, cardiomyocytes in suspension are gently centrifuged into a pellet, most of the supernatant is removed, and oxygen is excluded with an overlying layer of mineral oil. The cells quickly consume the residual oxygen in the pellet, resulting in a hypoxic environment. The advantage of this model is that metabolic waste products and cytokines can accumulate in the pellet, making the milieu very similar to that existing in ischemic tissue. While these nonbeating cells die very slowly in the pellet, they experience a rapid and predictable increase in osmotic fragility. Interestingly, preconditioning delays the appearance of fragility. In practice, aliquots of cardiomyocytes are removed from the pellet at regular intervals with a pipette and the osmotic fragility is measured by incubating the cells in hypotonic (85 mOsm) buffer containing a vital dye. Oxygenated myocytes tolerate this osmotic stress (no staining), but in the pellet the number of stained cells progressively increases until, by 2 hours, approximately 65% are stained. It is thought that this fragility contributes to necrosis since the ischemic myocyte in situ is also subjected to severe swelling. The pellet model appears to be an excellent mimic of the infarct model with respect to preconditioning. Cellular models not only eliminate the effects of noncardiac tissue, but also, because of their small volume, allow experiments to be performed with exotic agents and techniques that might be too expensive or otherwise impossible to use in a whole heart.

## PROTECTION IS TRIGGERED BY RECEPTORS

Early studies revealed that classic preconditioning did not involve opening of coronary collaterals, induction of antioxidants, synthesis of protective proteins, or changes in mitochondrial ATPases.<sup>2</sup> The first breakthrough came when it was demonstrated that protection was receptor-mediated.<sup>14</sup> During ischemia, numerous agents are released by the myocardium, including adenosine, catecholamines, angiotensin II, bradykinin, and endothelin.<sup>14</sup> All of these agents can occupy receptors on cardiac cells and, as will be explained below, all can contribute to preconditioning.

Adenosine is perhaps the prototypical byproduct of catabolism within the ischemic cell. It is produced by the heart when there is a net breakdown of ATP. Removal of the two high-energy phosphates from ATP leaves AMP. The latter is dephosphorylated by 5'-nucleotidase to produce free adenosine, which can easily exit the cell. Once in the interstitial space, adenosine can bind to surface receptors on the cardiomyocyte. In 1991, we reported that adenosine played an important role in ischemic preconditioning.<sup>14,15</sup> We observed that adenosine receptor blockers aborted preconditioning's protection in rabbits, but had little effect on nonpreconditioned hearts (*Figure 3*). Furthermore, a 5-minute intracoronary infusion of either adenosine or *R*(-)-*N*<sup>6</sup>-(2-phenylisopropyl) adenosine (*R*-PIA), a selective agonist for the adenosine A<sub>1</sub> receptor (A<sub>1</sub> receptors are responsible for the bradycardic effect of adenosine, while activation of A<sub>2</sub> receptors causes vasodilation), in lieu of the 5-minute preconditioning ischemia, mimicked protection (*Figure 3*). It seemed obvious to us at that time that adenosine production by the ischemic cardiomyocyte was central to this preconditioning phenomenon, and we concluded that adenosine, acting through its A<sub>1</sub> receptors, triggered preconditioning. The adenosine hypothesis has now been supported by many studies in rabbits, pigs, dogs, and even man.<sup>16,17</sup>

## WHY ADENOSINE WOULD NOT BE EFFECTIVE AS A PRECONDITIONING AGENT IN THE CLINIC

At first, it appeared that the mystery of the preconditioning phenomenon had been solved, and that it would be simple to reproduce preconditioning in a clinical setting by treating individuals with adenosine

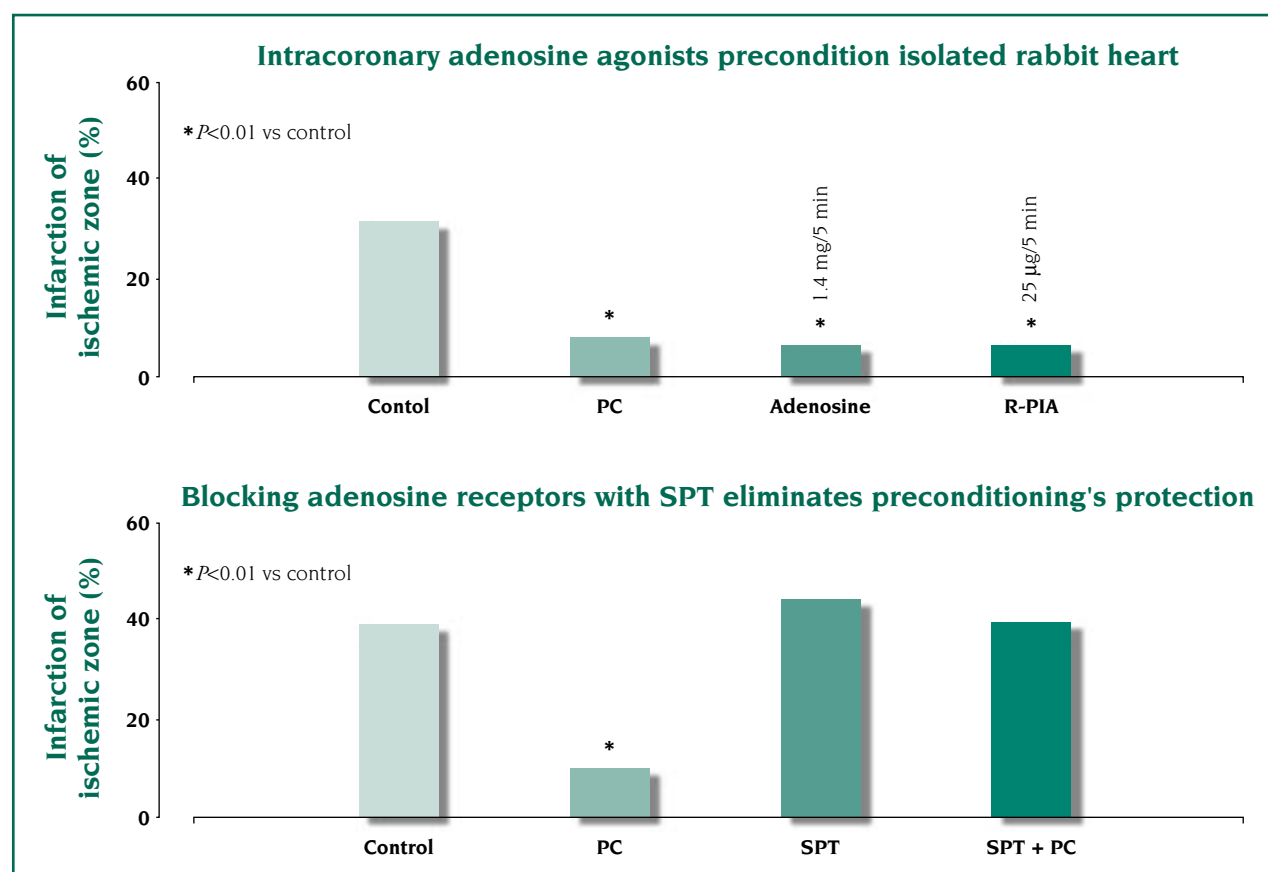


agonists. However, these early conclusions were much too naive. Three basic problems have confounded the clinical use of adenosine as a cardioprotectant.

Although there are reports that adenosine can be effective as an anti-infarct agent when given after the onset of ischemia, our experience and that of many others has been that adenosine can only induce salvage when given as a pretreatment.<sup>18</sup> The ischemic heart releases large amounts of adenosine within seconds after the onset of ischemia, and, as a result, the A<sub>1</sub> receptors are saturated. Consequently, it is not surprising that additional exogenous adenosine offers no further protection to the nonpreconditioned heart. Furthermore, pretreatment is seldom a possibility in the setting of acute myocardial infarction, and, therefore, adenosine as a preconditioning agent has been relegated to treatment of planned, iatrogenic

ischemia such as that seen during cardiac surgery or angioplasty.

Finally, prophylactic treatment with adenosine agonists has also been fraught with problems. The window of protection from a single dose of adenosine at best lasts for only 1 hour. If the adenosine is continuously infused, then the A<sub>1</sub> receptors quickly downregulate.<sup>14</sup> When we gave a rabbit a continuous infusion of an A<sub>1</sub>-selective adenosine agonist, the preconditioned state was lost within 72 hours of the onset of the infusion. Prolonged intermittent rather than continuous exposure to adenosine agonists holds more promise (see below). Because of these pitfalls, investigators have been looking beyond receptors and deeper into the signal transduction system with the hope that they will find a point amenable to intervention.



**Figure 3.** In the top panel, the effect on infarct size of 5-minute intracoronary infusions of adenosine and R(-)-N<sup>6</sup>-(2-phenylisopropyl)adenosine (R-PIA), an A<sub>1</sub>-selective adenosine agonist, in isolated, perfused rabbit hearts is compared to the effect of 5 minutes of ischemia (PC) before the standard 30-minute coronary occlusion. Whether the heart was preconditioned pharmacologically or with ischemia, infarction as a percentage of jeopardized myocardium or the ischemic zone was 25% of that observed in nonpreconditioned control hearts experiencing only the 30-minute coronary occlusion ( $P < 0.01$ ). These data strongly suggest that adenosine released during coronary occlusion participated in triggering protection.

To further document this proposal, the adenosine receptor antagonist 8-(p-sulfophenyl) theophylline (SPT) was administered to in situ rabbit heart preparations prior to the 5-minute preconditioning ischemia. As seen in the lower panel, this agent aborted the protective effect of brief ischemia (PC). Therefore, these data supported a central role for adenosine in preconditioning. Adapted from Liu et al.<sup>15</sup>

### PROTEIN KINASE C APPEARS TO BE PART OF THE SIGNAL TRANSDUCTION PATHWAY FOR PRECONDITIONING

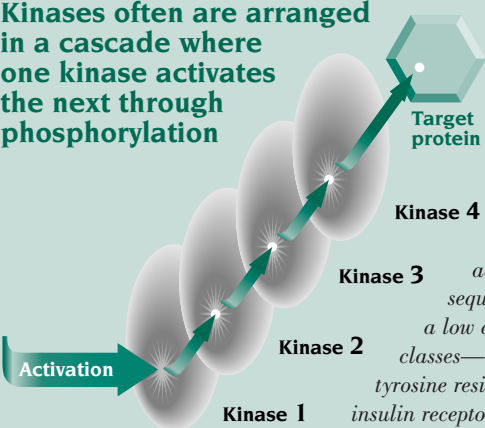
Adenosine binds to the extracellular domain of a complex membrane-spanning protein receptor, which, when occupied, activates a messenger protein, termed a G protein. Once activated, the G protein causes a number of events in the cell, including activation of protein kinases (*Box 1*). Current evidence suggests that adenosine and its receptors elicit protection through the activation of one particular protein kinase, protein kinase C (PKC)<sup>14,19</sup> (*Box 2*). Specific inhibitors of PKC will abort protection from an ischemic preconditioning protocol in rabbit and rat hearts, but have little effect on nonpreconditioned hearts. The same has been seen in isolated human cardiomyocytes.<sup>3</sup> Also, direct activators of PKC such as phorbol esters or diacylglycerols can mimic the protection of preconditioning in a variety of models, including human cardiomyocytes<sup>3</sup> and human atrial muscle strips.<sup>4</sup>

Once it became apparent that PKC was a critical component of the preconditioning pathway, we reasoned that any agonist capable of activating PKC should be equally capable of mimicking preconditioning's anti-infarct effect. PKC-coupled receptors on the cardiomyocyte include the angiotensin AT<sub>1</sub>, α<sub>1</sub>-adrenergic, bradykinin B<sub>2</sub>, and endothelin ET<sub>1</sub> receptors. Perhaps not surprisingly, all have been shown to be capable of mimicking preconditioning's protection in ischemic myocardium.<sup>14</sup> The only thing all of these receptors appear to have in common is their coupling to PKC.

### NOT ALL FINDINGS SUPPORT THE PROTEIN KINASE C HYPOTHESIS OF PRECONDITIONING

It should be noted, however, that attempts to confirm the PKC hypothesis in the dog and pig have, to date, not been universally successful. Increasingly, the evidence suggests that a true species difference

**Kinases often are arranged in a cascade where one kinase activates the next through phosphorylation**

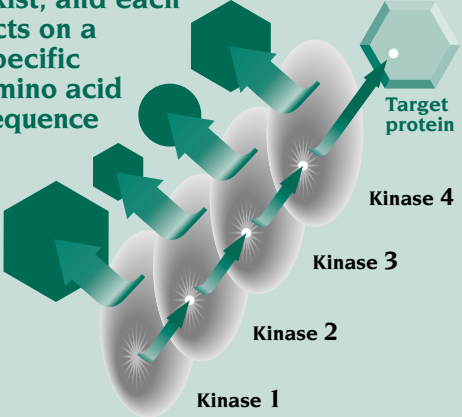


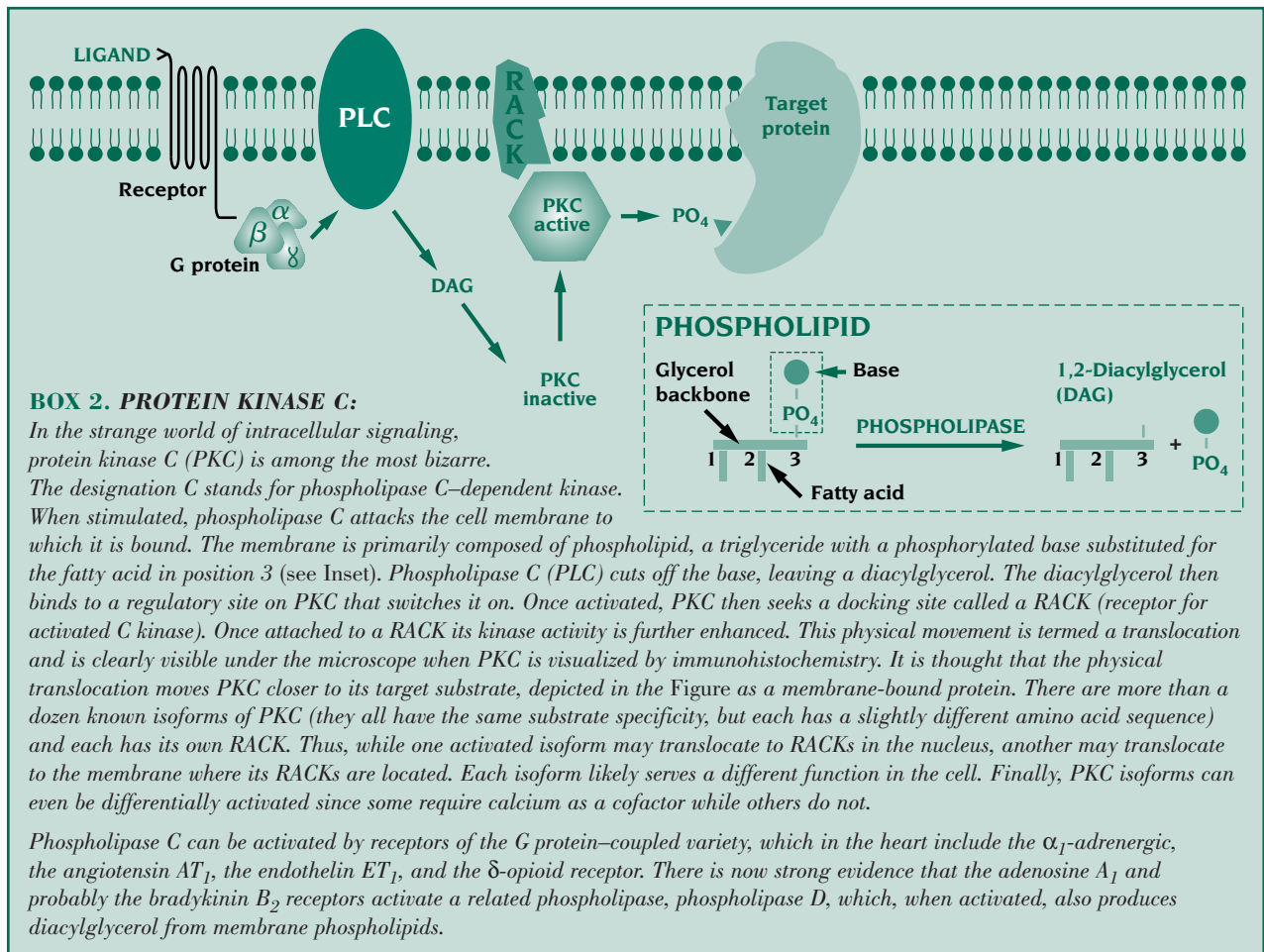
**BOX 1. PROTEIN KINASES: THE CELL'S NERVOUS SYSTEM:**

The cell regulates the function of many intracellular proteins through phosphorylation of key sites on the molecule. This phosphorylation is done by protein kinases, which take a phosphate from ATP and place it on an amino acid on the target protein. Once phosphorylated the protein's activity is altered. The number of kinases present in the cell are unknown, but probably is in the hundreds. Each protein kinase has a substrate specificity determined by a sequence of amino acids in the target protein. For example, protein kinase A (PKA), the kinase responsible for the increase in cardiac contractility following adrenergic stimulation, will only phosphorylate a serine residue if it occurs in the sequence Arg-Arg-X-Ser-X (where X indicates any amino acid). Other kinases have a low affinity for this particular sequence. Kinases can be divided into two broad classes—those that phosphorylate a serine or a threonine residue, and those that target a tyrosine residue. The latter family includes both membrane-bound receptors, such as the insulin receptor, which when bound to an insulin molecule will cause the receptor to autophosphorylate its own tyrosine residues, and soluble proteins inside the cell. Protein kinase C and the mitogen-activated kinases, on the other hand, belong to the former family of serine/threonine kinases. Receptor serine/ threonine kinases are so far unknown.

Protein kinases are often arranged in elaborate cascades in which one kinase will phosphorylate another kinase, which in turn phosphorylates yet another and so on until the end-effector is finally phosphorylated. While kinases put phosphate groups on the proteins, phosphatases take them off. Similar to the remarkable substrate specificity of kinases, phosphatases also have preferred substrates. Not surprisingly, there are many phosphatases in the cell, and these are also under careful regulation by cellular processes, including phosphorylation. Thus, the phosphorylation state of any protein depends on the balance between its kinases and its phosphatases. Because specific activators and inhibitors often exist for each of the kinases, modulation of a kinase or the corresponding phosphatase represents an excellent target for drug intervention.

**Phosphate groups are removed by phosphatases. Many phosphatases exist, and each acts on a specific amino acid sequence**





may be present, particularly in the pig.<sup>20</sup> However, because available data indicate that human myocardium exhibits a PKC-dependent form of ischemic preconditioning,<sup>3,4</sup> we believe that data from rats and rabbits may be more clinically pertinent than those from dogs and pigs. Nevertheless, the species differences cannot be ignored.

Some investigators have questioned the overall PKC hypothesis because of a lack of direct biochemical evidence for activation of PKC in preconditioned myocardium.<sup>21</sup> By necessity, the agonist/antagonist studies described above are dependent on the demonstrated specificity of these agents, and admittedly no enzyme substrate or blocker is truly completely specific for the intended target. However, it is currently technically impossible to directly measure PKC's activity in a cell since its activity is modulated by stimulating cofactors such as diacylglycerol, calcium, and phosphatidylserine, all of which are altered during processing of the tissue.

PKC exists in the cell not as a single protein but as many different proteins, all with a slightly different structure and each capable of phosphorylating substrate. These are termed isoforms. When activated, each isoform of PKC physically binds to its respective docking protein called a RACK (receptor for activated C-kinase) during its activation. This docking can be seen as a physical movement from the cytosol to specific structures within the cell. When activated, each isoform seems to translocate to a different intracellular structure such as the nucleus, the membrane, or a cytoskeletal element. Several studies have looked for such translocations in preconditioned myocardium in an attempt to determine if a particular isoform has been activated. Some studies find translocation in preconditioned hearts, while others do not, depending on the species examined and the technique employed. If PKC is involved in preconditioning, it is likely that only one of these isoforms actually participates, but nobody knows which one should be examined. Also, because

PKC's precise role, if any, is undefined, no one is sure exactly when the translocation occurs.

There are alternatives to the PKC hypothesis, and other second messengers have been suggested to mediate preconditioning's protection. For example, cyclic guanosine monophosphate (GMP) has been proposed to be responsible for the antiarrhythmic effect of preconditioning in dogs.<sup>8</sup> However, the evidence for this effect is inconclusive.

### **MULTIPLE MEDIATORS ENSURE THAT ISCHEMIA WILL PRECONDITION THE HEART**

The PKC hypothesis of preconditioning further broadens the possibilities of pharmacological application because many agonists of PKC-coupled receptors in addition to adenosine are released by the ischemic myocardium, and exogenous administration of each can trigger protection.<sup>14</sup> This causes the preconditioning mechanism to be highly redundant, teleologically an important adaptive survival mechanism. It can be shown in rabbit heart that bradykinin and opioid receptors participate equally with adenosine to trigger the protective state. For example, a bradykinin receptor antagonist will block protection from a single 5-minute occlusion, which is just above the threshold for protection.<sup>22</sup> Protection returns, however, if three 5-minute cycles of ischemia are used, which is presumably related to increased stimulation of the remaining receptors. We see similar behavior with opioid receptors. Other potential triggers, angiotensin II, norepinephrine, and endothelin, are also released by ischemic myocardium, and because their receptors are PKC-coupled, brief exogenous administration of any of these mimics preconditioning and protects myocardium from infarction during a coronary occlusion.<sup>14</sup> However, if a specific antagonist to the receptors for any of these three mediators is given to a rabbit, protection from a single 5-minute period of ischemia cannot be aborted. Therefore, presumably, these three agents are released in quantities too small to have a measurable effect in triggering preconditioning in the rabbit heart. In other species, these proportions may be different. As in rabbit, adenosine appears to be a physiological trigger in man.<sup>3,16,17</sup> Although adenosine was initially felt not to be a physiological trigger in rat,<sup>23</sup> it appears that adenosine is released by ischemic myocardium in amounts much greater than in the rabbit. As a result, a significantly higher dose of a competitive adenosine antagonist is required to block

the adenosine receptors in a rat heart.<sup>24</sup> If a higher dose of adenosine receptor inhibitor is used in a rat model, protection from ischemic preconditioning can also be aborted.

### **FREE RADICALS CONTRIBUTE TO THE PROTECTION AS WELL**

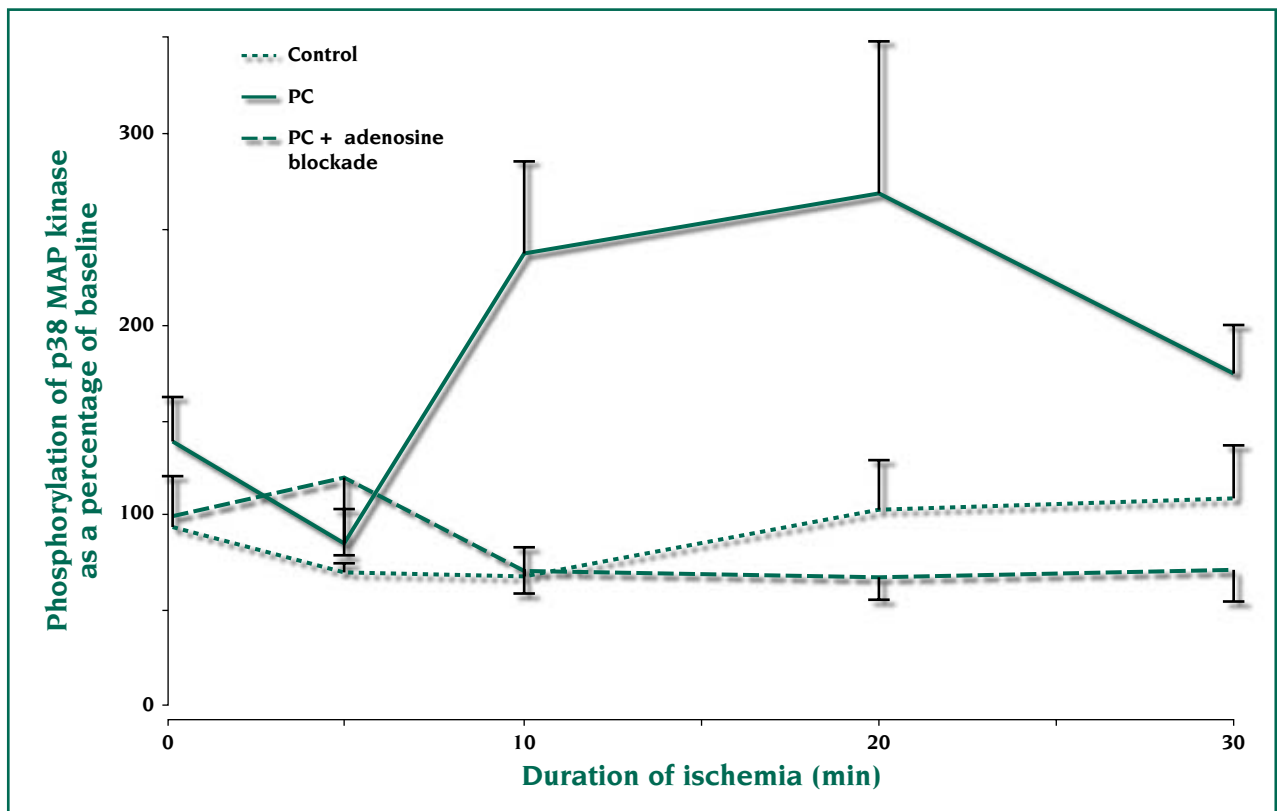
Free radicals are noteworthy for their touted toxic effects on cell organelles and membranes and the intracellular biochemical machinery. Paradoxically, an additional trigger of preconditioning is the free radicals generated when the heart is reperfused at the end of the preconditioning ischemia. Free radicals are known to directly stimulate PKC and can by themselves induce preconditioning.<sup>25</sup> In the rabbit, a free-radical scavenger can also block preconditioning's protection from a single 5-minute occlusion, but not from multiple preconditioning cycles.<sup>26</sup>

### **TYROSINE KINASES**

Although prevailing knowledge of cell signaling following receptor activation made it likely that PKC was part of the pathway leading to preconditioning's protection, it was not obvious what lay beyond. Recent experiments have shed light on the signal transduction pathway used in ischemic preconditioning distal to PKC. An understanding of this pathway is more than a mere academic pursuit. Selection of the best site for clinical intervention is dependent on such an appreciation. PKC belongs to a broad group of kinases that phosphorylate substrate proteins at either a serine or a threonine residue. In addition to serine/threonine kinases in the cell, there is also a class of kinases that phosphorylate tyrosine residues. At least one tyrosine kinase appears to be present in the rabbit's signal transduction pathway since tyrosine kinase blockers abort protection in preconditioned hearts, but have no effect on infarction in the nonpreconditioned heart.<sup>27</sup> Furthermore, this tyrosine kinase is thought to be downstream of PKC since blockade of tyrosine kinases in the rabbit heart blocks protection from direct activation of PKC by a phorbol ester.<sup>27</sup>

### **COULD P38 MAP KINASE BE INVOLVED?**

A group of kinases called the mitogen-activated protein (MAP) kinases is of great interest to molecular biologists. These kinases are part of a complex series of kinase



**Figure 4.** Phosphorylation of tyrosine 182, one of the activation sites of p38 MAP kinase, in biopsies of rabbit left ventricle, is presented on the vertical axis. Note that p38 MAP kinase is phosphorylated during ischemia only if the heart has been previously preconditioned. Blockade of protection with SPT [ $\beta$ -(*p*-sulfophenyl)theophylline], an adenosine  $A_1$  receptor antagonist, abolishes this increased phosphorylation. Reprinted from *J Mol Cell Cardiol* (1997;29:2383-2391), Copyright © 1997, Academic Press Limited (ref 29).

cascades that are intimately involved in gene expression in the cells. One member of this family is the p38 (p38 refers to a molecular weight of 38 kd) MAP kinase. This is sometimes referred to as a stress-activated MAP kinase since it is activated during cellular stresses including exposure to endotoxin, hydrogen peroxide, heat, and ischemia. A prime suspect for the implicated tyrosine kinase is the upstream activator of the p38 MAP kinase. p38 MAP kinase is activated by phosphorylation of both a tyrosine and a threonine residue. Once activated, it in turn phosphorylates MAPKAP kinase-2 (mitogen-activated protein kinase-activated protein kinase-2). MAPKAP kinase-2 then phosphorylates a 27-kd heat shock protein (HSP 27), which, when phosphorylated, promotes cytoskeletal actin filament polymerization.

Maulik et al<sup>28</sup> have found that ischemic preconditioning of the rat heart is associated with increased activity of p38 MAP kinase and MAPKAP kinase-2. These data support the hypothesis that a tyrosine kinase cascade is activated during preconditioning. Of course, these data do not prove whether this activation is critical to ultimate protection or whether it is an

epiphenomenon and is occurring as a consequence rather than a cause of the protection. In experiments in which we measured the phosphorylation state of p38 MAP kinase as an index of its activation, phosphorylation occurred during ischemia, but only if the heart had previously been preconditioned (Figure 4).<sup>29</sup> Furthermore, when protection in ischemically preconditioned hearts was blocked by an adenosine receptor antagonist, phosphorylation of p38 MAP kinase during ischemia no longer occurred.<sup>29</sup> Therefore, there was an obvious correlation between protection and p38 MAP kinase activation, strongly suggesting that the latter was a critical part of the pathway leading to protection rather than a simple epiphenomenon.

When p38 MAP kinase in isolated myocytes was directly activated with anisomycin, a compound that activates the p38 MAP kinase cascade without having effects on receptor tyrosine kinase, PKC, or the tyrosine kinase cascade (extracellular-regulated protein kinase [ERK] cascade) directly stimulated by PKC, the protection of ischemic preconditioning was mimicked.<sup>29</sup> Conversely, blockade of p38 MAP kinase with SB 203580, a selective,

stereospecific inhibitor of p38 MAP kinase without effect on any other known kinase or protein phosphatase, completely abolished preconditioning's protection in the isolated myocyte model.<sup>29</sup> These compelling data underscore the involvement of this stress-activated pathway and provide additional sites at which protection could be triggered. A major shortcoming of the p38 MAP kinase hypothesis is that most investigators fail to find a direct connection between activation of PKC and stimulation of p38 MAP kinase. Thus, the exact relationship between the two remains an enigma.

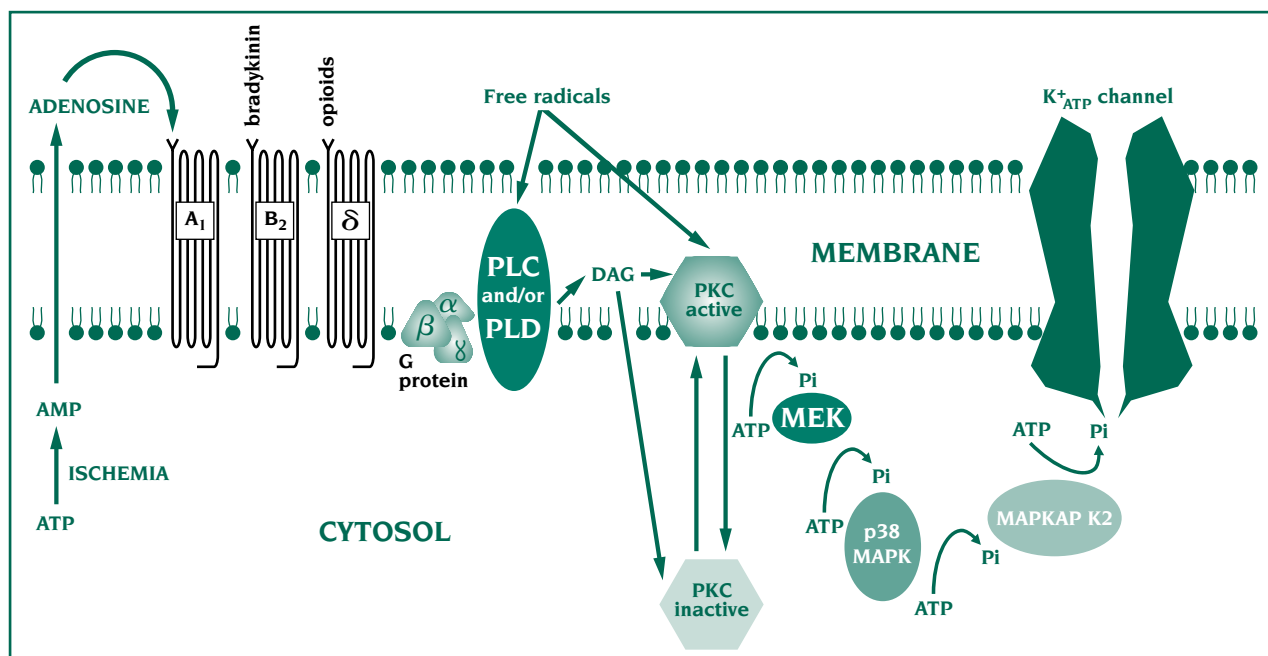
Figure 5 shows a simple diagram of the signal transduction pathways which we propose to be participating in ischemic preconditioning.

### COULD THE ATP-SENSITIVE POTASSIUM (K<sup>+</sup><sub>ATP</sub>) CHANNEL BE PRECONDITIONING'S END-EFFECTOR?

It is apparent that investigators have feverishly been looking for the end-effector—that protein or enzyme or ion channel which actually results in modification or remodeling of the cardiomyocyte to make it resistant

to the toxic effects of an ischemic milieu. There have been many suggestions regarding the possible identity of the end-effector, but none has yet been certified. Identification of the end-effector would undoubtedly accelerate prospects of clinical application.

The signal transduction pathway must act by phosphorylating some protein(s), which then become directly responsible for preconditioning's cardioprotective effect. The K<sup>+</sup><sub>ATP</sub> channel has repeatedly been proposed as the elusive end-effector. In canine heart, K<sup>+</sup><sub>ATP</sub> channel openers can mimic the protection of ischemic preconditioning, while channel blockers as glibenclamide and 5-hydroxydecanoate can abort the protection following brief ischemia (*see reference 30 for a recent review*). These observations have been made in many models including human atrial trabecular muscle.<sup>4</sup> In view of the results described above regarding the importance of activation of the p38 MAP kinase cascade to the protection of preconditioning, it is intriguing that recent unpublished studies from our laboratory using patch clamp measurements reveal that anisomycin also opens K<sup>+</sup><sub>ATP</sub> channels in rabbit cardiomyocytes. One problem with the K<sup>+</sup><sub>ATP</sub> hypothesis is the observation that K<sup>+</sup><sub>ATP</sub> blockers do not prevent



**Figure 5.** A proposed signal transduction pathway for ischemic preconditioning. Cell surface receptors, through their G proteins, activate phospholipases, which produce diacylglycerol, which in turn stimulates PKC. In addition, free radicals contribute by direct activation of PKC. PKC acts as a summing point for the signals from all of the activated receptors. We believe that PKC then protects by activating other kinases, leading to eventual phosphorylation of the end-effector (possibly the K<sup>+</sup><sub>ATP</sub> channel via the p38 MAP kinase cascade). Abbreviations: A<sub>1</sub>, adenosine receptor A<sub>1</sub>; AMP, adenosine monophosphate; ATP, adenosine triphosphate; B<sub>2</sub>, bradykinin receptor B<sub>2</sub>; DAG, diacylglycerol; Gp, G protein (α and βγ subunits); K<sup>+</sup><sub>ATP</sub> channel, ATP-sensitive potassium channel; MAPKAP K2, mitogen-activated protein-kinase-activated protein kinase-2; MEK, mitogen-activated protein kinase; p38 MAPK, mitogen-activated protein kinase of 38-kd molecular weight; Pi, inorganic phosphate; PKC, protein kinase C; PLC, phospholipase C; PLD, phospholipase D; δ, δ-opioid receptor.



preconditioning's protection in all animal models, most notably the rat.<sup>30</sup> Whether this is related to a failure of glibenclamide to adequately block those channels in that species or whether a different mechanism is present has not been resolved.

It is also unclear why opening  $K^+_{ATP}$  channels should be so protective. They could be exerting their effect on volume regulation of the myocytes, since opening of potassium channels along with chloride channels will oppose the osmotic swelling which threatens the ischemic myocyte. Initially, it was thought that  $K^+_{ATP}$  openers conserved energy by shortening the action potential, resulting in reduced calcium entry.<sup>30</sup> However,  $K^+_{ATP}$  openers even appear to protect nonbeating cardiomyocytes that have no action potentials.<sup>31</sup> An attractive hypothesis is that mitochondrial  $K^+_{ATP}$  channels may be responsible for the protection, which would explain why the latter is unrelated to effects on the action potential.<sup>32</sup> Opening of mitochondrial  $K^+_{ATP}$  channels could preserve mitochondrial function. Regardless of its mode of action, the  $K^+_{ATP}$  channel remains the most likely candidate for the ultimate end-effector. Direct openers of the  $K^+_{ATP}$  channel such as cromakalim and nicorandil are being investigated for their potential ability to protect ischemic myocardium. Because they tend to be powerful vascular smooth muscle relaxants, however, hypotension has been an undesirable side effect.

#### PERHAPS PRECONDITIONING STRENGTHENS THE CYTOSKELETON

Ganote and Armstrong have noted that during ischemia myocytes experience a predictable increase in their osmotic fragility, and have proposed that the latter is the result of changes in the cytoskeletal structure.<sup>33</sup> Additionally, they have observed that preconditioning myocardial cells with glucose-deficient medium, simulated ischemia, or adenosine analogs causes them to be more resistant to osmotic swelling at any time during ischemia.<sup>13</sup> Cells are filled with osmotically active proteins. To avoid swelling, the cell pumps out sodium, making extracellular sodium a counterbalancing osmolyte. During ischemia, the sodium pumps fail as ATP is depleted and the sodium gradient collapses. Furthermore, each mole of ATP is converted to 1 mole of AMP plus 2 moles of  $P_i$ . Thus, the osmotic pull of ATP is tripled. The net result is severe swelling in deeply ischemic tissue. Indeed, the mechanical disruption from swelling has even been proposed to be the lethal ischemic event.<sup>33</sup> Preconditioning could exert its final

effect by strengthening the cell's cytoskeleton to resist uncontrolled cell swelling and eventual sarcolemmal failure. Resistance to swelling and thus cell salvage may be the result of activation of the p38 MAP kinase cascade, another candidate for the end-effector. Phosphorylation of p38 MAP kinase and then MAPKAP kinase-2 sets the stage for phosphorylation of HSP 27. Whereas the unphosphorylated HSP 27 acts to inhibit polymerization of actin filaments, phosphorylated HSP 27 actually promotes actin polymerization, which should strengthen the cytoskeleton, making it more resistant to rupture. In this paradigm, protection does not derive from production of new HSP 27, but rather from phosphorylation of that already present in the cell.

#### 5'-NUCLEOTIDASE HAS BEEN PROPOSED AS AN END-EFFECTOR

Kitakaze and colleagues have proposed that increased 5'-nucleotidase activity is responsible for preconditioning's protection.<sup>34</sup> This enzyme dephosphorylates AMP (ATP's degradation product during ischemia) to adenosine, which is then free to leave the cardiomyocyte. The theory holds that preconditioned hearts produce more adenosine during ischemia, which then protects them by an as yet unidentified mechanism. Activation of PKC has been shown to increase the activity of 5'-nucleotidase, and in dog heart more adenosine was reported to be present in the coronary sinus during ischemia if the heart had been preconditioned.<sup>34</sup> Unfortunately, not all investigators have found that preconditioned hearts actually release more adenosine than their nonpreconditioned counterparts.<sup>35</sup> Furthermore, in one study, augmenting adenosine levels by two orders of magnitude with an adenosine deaminase inhibitor during regional ischemia in a canine model failed to mimic preconditioning's protection.<sup>36</sup> Thus, it has been difficult to prove a cause-and-effect relationship between the increased 5'-nucleotidase activity and preconditioning's protection. Also, the theory still does not explain why the additional adenosine would be so protective.

#### COULD PRECONDITIONING ACT TO CONSERVE ENERGY STORES DURING ISCHEMIA?

The oldest proposed theory to account for the observed protection is slowing of ATP utilization in ischemic myocardium that has been preconditioned, which might imply that a metabolic enzyme is involved. Unfortunately,

preserved ATP in the preconditioned heart has not been a universal finding. In preconditioned rat hearts, ATP actually falls more rapidly than in nonpreconditioned hearts.<sup>37</sup> Nevertheless, many other studies do indicate preservation of energetics in preconditioned hearts, and thus this hypothesis cannot be excluded at this time. Other theories that are being considered include activation of the vacuolar proton ATPase and even prevention of programmed cell death (apoptosis). Certainly, identification of the end-effector must receive high priority as our understanding of preconditioning—and more fundamentally, how ischemia kills myocardium—will remain incomplete without it.

### **PHARMACOLOGICAL PRECONDITIONING**

As pointed out above, receptor agonists are not well suited to protect hearts in the setting of acute myocardial infarction because of the need for pretreatment. In addition, most of the agonists to the receptors identified as capable of preconditioning the heart cause severe peripheral vascular effects, eg, hypertension (norepinephrine, angiotensin, endothelin) or hypotension (bradykinin and adenosine), which would prevent their parenteral administration. As a result they could only be effective if given by an intracoronary route. Recently, we have demonstrated that a 5-minute intravenous infusion of a cocktail of norepinephrine and adenosine (1:200) can fully precondition the rabbit heart.<sup>38</sup> Because of the diametrically opposed hemodynamic effects of these two agents, only a slight bradycardia was seen in the rabbits. This combination could be used to precondition hearts before revascularization surgery, especially those procedures performed with limited access techniques in which cardioplegic agents cannot be used. Of course, this cocktail is still not protective if given after ischemia has started, thus precluding its use in the setting of acute myocardial infarction.

Recently, we have examined a different class of compounds, the phosphatase inhibitors. In cell signaling, substrate proteins are phosphorylated by kinases. However, to extinguish the signal, the phosphate group must subsequently be removed by a phosphatase. The latter enzymes are critical to the successful functioning of the cell. In the cell, there are many phosphatases present, each with its own substrate specificity. Inhibition of a phosphatase should have an effect similar to that of stimulation of the associated kinase, ie, maintenance of a given substrate in its

phosphorylated form. Ganote and Armstrong<sup>33</sup> noted some time ago that okadaic acid, a protein phosphatase 2A inhibitor, was very protective of cardiomyocytes during simulated ischemia. We have recently tested fostriecin, a highly selective inhibitor of protein phosphatase 2A, in our isolated rabbit heart model.<sup>39</sup> Pretreatment of hearts with fostriecin in lieu of brief ischemia was as protective as ischemic preconditioning in the reduction of infarct size. Furthermore, when fostriecin infusion was started in the isolated rabbit heart model 10 minutes after ischemia had begun, protection was still evident.<sup>39</sup> This agent, therefore, is one of the first that we have seen to have efficacy when administered after the onset of ischemia. This observation is important since most individuals with acute myocardial infarction can only be treated after onset of the infarction process.

It is not known at which step fostriecin acts to confer this protection, but protein phosphatase 2A is known to deactivate p38 MAP kinase and affect phosphorylation of MAPKAP kinase-2 and HSP 27. Therefore, a phosphatase inhibitor may act to turn on the p38 MAP kinase cascade or some other protective kinase system in the heart. The requirement for pretreatment with receptor agonists such as adenosine is believed to result from delays in the signal transduction pathways. As a result, the end-effector fails to be activated in time to protect the nonpreconditioned heart. If that is the case, then intervention at a point further down the signal transduction pathway could bypass the delay point, and protection could theoretically be achieved even when the triggering agent is given after the onset of ischemia. Intuitively, in this paradigm, the earlier the agent were given, the more effective it would be. Fostriecin has been used in man with acceptable short-term toxicity, and thus could form the basis of an important new therapy.

### **WHAT IS THE MECHANISM OF PRECONDITIONING IN MAN?**

As explained in the article by Kloner in this issue, there is convincing evidence that human hearts can be preconditioned. In studies of excised human myocardial tissue and cells as well as whole heart responses monitored during coronary angioplasty, mechanisms of preconditioning remarkably similar to those observed in experimental animals have been documented.

Ikonomidis et al<sup>3</sup> studied cultures of human ventricular cardiomyocytes in which ischemia was simulated by



flushing the cells with 100% nitrogen. Separate cultures were preconditioned with a period of anoxia followed by reoxygenation. Preconditioning reduced the number of dead cells following 90 minutes of simulated ischemia. Interestingly, no effect on the ATP content during ischemia was found. Ikonomidis et al also found that addition of an adenosine receptor blocker abolished preconditioning's protective effect, while incubation of the myocytes in buffer supplemented with adenosine or the A<sub>1</sub> selective adenosine agonist R-PIA induced protection similar to that seen with simulated ischemia. Finally, PKC blockade abolished preconditioning's protection, and direct PKC stimulation with a phorbol ester mimicked it.

It is also possible to precondition human right atrial trabeculae.<sup>4</sup> Ischemia was simulated by suspending the muscle in a hypoxic bath and rapidly pacing it for 90 minutes followed by 120 minutes of reoxygenation. Preconditioning with 3 minutes of simulated ischemia followed by 10 minutes of recovery caused a greater return of developed tension after reoxygenation. Exposure of the muscle to an adenosine receptor agonist in lieu of brief simulated ischemia resulted in a similar protective effect. Preincubation of atrial trabeculae with the K<sup>+</sup><sub>ATP</sub> channel opener cromakalim or a PKC activator also improved postischemic function. Finally, preconditioning's protection could be blocked by a PKC antagonist or a K<sup>+</sup><sub>ATP</sub> blocker. Thus, human myocardium can be preconditioned, and the cellular mechanisms appear to be identical to those seen in rabbit heart.

Yellon et al<sup>40</sup> obtained biopsies from the anterior free wall of the left ventricle during coronary artery bypass surgery. Hearts were preconditioned with two episodes of 3 minutes of global ischemia during cross-clamping of the aorta. Each ischemic period was followed by 2 minutes of reperfusion. During this preconditioning protocol, the hearts were paced. These preliminary cross-clamping periods were absent in control hearts. All patients experienced 10 minutes of global ischemia caused by cross-clamping of the aorta with electrical ventricular fibrillation. Myocardial biopsies were analyzed for ATP content. ATP content after 10 minutes of ischemia was higher in the preconditioned group (12.0±1.1 μmol/g dry weight) compared with controls (6.8±0.2 μmol/g dry weight, *P*<0.05). This study suggests that ischemic preconditioning slows the rate of ATP depletion during ischemia in the human heart.

Deutsch and colleagues<sup>41</sup> examined the ECG tracing recorded in angioplasty patients during serial balloon

inflations. They noted that the ST-segment rose more rapidly during the first coronary occlusion than in subsequent occlusions, and attributed this change to possible preconditioning. Less pain and coronary sinus lactate production were also observed during the later occlusions. It was subsequently suggested that the change in the ST-segments may have reflected improved collateral flow in the subsequent coronary occlusions rather than any preconditioning effect. The ST-segment was therefore examined in the ischemic pig heart, which has a negligible collateral circulation. Indeed, ST-segment shifts evolved much more slowly during ischemia if the heart had previously been preconditioned.<sup>42</sup> We made a similar observation in preconditioned rabbit heart, also devoid of significant coronary collateral vessels.<sup>42</sup> Furthermore, blockade of protection in the rabbit with an adenosine receptor blocker abolished the beneficial effect of ischemic preconditioning on the ST-segment.<sup>42</sup> Thus, a reduced rate of rise of the ST-segment during ischemia appears to be a true property of the preconditioned heart.

Tomai and colleagues took this approach one step further. They reported that the adenosine receptor blocker bamiphylline could abolish the changes in the ST-segments during serial balloon inflations in angioplasty patients.<sup>16</sup> A similar effect on the ST-segment was seen in a subsequent study when they gave the K<sup>+</sup><sub>ATP</sub> blocker glibenclamide to patients during angioplasty. Conversely, several reports indicate that intracoronary adenosine in angioplasty patients caused changes in the ST-segment consistent with those seen with ischemic preconditioning.<sup>17</sup> The setting of angioplasty has emerged as a powerful tool for testing preconditioning-mimetic agents in man.

### **COULD PRECONDITIONING REDUCE MORTALITY IN MAN?**

Because of the sharp boundaries in perfusion between adjacent coronary branches and the transmural gradient in collateral blood flow, salvage from a preconditioning intervention would occur principally as a shrinkage of the infarct in the transmural direction rather than contraction of the lateral borders. Thus, an infarct originally stated to be transmural would become subendocardial. Little is known as to how effective such salvage would be in preserving global pump function.

In rabbits and dogs, preconditioned hearts behave as if the ischemic period were shortened by only 20 minutes. Because the ultimate infarct size would still depend

on the rapidity with which the occluded artery was reperfused, a 20-minute delay might be insignificant in the clinical setting since recanalization is typically accomplished several hours after the onset of symptoms. In rabbits, approximately 35% of the ischemic zone will infarct after 30 minutes of ischemia. The rate of infarction in humans is unknown, but studies on baboons reveal that infarction progresses at a much slower pace, almost one sixth that of the rabbit. Baboons can also be preconditioned to cause a further slowing of the infarction process (S. Vatner, personal communication). If human myocardium is like that of the baboon, then preconditioning may have a much more profound effect in the clinical setting than that predicted by experiments in laboratory animals.

To address the above issue, Kloner et al<sup>43</sup> examined the TIMI (Thrombolysis In Myocardial Infarction) database and found that patients who had one or more episodes of angina prior to an acute myocardial infarction had a lower incidence of both in-hospital mortality and congestive heart failure than those who did not report any antecedent angina. They concluded that the anginal attacks preceding the coronary thrombotic event preconditioned and thus protected the hearts. These observations have been echoed by other retrospective evaluations of populations suffering myocardial infarctions (see the article by Kloner in this issue).

### **THE "SECOND WINDOW OF PROTECTION"**

A substantial body of evidence has documented that preconditioning's early protective phase is followed by a delayed phase of protection occurring many hours later. This delayed phase of protection has been termed the "second window of protection."<sup>6</sup> The second window of protection was first reported in 1993 in both a rabbit and a canine model as an anti-infarct effect appearing 24 hours after a preconditioning stimulus consisting of repetitive cycles of coronary occlusion. This anti-infarct effect has subsequently been confirmed in a number of laboratories. In the rabbit, this delayed protection from a single preconditioning episode lasts between 2 and 3 days. Activation of PKC also appears to be involved in triggering the second window. PKC inhibitors given just prior to the preconditioning stimulus abort the protection against both infarction<sup>6</sup> and stunning<sup>44</sup> in the rabbit.

It has been assumed that the protection is related to the expression of new proteins. A large number of

proteins including proto-oncogenes, intracellular antioxidants, and heat shock proteins appear after a sublethal ischemic insult. The slow onset of appearance (12 to 24 h) and long duration (48 to 72 h) of this second window would be consistent with gene expression. Heat stress proteins were first considered as mediators of the second window. It has long been appreciated that organisms exposed to thermal stress acquire a tolerance to heating by expression of a family of heat stress proteins. Not surprisingly, heating anesthetized rats and rabbits was seen to induce both an expression of heat stress proteins, in particular the 70-kd HSP 70, and an increased tolerance to ischemia. The myocardial content of HSP 70 is similarly elevated 24 hours after preconditioning and has been proposed to account for the protection.<sup>45</sup> Others have proposed that protection is related to the expression of manganese superoxide dismutase (SOD), an antioxidant found in the heart's mitochondria.<sup>46</sup> While overexpression of HSP 70 can elicit protection of mouse hearts,<sup>6</sup> it has yet to be proven that either HSP 70 or MnSOD is directly responsible for the protection of the second window of preconditioning.

A third candidate for the protective end-effector of this "second window" phenomenon is nitric oxide synthase (NOS). Bolli's group reports that endogenously produced nitric oxide protects against stunning 24 hours after ischemic preconditioning in the rabbit.<sup>47</sup> When an NOS inhibitor was administered just prior to the ischemic insult on the second day of the protocol, it abolished the antistunning effect, suggesting that protection involves increased production of nitric oxide. Induction of NOS would be the logical explanation.

### **THE SECOND WINDOW MAY ALLOW PROPHYLACTIC TREATMENT OF HIGH-RISK PATIENTS**

The attractiveness of the second window is that it might be more amenable to prophylactic therapy. The cell-signaling pathways that trigger the second window appear to be similar to those used by classic preconditioning.<sup>6</sup> A single intravenous bolus of an adenosine A<sub>1</sub> receptor agonist offers significant protection against infarction from a subsequent lethal ischemic insult in rabbit myocardium starting 24 hours after drug administration and continuing for 48 to 72 hours.<sup>6</sup> More importantly, Yellon's group recently found that the protection could be renewed with no sign of receptor downregulation when a single bolus was injected every 2 days.<sup>48</sup>



Several years ago it was found that endotoxin stress could cause the induction of tolerance to ischemia just as was seen with heat stress. Subsequent studies suggested that the toxicity of endotoxin and its ability to trigger a second window-like protection were unrelated. As a result, a derivative, monophosphoryl lipid A, which has mild toxicity, but appears capable of inducing the second window, was synthesized.<sup>49</sup> Development of this interesting drug is continuing.

The second window of protection also seems to exert protection against ischemia-induced arrhythmias. A delayed antiarrhythmic effect following pacing-induced preconditioning has been reported for the dog heart.<sup>8</sup> In that model, the antiarrhythmic protection was almost completely lost after 24 hours in contrast to the anti-infarct effect in the rabbit, which lasts much longer. The explanation for the difference is not clear. It is not known whether the mechanism that protects against arrhythmias is the same as that protecting against infarction or stunning. Thus, each form of protection may have a completely different mediator. The other possibility is that the mechanisms are similar but that they wane faster in dog than in rabbit heart. A second window of protection against arrhythmias has only been reported to occur in dogs. Anesthetized rabbits have too few arrhythmias to detect any antiarrhythmic effect from preconditioning. Conscious rabbits, however, have a very high incidence of fibrillation with a coronary occlusion. We ischemically preconditioned conscious, instrumented rabbits 1 day prior to a coronary occlusion and experienced a striking reduction of ischemia-induced ventricular fibrillation, but the difference did not reach significance, possibly because of the relatively small numbers of animals studied.<sup>7</sup>

It is unknown whether a second window of protection occurs in humans, but it certainly does not appear in all animal species. The pig heart could not be protected against either infarction or stunning 24 hours after preconditioning.<sup>50</sup> Yet the pig heart exhibits a good classic preconditioning effect against infarction. A second window phenomenon could, however, explain why a decreased mortality occurs in infarct patients with antecedent angina even when the anginal episode was days prior to the coronary occlusion.<sup>43</sup> Patients having a history of angina in the 24-hour period prior to infarction have been studied.<sup>51</sup> The mean time between the last episode of angina and onset of infarction was around 11 hours, a time that clearly falls outside of the time frame of experimentally defined classic preconditioning. Yet the patients in whom angina heralded the infarction had a better prognosis

than those without angina. In another study, patients having had their last episode of angina a mean of 25 hours before onset of infarction clearly benefited.<sup>52</sup> Although the greatest benefit was seen in patients who had experienced angina closer to the onset of infarction, none of these patients experienced their last episode of angina within the time frame of classic preconditioning reported for animals, which is 60 to 90 minutes.

## CONCLUSION

Ischemic preconditioning demonstrates that preservation of ischemic myocardium is at least theoretically possible. Research in this area has already identified a wide variety of agents that can be used to precondition the heart pharmacologically. In situations such as surgery or angioplasty in which ischemia is anticipated, it is possible to protect the heart with one of the receptor agonists or even ischemia itself. As limited-access myocardial revascularization grafting gains in popularity, preconditioning-mimetics should play an increasingly important role since standard cardioplegia cannot be used in this setting. The largest target population, however, remains the patients presenting with acute myocardial infarction. Treatment could take the form of prophylactically keeping high-risk patients in the second window of protection, perhaps by periodic treatment with an A<sub>1</sub>-selective adenosine agonist or monophosphoryl lipid A. The other approach is to identify agents that can protect even after ischemia has begun. These might include a phosphatase inhibitor such as fostriecin. Because of its relatively mild side effects, an agent like fostriecin might be administered by emergency medical service personnel to any patient suspected of having a coronary thrombus. As our knowledge of preconditioning's mechanism grows, more and more strategies for protecting the ischemic myocardium are sure to emerge.

After this overview of our current knowledge on preconditioning, this issue of *Dialogues* will proceed to highlight three major aspects: Robert Kloner poses the essential question **"What is the evidence that preconditioning occurs in man?"** A related aspect concerns the "second window of preconditioning." Garrett Gross analyzes this concept and answers the question: **"Does the second window of preconditioning have any potential for clinical exploitation?"** A firm conceptual footing being thus established, Derek Yellon and Gary Baxter then turn to what can be reaped in terms of practical applications: **"Can acute preconditioning be mimicked and exploited with pharmacological agents?"**

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