Preconditioning

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Preconditioning: what it is and how it works

James M. Downey, PhD*; Michel V. Cohen, MD†

**Professor of Physiology, Department of Physiology; †Professor of Medicine, Department of Internal Medicine*
College of Medicine (MSB 3024) - University of South Alabama - Mobile, Ala 36688 - USA

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_1 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^+_{ATP}) 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 β-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.[1] 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,[2] including dog, rat, rabbit, and pig, as well as in human isolated cardiomyocytes[3] and atrial muscle.[4] 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.

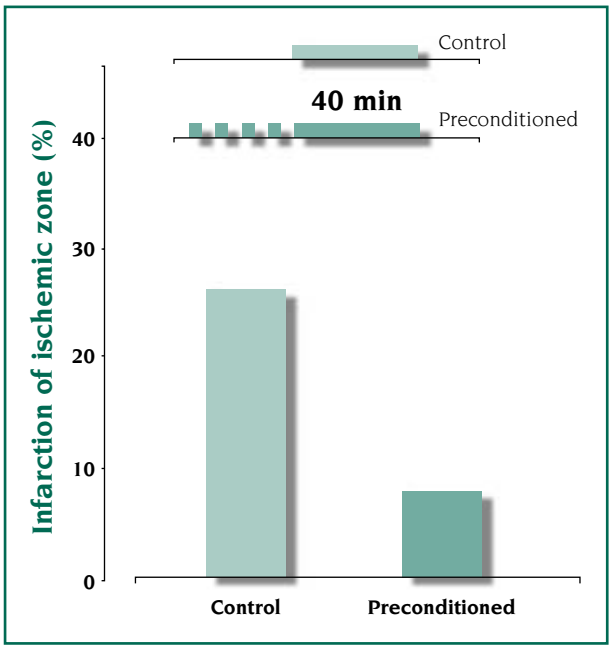


Figure 1. *In this pioneering study by Murry et al,[1] infarct size as a percentage of the ischemic zone was measured in dogs after either a 40-minute coronary occlusion (control) or a 40-minute occlusion preceded by four cycles of 5-minute occlusion/5-minute reperfusion (preconditioned) (see key in 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.*

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.[2] 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

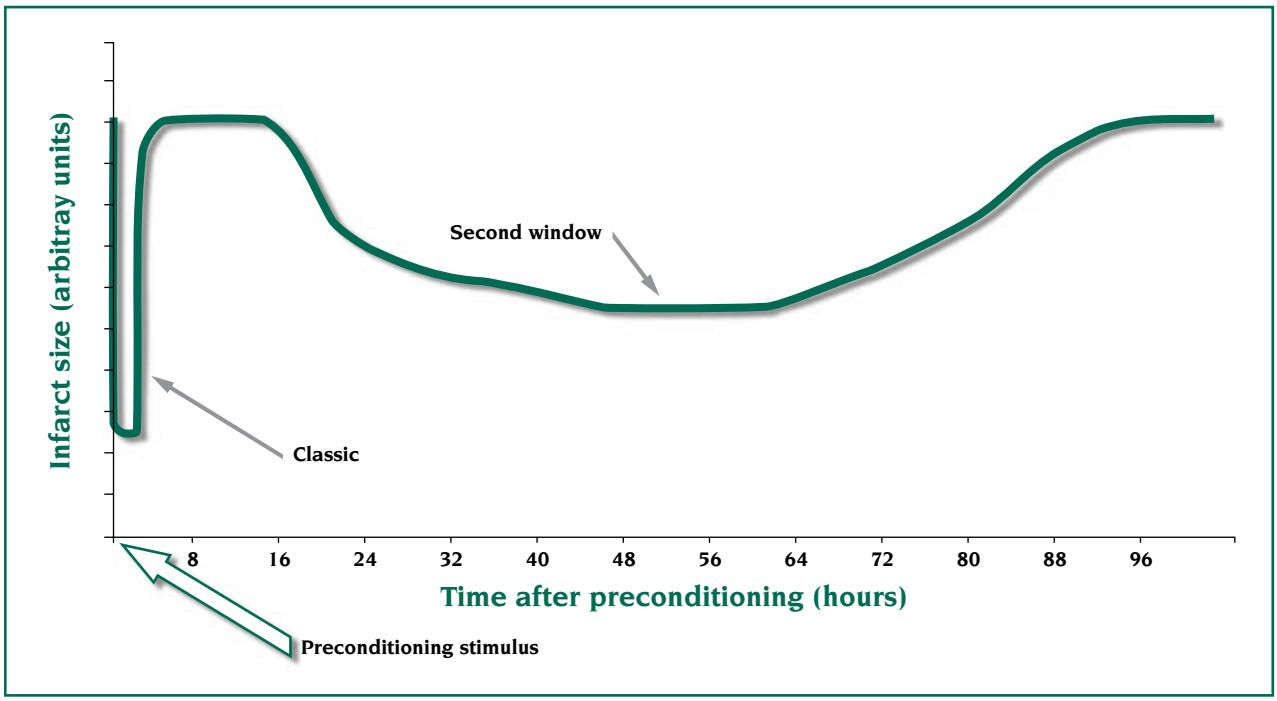


Figure 2. *Schematic representation of the timing and effectiveness of the classic and second window of preconditioning. Whereas myocardial protection related to the former is seen for only several hours after the preconditioning stimulus, protection of the second window appears between 16 to 24 hours 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.[2] In conscious rabbits, protection may last as long as 4 hours.[5] Interestingly, a second window of protection reappears 24 hours after preconditioning and may persist for as long as 2 to 3 days.[6]
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.[7] *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[8] and rat,[9] and perhaps the conscious rabbit model.[10] 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.[8] Classic preconditioning seems to offer little protection against stunned myocardium.[11] However, the second window of protection is associated with a strong antistunning effect in the rabbit heart.[12] 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.[4] 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,[1] 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[13] 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.[2] The first breakthrough came when it was demonstrated that protection was receptor-mediated.[14] During ischemia, numerous agents are released by the myocardium, including adenosine, catecholamines, angiotensin II, bradykinin, and endothelin.[14] 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.[14,15] 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^6-(2-phenylisopropyl) adenosine (R-PIA), a selective agonist for the adenosine A_1 receptor (A_1 receptors are responsible for the bradycardic effect of adenosine, while activation of A_2 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_1 receptors, triggered preconditioning. The adenosine hypothesis has now been supported by many studies in rabbits, pigs, dogs, and even man.[16,17]

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.[18] The ischemic heart releases large amounts of adenosine within seconds after the onset of ischemia, and, as a result, the A_1 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_1 receptors quickly downregulate.[14] When we gave a rabbit a continuous infusion of an A_1-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.

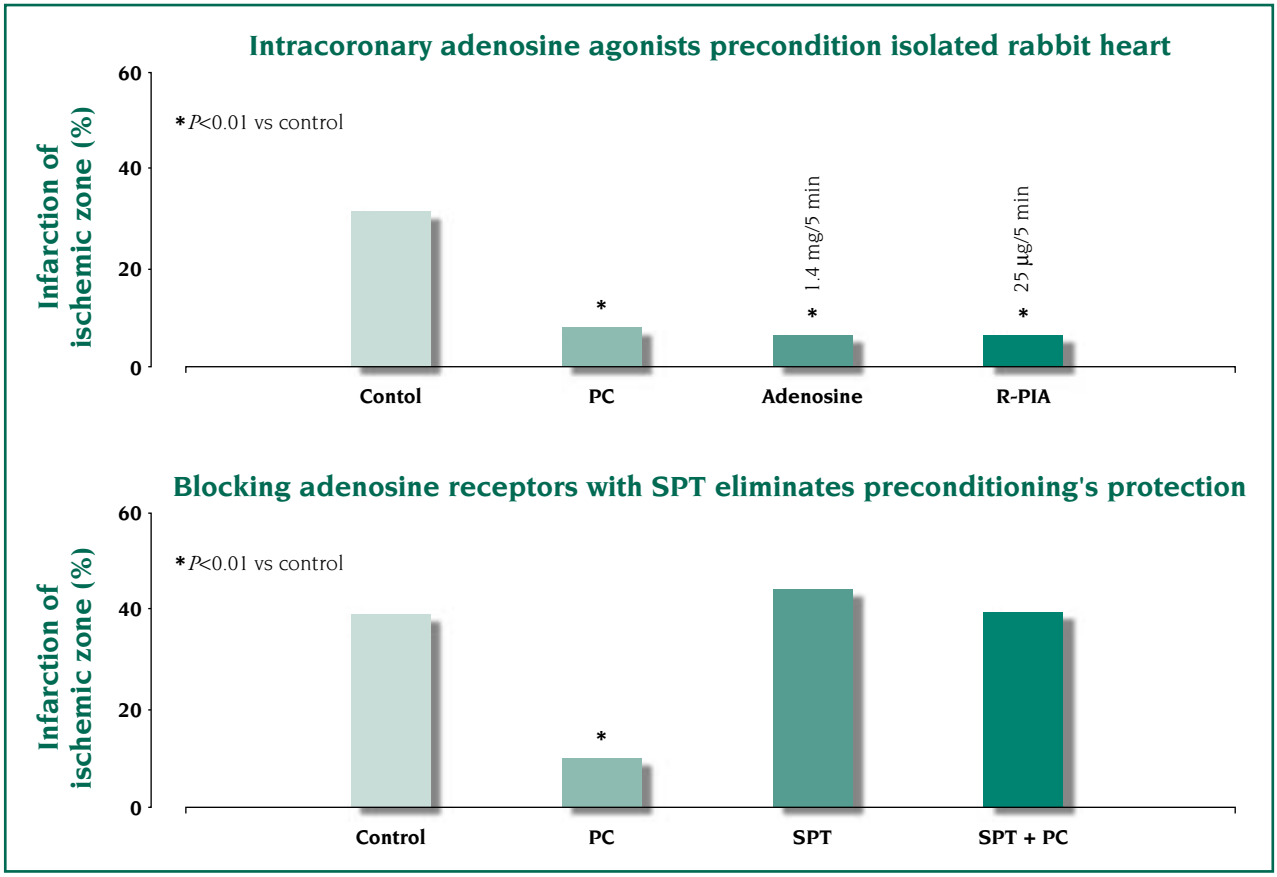


Figure 3. *In the top panel, the effect on infarct size of 5-minute intracoronary infusions of adenosine and* R(-)-N^6*-(2-phenylisopropyl)adenosine (R-PIA), an* A_1*-selective adenosine agonist, in isolated, perfused rabbit hearts is compared to the effect of 5 minutes of ischemia (PC) before the standard 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.*[15]

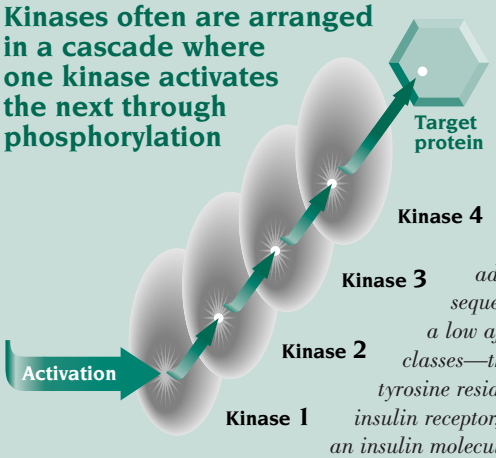
PROTEIN KINASE C APPEARS TO BE PART OF THE SIGNAL TRANSDUCTION PATHWAY FOR PRECONDITIONING

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)[14,19] *(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.[3] 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[3] and human atrial muscle strips.[4]

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_1, α_1-adrenergic, bradykinin B_2, and endothelin ET_1 receptors. Perhaps not surprisingly, all have been shown to be capable of mimicking preconditioning's protection in ischemic myocardium.[14] 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

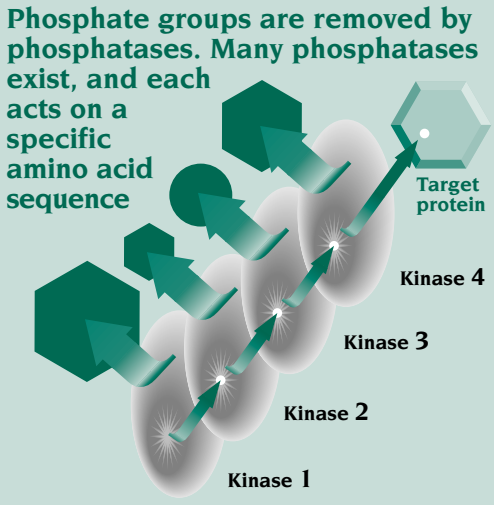
It should be noted, however, that attempts to confirm the PKC hypothesis in the dog and pig have, to date, not been universally successful. Increasingly, the evidence suggests that a true species difference

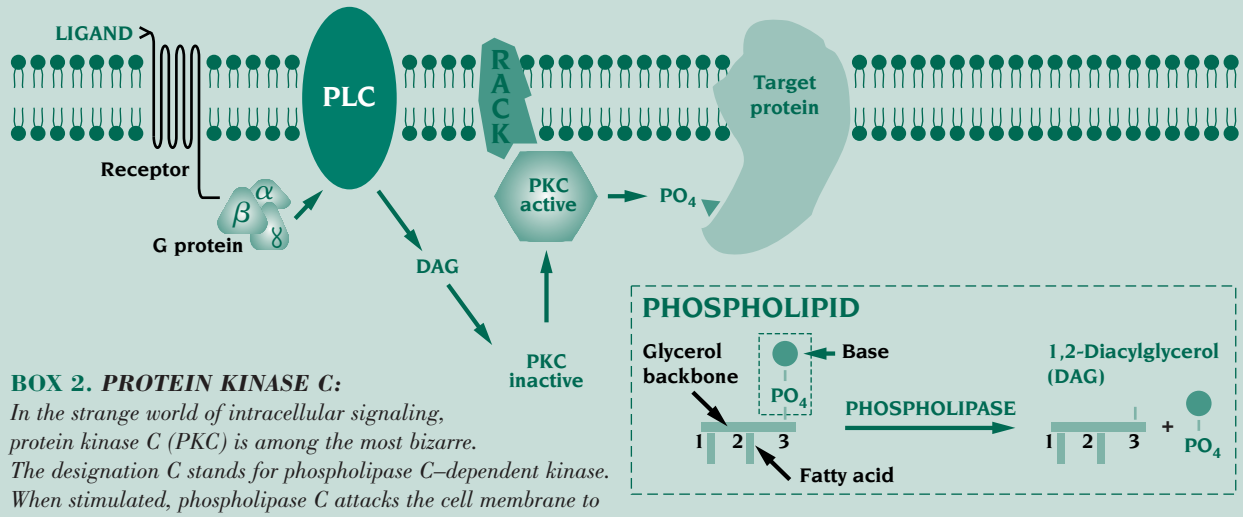


BOX 1. *PROTEIN KINASES: THE CELL'S NERVOUS SYSTEM:*

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.





BOX 2. *PROTEIN KINASE C:*

In the strange world of intracellular signaling, protein kinase C (PKC) is among the most bizarre. The designation C stands for phospholipase C–dependent kinase. When stimulated, phospholipase C attacks the cell membrane to which it is bound. The membrane is primarily composed of phospholipid, a triglyceride with a phosphorylated base substituted for the fatty acid in position 3 (see Inset). *Phospholipase C (PLC) cuts off the base, leaving a diacylglycerol. The diacylglycerol then binds to a regulatory site on PKC that switches it on. Once activated, PKC then seeks a docking site called a RACK (receptor for activated C kinase). Once attached to a RACK its kinase activity is further enhanced. This physical movement is termed a translocation and is clearly visible under the microscope when PKC is visualized by immunohistochemistry. It is thought that the physical translocation moves PKC closer to its target substrate, depicted in the* Figure *as a membrane-bound protein. There are more than a dozen known isoforms of PKC (they all have the same substrate specificity, but each has a slightly different amino acid sequence) and each has its own RACK. Thus, while one activated isoform may translocate to RACKs in the nucleus, another may translocate to the membrane where its RACKs are located. Each isoform likely serves a different function in the cell. Finally, PKC isoforms can even be differentially activated since some require calcium as a cofactor while others do not.*

Phospholipase C can be activated by receptors of the G protein–coupled variety, which in the heart include the α_1-adrenergic, the angiotensin AT_1, the endothelin ET_1, and the δ-opioid receptor. There is now strong evidence that the adenosine A_1 and probably the bradykinin B_2 receptors activate a related phospholipase, phospholipase D, which, when activated, also produces diacylglycerol from membrane phospholipids.

may be present, particularly in the pig.[20] However, because available data indicate that human myocardium exhibits a PKC-dependent form of ischemic preconditioning,[3,4] 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.[21] 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.[8] 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.[14] 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.[22] 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.[14] 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.[3,16,17] Although adenosine was initially felt not to be a physiological trigger in rat,[23] 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.[24] 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.[25] 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.[26]

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.[27] 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.[27]

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

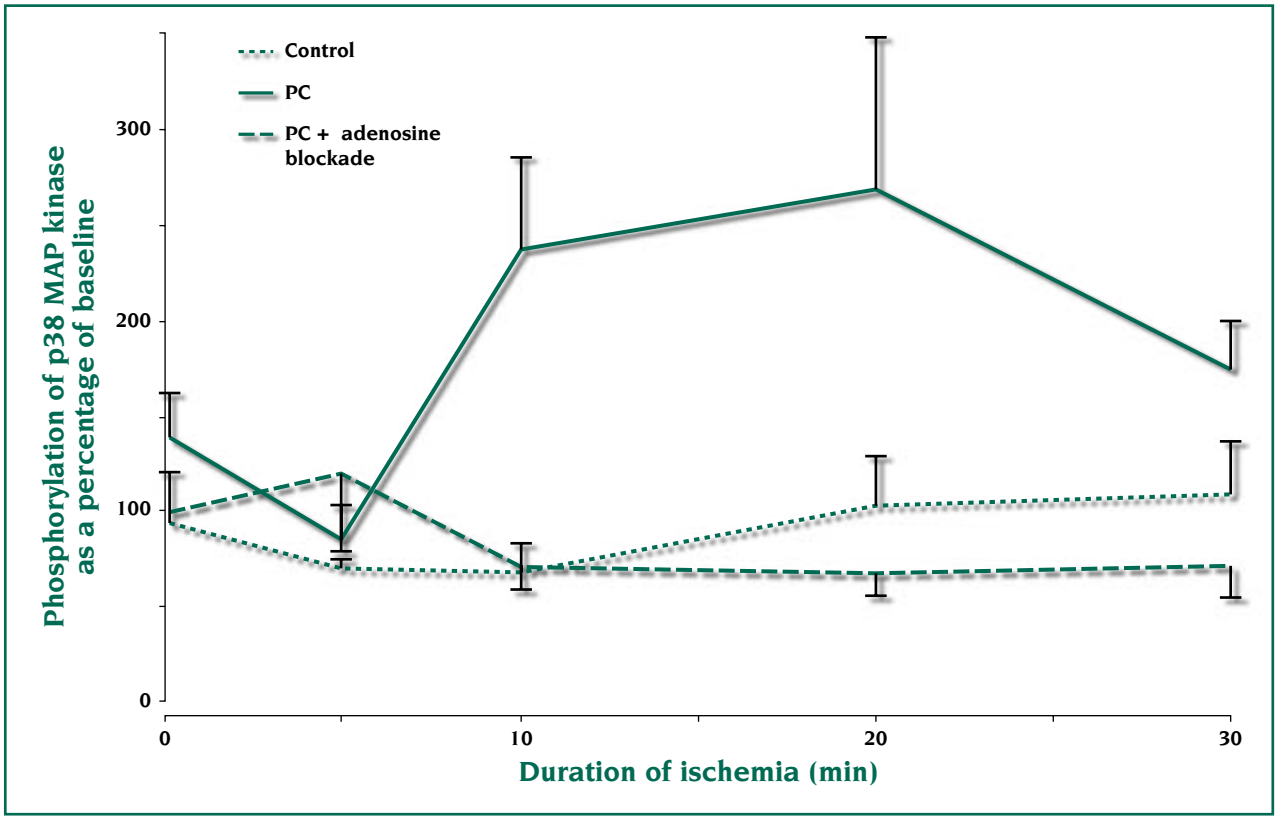


Figure 4. *Phosphorylation of tyrosine 182, one of the activation sites of p38 MAP kinase, in biopsies of rabbit left ventricle, is presented on the vertical axis. Note that p38 MAP kinase is phosphorylated during ischemia only if the heart has been previously preconditioned. Blockade of protection with SPT [8-(*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[28] 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)*.[29] Furthermore, when protection in ischemically preconditioned hearts was blocked by an adenosine receptor antagonist, phosphorylation of p38 MAP kinase during ischemia no longer occurred.[29] 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.[29] 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.[29] 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^+_{ATP}) 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^+_{ATP} channel has repeatedly been proposed as the elusive end-effector. In canine heart, K^+_{ATP} 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.[4] 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^+_{ATP} channels in rabbit cardiomyocytes. One problem with the K^+_{ATP} hypothesis is the observation that K^+_{ATP} blockers do not prevent

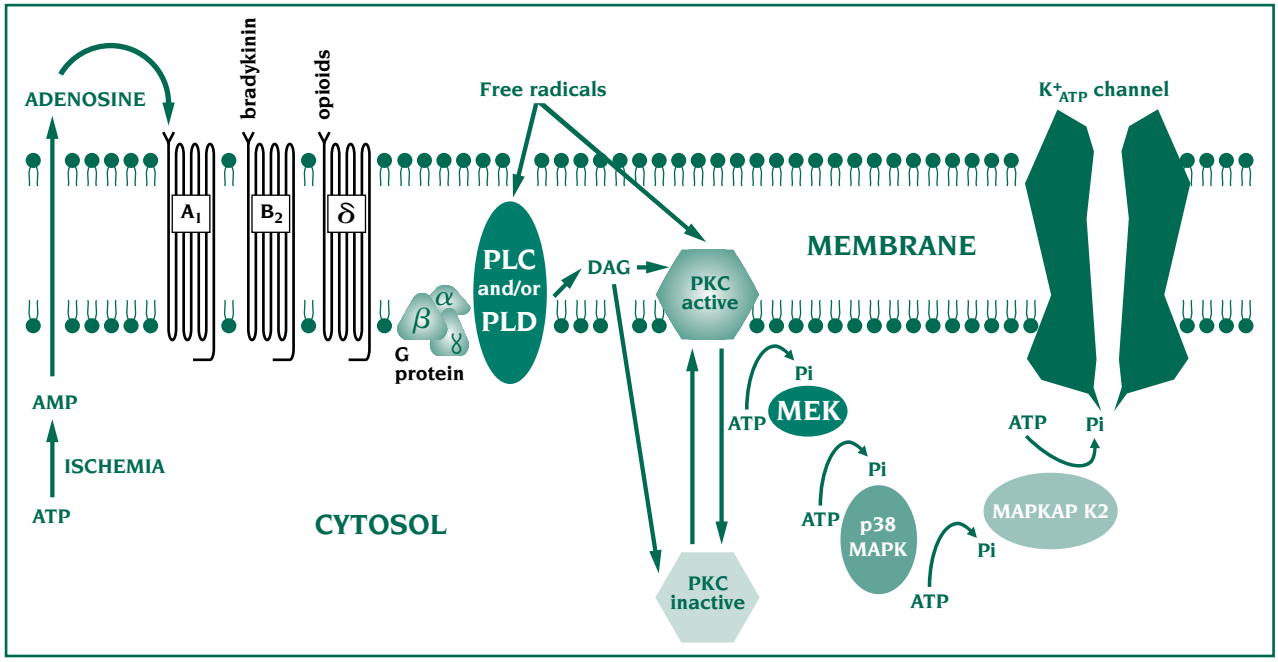


Figure 5. *A proposed signal transduction pathway for ischemic preconditioning. Cell surface receptors, through their G proteins, activate phospholipases, which produce diacylglycerol, which in turn stimulates PKC. In addition, free radicals contribute by direct activation of PKC. PKC acts as a summing 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^+_{ATP} channel via the p38 MAP kinase cascade). Abbreviations: A_1, adenosine receptor A_1; AMP, adenosine monophosphate; ATP, adenosine triphosphate; B_2, bradykinin receptor B_2; DAG, diacylglycerol; Gp, G protein (α and βγ subunits); K^+_{ATP} 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.[30] 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.[30] However, K^+_{ATP} openers even appear to protect nonbeating cardiomyocytes that have no action potentials.[31] 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.[32] 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.[33] 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.[13] 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.[33] 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.[34] 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.[34] Unfortunately, not all investigators have found that preconditioned hearts actually release more adenosine than their nonpreconditioned counterparts.[35] 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.[36] 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.[37] 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.[38] 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[33] 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.[39] 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.[39] 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[3] 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_1 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.[4] 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^+_{ATP} 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^+_{ATP} blocker. Thus, human myocardium can be preconditioned, and the cellular mechanisms appear to be identical to those seen in rabbit heart.

Yellon et al[40] 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[41] 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.[42] We made a similar observation in preconditioned rabbit heart, also devoid of significant coronary collateral vessels.[42] Furthermore, blockade of protection in the rabbit with an adenosine receptor blocker abolished the beneficial effect of ischemic preconditioning on the ST-segment.[42] 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.[16] A similar effect on the ST-segment was seen in a subsequent study when they gave the K^+_{ATP} 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.[17] 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[43] 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."[6] 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[6] and stunning[44] 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.[45] Others have proposed that protection is related to the expression of manganese superoxide dismutase (SOD), an antioxidant found in the heart's mitochondria.[46] While overexpression of HSP 70 can elicit protection of mouse hearts,[6] 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.[47] 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.[6] A single intravenous bolus of an adenosine A_1 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.[6] 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.[48]

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.[49] 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.[8] 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.[7]

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.[50] 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.[43] Patients having a history of angina in the 24-hour period prior to infarction have been studied.[51] 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.[52] 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_1-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?"**

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. Lawson CS, Downey JM.
Preconditioning: state of the art myocardial protection.
Cardiovasc Res. 1993;27:542-550.

3. Ikonomidis JS, Shirai T, Weisel RD, et al.
Preconditioning cultured human pediatric myocytes requires adenosine and protein kinase C.
Am J Physiol. 1997;272(3, pt 2):H-1220–H-1230.

4. Speechly-Dick ME, Grover GJ, Yellon DM.
Does ischemic preconditioning in the human involve protein kinase C and the ATP-dependent K^+ channel? Studies of contractile function after simulated ischemia in an atrial in vitro model.
Circ Res. 1995;77:1030-1035.

5. Burckhartt B, Yang XM, Tsuchida A, Mullane KM, Downey JM, Cohen MV.
Acadesine extends the window of protection afforded by ischaemic preconditioning in conscious rabbits.
Cardiovasc Res. 1995;29:653-657.

6. Yellon DM, Baxter GF.
A "second window of protection" or delayed preconditioning phenomenon: future horizons for myocardial protection?
J Mol Cell Cardiol. 1995;27:1023-1034.

7. Yang XM, Baxter GF, Heads RJ, Yellon DM, Downey JM, Cohen MV.
Infarct limitation of the second window of protection in a conscious rabbit model.
Cardiovasc Res. 1996;31:777-783.

8. Parratt JR, Vegh A, Kaszala K, Papp JG.
Protection by preconditioning and cardiac pacing against ventricular arrhythmias resulting from ischemia and reperfusion.
Ann N Y Acad Sci. 1996;793:98-107.

9. Shiki K, Hearse DJ.
Preconditioning of ischemic myocardium: reperfusion-induced arrhythmias.
Am J Physiol. 1987;253(6, pt 2):H-1470–H-1476.

10. Cohen MV, Yang XM, Downey JM.
Conscious rabbits become tolerant to multiple episodes of ischemic preconditioning.
Circ Res. 1994;74:998-1004.

11. Ovize M, Przyklenk K, Hale SL, Kloner RA.
Preconditioning does not attenuate myocardial stunning.
Circulation. 1992;85:2247-2254.

12. Sun JZ, Tang XL, Knowlton AA, Park SW, Qiu Y, Bolli R.
Late preconditioning against myocardial stunning: an endogenous protective mechanism that confers resistance to postischemic dysfunction 24 h after brief ischemia in conscious pigs.
J Clin Invest. 1995;95:388-403.

13. Armstrong S, Ganote CE.
Preconditioning of isolated rabbit cardiomyocytes: effects of glycolytic blockade, phorbol esters, and ischaemia.
Cardiovasc Res. 1994;28:1700-1706.

14. Cohen MV, Downey JM.
Myocardial preconditioning promises to be a novel approach to the treatment of ischemic heart disease.
Annu Rev Med. 1996;47:21-29.

15. Liu GS, Thornton J, Van Winkle DM, Stanley AWH, Olsson RA, Downey JM.
Protection against infarction afforded by preconditioning is mediated by A_1 adenosine receptors in rabbit heart.
Circulation. 1991;84:350-356.

16. Tomai F, Crea F, Gaspardone A, et al.
Effects of A_1 adenosine receptor blockade by bamiphylline on ischaemic preconditioning during coronary angioplasty.
Eur Heart J. 1996;17:846-853.

17. Leesar MA, Stoddard M, Ahmed M, Broadbent J, Bolli R.
Preconditioning of human myocardium with adenosine during coronary angioplasty.
Circulation. 1997;95:2500-2507.

18. Thornton JD, Liu GS, Olsson RA, Downey JM.
Intravenous pretreatment with A_1-selective adenosine analogues protects the heart against infarction.
Circulation. 1992;85:659-665.

19. Ytrehus K, Liu Y, Downey JM.
Preconditioning protects ischemic rabbit heart by protein kinase C activation.
Am J Physiol. 1994;266(3, pt 2):H-1145–H-1152.

20. Vahlhaus C, Schulz R, Post H, Onallah R, Heusch G.
No prevention of ischemic preconditioning by the protein kinase C inhibitor staurosporine in swine.
Circ Res. 1996;79:407-414.

21. Brooks G, Hearse DJ.
Role of protein kinase C in ischemic preconditioning: player or spectator?
Circ Res. 1996;79:627-630.

22. Goto M, Liu Y, Yang XM, Ardell JL, Cohen MV, Downey JM.
Role of bradykinin in protection of ischemic preconditioning in rabbit hearts.
Circ Res. 1995;77:611-621.

23. Li Y, Kloner RA.
The cardioprotective effects of ischemic "preconditioning" are not mediated by adenosine receptors in rat hearts.
Circulation. 1993;87:1642-1648.

24. Headrick JP.
Ischemic preconditioning: bioenergetic and metabolic changes and the role of endogenous adenosine.
J Mol Cell Cardiol. 1996;28:1227-1240.

25. Tritto I, D'Andrea D, Eramo N, et al.
Oxygen radicals can induce preconditioning in rabbit hearts.
Circ Res. 1997;80:743-748.

26. Baines CP, Goto M, Downey JM.
Oxygen radicals released during ischemic preconditioning contribute to cardioprotection in the rabbit myocardium.
J Mol Cell Cardiol. 1997;29:207-216.

27. Baines CP, Wang L, Cohen MV, Downey JM.
Protein tyrosine kinase is downstream of protein kinase C for ischemic preconditioning's anti-infarct effect in the rabbit heart.
J Mol Cell Cardiol. In press.

28. Maulik N, Watanabe M, Zu YL, et al.
Ischemic preconditioning triggers the activation of MAP kinases and MAPKAP kinase 2 in rat hearts.
FEBS Lett. 1996;396:233-237.

29. Weinbrenner C, Liu GS, Cohen MV, Downey JM.
Phosphorylation of tyrosine 182 of p38 mitogen–activated protein kinase correlates with the protection of preconditioning in the rabbit heart.
J Mol Cell Cardiol. 1997;29:2383-2391.

30. Gross GJ.
ATP-sensitive potassium channels and myocardial preconditioning.
Basic Res Cardiol. 1995;90:85-88.

31. Armstrong SC, Liu GS, Downey JM, Ganote CE.
Potassium channels and preconditioning of isolated rabbit cardiomyocytes: effects of glyburide and pinacidil.
J Mol Cell Cardiol. 1995;27:1765-1774.

32. Marban E, O'Rourke B, Liu Y.
Role of K_{ATP} channels in the mechanism of ischemic preconditioning.
J Mol Cell Cardiol. 1997;29:A36. Abstract.

33. Ganote C, Armstrong S.
Ischaemia and the myocyte cytoskeleton: review and speculation.
Cardiovasc Res. 1993;27:1387-1403.

34. Kitakaze M, Hori M, Takashima S, Sato H, Inoue M, Kamada T.
Ischemic preconditioning increases adenosine release and 5'-nucleotidase activity during myocardial ischemia and reperfusion in dogs: implications for myocardial salvage.
Circulation. 1993;87:208-215.

35. Van Wylen DGL.
Effect of ischemic preconditioning on interstitial purine metabolite and lactate accumulation during myocardial ischemia.
Circulation. 1994;89:2283-2289.

36. Silva PH, Dillon D, Van Wylen DGL.
Adenosine deaminase inhibition augments interstitial adenosine but does not attenuate myocardial infarction.
Cardiovasc Res. 1995;29:616-623.

37. Kolocassides KG, Seymour AML, Galiñanes M, Hearse DJ.
Paradoxical effect of ischemic preconditioning on ischemic contracture? NMR studies of energy metabolism and intracellular pH in the rat heart.
J Mol Cell Cardiol. 1996;28:1045-1057.

38. Cohen MV, Thornton JD, Thornton CS, Sato H, Miki T, Downey JM.
Intravenous coinfusion of adenosine and norepinephrine preconditions the heart without adverse hemodynamic effects.
J Thorac Cardiovasc Surg. 1997;114:236-242.

39. Weinbrenner C, Baines CP, Liu FS, et al.
Fostriecin, an inhibitor of protein phosphatase 2A, limits myocardial infarct size even when administered after onset of ischemia.
Circulation. In press.

40. Yellon DM, Alkhulaifi AM, Pugsley WB.
Preconditioning the human myocardium.
Lancet. 1993;342:276-277.

41. Deutsch E, Berger M, Kussmaul WG, Hirshfeld JW Jr, Herrmann HC, Laskey WK.
Adaptation to ischemia during percutaneous transluminal coronary angioplasty: clinical, hemodynamic, and metabolic features.
Circulation. 1990;82:2044-2051.

42. Downey JM, Cohen MV.
Preconditioning: markers vs epiphenomena.
Basic Res Cardiol. 1996;91:35-37.

43. Kloner RA, Shook T, Przyklenk K, et al.
Previous angina alters in-hospital outcome in TIMI 4: a clinical correlate to preconditioning?
Circulation. 1995;91:37-45.

---

44. Qiu Y, Tang XL, Rizvi A, et al.
Protein kinase C mediates late preconditioning against myocardial stunning in conscious rabbits.
Circulation. 1996;94(suppl I):I-184. Abstract.

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45. 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.

---

46. Hoshida S, Kuzuya T, Fuji H, et al.
Sublethal ischemia alters myocardial antioxidant activity in canine heart.
Am J Physiol. 1993;264(1, pt 2):H-33–H-39.

---

47. Bolli R, Bhatti ZA, Tang XL, et al.
Evidence that late preconditioning against myocardial stunning in conscious rabbits is triggered by the generation of nitric oxide.
Circ Res. 1997;81:42-52.

---

48. Dana A, Baxter GF, Walker JM, Yellon DM.
Prolonging the delayed phase of myocardial protection: anti-infarct effects of adenosine A_1 receptor activation are maintained despite repetitive dosing.
Heart. 1997;77(suppl I):P23. Abstract.

---

49. Elliott GT, Comerford ML, Smith JR, Zhao L.
Myocardial ischemia/reperfusion protection using monophosphoryl lipid a is abrogated by the ATP-sensitive potassium channel blocker, glibenclamide.
Cardiovasc Res. 1996;32:1071-1080.

---

50. Qiu Y, Tang XL, Park SW, Sun JZ, Kalya A, Bolli R.
The early and late phases of ischemic preconditioning: a comparative analysis of their effects on infarct size, myocardial stunning, and arrhythmias in conscious pigs undergoing a 40-min coronary occlusion.
Circ Res. 1997;80:730-742.

---

51. Ottani F, Galvani M, Ferrini D, et al.
Prodromal angina limits infarct size: a role for ischemic preconditioning.
Circulation. 1995;91:291-297.

---

52. Nakagawa Y, Ito H, Kitakaze M, et al.
Effect of angina pectoris on myocardial protection in patients with reperfused anterior wall myocardial infarction: retrospective clinical evidence of "preconditioning."
J Am Coll Cardiol. 1995;25:1076-1083.

Preconditioning

Expert Answers to Three Key Questions

1

What is the evidence that preconditioning occurs in man?

R.A. Kloner

2

What is the second window of preconditioning and does it have any potential for clinical exploitation?

G.J. Gross

3

Can acute preconditioning be mimicked and exploited with pharmacological agents?

D.M. Yellon, G.F. Baxter

What is the evidence that preconditioning occurs in man?

Robert A. Kloner, MD, PhD

Director of Research - Heart Institute - Good Samaritan Hospital - Professor of Medicine - University of Southern California - Los Angeles, Calif - USA

Preconditioning has been shown to be a powerful technique for reducing infarct size in every animal model tested. There is now evidence that the human heart can be preconditioned by ischemia and pharmacologic agents. Humans can adapt rapidly to brief ischemic insults, as shown by "warm-up" or "walk-through" angina pectoris. In vitro analysis of human tissue, angioplasty literature, and studies of preinfarction angina have also shown the reality of preconditioning in humans. The mechanisms whereby preinfarction angina confers benefit has been an area of considerable discussion. Preconditioning-like drugs might be administered to produce a cardioprotective effect in patients undergoing open heart procedures, or in patients with unstable angina pectoris or threatened myocardial infarction. Whether these agents could play any role during evolving myocardial infarction is less clear.

Cardiovascular disease remains the major killer of men and women in the Western world. In the United States, 1.5 million people a year develop myocardial infarctions; 500 000 die of myocardial infarctions; and 250 000 die within the first hour of onset of symptoms. There is no question that major progress has been made in the treatment of myocardial infarction and coronary artery disease. Lifestyle modifications with reduction in risk factors may play an important role, but so does better therapy for coronary artery disease. Decrease in in-hospital mortality for acute myocardial infarction is related to early reperfusion by either thrombolytic therapy or angioplasty. Early reperfusion salvages myocardium and improves cardiac function and survival. However, despite these advances in the early treatment, in-hospital mortality still averages about 7%. What other maneuvers and treatments might salvage ischemic myocardium?

Over the course of 25 years there have been numerous attempts at trying to reduce myocardial infarct size. Various drug therapies have been attempted, including β-blockers, calcium channel blockers, fluorocarbons, anti-inflammatory agents, and antineutrophil agents, with varying success, in both experimental as well as clinical trials. In experimental studies, there only have been a small number of maneuvers that have consistently reduced myocardial infarct size. These include early coronary reperfusion, ischemic preconditioning, and hypothermia. Preconditioning has been shown to be such a powerful technique for reducing myocardial infarct size in every animal model tested that there has been considerable interest in the possibility that it could be applied to the ischemic human myocardium. If the mechanism whereby ischemic preconditioning worked could be identified, then pharmacologic agents involved in possible mechanisms might be applied to human coronary events. The purpose of this review is to discuss the evidence that the human heart can be preconditioned in relationship to both ischemic and pharmacologic preconditioning.

EVIDENCE FROM THE ANGIOPLASTY LITERATURE

One of the earliest findings suggesting that preconditioning might occur in man actually comes from the angioplasty literature. Several clinical reports described that sequential angioplasty balloon inflations were associated with decreasing chest pain, decreasing degrees of ST-segment elevation, and reduced amount of lactate production, on subsequent

compared to the initial inflation.[1,2] This observation was made without any increase in coronary flow in one study,[1] but may have been associated with recruitment of coronary collaterals in some, but not all, patients in another study.[2] Over the past few years, there has been growing evidence that some of the same pathways involved in infarct size reduction in animal studies may also play a role in ischemic preconditioning during angioplasty procedures in man. For example, the K_{ATP} channel blocker glibenclamide will block the beneficial effects of repetitive balloon inflation in humans, just as it blocks the ability of ischemic preconditioning to reduce infarct size in animal models.[3,4] Infusion of adenosine can mimic the cardioprotective effects of repetitive balloon inflation, just as adenosine agonists can reduce myocardial infarct size in animal experiments.[5,6]

Adenosine antagonists will block the beneficial effects of repetitive balloon inflation, just as they block the ability of ischemic preconditioning to reduce myocardial infarct size in some animal models.[7] There is a practical clinical implication that may be derived from the preconditioning-like effect of repetitive coronary artery angioplasty balloon inflation. Patients with coronary stenoses within arteries that supply large territories of the ventricles and patients with difficult and complex lesions might benefit from repetitive balloon inflations in order to precondition the myocardium prior to lengthy angioplasty procedures. In general, the duration of the initial inflation needed to achieve a protective effect appears to be 90 seconds.[1] Alternatively, pretreatment prior to angioplasty with an agent such as adenosine or perhaps a K_{ATP} channel opener might achieve the same benefit.

EVIDENCE FROM PREINFRACTION ANGINA STUDIES

Another clinical observation that supports the notion that ischemic preconditioning occurs in man comes from studies of preinfarction angina. Experimental studies have clearly demonstrated that brief periods of nonlethal ischemia (usually of the order of 2 to 10 min) prior to a prolonged episode of ischemia (usually of the order of 30 to 90 min), will markedly reduce the size of the myocardial infarction. Many patients are known to have anginal attacks prior to their myocardial infarction. Assuming that these anginal attacks are brief (less than 20 min), then one could postulate that these brief nonlethal episodes of ischemia prior to myocardial infarction might actually be protective in humans. While this concept is seductive, there are some complicating features of patients with histories of angina prior to myocardial infarction. These patients tend to have more multivessel coronary disease, may have more risk factors, and may be on more antianginal medicines than patients without prior histories of angina pectoris. Indeed, some studies have suggested that patients with long histories of prior angina have a poorer long-term prognosis.[8]

MILIS, TIMI 4 and 9, GUSTO, and other studies

This was especially the case in studies from the prethrombolytic era. We performed an analysis on patients who had entered one such prethrombolytic trial called the Multicenter Investigation of the Limitation of Infarct Size (MILIS) trial,[9] which showed that there was no overall reduction in infarct size assessed by creatine kinase (CK) curves in patients with versus patients without a history of preinfarction angina. This came as no surprise, since animal studies have shown that ischemic preconditioning will only reduce myocardial infarct size if there is reasonably early reperfusion—within 60 to 90 min of the prolonged coronary occlusion. Thus, ischemic preconditioning would not be expected to work in the situation of permanent coronary occlusion. However, in the MILIS trial, there was a group of patients who probably had early spontaneous reperfusion, as suggested by early peaking of their CK curves. In this group of patients, those who had histories of preinfarction angina had lower total CKs, suggesting smaller infarct sizes compared with patients with early reperfusion, but no history of preinfarct angina.

In the Thrombolysis In Myocardial Infarction (TIMI) 4 trial,[10] which was a thrombolytic trial, we observed that patients who had a history of preinfarct angina had lower total CK release as well as fewer Q waves on their ECG, compared with patients who had no history of preinfarct angina. Patients with preinfarction angina also had better in-hospital survival and less congestive heart failure and/or shock. Coronary angiograms did not reveal more collaterals in the preinfarct angina group. The benefits of preinfarction angina were not related to differences in medicines between the two groups. The benefit of preinfarction angina occurred despite the fact that patients with preinfarction angina had more multivessel coronary disease and had a longer duration from onset of angina to administration of thrombolytic. There was a downside to having preinfarction angina—these patients had a trend toward more postinfarction angina.

This probably was due to the fact that these patients had more multivessel coronary disease.

Recently, we analyzed the findings of another large thrombolytic trial, the TIMI 9 trial.[11] In this trial, the benefits of preinfarction angina were only manifest when the history of preinfarct angina occurred within 24 hours of onset of the infarction. Patients who had preinfarction angina during this time were more likely to have lower peak CKs and a lower incidence of in-hospital death, shock, and/or heart failure. However, in patients who had onset of angina more than 24 h prior to onset of infarction, there was no protective effect. This trial suggested that for preinfarct angina to have a beneficial effect there must be a close temporal relationship between the brief ischemic episodes and the myocardial infarction. A time course of 24 h would allow for mechanisms of either classic preconditioning or the second window of protection to play a role. The benefits of preinfarction angina were not related to antianginal medication.

Over the last 3 years, there have been a number of published studies that have confirmed the concept that preinfarction angina is protective. These include reports that preinfarction angina reduces myocardial infarct size as measured by CK curves, improves ventricular function, reduces the frequency of clinical congestive heart failure, reduces arrhythmias, and reduces the likelihood of ventricular rupture.[12-15] One preliminary study suggested that preinfarct angina that occurs within 24 h of infarction improves long-term survival.[16] Not all such studies have been positive. For example, a preliminary study from the GUSTO group (Global Utilization of Streptokinase and TPA for Occluded arteries) did not observe a benefit of preinfarction angina.[17] However, their study included patients with angina that may have been more remote from the time of infarction.

What are the possible mechanisms?

The mechanisms whereby preinfarction angina confer early benefit in these studies have been an area of considerable discussion. Obviously, one likely explanation is that preconditioning caused the benefit. Another potential explanation is that intramural collaterals developed in patients with previous angina. Small vessels might not be visualized on angiography, so that studies would not necessarily describe an increase in collaterization among patients with previous angina. An intriguing new theory regarding the benefit of preinfarction angina was suggested by Andreotti et al.[18] They showed, in a small cohort of patients, that previous angina was associated with earlier and more complete reperfusion by thrombolysis. This unique concept suggests that ischemic preconditioning may protect the large epicardial coronary arteries, making them more susceptible to thrombolytic agents. Hata and Przyklenk[19] in our laboratory have observed a similar finding in a canine model of partial coronary artery stenosis, in which brief episodes of ischemia improve vessel patency and reduce cyclic flow variation due to transient platelet plugs.

Clinical and therapeutic implications

Could the knowledge that preinfarction angina confers beneficial effects have practical clinical or therapeutic implications? Patients with myocardial infarction who present with a history of angina of recent onset may have early in-hospital benefits. However, the clinician should know that these patients may have more multivessel coronary artery disease, and therefore may be more likely to present with recurring ischemia. It is tempting to think that preconditioning-like drugs (such as adenosine, adenosine agonists, and K_{ATP} channel openers) might be administered early during the course of myocardial infarction and produce a cardioprotective effect as occurs with preconditioning. Whether these drugs could be administered early enough to have a benefit in patients already evolving substantial tissue necrosis is not yet known. However, it might be possible to administer preconditioning-like drugs that stimulate the pathways of preconditioning without causing ischemia in patients with either threatened acute myocardial infarction or in patients with unstable angina pectoris. It is more likely that the practical benefits of giving ischemic preconditioning-like drugs will be most useful in controlled clinical settings, in which the drugs can be given in a pretreatment fashion prior to a known ischemic insult. An obvious situation to consider would be administration of such agents prior to coronary artery bypass surgery or any cardiopulmonary bypass procedure. In a recent report, Mentzer et al[20] attempted pharmacologic preconditioning in patients undergoing open heart procedures. Placebo or various doses of adenosine were given to the patients prior to undergoing cardiopulmonary bypass. Patients who received high doses of adenosine had improved regional wall motion and had less need for inotropic support. This is one of the first studies of which I am aware where a preconditioning-mimetic agent was shown to have direct clinical benefit.

EVIDENCE FROM "WARM-UP" OR "WALK-THROUGH" ANGINA

Another clinical situation in which preconditioning may occur is in the situation of so-called "warm-up" or "walk-through" angina pectoris. Most clinicians have seen the patient who develops angina while walking, stops for a few minutes, and then continues to walk without chest pain. This "warm-up" phenomenon may be related to ischemic preconditioning. Patients are able to exercise for a longer duration before developing ischemia during a second stress test compared to a first test, if there is a brief rest period between the first and second tests.[21] In another related study, patients with coronary artery disease who were undergoing cardiac catheterization were rapidly paced during two finite periods, with a period of nonpacing in between. There was less ST-segment deviation and less lactate production during the second compared to the first pacing period.[22] Interestingly, there was no increase in coronary flow during the second test compared to the first test. There was, however, a reduction in myocardial oxygen demand during the second test compared to the first, suggesting a possible mechanism for this benefit. Maybaum et al[23] reported the effect of three sequential exercise tests, separated by a period of 30 min. They observed a longer time to development of ST-segment depression during the second and third tests compared to the first. In addition, the exercise duration was improved in the second and third tests and these were associated with a higher rate-pressure product. In summary, it does appear that humans can adapt rapidly to brief ischemic insults. A practical take-home message for the clinician is to suggest a warm-up phase of exercise in those coronary disease patients that are physically active. It is also useful to know that exercise tolerance in these patients may vary from time to time depending on whether there was a warm-up phase.

EVIDENCE FROM IN VITRO ANALYSIS OF HUMAN MYOCARDIAL TISSUE

While this review has focused on examples of clinical preconditioning, there are a number of studies that support the concept that the human heart can be preconditioned that have relied upon in vitro analysis of human tissue. Ikonomidis et al[24] observed enhanced survival of cultured human cardiomyocytes exposed to simulated prolonged ischemia when they were preconditioned with prior episodes of brief ischemia. Yellon's group performed a series of studies in human atrial trabeculae that were exposed to ischemia and reperfusion, and measured contractile function of these strips of muscle.[25,26] They observed that adenosine receptor activation enhanced recovery of function from simulated ischemia, as did the K_{ATP} channel opener cromakalim and stimulation of protein kinase C.

POTENTIAL APPLICATIONS OF PRECONDITIONING IN MAN

In some but not all animal models preconditioning appears to have a beneficial effect on arrhythmias. Takano et al[27] suggested that this may hold true in humans. They investigated the incidence of ventricular arrhythmias during ischemic attacks in relationship to the previous ischemic period. The incidence of ventricular premature beats, couplets, and ventricular tachycardia was reduced when the previous ischemic attack occurred within 5 h, compared to when the previous attack occurred at an interval that was greater than 5 h.

One of the first studies that suggested that human cardiac tissue could be preconditioned comes from a study in which serial biopsies were obtained from ventricular myocardium for ATP levels. In this study, patients undergoing coronary bypass surgery received a 10-min period of ischemia induced by aortic cross-clamp defibrillation. Some of the patients were preconditioned with two episodes of 3 min of ischemia induced by aortic cross-clamping. Patients who were preconditioned demonstrated less degradation of ATP during the test period of ischemia.[28] The same group showed that ischemic preconditioning prior to placement of a coronary artery bypass graft reduced serum troponin T concentrations.[29] Could ischemic preconditioning be used to protect the myocardium during cardiopulmonary bypass procedures? While various cardioplegic techniques are commonly used, they are often associated with some degree of myocardial stunning.[30] There is a need for better cardioprotective techniques. As already mentioned, one study suggested that pretreatment with the preconditioning-mimetic adenosine could improve postoperative cardiac function, despite the use of cardioplegics. The author has heard anecdotes in which a cardiac surgeon has preconditioned a territory of the anterior wall of the left ventricle by actually clamping the coronary artery for a few min, reperfusing, and then proceeding to a coronary bypass of the artery. Certainly, with the advent of minimally invasive surgery, there is a need to consider newer ways of protecting the heart during the period of the anastomosis.

CONCLUSION

Based on the studies discussed above as well as numerous other studies (for more detailed reviews, see references 31, 32, and 33), it is very likely that the human heart can be preconditioned. What remains to be determined from a clinical standpoint is whether the basic science information resulting from the exploration of the mechanism of ischemic preconditioning will result in preconditioning-mimetic drugs that do not cause ischemia but do cause cardioprotection. Likely clinical situations in which preconditioning could be harnessed to protect the ischemic myocardium include pretreatment with preconditioning-mimetics prior to minimally invasive cardiac surgery, routine cardiac surgery, prior to difficult coronary angioplasty cases, and administration of such agents to donor hearts to preserve them prior to heart transplant. It is also conceivable that preconditioning-mimetics might be assessed for efficiency in patients with threatened myocardial infarction or unstable angina pectoris. Whether these agents could play any role during evolving myocardial infarction is less certain. What is certain is that ischemic preconditioning is one of the most powerful techniques that experimentalists have observed for reducing myocardial infarct size. Understanding its mechanism will improve our overall understanding of myocardial ischemia and hopefully provide future therapies.

REFERENCES

1. Deutsch E, Berger M, Kussmaul WG, et al.
Adaption to ischemia during percutaneous transluminal coronary angioplasty. Clinical, hemodynamic, and metabolic features.
Circulation. 1990;82:2044-2051.

2. Cribier A, Korsatz L, Koning R, et al.
Improved myocardial ischemic response and enhanced collateral circulation with long repetitive coronary occlusion during angioplasty: a prospective study.
J Am Coll Cardiol. 1992;20:578-586.

3. Tomai F, Crea F, Gaspardone A, et al.
Ischemic preconditioning during coronary angioplasty is prevented by glibenclamide, a selective ATP-sensitive K^+ channel blocker.
Circulation. 1994;90:700-705.

4. Gross GJ, Auchampach JA.
Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs.
Circ Res. 1992;70:223-233.

5. Leesar MA, Stoddard M, Ahmed M, Broadbent J, Bolli R.
Preconditioning of human myocardium with adenosine during coronary angioplasty.
Circulation. 1997;95:2500-2507.

6. Thornton JD, Liu GS, Olsson RA, Downey JM.
Intravenous pretreatment with A_1-selective adenosine analogues protects the heart against infarction.
Circulation. 1992;85:659-665.

7. Tomai F, Crea F, Gaspardone A, et al.
Effects of A_1 adenosine receptor blockade by bamiphylline on ischemic preconditioning during coronary angioplasty.
Eur Heart J. 1996;17:846-853.

8. Behar S, Reicher RH, Abinader E, et al.
The prognostic significance of angina pectoris preceding the occurrence of a first acute myocardial infarction in 4166 consecutive hospitalized patients.
Am Heart J. 1992;123:1481-1486.

9. Kloner RA, Muller J, Davis V.
Effects of previous angina pectoris in patients with first acute myocardial infarction not receiving thrombolytics.
Am J Cardiol. 1995;75:615-617.

10. Kloner RA, Shook T, Przyklenk K, et al.
Previous angina alters in-hospital outcome in TIMI 4: a clinical correlate to preconditioning?
Circulation. 1995;91:37-45.

11. Kloner RA, Shook T, Antman EM, et al,
TIMI 9 Investigators. A prospective analysis on the potential preconditioning effect of preinfarct angina in TIMI 9.
Circulation. 1996;94(suppl I):I-611.

12. Ottani F, Galvani M, Ferrini D, et al.
Prodromal angina limits infarct size. A role for ischemic preconditioning.
Circulation. 1995;91:291-297.

13. Nakagawa Y, Ito H, Kitakaze M, et al.
Effect of angina pectoris on myocardial protection in patients with reperfused anterior wall myocardial infarction: retrospective clinical evidence of "preconditioning."
J Am Coll Cardiol. 1995;25:1076-1083.

14. Anzai T, Yoshikawa T, Asakura Y, et al.
Effect on short-term prognosis and left ventricular function of angina pectoris prior to first Q-wave anterior wall acute myocardial infarction.
Am J Cardiol. 1994;74:755-759.

15. Anzai T, Yoskikawa T, Asakura Y, et al.
Preinfarction angina as a major predictor of left ventricular function and long-term prognosis after a first Q-wave myocardial infarction.
J Am Coll Cardiol. 1995;26:319-327.

16. Ishihara M, Sato H, Kawagoe T, Sakai K.
Implication of prodromal angina pectoris on long-term prognosis after acute myocardial infarction.
Circulation. 1996;94(suppl 1):I-611–I-612.

17. Migrino RQ, Ross AM, Betriu A, et al.

Preinfarction angina is not associated with improved outcome after thrombolytic therapy for myocardial infarction.

Circulation. 1996;94(suppl I):I-611.

---

18. Andreotti F, Pasceri V, Hackett DR, Davies GJ, Harder AW, Maseri A.

Preinfarction angina as a predictor of more rapid coronary thrombolysis in patients with acute myocardial infarction.

N Engl J Med. 1996;334:7-12.

---

19. Hata K, Kloner RA, Przyklenk K.

Brief antecedent ischemia attenuates platelet-mediated thrombosis in damaged and stenotic canine coronary arteries.

Circulation. 1996;94(suppl I):I-538.

---

20. Mentzer RM Jr, Rahko PS, Molina-Viamonte V, et al.

Safety, tolerance, and efficacy of adenosine as an additive to blood cardioplegia in human during coronary artery bypass surgery.

Am J Cardiol. 1997;79(suppl 12A):38-43.

---

21. Okazaki Y, Kodama K, Sato H, et al.

Attenuation of increased regional myocardial oxygen consumption during exercise as a major cause of warm-up phenomenon.

J Am Coll Cardiol. 1993;21:1597-1604.

---

22. Williams DO, Bass TA, Gerwitz H, et al.

Adaptation to the stress of tachycardia in patients with coronary artery disease: insight into the mechanism of the warm-up phenomenon.

Circulation. 1985;71:687-692.

---

23. Maybaum S, Ilan M, Mogilevsksy J, Tzivoni D.

Improvement in ischemic parameters during repeated exercise testing: a possible model for myocardial preconditioning.

Am J Cardiol. 1996;78:1087-1091.

---

24. Ikonomidis JS, Tumiati LC, Weisel RD, et al.

Preconditioning human ventricular cardiomyocytes with brief periods of stimulated ischemia.

Cardiovasc Res. 1994;28:1285-1291.

---

25. Walker DM, Walker JM, Pugsley WB, et al.

Preconditioning in isolated superfused human muscle.

J Mol Cell Cardiol. 1995;27:1349-1356,

---

26. Speechly-Dick ME, Grover GJ, Yellon DM.

Does ischemic preconditioning in the human involve protein kinase C and the ATP-dependent K^+ channel? Studies of contractile function following simulated ischemia in an atrial in-vivo model.

Circ Res. 1995;77:1030-1035.

---

27. Takano H, Kusaina Y, Saito T, et al.

Antiarrhythmic effect of ischemic preconditioning in patients with variant angina.

J Am Coll Cardiol. 1994;23:80A. Abstract.

---

28. Yellon DM, Alkhulaifi AM, Pugsley WB.

Preconditioning the human myocardium.

Lancet. 1993;342:276-277.

---

29. Jenkins DP, Pugsley WB, Alkhulaifi AM, Kemp M, Hooper J, Yellon DM.

Ischaemic preconditioning reduces troponin T release in patients undergoing coronary artery bypass surgery.

Heart. 1997;77:314-318.

---

30. Kloner RA, Przyklenk K, Kay GL.

Clinical evidence for stunned myocardium after coronary artery bypass surgery.

J Card Surg. 1994;9(suppl):397-402.

---

31. Kloner RA, Przyklenk K, Shook T, et al.

Clinical aspects of preconditioning and implication for the cardiac surgeon.

J Card Surg. 1995;10:369-375.

---

32. Kloner RA, Yellon DM.

Does ischemic preconditioning occur in patients?

J Am Coll Cardiol. 1994;24:1133-1142.

---

33. Kloner RA, Yellon DM.

Cardiac preconditioning in humans. In: Yellon D, Rahimtoola S, Opie L, eds.

New Ischemic Syndromes. Author's Publishing House, Lippincott-Raven Publishers. 1997:76-89.

What is the second window of preconditioning and does it have any potential for clinical exploitation?

Garrett J. Gross, PhD

Department of Pharmacology and Toxicology - Medical College of Wisconsin - 8701 Watertown Plank Road - Milwaukee, Wis 53226 - USA

Although classic ischemic preconditioning has been shown to produce a marked reduction in myocardial infarct size in all species tested, its clinical potential is limited by its relatively short-lived duration (30 to 120 min). However, recent evidence has demonstrated that a delayed protective effect reappears 12 to 24 h following acute preconditioning, and this has been termed the second window of protection. Delayed preconditioning differs from classic preconditioning in that it has been shown to produce a cardioprotective effect against both infarction and myocardial stunning. The mechanisms responsible for the cardioprotective effect of classic and delayed preconditioning in reducing infarct size have many similarities, buth those responsible for delayed preconditioning against stunning may be different.
Several pharmacological agents have been shown to produce delayed preconditioning, which suggests that it may be exploited clinically.

Before discussing the potential for clinical exploitation of the second window of preconditioning, this phenomenon will first be defined and evidence will be presented both for and against its existence in animal models using infarct size reduction and postischemic dysfunction or myocardial stunning as end points of its efficacy as a cardioprotective intervention. Subsequently, potential mechanisms thought to be responsible for the cardioprotective effect of delayed preconditioning will be discussed as well as how these mechanisms might be utilized in the clinical setting for therapeutic benefit. Finally, evidence will be presented which shows that several pharmacological agents produce delayed preconditioning in animal models and may eventually be exploited clinically to protect the heart against infarction or stunning following specific surgical procedures. A summary of the important characteristics of delayed preconditioning and potential mediators of this phenomenon is outlined in *Table I.*

DELAYED PRECONDITIONING AGAINST INFARCTION
Time window of protection
• 24 to 72 h (rabbits)
Efficacy of infarct size reduction
• 30% to 50% (rabbits) • 46% (dogs)
Potential mediators of protection
• Heat shock proteins (HSPs) • Antioxidant enzyme upregulation • Adenosine A_1 receptors • Protein kinase C (PKC)
DELAYED PRECONDITIONING AGAINST STUNNING
Time window of protection
• 6 to 72 h (pigs, rabbits)
Efficacy of reduction in the total deficit in wall thickening
• 50% to 60% (pigs, rabbits)
Potential mediators of protection
• Heat shock proteins (HSPs) • Adenosine A_1 receptors • Oxygen-derived free radicals (OFRs) • Nitric oxide
PHARMACOLOGICAL INDUCERS OF DELAYED PRECONDITIONING
Infarct size reduction
• Adenosine A_1 agonist (CCPA) • Monophosphoryl lipid A (MLA)
Attenuation of stunning
• Monophosphoryl lipid A (MLA)

Table I. *Characteristics of the second window of protection or delayed preconditioning.*

DEFINITION OF AND EVIDENCE FOR AND AGAINST THE SECOND WINDOW OF PRECONDITIONING AGAINST INFARCTION

Delayed preconditioning against infarction was first demonstrated in 1993 by Kuzuya et al[1] and Marber et al[2] in anesthetized dogs and rabbits, respectively. These investigators demonstrated that four 5-min periods of ischemia interspersed with 10 min of reperfusion produced a 40% to 50% reduction in myocardial infarct size 24 h after the sublethal ischemic insult. Subsequently, Baxter et al[3] showed that anesthetized rabbits subjected to one, two, or four 5-min preconditioning episodes demonstrated equivalent reductions (40% to 50%) in infarct size when subjected to 30 min of coronary artery occlusion and 2 h of reperfusion 48 h later. The cardioprotective effect of the four 5-min occlusions was still present at 72 but not at 96 h.
This delayed protective effect was termed the second window of protection (or SWOP) by Yellon and Baxter[4] to clearly delineate it from early or classic preconditioning, which only has a time window of between 30 and 120 min, following sublethal ischemia. Since SWOP was initially demonstrated in anesthetized animals where the results obtained may have been influenced by surgical trauma, anesthesia, temperature, and other neurohormonal factors, Yang and coworkers[5] recently performed a study in conscious rabbits and found that four 5-min periods of ischemia interspersed with 10 min of reperfusion also resulted in a delayed reduction in infarct size (33%) 24 h later.

In contrast, several studies have shown that ischemic preconditioning does not produce SWOP in rabbits, pigs, and intact rats.[4] More specifically, Tanaka et al[6] found that 5 min of ischemia followed by 5 min, 2 h, or 24 h of reperfusion did not result in a reduction in infarct size in anesthetized rabbits in spite of an elevation in cardiac heat shock proteins. Similarly, Qiu et al[7] found that ten 2-min coronary artery occlusions produced a marked (80%) reduction in infarct size when studying classic preconditioning in conscious pigs. However, this same protocol produced no reduction in infarct size 24 h later. Similar results were obtained in intact anesthetized rats using infarct size as the end point of injury.[4] Thus, although the second window of protection against infarction has been clearly demonstrated in dogs and rabbits, its efficacy to reduce infarct size is considerably less than that observed with classic preconditioning. In the instances in which it occurs, however, its long (24 to 72 h) time window of protection makes it clearly superior to that of classic preconditioning when considering the clinical potential of the two phases of preconditioning. On the other hand, the relatively smaller reductions in infarct size observed following SWOP as compared to classic preconditioning may be partially responsible for the positive effects obtained in some species as opposed to negative ones obtained in other species.
Since SWOP appears to have a less efficacious effect as compared to early classic preconditioning, this may limit its effectiveness in reducing infarct size to shorter periods of ischemia, such as 30 min, a time frame of ischemia where most patients suffering from an acute myocardial infarction have not yet received reperfusion therapy. Thus, although the time window of protection following SWOP is very attractive, its limited efficacy may curtail its clinical potential unless adjunctive methods are found which will enhance its cardioprotective effects.

EVIDENCE FOR DELAYED PROTECTION AGAINST MYOCARDIAL STUNNING

There is little evidence to suggest that classic preconditioning protects against regional stunning following a short interval of reperfusion after a single brief ischemic insult. However, recent work from Bolli's laboratory[8] has clearly demonstrated ischemia-induced delayed preconditioning against stunning in conscious pigs. These investigators found that a protocol of ten 2-min occlusion / 2-min reperfusion cycles induced severe postischemic dysfunction on day one of a sequence, but when the same sequence was reproduced 24 h later the severity of stunning was reduced by approximately 50%. This resistance to stunning persisted for 6 to 72 h. Recently, the same group observed a similar effect in conscious rabbits subjected to six 4-min occlusions separated by 4 min of reperfusion. Thus, it appears that delayed preconditioning against stunning is very reproducible in the conscious pig and rabbit, and has a powerful effect to attenuate severe stunning.

POTENTIAL MECHANISMS FOR DELAYED PRECONDITIONING AGAINST INFARCTION

Heat shock proteins (HSPs)

In the initial study by Marber et al,[2] four 5-min episodes of ischemia or heat stress produced by elevating whole body temperature to 42°C for 15 min in rabbits both resulted in a significant reduction in infarct size and an elevation in myocardial heat shock protein 72 (HSP 72) as assessed by Western blotting 24 h after the initial stress. HSP 60 was also significantly elevated by ischemic preconditioning. These results suggest that increases in HSPs may

be involved in the delayed cardioprotective adaptation to thermal or ischemic stress. However, since a number of other investigators using similar protocols have shown cardioprotection in the absence of elevations in stress proteins, or conversely, an elevation in stress proteins in the absence of a cardioprotective effect, it is still unclear what is the precise cause and effect relationship between these two events.

Antioxidant enzymes

A number of studies have demonstrated increases in antioxidant enzyme activity following delayed preconditioning in intact animals as well as in isolated cardiac myocytes. More specifically, Hoshida et al[9] examined antioxidant enzyme activity in dog hearts subjected to four 5-min periods of regional ischemia and reperfusion, and found that mitochondrial manganese superoxide dismutase (Mn-SOD) was significantly increased at 24 h, but not at 3 h following sublethal ischemia. No increases were observed in Cu,Zn-SOD activity at any time point. These increases in Mn-SOD activity correlated temporally with the delayed reduction in infarct size observed. However, as in the case with HSPs, it is not clear whether there is a direct link between the increases in antioxidant enzyme activity and myocardial protection.

Adenosine A_1 receptors

A strong case can be made for an important role for adenosine acting on its A_1 receptor in triggering the cardioprotective effect of delayed preconditioning to reduce infarct size in rabbits. Yellon and Baxter[4] have shown that blockade of adenosine receptors by the administration of the nonselective antagonist 8-(*p*-sulfophenyl)theophylline (SPT) during the preconditioning period prevented the delayed reduction in infarct size normally observed following four 5-min episodes of sublethal ischemia. Furthermore, these investigators[4] found that transient activation of the A_1 adenosine receptor by the specific agonist 2-chlorocyclopentyl adenosine (CCPA) resulted in a delayed reduction in infarct size 24 h later, similar to that produced by ischemic preconditioning. Similar to ischemia, the reductions in infarct size were approximately 40% to 50% and the highest dose (100 µg/kg) produced the largest decrease. Although CCPA produced hypotension and bradycardia when initially administered, there were no differences in systemic hemodynamics between drug-treated and control groups the following day when the infarct study was completed. These results are promising and suggest that a selective adenosine A_1 agonist may be able to protect against ischemic and/or reperfusion injury, which may occur during certain surgical procedures such as coronary artery bypass grafting. However, further studies are necessary in other species, including man, to confirm that adenosine A_1 receptor activation is a universal mediator of delayed preconditioning against infarction similar to that observed following classic ischemic preconditioning.

Protein kinase C (PKC)

Several studies, performed primarily in anesthetized and conscious rabbits, suggest that activation and translocation of PKC is an integral part of the cardioprotective effect of classic preconditioning.
Since adenosine has been shown to activate PKC in rabbit hearts, Yellon's group[4] hypothesized that PKC may also be involved in the second window of protection. In this regard, these investigators administered the selective PKC inhibitor chelerythrine immediately prior to four cycles of ischemic preconditioning and found that the delayed protection against infarction 24 h later was blocked. The same investigators also found that administration of 1,2-dioctanoyl-*sn*-glycerol (DiC8), an analogue of diacylglycerol, the normal physiological activator of PKC, produced a delayed cardioprotective effect to reduce infarct size similar to that of ischemic preconditioning.[4] These results suggest that activation of PKC is involved in both classic and delayed preconditioning against infarction. However, since direct activation of PKC has also been shown to produce deleterious effects in the heart and coronary vasculature, this may not be particularly attractive as a target for mimicking delayed preconditioning pharmacologically.

POTENTIAL MECHANISMS FOR DELAYED PRECONDITIONING AGAINST STUNNING

Heat shock proteins (HSPs)

In the first study demonstrating a second window of protection against myocardial stunning in conscious pigs, Sun et al[8] found that there was an increase in the mRNA for HSP 70 2 h after ten 2-min occlusion / 2 min reperfusion cycles used to produce acute myocardial stunning and delayed preconditioning against stunning 24 h later. Concomitantly, these authors found an increase in immunohistochemical staining for HSP 70 in the cell nucleus, and, 24 h after preconditioning, Western dot-blot analysis also showed an increase in HSP 70. Thus, as is the case in delayed preconditioning against infarction, there appears to be an association between the induction of HSPs and the cardioprotective

effect of ischemic preconditioning to attenuate myocardial stunning, but the data do not unequivocally confirm a cause and effect relationship between HSP induction and delayed preconditioning to limit postischemic dysfunction. In the same study,[8] the authors also demonstrated that the nonselective adenosine receptor antagonist SPT did not block this type of preconditioning, which suggested that this phenomenon may have a different mechanism responsible for its cardioprotective effect than that of classic or delayed preconditioning against infarction.

Adenosine A_1 receptors

To further study the role of adenosine receptor activation in delayed preconditioning against stunning, Maldonado et al[10] recently used a newly developed conscious rabbit model in which they produced preconditioning by six 4-min coronary artery occlusion/4-min reperfusion cycles. They found that pretreatment with two nonselective adenosine receptor antagonists, SPT or PD-115199, prior to preconditioning failed to block its cardioprotective effect and that treatment with the selective A_1 adenosine receptor agonist CCPA the day prior to stunning failed to mimic delayed preconditioning against stunning. These results clearly suggest that adenosine receptors are not involved in this phenomenon and that agonists of these receptors would not be useful targets for producing delayed preconditioning against myocardial stunning.

Oxygen-derived free radicals

Considerable evidence exists to suggest that oxygen free radicals are partially responsible for the acute postischemic dysfunction observed following single or multiple brief periods of ischemia. However, Bolli's group recently demonstrated that oxygen radicals not only contribute to acute myocardial stunning, but are important in triggering delayed preconditioning against stunning. In support of this hypothesis, Sun et al[11] showed that a cocktail of free radical scavengers, superoxide dismutase, catalase, and *N*-2-mercaptopropionyl glycine, attenuated stunning on D1 of a 3-day cycle of ten 2-min occlusion/2 min reperfusion periods, in conscious pigs and this resulted in the absence of delayed preconditioning on D2, but not on D3. Nisoldipine, a calcium channel blocker, also attenuated stunning on D1, but did not block preconditioning on D2, which suggested that the attenuation of stunning per se on D1 was not responsible for the lack of delayed preconditioning on D2, but that late preconditioning was specifically mediated by the generation of oxygen-derived free radicals. These results suggest that treatment of acute myocardial stunning by antioxidants may only produce a transient improvement in postischemic function while resulting in a long-term decrement in function. Thus, the possible use of oxygen radical scavengers as therapy for myocardial stunning may need to be reassessed in light of these intriguing new findings.

Nitric oxide (NO)

Recent results by Bolli et al[12] suggest that NO generation also plays an important role in delayed preconditioning against myocardial stunning in conscious rabbits. These investigators showed that administration of the nitric oxide synthase (NOS) inhibitor, N^{ω}-nitro-L-arginine (L-NA) prevented delayed preconditioning against stunning 24 h after an initial sequence of six 4-min occlusion/4-min reperfusion cycles performed the previous day. Since NO is a free radical and is known to form the peroxynitrite anion in the presence of the superoxide anion, it is possible that NO is forming secondary free radicals that are responsible for the delayed preconditioning effect observed following the stunning protocol performed on D1 of a 3-day sequence. Whether L-arginine, the natural substrate for NO, or NO donors can be utilized to mimic the effect of delayed preconditioning against stunning as a therapeutic modality remains to be determined.

Pharmacological approaches to mimicking the second window of preconditioning

The major question that remains is whether pharmacological agents can be developed that can safely mimic the delayed phase of preconditioning against infarction and/or myocardial stunning. Evidence has already been presented which suggests that adenosine A_1 receptor activation mimics the second window of protection produced by ischemia against infarction in the rabbit heart.[4] However, this same receptor does not appear to be an important component in delayed preconditioning against myocardial stunning.[10] This lack of efficacy of adenosine A_1 receptor activation to produce a delayed preconditioning-like effect against stunning and the associated hemodynamic side effects associated with the administration of such agents most likely precludes their widespread use in the prevention or attenuation of myocardial ischemia-reperfusion injury.

On the other hand, recent results from our laboratory have shown that small doses of monophosphoryl lipid A (MLA), a nontoxic analogue of endotoxin, produces a potent cardioprotective effect against both infarction and stunning 24 h after

its administration to dogs.[13,14] These effects occurred independently of any hemodynamic changes and appear to be mediated via nitric oxide and the adenosine triphosphate–regulated potassium channel (K_{ATP} channel). Preliminary data suggest that MLA may be administered safely to human subjects and the results of a pending clinical trial in which this agent will be given 24 h prior to coronary artery bypass surgery are awaited with interest.

CONCLUSIONS

Convincing evidence has been presented which suggests that brief periods of ischemia, thermal stress, and several pharmacological agents can produce a delayed long-lasting cardioprotective effect against myocardial infarction and/or stunning. These results are intriguing and suggest that this second window of protection may be amenable to the clinical arena, although more studies are necessary to better understand the precise signaling pathways involved in this delayed cardioprotective effect so that more specific pharmacological interventions can be designed that target selective processes safely and effectively.

Acknowledgments

The excellent secretarial assistance of Donna Sloane is gratefully appreciated.

REFERENCES

1. Kuzuya T, Hoshida S, Yamashita N, et al.
Delayed effects of sublethal ischemia on the acquisition of tolerance to ischemia.
Circ Res. 1993;72:1293-1299.

2. 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.

3. Baxter GF, Gama FM, Yellon DM.
Characterization of the infarct-limiting effect of delayed preconditioning: time course and dose-dependency studies in rabbit myocardium.
Basic Res Cardiol. 1997;92:159-167.

4. Yellon DM, Baxter GF.
A "second window of protection" or delayed preconditioning phenomenon: future horizons for myocardial protection?
J Mol Cell Cardiol. 1995;27:1023-1034.

5. Yang, XM, Baxter GF, Heads RJ, Yellon DM, Downey JM, Cohen MV.
Infarct limitation of the second window of protection in a conscious rabbit model.
Cardiovasc Res. 1996;31:777-783.

6. Tanaka M, Fujiwara H, Yamasaki K, et al.
Ischemic preconditioning elevates cardiac stress protein but does not limit infarct size 24 or 48 h later in rabbits.
Am J Physiol. 1994;267(4, pt 2): H-1476–H-1482.

7. Qiu Y, Tang XL, Park SW, Sun JZ, Kalya A, Bolli R.
The early and late phases of ischemic preconditioning. A comparative analysis of their effects on infarct size, myocardial stunning and arrhythmias in conscious pigs undergoing a 40-minute coronary occlusion.
Circ Res. 1997;80:730-742.

8. Sun JZ, Tang XL, Knowlton AA, Park SW, Qiu Y, Bolli R.
Late preconditioning against myocardial stunning. An endogenous protective mechanism that confers resistance to postischemic dysfunction 24 h after brief ischemia in conscious pigs.
J Clin Invest. 1995;95:388-403.

9. Hoshida S, Kuzuya T, Fuji H, et al.
Sublethal ischemia alters myocardial antioxidant activity in canine heart.
Am J Physiol. 1993;264(1, pt 2): H-33–H-39.

10. Maldonado C, Qiu Y, Tang XL, Cohen MV, Auchampach J, Bolli R.
Role of adenosine receptors in late preconditioning against myocardial stunning in conscious rabbits.
Am J Physiol. 1997;273(3, pt 2): H-1324–H-1332.

11. Sun JZ, Tang XL, 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.

12. Bolli R, Bhatti ZA, Tang XL, et al.
Evidence that late preconditioning against myocardial stunning in conscious rabbits is triggered by the generation of nitric oxide.
Circ Res. 1997;81:42-52.

13. Yao Z, Auchampach JA, Pieper GM, Gross GJ.
Cardioprotective effects of monophosphoryl lipid A, a novel endotoxin analogue in dogs.
Cardiovasc Res. 1993;27:832-838.

14. Yao Z, Elliott GT, Gross GJ.
Monophosphoryl lipid A (MLA) preserves myocardial contractile function following multiple, brief periods of coronary occlusion in dogs.
Pharmacology. 1995;51:152-159.

Can acute preconditioning be mimicked and exploited with pharmacological agents?

Derek M. Yellon, DSc; Gary F. Baxter, PhD

The Hatter Institute and Center for Cardiology - University College London Hospitals and Medical School - UCL Hospital - Grafton Way London WC1E 6DB - UK

Our understanding of preconditioning mechanisms suggests that several pathways may be amenable to pharmacological manipulation in patients at risk of myocardial ischemic episodes. Potential approaches include stimulation of the adenosine A_1 receptor and opening of the ATP-sensitive K^+ channel, both of which have been implicated in experimental preconditioning. Identification of suitable patient groups is important since any preconditioning therapy must be administered before the myocardial ischemic episode. We suggest that two populations may be amenable to such therapy initially: patients undergoing elective procedures involving scheduled ischemia, such as coronary artery bypass grafting, and patients with unstable angina, who are at risk of subsequent ischemic events. Clinical trials sensitive enough to detect clinical benefit should be designed to test such therapies in the context of other therapeutic strategies.

"...what drugs, what charms,
what conjuration,
and what mighty magic..."

William Shakespeare,
Othello, act 1, scene 3.

The search for pharmacological agents that will render the myocardium tolerant of ischemia and, more specifically, delay the evolution of irreversible cell injury has, until recently, proved to be a disappointing endeavor. It is arguably the case that the pharmacological evaluation of traditional "anti-ischemic" agents has yielded an equivocal literature and perhaps engendered a philosophy of despair in some. This sense of disappointment is justified by the numerous investigations conducted in the 1970s and 1980s with agents such as L-type calcium channel blockers, β-adrenoceptor blockers, nitrates, anti-inflammatory drugs, and oxyradical scavengers. On the other hand, more recent advances in our understanding of the basic pathophysiology of myocardial ischemia-reperfusion have provided new avenues for exploration of cardioprotective measures. Prominent among these newer approaches is an understanding of how myocardium can adapt to stresses such as ischemia.

Ischemic preconditioning of myocardium undoubtedly protects the myocardium against a subsequent, longer ischemic period. The protection conferred by preconditioning is powerful and reproducible. Importantly, the available evidence suggests that the phenomenon is demonstrable in human myocardium (reviewed by Bob Kloner in this issue). Inevitably, therefore, the question arises: can preconditioning be mimicked and exploited to therapeutic advantage? We would answer this question with a positive response. The reason for our optimism lies in the vast number of new theoretical approaches to cardioprotection that preconditioning opens up by virtue of its apparent complexity. The lush landscape of intracellular pathways and cellular interactions painted by current research, complete with the odd brush stroke of contradiction and scientific controversy, may in time be harmonized into a picture of exquisite simplicity. However, for the moment we can say positively, perhaps for the first time, that various pharmacological interventions tested during experimental investigation of the preconditioning response are reproducibly cardioprotective and may thus serve as templates for the development of novel therapeutic approaches. We believe that clarification of two important subsidiary questions is required for the successful translation of these impressive laboratory

findings into clinical use: how might we activate endogenous cardioprotective pathways pharmacologically? And, which patients would be best suited to receive therapeutic approaches based on preconditioning? To respond, we confine ourselves specifically to classic preconditioning (although, for the record, we would state here our belief that delayed or "second window" preconditioning may in time prove to be equally, if not more, valuable).

HOW MIGHT WE ACTIVATE ENDOGENOUS CARDIOPROTECTIVE PATHWAYS PHARMACOLOGICALLY?

Direct activation of the cellular pathways involved in ischemic preconditioning by pharmacological manipulation would allow improved myocardial protection without the need for an ischemic preconditioning insult. A clear understanding of the mechanisms involved in either form of protection (early or late) is essential to allow a reasoned approach to drug design, and appreciation of these mechanisms is developing continually. Based on our present knowledge, there are several classes of pharmacological agents that may be able to mimic the protection conferred by ischemic preconditioning and provide some basis for optimism that a beneficial and clinically detectable improvement in myocardial protection may be possible. Some feasible pharmacological approaches are discussed below.

Mimic the trigger

Adenosine released during ischemia is one of the most well-researched candidates for the role of a preconditioning trigger, and adenosine A_1 receptor agonists, several of which are in development, represent a promising therapy for improved myocardial resistance to ischemia. There is a potential problem with this approach. Experiments in Downey's laboratory suggest that continuous adenosine A_1 receptor activation with high-dose chronic infusion of A_1 agonist leads to downregulation of the signaling mechanism and loss of protection.[1] However, more encouraging data have been obtained recently in our own laboratory using a more subtle dosing schedule. An A_1 agonist was administered to rabbits by intermittent dosing over a 10-day period, and the persistence of myocardial protection was assessed 48 hours after the final dose, an effect more clearly associated with delayed preconditioning than classic preconditioning.[2]

The potential role of mediators derived from the endothelium has also been investigated. In anesthetized dogs, the antiarrhythmic effect of preconditioning can be attenuated or abolished by an inhibitor of the L-arginine nitric oxide (NO) pathway and by intracoronary administration of methylene blue (preventing the effects of released NO on soluble guanylate cyclase).[3] There is evidence that bradykinin synthesis and release is markedly increased during an ischemic insult from studies in which coronary sinus blood is sampled during coronary angioplasty. Bradykinin has been postulated as a trigger for the release of NO and prostacyclin. Various attempts have been made to synthesize stable analogues of prostacyclin (eg, iloprost), and drugs known as "nitric oxide donors" have been studied for many years in an attempt to ameliorate ischemic injury. It seems more likely, however, that progress in cardioprotection will result from manipulation of cellular mediators downstream of bradykinin and NO/prostacyclin release.

Modulate the trigger

If the release, transport, uptake, or metabolism of the putative trigger could be deliberately modified, then a tissue-targeted effect would be more feasible, and systemic side effects avoided. This approach has been used in order to increase the amount of locally synthesized adenosine present during myocardial ischemia. Nucleoside transport inhibitors have already been shown to potentiate the antiarrhythmic effects of adenosine. The time threshold for the limitation in myocardial infarct size following classic preconditioning is lowered by draflazine (R 75231).[4] Draflazine has also been shown to potentiate the effects of preconditioning and reduce the severity of ischemia-induced arrhythmias in the pig. Acadesine, another agent that regulates local adenosine levels,[5] has been shown to have protective properties under various conditions, including cardiac surgery.

It is conceivable that this approach to the pharmacological induction of preconditioning has been utilized unwittingly, specifically with the widespread use of angiotensin-converting enzyme (ACE) inhibitors. There is considerable evidence that bradykinin is involved as a trigger in the preconditioning response. Since ACE inhibitors prevent the breakdown of bradykinin and thereby potentiate its activity, it has been suggested that these drugs may be able to potentiate the preconditioning effect of ischemia in which bradykinin is released. Thus, a beneficial effect of ACE inhibitors in combination with "subthreshold" preconditioning ischemia has been demonstrated in isolated human myocardium, and this appears to be a B_2 receptor–mediated action,[6] but this potentially very useful property of ACE inhibitors remains to be rigorously assessed in patients.

Activate the intracellular signal

The mechanism by which the triggers mentioned above interact with the cell to cause generation of an intracellular message is thought to involve coupling of the receptor to G proteins. Surprisingly, there has been little published work examining the possibility of deliberate upregulation of G protein function or quantity in cardiac muscle in order to enhance myocardial resistance to ischemia. Inhibitory G proteins couple to protein kinase C (PKC), and the possibility of manipulating PKC activity directly is a further theoretical approach. It seems likely that certain isoforms have more importance than others in the mechanisms of classic and delayed preconditioning, necessitating specific targeting of these isoforms to avoid unwanted more generalized effects associated with global PKC activation. At present, however, no selective pharmacological agents are available. Similar considerations may be extended to other downstream kinases (especially the mitogen-activated protein kinases), which are receiving attention at present in mechanistic studies of preconditioning. For example, the c-jun kinase activator anisomycin confers acute cardioprotection; nontoxic analogues of this agent are being developed.

Although the PKC hypothesis has many enthusiastic supporters, another intracellular pathway has been proposed to explain the marked antiarrhythmic effects of preconditioning. We have already mentioned that bradykinin is released from endothelium during ischemic episodes. Bradykinin is thought to act via B_2 receptors to activate nitric oxide synthase in endothelial cells. This leads to stimulation of soluble guanylate cyclase in myocytes with a resultant increase in intracellular cyclic guanosine monophosphate (cGMP). Elevated GMP may reduce calcium influx, stimulate cGMP phosphodiesterase (PDE), and therefore reduce cyclic adenosine monophosphate (cAMP) levels, and decrease myocardial oxygen demand via nitric oxide–mediated reduction in myocardial contractility. Clearly, this pathway may be amenable to pharmacological manipulation.[7] Selective inhibitors of PDE (eg, zaprinast) might prevent breakdown of cGMP. Activation of the PDE enzymes responsible for breakdown of cAMP might also be possible, and it is interesting to note that prostacyclin derivatives have early and late myocardial protective effects and are also selective activators of specific isotypes of PDE.

Modulate the end-effector

The impressive experimental evidence implicating the K_{ATP} channel in the mechanism of classic preconditioning, possibly as the end-effector of protection, would suggest that there are possibilities for therapeutic exploitation. Several K_{ATP} channel openers are under development as anti-ischemic agents at present[8] and the hybrid nitrate–K_{ATP} channel opener nicorandil is licensed in some countries. The potential of this approach to cardioprotection may be greatly enhanced by ongoing work examining the role of mitochondrial rather than sarcolemmal K_{ATP} channels in the mechanism of classic preconditioning. Targeting the organelle that is specifically involved in cellular respiration has obvious theoretical advantages as well as avoiding unwanted effects on sarcolemmal transmembrane potential.

WHICH PATIENTS WOULD BE BEST SUITED TO RECEIVE THERAPEUTIC APPROACHES BASED ON PRECONDITIONING?

Evidence outlined elsewhere in this issue implies that human myocardium is amenable to preconditioning and that preconditioning may occur as a natural feature of some ischemic syndromes. However, even with the development of pharmacological agents that can mimic or evoke the protection of ischemic preconditioning, the timing of administration will be critical. Prompt reperfusion will always remain the most effective method of limiting ischemic injury, and is, therefore, the most important determinant of prognosis. However, there are certain situations in which the timing of treatment before the onset of ischemia can be controlled to some extent.

The patient at risk of myocardial infarction

Identification and risk-stratification of patients for myocardial infarction is an area that lies outside the scope of this article. However, we would suggest that it is possible to identify some groups of patients with coronary disease who might benefit from augmented myocardial tolerance to ischemia during a subsequent ischemic event. For example, patients presenting with unstable angina are at higher risk of ischemic events, including myocardial infarction during 12 months' follow-up. These would form a reasonably well-defined group for preemptive treatment. A therapy that stimulated or augmented cellular preconditioning mechanisms over a period of several days or weeks could maintain the myocardium in a protected state. Although newer antiplatelet therapies such as the glycoprotein IIb/ IIIa inhibitors

may provide some protection against subsequent thrombotic occlusion (albeit limited), a preconditioning treatment could provide additional cover by enhancing tissue tolerance and slowing the rate of necrosis in the event of the patient suffering an acute myocardial infarction. Essentially, an effective anti-ischemic treatment would "buy time" for the administration of revascularization therapies. A major theoretical hurdle is maintaining myocardium in a protected state by prolonged treatment. The K_{ATP} channel openers present one feasible option. Another may be the use of adenosine A_1 agonists, which are under development, and, as we have already mentioned, tachyphylaxis to these agents need not occur with appropriate and sensible dosing schedules that maintain delayed protection.[2]

Patients undergoing elective procedures involving ischemia

Preconditioning strategies might also be applied prior to a planned procedure involving a potentially injurious ischemic insult. An example is coronary artery bypass graft (CABG) surgery. Highly effective strategies for myocardial preservation have already been developed, eg, various cardioplegic solutions, topical and systemic hypothermia, and intermittent aortic cross-clamping with ventricular fibrillation. In general, use of cardioplegic techniques includes rapid diastolic arrest, membrane stabilization, hyperosmolarity (to prevent intracellular edema), acid-buffering, and hypothermia. Additional strategies, such as continuous coronary perfusion, warm instead of cold cardioplegia (to avoid cold injury), and use of blood instead of crystalloid solutions (to improve oxygen delivery), have all added to the choices available to the cardiac surgeon. Having said that present cardioprotective measures are highly effective at minimizing irreversible injury that might occur during these periods of imposed ischemia, they are not without their limitations. Even with carefully controlled intraoperative ischemic periods and hypothermia, sensitive markers of tissue injury such as troponin-T and troponin-I indicate that discrete necrosis occurs. Moreover, as surgeons undertake more complex and higher-risk operations, so the need for better preservation methods increases. In CABG, the administration of an agent prior to surgery that could enhance myocardial defenses would reduce susceptibility to focal necrosis during surgery and permit the extension of the intraoperative ischemic period. High-risk patients with poor preoperative left ventricular function might certainly benefit if the degree of protection could be improved by invoking endogenous cellular adaptive mechanisms. The possibility that organ preservation prior to transplantation could benefit from similar protection is also of interest.

CONCLUSION

We feel that exploitation of endogenous cardioprotective mechanisms may be possible in the context of carefully conducted clinical studies. There have been significant advances in our understanding of the mechanisms underlying ischemia-reperfusion injury as a result of preconditioning research, and potential pharmacological approaches to protection seem feasible. However, further development of pharmacological therapies must be based on sound experimental investigation. Such therapies should be pursued in the context of efficacy and tolerability of other therapeutic strategies.

REFERENCES

1. Tsuchida A, Thompson R, Olsson RA, Downey JM.
The anti-infarct effect of an adenosine A_1-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.

2. Dana A, Baxter GF, Walker JM, Yellon DM.
Prolonging the delayed phase of cardioprotection by repetitive adenosine A_1 receptor activation.
J Mol Cell Cardiol. 1997;29:A67. Abstract.

3. Vegh A, Szekeres L, Parratt JR.
Preconditioning of ischaemic myocardium: involvement of the L-arginine nitric oxide pathway.
Br J Pharmacol. 1992;107:648-652.

4. Itoya M, Miura T, Urabe K, Ishimoto R, Iimura O.
Nucleoside transport inhibitors enhance the infarct size limiting effect of preconditioning.
Circulation. 1992;86(suppl I):I-125. Abstract.

5. Mangano DT.
Effects of acadesine on myocardial infarction, stroke, and death following surgery. A meta-analysis of the 5 international randomized trials. The Multicenter Study of Perioperative Ischemia (McSPI) Research Group.
JAMA. 1997;277:325-332.

6. Morris SD, Yellon DM.
Angiotensin-converting enzyme inhibitors potentiate preconditioning through bradykinin B_2 receptor activation in human heart.
J Am Coll Cardiol. 1997;29:1599-1606.

7. Parratt JR.
Protection of the heart by ischaemic preconditioning: mechanisms and possibilities for pharmacological exploitation.
Trends Pharmacol Sci. 1994;15:19-25.

8. Hearse DJ.
Activation of ATP-sensitive potassium channels: a novel pharmacological approach to myocardial protection?
Cardiovasc Res. 1995;30:1-17.

Preconditioning

Summaries of Ten Seminal Papers

1

Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium

C.E. Murry and others. *Circulation*. 1986

2

Protection against infarction afforded by preconditioning is mediated by A_1 adenosine receptors in rabbit heart

G.S. Liu and others. *Circulation*. 1991

3

Preconditioning protects ischemic rabbit heart by protein kinase C activation

K. Ytrehus and others. *Am J Physiol*. 1994

4

Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs

G.J. Gross and J.A. Auchampach. *Circ Res*. 1992

5

Adaptation to ischemia during percutaneous transluminal coronary angioplasty: clinical, hemodynamic, and metabolic features

E. Deutsch and others. *Circulation*. 1990

6

Preconditioning of ischemic myocardium: reperfusion-induced arrhythmias

K. Shiki and D.J. Hearse. *Am J Physiol*. 1987

7

Myocardial infarct size-limiting effect of ischemic preconditioning: its natural decay and the effect of repetitive preconditioning

T. Miura. *Cardiovasc Pathol*. 1992

8

Preconditioning cultured human pediatric myocytes requires adenosine and protein kinase C

J.S. Ikonomides et al. *Am J Physiol*. 1997

9

Previous angina alters in-hospital outcome in TIMI 4: a clinical correlate to preconditioning?

R.A. Kloner and others. *Circulation*. 1995

10

Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction

M.S. Marber and others. *Circulation*. 1993

Summaries prepared by **Kim Gallagher, PhD**
Parke Davis Pharmaceutical Research - Division of Warner Lambert Company
2800 Plymouth Road - Ann Arbor - Mich 48105 - USA
Events selected by **Dr P.B. Garlick** - Division of Radiological Sciences - Guy's Hospital - London SE1 9RT - UK

Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium

C.E.Murry, R.B. Jennings, K.A. Reimer

Circulation. 1986;74:1124-1136

This was the first preconditioning paper. For quite a while, investigators had been noticing that repeated short coronary occlusions did not produce as much myocardial damage or as many arrhythmias or the same amount of ATP depletion as one long sustained occlusion. Although these were intriguing observations, some even published, they remained in the realm of anecdote until Murry et al carried out this infarct study in dogs. They hypothesized that repeated brief (5-minute) ischemic episodes might protect the heart from a subsequent sustained bout of ischemia (40 minutes or more of coronary occlusion). The idea was to "precondition" the myocardium with four coronary occlusions, each 5 minutes in length and separated by 5 minutes of reperfusion. Following the preconditioning, one group of dogs underwent 40 minutes of coronary occlusion vs 3 hours in a second group. In control studies, the dogs underwent the sustained coronary occlusions without preconditioning.

The principal end point was histologic infarct size: this was reduced by an impressive 75% in the 40-minute occlusion group, compared to controls, whereas no significant difference was noted in the 3-hour occlusion group. The absence of any significant difference between control and preconditioned groups in terms of collateral blood flow and hemodynamics meant that the infarct reduction size in the 40-minute occlusion group was attributable to the preconditioning, and not to some artifact in the study.

These results set the stage for all subsequent studies on preconditioning and were striking for a number of reasons: (i) they documented definitively that ischemia could indeed beget something that protected the myocardium; (ii) the magnitude of infarct size reduction (75%) by far outstripped that demonstrated with a boatload of pharmacologic agents over the previous 20 years (generally averaging about 50%); (iii) they were produced by as rigorous a pair of senior researchers (Reimer and Jennings) as any in the myocardial ischemia field: if they found something that consistently and dramatically reduced infarct size, it was very likely real; and (iv) the fact that the lead author of such an important study was a medical student at the time (albeit with distinguished seniors looking over his shoulders) had a certain appeal.

There was another appealing element. In 1986, the experimental myocardial ischemia business was ripe for change. For several years, the main focus of activity had been on oxygen free radicals and radical scavenging interventions, but this was now running out of steam. Preconditioning came along at a good time to seize the attention of investigators.

This study, like all good research, prompted a lot of questions. First and foremost, what did the brief periods of ischemia do to protect the heart? Murry et al suggested that the protection may have been due to reduced ATP depletion and/or reduced catabolite accumulation, but readily acknowledged that there were other possibilities and, frankly, that they did not know for sure. Another question was why preconditioning worked for the 40-minute occlusions, but not the 3-hour occlusions. Did the preconditioning "effect" wear off or was it overwhelmed if the ischemic episode lasted long enough? Was preconditioning unique to the dog or was it evident in other species? What about humans? Do anginal episodes, as Murry et al suggested, precondition human hearts? Was there a way, as one eminent researcher later asked, to put preconditioning into a bottle? Preconditioning was off to a very good start.

1986

The nuclear reactor at Chernobyl is damaged by an explosion; the Swedish Prime Minister Olof Palme is assassinated; and "Bad guy" Hollywood actor James Cagney dies, aged 86

Protection against infarction afforded by preconditioning is mediated by A_1 adenosine receptors in rabbit heart

G.S. Liu, J. Thornton, D.M. Van Winkle, A.W.H. Stanley, R.A. Olsson, J.M. Downey

Circulation. 1991;84:350-356

Although preconditioning had been identified 5 years prior to this study, its mechanism remained to be determined. It was about time to move out of the "descriptive phase" and into a "mechanistic" one, but the transition was not proving easy. Studies had already shown that preconditioning did not depend on mechanical stunning *(personal disappointment there—I was sure that it was)*, opening of coronary collaterals, or acute expression of protective proteins. Likewise, biochemical explanations tied to glycolysis or ATP metabolism had not held up very well. Liu et al postulated that adenosine might be important. There was a solid rationale behind this idea since adenosine is released in large amounts by ischemic cardiac myocytes and had been demonstrated to have cardioprotective actions.

Rabbits were chosen because they have few native collaterals (eliminating the need to measure collateral blood flow, an absolute necessity in dog studies). The infarcts were produced by 30-minute coronary occlusions followed by 3 hours of reperfusion. Infarct size was quantified using a staining method based on triphenyl tetrazolium chloride. Without intervention, this regimen produced an infarct size that averaged 39%±4% of the region at risk in the rabbit hearts.

To precondition the myocardium, Liu et al preceded the 30-minute coronary occlusion with a single 5-minute episode of ischemia and 10 minutes of reperfusion. This preconditioning dramatically reduced infarct size. If endogenous adenosine was important, blockade of adenosine receptors should eliminate the protection produced by preconditioning. Two nonselective adenosine antagonists (8-*p*-sulfophenyl theophylline [SPT] and PD 115199) were used, and neither had a significant effect on infarct size in nonpreconditioned rabbits. When infused during the preconditioning occlusion, however, the protective effect of preconditioning was abolished and infarct size was not significantly different from controls, supporting the conclusion that endogenous adenosine played an important role in preconditioning.

A second series of experiments was performed in isolated blood-perfused rabbit hearts. If endogenous adenosine initiated preconditioning, then exogenous adenosine should be able to produce a similar effect. In whole animals, however, intravenous adenosine did not reduce infarct size, but the short half-life, dilutional effect of intravenous administration, and hypotensive effect of adenosine made these findings difficult to interpret clearly. In the isolated heart, adenosine could be delivered directly to the heart and perfusion pressure could be sustained at physiologic levels. Control infarcts in the isolated hearts averaged 32%±4% of the region at risk. Preconditioning with a 5-minute occlusion reduced infarct size to 8%±3%. Intracoronary adenosine was equally effective (infarct size 7%±1%), as was the specific adenosine A_1 agonist, R-PIA (N^6-1-[phenyl-2*R*-isopropyl adenosine]), which reduced infarct size to the same degree (8%±3%). Thus, in the right experimental circumstances, adenosine closely simulated the effects of preconditioning on infarct size, complementing the data obtained with the adenosine antagonists.

Based on these findings, Liu et al proposed that the preconditioning effect was mediated by the rapid accumulation of adenosine during the preconditioning occlusion, stimulating adenosine A_1 receptors on cardiac myocytes. This started a sequence of events that somehow made the myocardium more resistant to ischemic damage. The "adenosine hypothesis" prompted a bumper crop of new questions. Was this *the* mechanism, in all species, and under all conditions? What happens intracellularly after the adenosine A_1 receptors are activated? Could this mechanism be exploited therapeutically? Despite a lot of progress in the 6 years since Liu et al published this paper, we are still looking for complete answers to these questions.

1991

Kevin Costner's "Dances with Wolves"
wins the Best Picture Oscar;
Richard Ernst wins the Nobel Prize for Chemistry,
for his work on NMR;
and Boris Yeltsin is elected Russian President

Preconditioning protects ischemic rabbit heart by protein kinase C activation

K. Ytrehus, Y. Liu, J.M. Downey

Am J Physiol. 1994;266(3, pt 2):H-1145–H-1152

Substantial evidence supported the idea that adenosine receptor stimulation "switched on" preconditioning, but making the leap from adenosine receptor activation to tougher, more ischemia-resistant myocytes was a long one. The next steps in the process remained to be determined. There was good reason to think that protein kinase C (PKC) was involved. Adenosine and other inhibitory G protein–linked receptors mimicked the effects of preconditioning well. G proteins, in turn, can activate phospholipase C (PLC), which leads to production of the second messengers diacylglycerol (DAG) and D-myoinositol 1,4,5-triphosphate. DAG is an activator of PKC, which phosphorylates proteins, activating them or modifying their activity. This signaling pathway is a fundamental one in cellular biology, so it seemed reasonable that it might play a role in preconditioning, too.

The hypothesis was tested in whole animals (rabbits), using a classic pharmacologic approach. If PKC played a role in preconditioning, then PKC inhibitors would be predicted to block the protective effects of preconditioning. Likewise, activators of PKC should simulate the protective effects of preconditioning. The PKC inhibitors staurosporine or polymyxin B were administered to anesthetized rabbits before a standard 30-minute coronary occlusion, 3-hour reperfusion protocol designed to produce myocardial infarcts. Control (nonpreconditioned) hearts had infarcts that averaged 38%±3% of the region at risk. Infarct size in nonpreconditioned staurosporine and polymyxin B–treated rabbits was 41%±3% and 42%±7%, respectively, indicating that the PKC inhibitors had no significant effect on infarct size in the absence of preconditioning. A 5-minute preconditioning occlusion followed by 10 minutes of reperfusion reduced infarct size to 7%±3%. When staurosporine or polymyxin B was administered after preconditioning, but before the 30-minute occlusion, the protective effects of preconditioning were eliminated and infarcts were 36%±3% and 41%±3%, respectively.

The next part of the study was conducted in isolated hearts. The objective was to use activators of PKC to determine if they produced infarct size reduction similar to preconditioning. Thirty-minute occlusions produced infarcts that were 28%±5% of the region at risk in controls. The PKC activators 4β-phorbol-12-myristate-13-acetate or 1-oleyl-2-acetyl glycerol were infused for 5 minutes followed by a 10-minute washout. They reduced infarct size to 6%±1% and 12%±3%, respectively, similar to preconditioning (12%±2%). Polymyxin B was also used in the isolated heart model and, consistent with the PKC hypothesis, it eliminated preconditioning-induced protection, producing infarcts of 33%±5%.

This was one the first papers to move preconditioning to the "inside" of the myocyte even though it was a whole animal study. The data were consistent with a role for PKC in ischemic preconditioning. The mechanism of preconditioning looked like it included the signaling pathway adenosine receptor to inhibitory G protein to PLC to DAG to PKC. If this axis was correct, some very intriguing questions arose. For example, what protein(s) did PKC phosphorylate? Which of the numerous isoforms of PKC was involved? Was this signaling pathway unique to the rabbit? These questions have not yet been answered definitively nor has the PKC hypothesis been uniformly accepted. The paper by Ytrehus et al prompted a substantial number of subsequent studies, making it a seminal report, but conflicting results were obtained in more than a few of them, leading to a controversy that is still simmering and occasionally comes to an entertaining boil.

1994

A student fires two blank pistol shots
at Prince Charles in Sydney;
Spanish cyclist Miguel Indurain
wins the Tour de France;
and philosopher and author
Karl Popper dies, aged 92

Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs

G.J. Gross, J.A. Auchampach

Circ Res. 1992;70:223-233

Even though the "adenosine hypothesis" looked like a good explanation for preconditioning, it only explained how the effect was switched on, not what happened after the switch was thrown. Studies in isolated ventricular myocytes suggested that adenosine A_1 receptors were coupled to K_{ATP} channels. In addition, K_{ATP} channel openers such as pinacidil and nicorandil were successful in reducing experimental infarct size. Consequently, a reasonable rationale existed to hypothesize a role for K_{ATP} channels in preconditioning. Maybe opening of K_{ATP} channels was the next step after adenosine receptor activation. If so, blockade of K_{ATP} channels should block the protective effects of preconditioning. Likewise, the effects of preconditioning should be mimicked by a K_{ATP} channel opener.

This is what Gross and Auchampach set out to establish in anesthetized dogs, using myocardial infarct size as the principal end point. Infarcts were produced using a standardized protocol and infarct size was delineated with triphenyl tetrazolium chloride. To account for potential variability in collateral blood flow (a common problem in dogs), collateral blood flow was measured during the occlusions. Hearts were preconditioned by a single 5-minute coronary occlusion followed by 10 minutes of reperfusion. Glibenclamide was used to block K_{ATP} channels. To open K^+ channels, the potassium channel opener RP 52891 was used at a dosage that did not produce arterial hypotension.

Infarct size after 60 minutes' coronary occlusion was similar in controls (28%±6% of the region at risk) and when glibenclamide was administered without any other interventions (31%±6%), but was strikingly reduced after a single 5-minute preconditioning occlusion (6%±2%). This myocardial protection was eliminated when glibenclamide was administered 10 minutes before (infarct size 28%±6%) or immediately after (31%±8%) the preconditioning occlusion. The potassium channel opener RP 52891, administered before the 60-minute occlusion, reduced infarct size significantly to 13%±3%. Although not quite as spectacular as preconditioning, RP 52891 was effective, consistent with the idea that opening K_{ATP} channels was protective.

This paper was important because it presented a novel explanation for the activation of preconditioning. Although some people saw the K_{ATP} channel and adenosine hypotheses as "competing," they were probably better viewed as complementary. Adenosine receptor activation could lead to K_{ATP} channels opening up, an effect potentially enhanced by reductions in intracellular ATP during the preconditioning occlusion. Unfortunately, the data linking adenosine receptors and K_{ATP} channels were generated in isolated rat ventricular myocytes. Rats can be ischemically preconditioned, but preconditioning is unrelated to adenosine in this particular species. This certainly did not rule out a link between adenosine receptors and K_{ATP} channels in dogs, but it did complicate efforts to come up with a unified hypothesis.

As readily acknowledged by the authors, it remained to be seen how opening the channels actually protected myocardial cells. They suggested that the reduced action potential duration and attenuation of membrane depolarization secondary to K_{ATP} channel opening could lead to reductions in cytosolic free calcium concentration, rapid elimination of contractile activity, and decreased ATP utilization. A potential effect on neutrophil activation or migration was also suggested. The authors cautioned that glibenclamide influences insulin and glucose levels, so that there may have been effects exerted on the heart by glibenclamide that were indirect and independent of preconditioning. Despite this disclaimer, the paper by Gross and Auchampach prompted a number of other investigators to follow up their lead regarding K_{ATP} channels. Although there were some conflicting reports at first, the K_{ATP} channel hypothesis has stood the test of time well.

1992

A 12-year-old American boy wins a "divorce" from his parents for neglect; the Booker Prize is awarded to Michael Ondaatje for "The English Patient"; and Czech statesman Alexander Dubček dies, aged 70

Adaptation to ischemia during percutaneous transluminal coronary angioplasty: clinical, hemodynamic, and metabolic features

E. Deutsch, M. Berger, W.G. Kussmaul, J.W. Hirshfeld Jr, H.C. Herrmann, W.K. Laskey

Circulation. 1990;82:2044-2051

Does preconditioning occur in humans? That was the question posed in this paper published in 1990. This was 4 years after the first preconditioning paper (or, at least, the first paper to call preconditioning by its name). Although relatively few papers on preconditioning had been published up to this point, there was a lot of excitement in the myocardial ischemia research community. Preconditioning represented something brand new: it tapped into the myocardial cells' own means of protecting themselves from injury. Deutsch et al cleverly took advantage of the "human acute myocardial ischemia model" to try to find out if this novel, endogenous myocardial protective mechanism could be demonstrated in humans.

The human model, of course, is the angioplasty patient. In 12 patients with clinically stable, isolated obstructive disease who were undergoing percutaneous transluminal coronary angioplasty, Deutsch et al did two sequential 90-second balloon inflations, separated by at least 5 minutes of reperfusion, to occlude the left anterior descending artery. They measured ST-segment shifts, pulmonary pressures, and great cardiac vein flow during the occlusions to test the hypothesis that evidence of ischemia would be reduced during the second occlusion. In a second group of 7 patients, the same experimental protocol was followed, but lactate measurements were made to see if lactate production was attenuated during the second balloon inflation.

During the second balloon inflations, the patients reported less anginal discomfort. Electrocardiographically, ST-segments shifted significantly less (0.21±0.07 mV vs 0.44±0.13 mV) and hemodynamically, mean pulmonary artery pressures were significantly lower (20±2 mm Hg vs 25±1 mm Hg). Cardiac vein flow was also significantly lower (83±2 mL/min vs 96±1 mL/min) and there was less myocardial lactate production (lactate extraction ratio, -0.03±0.02 vs -0.11±0.03) during the second balloon inflation. Thus, the patients appeared to have less severe ischemia during the second coronary occlusion compared to the first. Angina, ST-segments, coronary venous flow, and lactate production were all reduced, supporting the conclusion that the first balloon inflation preconditioned the myocardium, reducing the severity and/or consequences of the ischemia during the second balloon inflation. It looked like preconditioning worked in humans, too.

The paper by Deutsch et al got a lot of people focused on preconditioning. Here was something novel, very effective, and it happened in humans. Would it have significant impact on how patients were treated? Only time would tell, but, in 1990, preconditioning was clearly the thing on which to work in myocardial protection. A lot of interventions had been studied over the previous 30 years and very few had paid off. Now, right at the start, was evidence that preconditioning was relevant in humans. There was something "built-in" to human myocardial cells, similar to dog and rat and rabbit myocardial cells, that could be switched on somehow to make them resist the effects of ischemia. It remained (remains?) to be determined what is "built-in" and how to switch it on or off, but the point is that Deutsch et al had provided a strong incentive to find out.

Not everyone, however, was convinced that what had been shown was actually preconditioning. The main reservation was the idea that collateral flow was recruited by the first coronary occlusion. If collateral flow increased, the severity of ischemia would be less during the second occlusion, accounting for the lower ST-segments, etc. Deutsch et al anticipated this criticism, contending that the data on cardiac vein flows and wedge pressures were inconsistent with augmented collateral perfusion. Despite this explanation, a fair amount of skepticism lingered on the fringes. Rather than turning researchers off to preconditioning, however, I think "loose ends" like this actually encouraged additional work in the area.

1990

American Larry Khan retains his title as World Tiddlywinks Champion, in London; Queen Elizabeth, the Queen Mother, celebrates her 90th birthday; and French cellist Paul Tortelier dies, aged 76

Preconditioning of ischemic myocardium: reperfusion-induced arrhythmias

K. Shiki, D.J. Hearse

Am J Physiol. 1987;253(6, pt 2):H-1470–H-1476

When this paper was published, preconditioning was still a fairly young concept, generally associated with infarct size reduction in dogs. Whether or not preconditioning protected the myocardium in species other than the dog and from consequences of myocardial ischemia other than infarction remained unknown.

Shiki and Hearse were among the first to delve into this matter by studying rats in which they produced reversible coronary occlusions by means of a ligature passed as snare around the left anterior descending artery. The main end points were: (i) incidence of premature ventricular contractions (PVCs); (ii) incidence of ventricular fibrillation (VF); (iii) incidence and duration of ventricular tachycardia (VT); and (iv) time in normal sinus rhythm. Releasing the snare and reperfusing the myocardium after a few minutes of ischemia produced little or no infarction, but did lead to a storm of arrhythmias. The question was: could ischemic preconditioning quiet the storm?

To answer this question, two occlusions were performed in the rats. The first one produced the expected storm of arrhythmias, but, it was hypothesized, would also precondition the myocardium. The second one was performed to see if the arrhythmic tempest was modified in any way. The interval between the two occlusions was varied between 10 minutes and 3 days to get a handle on the duration of any antiarrhythmic preconditioning effect. After the first occlusion, VT occurred in 100% of the rats, 83% had VF, and the number of PVCs in the first 3 minutes of reperfusion averaged about 680. After the second occlusion, the incidence of VT and VF and the number of PVCs were reduced dramatically. Thus, when the recovery period between the two occlusions was 10 minutes, no hearts underwent VF, VT was reduced to 17%, and there were only 4 PVCs. When the duration of the recovery period was increased, the incidence of arrhythmias gradually increased until, at an interval of 3 days, there was no longer a difference between the first and second episodes of reperfusion-induced arrhythmias.

Another series of experiments sought to find out if there was a "dose-response" relationship between ischemia time and reperfusion-induced arrhythmias associated with the first (preconditioning) occlusion and protection from arrhythmias after the second occlusion. What Shiki and Hearse observed was an inverse relationship between arrhythmias in the first and second occlusions. When the first occlusion was 0.5 minute in length, few arrhythmias were produced and the effect on electrical vulnerability after the second occlusion was nil. First occlusions of longer duration (up to 5 minutes) produced increasingly severe arrhythmic storms, but preconditioned more effectively because the incidence of arrhythmias after the second occlusion decreased in what looked to be a "dose-dependent" manner.

These data were important as: (i) they showed that ischemic preconditioning worked in another species besides the dog; rats may not be the most pleasant of creatures, but they are another species, and if something happens in more than one species maybe it happens in all species; (ii) Shiki and Hearse showed that ischemic preconditioning influenced an end point other than infarct size: demonstrating that preconditioning reduced vulnerability to reperfusion-induced arrhythmias suggested that preconditioning had a wider scope than prevention or delay of necrosis; (iii) the rapid induction of protection followed by a gradual decay provided important information on the "kinetics" of preconditioning; (iv) the dose-response effect evident in the second series of experiments suggested that the preconditioning effect was titratable. Although this particular conclusion has not held up as well as the others, it does little to reduce the impact of this important study.

1987

19-year-old West German Mathias Rust lands his light aircraft in Red Square; the year is shortened by 1 s to allow for adjustments in the Gregorian calendar; and US artist Andy Warhol dies, aged 58

Myocardial infarct size-limiting effect of ischemic preconditioning: its natural decay and the effect of repetitive preconditioning

T. Miura, T. Adachi, T. Ogawa, T. Iwamoto, A. Tsuchida, O. Iimura

Cardiovasc Pathol. 1992;1:147-154

Although impressive resistance to infarction, arrhythmias, and functional deficits had been documented in a number of preconditioning studies, it was important to determine if these effects lasted long enough to be useful clinically.

Miura et al subjected rabbits to 30-minute coronary occlusions to produce myocardial infarction, followed by 72 hours of reperfusion, after which the infarcts were identified and quantitated histologically. Without preconditioning, infarcts averaged 44%±4% of the region at risk. When the 30-minute occlusion was preceded by a standard preconditioning occlusion of 5 minutes' duration, followed by 5 minutes of reperfusion, infarct size was significantly reduced to 21%±3%, demonstrating the protective effect of preconditioning.

To determine how preconditioning-induced protection decayed, Miura et al prolonged the reperfusion (or recovery) period between the preconditioning and 30-minute occlusions to 15, 25, or 35 minutes. Significantly smaller infarcts (27%±4%), compared to controls, were still evident when the recovery period was 15 minutes. However, after 25 or 35 minutes, infarct sizes were not significantly different from controls (30%±6% and 36%±4%, respectively). This suggested that preconditioning-induced protection against infarction lasted less than half-an-hour.

To find out if protection could be augmented with repetitive preconditioning, 5-minute occlusions were used twice or four times before a 30-minute occlusion in additional groups of rabbits. Each 5-minute occlusion was separated by 5 minutes of reperfusion. A tendency towards smaller infarcts was observed (two cycles, 16%±4%; four cycles, 14%±3%), but there was no significant difference with those seen after a single preconditioning occlusion (21%±3%). Consequently, it appeared that recurrent preconditioning produced, at best, a quite modest additive effect.

The last part of the study focused on the time course of myocardial stunning. If myocardial stunning played an important role in preconditioning, it followed that the time courses of stunning and protection due to preconditioning should be similar. To measure myocardial stunning, Miura et al used miniature Doppler probes placed on the left ventricle to monitor changes in wall thickness. A 5-minute occlusion was performed (simulating a preconditioning occlusion) during which systolic wall thickening was replaced by systolic thinning. Five minutes after reperfusion, systolic wall thickening was 64%±9% of baseline values, demonstrating that the myocardium was mechanically stunned. At 35 minutes after reperfusion (ie, after preconditioning-induced protection against infarction had subsided), the myocardium was still stunned with systolic wall thickening recovered to only 73%±7% of baseline values. Therefore, the time courses of stunning and myocardial protection did not correspond, supporting the conclusion that stunning did not contribute importantly to myocardial preconditioning.

The results on repetitive preconditioning and stunning were solid, providing strong support for conclusions advanced in previous studies. The most important finding was on the duration of preconditioning-induced protection. It had been clear from the first studies on preconditioning by Murry et al and Shiki and Hearse that preconditioning did not last forever, and subsequent preliminary reports had reinforced this view. In the present study, Miura et al showed that preconditioning not only did not last forever, it did not last very long at all.

Useful though this information was from a mechanistic standpoint, it was also a bit disheartening especially to those of us trying to "bottle" preconditioning and apply it clinically: preconditioning may be intense, but it did not appear to have much staying power.

1992

Anthony Hopkins "has an old friend for dinner"
in "Silence of the Lambs";
Queen Elizabeth II describes
the year as her annus horribilis;
and German statesman Willy Brandt dies aged 78

Preconditioning cultured human pediatric myocytes requires adenosine and protein kinase C

J.S. Ikonomidis, T. Shirai, R.D. Weisel, B. Derylo, V. Rao, C.I. Whiteside, D.A.G. Mickle, R.K. Li

Am J Physiol. 1997;272(3, pt 2):H-1220–H-1230

This group had shown earlier that it was possible to culture human pediatric myocytes and simulate "ischemia" by subjecting the cells to a low-volume, anoxic environment for 20 minutes. Following this by 20 minutes of "reperfusion" (washout really) preconditioned the myocytes, reducing the damage produced by a subsequent exposure to 90 minutes of "ischemia" and 30 minutes of "reperfusion." The present study sought to evaluate the potential roles of adenosine and protein kinase C (PKC) in this model of preconditioning.

Ikonomidis et al first showed that the "ischemic" cells in culture released something into the supernatant that was protective: the supernatant taken from one "ischemic" culture reduced damage in a second culture, indicating that the protective substance was transferable. Adenosine was detected in the supernatant at high enough concentration (approx. 14 nmol/L) to produce adenosine receptor activation. Although this finding might seem anticlimactic given how well entrenched the adenosine hypothesis was, it must be remembered that this study was done in *human* cells, so this was the first indication that human preconditioning involved adenosine. Animal experiments had demonstrated significant species differences in preconditioning, so there was no guarantee that human preconditioning could be "switched on" with adenosine.

The next step was to use the adenosine receptor antagonist 8-(*p*-sulfophenyl)theophylline (SPT, 100 µmol/L) to abolish the protective effects of preconditioning. The adenosine receptor agonist *R*(-)-N^6-(2-phenyisopropyl)adenosine (PIA, 100 µmol/L) reduced ischemic damage as well, consistent with the hypothesis. Exogenous adenosine did the same when it was added to the supernatant in various concentrations. Curiously, however, high concentrations (10-50 µmol/L) of exogenous adenosine were required to significantly reduce damage, and a biphasic response was observed, such that higher concentrations did not reduce damage at all. Although the somewhat peculiar effects of exogenous adenosine remained unexplained, the adenosine hypothesis had passed the conventional tests in this unconventional model.

Ikonomidis et al then examined the role of PKC in the signaling pathway of preconditioning. Preconditioning and preincubation with the PKC activator 4β-phorbol-12-myristate-13-acetate (PMA, 1 µmol/L) both reduced ischemic damage, and both also led to translocation of PKC from the cytoplasm to the cell membrane and perinuclear areas, and to increased PKC phosphorylation rates. Preconditioning-induced protection was blocked by the PKC inhibitors calphostin C (200 nmol/L) or chelerythrine (1 µmol/L). Calphostin C also abolished the protective effects of exogenous adenosine and eliminated the increase in PKC phosphorylation rate. These data strongly favored a role for PKC in preconditioning. Ikonomidis et al had used direct measurements of PKC activation (translocation and phosphorylation) and inhibitors that were selective for PKC over other types of kinases, so this was an impressively thorough test of the PKC hypothesis.

It could be argued that these results might be unique to human pediatric myocytes in culture, and any cells in culture are a little suspect given the dedifferentiation they undergo. However, even if such cells provide information that is quantitatively off the mark, the basic findings are probably directionally correct. Preconditioning appears to be a fundamental aspect of cell biology, not restricted to the heart, so the mechanisms that initiate and sustain it are unlikely to be lost due to culture. This study provided the first information on the mechanism of human preconditioning, at least of the "first window" variety. Which PKC isoforms are activated and which proteins are phosphorylated, leading to increased resistance to ischemia, remains to be determined, as is the manner in which preconditioning can be "bottled" and exploited clinically.

1997

18 years of Conservative rule in Britain end with Tony Blair's landslide victory; Britain gives Hong Kong back to China; and 21-year-old Tiger Woods wins the US Masters golf championship

Previous angina alters in-hospital outcome in TIMI 4: a clinical correlate of preconditioning?

R.A. Kloner, T. Shook, K. Przyklenk, V.G. Davis, L. Junio, R.V. Matthews, S. Burstein, C.M. Gibson, W.K. Poole, C.P. Cannon, C.H. McCabe, E. Braunwald, and the TIMI 4 investigators

Circulation. 1995;91:37-47

In their landmark 1986 paper, *(see page 216)*, Murry et al postulated that myocardial ischemia, which produced angina in human patients, might also be preconditioning hearts to reduce or delay myocardial injury due to a subsequent sustained occlusion. Nine years later, Kloner et al tested this hypothesis by retrospectively analyzing the effect of previous angina on in-hospital outcomes of patients with acute myocardial infarction enrolled in the TIMI (Thrombolysis In Myocardial Infarction) 4 trial. Evidence had already accumulated that preconditioning could be induced in humans, but the possibility that angina might do so was less conclusive.

Three different thrombolytic treatment regimens for acute myocardial infarction were evaluated in TIMI 4. To determine if a history of previous angina affected patient outcome, data regarding previous history of angina, in-hospital outcome, and 6-week follow-up were collected from case report forms of 218 patients with a history of previous angina before acute myocardial infarction and 198 patients who did not have previous angina.

The patients with previous angina were less likely to die in hospital (3% vs 8%, P=0.03) or develop severe congestive heart failure or shock (1% vs 7%, P=0.006). When the end points were combined, the percentages also favored the patients with previous angina (4% vs 12%, P=0.004). Patients with a history of angina also appeared to have smaller infarcts based on creatine kinase (119 vs 154 CK integrated units, P=0.01) and were less likely to have Q waves (57% vs 69%, P=0.01). Similar results were obtained when the subset of patients experiencing angina 48 hours before infarction were compared with those who did not. There was no difference between patients with angina and those without in terms of angiographically detectable collaterals. Despite all of this "good" news regarding angina, there was some bad. Consistent with other clinical findings, patients with a history of previous angina had a trend for more recurrent ischemic pain, suggesting that "good" effects induced by angina were not sustained after the patients left the hospital.

There were three explanations for the better outcomes in the group of patients with angina. One was more collaterals in the angina patients. However, angiography showed that the angina group had no more and maybe even fewer epicardial collaterals than the nonangina patients. This did not rule out a difference in angiographically invisible microvascular collaterals, but their potential significance is uncertain even if they were present. A second explanation was a difference in antianginal medications between the two groups. Not unexpectedly, the angina patients did take more antianginal medications, and some antianginals reduce injury secondary to ischemia, at least experimentally. But when the statistical effect of antianginal medications was factored into the comparisons between angina and nonangina patients, this could not account for the difference in patient outcomes alone. That left the third possible explanation, preconditioning. Kloner et al suggested that the ischemia which produced angina also preconditioned the myocardium, reducing the extent of damage when the patients later had ischemia that lasted long enough to cause myocardial infarctions. True, there were limitations to this retrospective study (freely acknowledged by Kloner et al), but the general conclusion that angina and preconditioning could occur at the same time seemed quite reasonable.

This conclusion went along with a preconceived notion many of us entertained, but even preconceived notions are correct, occasionally. More importantly, if angina could do something good for the human heart, a lot of scientists were encouraged to think that they could recreate the effect with less risky (and uncomfortable) interventions.

1995

Hurricane Luis devastates the French/Dutch Caribbean island of Saint Martin/Sint Maarten; statues of the Hindu god Ganesh start drinking milk; and 200 heads of state attend the UN's 50th birthday party in New York

Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction

M.S. Marber, D.S. Latchman, J.M. Walker, D.M. Yellon

Circulation. 1993;88:1264-1272

Until this study by Marber et al, most work on preconditioning had concentrated on myocardial protection in the first couple of hours after inducing preconditioning, and it was clear that the protection did not last very long. Studies in rabbit and rat hearts had shown that a brief episode of heat shock increased heat shock protein (HSP) expression 24 hours later, and this was associated with reductions in infarct size or other types of myocardial injury. If brief bouts of ischemia induced HSPs, expression of which would take 24 hours or so, could there be a second, delayed period in which the myocardium was protected from ischemic change?

Marber et al studied rabbits exposed to hyperthermia (42°C for 15 minutes) or regional myocardial ischemia (four 5-minute preconditioning occlusions); 24-hours later, the rabbits underwent a protocol designed to produce myocardial infarction, the principal end point of the study, or to measure HSP expression (HSP 72 and HSP 60) with Western blot analysis. Thermal stress increased HSP 72 expression eightfold compared to sham controls, and ischemic preconditioning occlusions increased expression sevenfold. HSP 60 expression was elevated 1.5-2.0-fold by ischemia, but not by thermal stress. Infarcts were produced by 30-minute coronary occlusions followed by 2 hours of reperfusion, and infarct size was delineated with triphenyl tetrazolium chloride.

Both thermal stress and ischemic preconditioning significantly reduced infarct size. Thermally stressed rabbits had infarcts averaging 33%±4% of the region at risk compared to 57%±7% in controls; in ischemically preconditioned rabbits infarcts were 29%±5% vs 52%±5% in controls with sham thoracotomies. Thus, both interventions induced HSP 72 expression and significantly reduced infarct size 24 hours later, and elevations in HSP 72 were similar as were the extents of infarct size reduction. HSP 60 did not appear to be important, but an association between HSP 72 and infarct size reduction was clear, strongly suggesting (if not quite proving) a cause-and-effect relationship.

In addition to providing a good example of the principle of cross-tolerance, this study indicated that preconditioning was biphasic. It was already well established that the early phase ("classic" preconditioning), involving activation of receptors and opening of K_{ATP} channels, was induced rapidly, but lasted only 30 to 120 minutes depending on the species. The delayed phase was evident 24 hours later, associated with HSP 72 induction, and characterized by somewhat less striking infarct size reduction than the early phase. Marber et al coined the catchy terms "first window" and "second window" of myocardial protection to describe the two phases.

The first and second window idea caught on very rapidly and promoted renewed interest in preconditioning. The first window had been characterized thoroughly even if the mechanistic story remained incomplete. The main problem with the first window was the fact that it was "narrow." The second window, on the other hand, held out the promise of being "wider" in terms of duration (if not intensity of myocardial protection). Although Marber et al targeted two stress proteins (HSP 72 and HSP 60) in their study, no one was under the illusion that these were the only proteins potentially induced by preconditioning. There were other HSPs, antioxidant enzymes, and transcription factors to examine. The characteristics of the second window (particularly how wide it really was) also needed clarification. Therefore, it is not surprising that the second window idea provided a major boost to basic research into preconditioning.

1993

Prince Sihanouk returns to Cambodia;
Japan's Crown Prince Naruhito weds Masako, his Oxford-educated bride;
and Briton Mark Nyman wins the World Scrabble championship with the word "wet"

Preconditioning

Bibliography of One Hundred Key Papers

Ardell JL, Yang XM, Barron BA, Downey JM, Cohen MV.

Endogenous myocardial norepinephrine is not essential for ischemic preconditioning in rabbit heart.

Am J Physiol. 1996;270(3, pt 2):H-1078–H-1084.

Armstrong S, Downey JM, Ganote CE.

Preconditioning of isolated rabbit cardiomyocytes: induction by metabolic stress and blockade by the adenosine antagonist SPT and calphostin C, a protein kinase C inhibitor.

Cardiovasc Res. 1994;28:72-77.

Armstrong SC, Hoover DB, Delacey MH, Ganote CE.

Translocation of PKC, protein phosphatase inhibition and preconditioning of rabbit cardiomyocytes.

J Mol Cell Cardiol. 1996;28:1479-1492.

Armstrong SC, Kao R, Gao W, et al.

Comparison of in vitro preconditioning responses of isolated pig and rabbit cardiomyocytes: effects of a protein phosphatase inhibitor, fostriecin.

J Mol Cell Cardiol. 1997;29:3009-3024.

Armstrong SC, Liu GS, Downey JM, Ganote CE.

Potassium channels and preconditioning of isolated rabbit cardiomyocytes: effects of glyburide and pinacidil.

J Mol Cell Cardiol. 1995;27:1765-1774.

Asimakis GK.

Myocardial glycogen depletion cannot explain the cardioprotective effects of ischemic preconditioning in the rat heart.

J Mol Cell Cardiol. 1996;28:563-570.

Auchampach JA, Gross GJ.

Adenosine A_1 receptors, K_{ATP} channels, and ischemic preconditioning in dogs.

Am J Physiol. 1993;264(5, pt 2):H-1327–H-1336.

Baines CP, Goto M, Downey JM.

Oxygen radicals released during ischemic preconditioning contribute to cardioprotection in the rabbit myocardium.

J Mol Cell Cardiol. 1997;29:207-216.

Banerjee A, Locke-Winter C, Rogers KB, et al.

Preconditioning against myocardial dysfunction after ischemia and reperfusion by an α_1-adrenergic mechanism.

Circ Res. 1993;73:656-670.

Barbosa V, Sievers RE, Zaugg CE, Wolfe CL.

Preconditioning ischemia time determines the degree of glycogen depletion and infarct size reduction in rat hearts.

Am Heart J. 1996;131:224-230.

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.

Baxter GF, Goma FM, Yellon DM.

Characterisation of the infarct-limiting effect of delayed preconditioning: time course and dose-dependency studies in rabbit myocardium.

Basic Res Cardiol. 1997;92:159-167.

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.

Birnbaum Y, Hale SL, Kloner RA.

Progressive decrease in the ST segment elevation during ischemic preconditioning: is it related to recruitment of collateral vessels?

J Mol Cell Cardiol. 1996;28:1493-1499.

Bolli R, Bhatti ZA, Tang XL, et al.

Evidence that late preconditioning against myocardial stunning in conscious rabbits is triggered by the generation of nitric oxide.

Circ Res. 1997;81:42-52.

Brooks G, Hearse DJ.

Role of protein kinase C in ischemic preconditioning: player or spectator?

Circ Res. 1996;79:627-630.

Bugge E, Ytrehus K.

Bradykinin protects against infarction but does not mediate ischemic preconditioning in the isolated rat heart.

J Mol Cell Cardiol. 1996;28:2333-2341.

Cave AC.

Preconditioning induced protection against post-ischaemic contractile dysfunction: characteristics and mechanisms.

J Mol Cell Cardiol. 1995;27:969-979.

Chen W, Wetsel W, Steenbergen C, Murphy E.

Effect of ischemic preconditioning and PKC activation on acidification during ischemia in rat heart.

J Mol Cell Cardiol. 1996;28:871-880.

Cohen MV, Liu Y, Liu GS, et al.

Phospholipase D plays a role in ischemic preconditioning in rabbit heart.

Circulation. 1996;94:1713-1718.

Cohen MV, Thornton JD, Thornton CS, Sato H, Miki T, Downey JM.

Intravenous co-infusion of adenosine and norepinephrine preconditions the heart without adverse hemodynamic effects.

J Thorac Cardiovasc Surg. 1997;114:236-242.

Cohen MV, Yang XM, Downey JM.

Conscious rabbits become tolerant to multiple episodes of ischemic preconditioning.

Circ Res. 1994;74:998-1004.

Deutsch E, Berger M, Kussmaul WG, Hirshfeld JW Jr, Herrmann HC, Laskey WK.

Adaptation to ischemia during percutaneous transluminal coronary angioplasty: clinical, hemodynamic, and metabolic features.

Circulation. 1990;82:2044-2051.

Goto M, Cohen MV, Van Wylen DGL, Downey JM.

Attenuated purine production during subsequent ischemia in preconditioned rabbit myocardium is unrelated to the mechanism of protection.

J Mol Cell Cardiol. 1996;28:447-454.

Goto M, Liu Y, Yang XM, Ardell JL, Cohen MV, Downey JM.

Role of bradykinin in protection of ischemic preconditioning in rabbit hearts.

Circ Res. 1995;77:611-621.

Gottlieb RA, Gruol DL, Zhu JY, Engler RL.

Preconditioning in rabbit cardiomyocytes: role of pH, vacuolar proton ATPase, and apoptosis.

J Clin Invest. 1996;97:2391-2398.

Gross GJ, Auchampach JA.	*Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs.* ***Circ Res.*** 1992;70:223-233.
Headrick JP.	*Ischemic preconditioning: bioenergetic and metabolic changes and the role of endogenous adenosine.* ***J Mol Cell Cardiol.*** 1996;28:1227-1240.
Ikonomidis JS, Shirai T, Weisel RD, et al.	*Preconditioning cultured human pediatric myocytes requires adenosine and protein kinase C.* ***Am J Physiol.*** 1997;272(3, pt 2):H-1220–H-1230.
Jennings RB, Murry CE, Reimer KA.	*Energy metabolism in preconditioned and control myocardium: effect of total ischemia.* ***J Mol Cell Cardiol.*** 1991;23:1449-1458.
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.
King LM, Opie LH.	*Does preconditioning act by glycogen depletion in the isolated rat heart?* ***J Mol Cell Cardiol.*** 1996;28:2305-2321.
Kitakaze M, Hori M, Morioka T, et al.	*Alpha$_1$-adrenoceptor activation mediates the infarct size-limiting effect of ischemic preconditioning through augmentation of 5'-nucleotidase activity.* ***J Clin Invest.*** 1994;93:2197-2205.
Kitakaze M, Node K, Minamino T, et al.	*Role of activation of protein kinase C in the infarct size-limiting effect of ischemic preconditioning through activation of ecto-5'-nucleotidase.* ***Circulation.*** 1996;93:781-791.
Kloner RA, Shook T, Przyklenk K, et al.	*Previous angina alters in-hospital outcome in TIMI 4: a clinical correlate to preconditioning?* ***Circulation.*** 1995;91:37-45.
Kolocassides KG, Galiñanes M, Hearse DJ.	*Preconditioning accelerates contracture and ATP depletion in blood-perfused rat hearts.* ***Am J Physiol.*** 1995;269(4, pt 2):H-1415–H-1420.
Kolocassides KG, Seymour AML, Galiñanes M, Hearse DJ.	*Paradoxical effect of ischemic preconditioning on ischemic contracture? NMR studies of energy metabolism and intracellular pH in the rat heart.* ***J Mol Cell Cardiol.*** 1996;28:1045-1057.
Kuzuya T, Hoshida S, Yamashita N, et al.	*Delayed effects of sublethal ischemia on the acquisition of tolerance to ischemia.* ***Circ Res.*** 1993;72:1293-1299.
Leesar MA, Stoddard M, Ahmed M, Broadbent J, Bolli R.	*Preconditioning of human myocardium with adenosine during coronary angioplasty.* ***Circulation.*** 1997;95:2500-2507.
Li GC, Vasquez JA, Gallagher KP, Lucchesi BR.	*Myocardial protection with preconditioning.* ***Circulation.*** 1990;82:609-619.

Li Y, Kloner RA.

Does protein kinase C play a role in ischemic preconditioning in rat hearts?

Am J Physiol. 1995;268(1, pt 2):H-426–H-431.

Liu GS, Richards SC, Olsson RA, Mullane K, Walsh RS, Downey JM.

Evidence that the adenosine A_3 receptor may mediate the protection afforded by preconditioning in the isolated rabbit heart.

Cardiovasc Res. 1994;28:1057-1061.

Liu GS, Thornton J, Van Winkle DM, Stanley AWH, Olsson RA, Downey JM.

Protection against infarction afforded by preconditioning is mediated by A_1 adenosine receptors in rabbit heart.

Circulation. 1991;84:350-356.

Liu Y, Downey JM.

Ischemic preconditioning protects against infarction in rat heart.

Am J Physiol. 1992;263(4, pt 2):H-1107–H-1112.

Liu Y, Gao WD, O'Rourke B, Marban E.

Synergistic modulation of ATP-sensitive K^+ currents by protein kinase C and adenosine: implications for ischemic preconditioning.

Circ Res. 1996;78:443-454.

Liu Y, Tsuchida A, Cohen MV, Downey JM.

Pretreatment with angiotensin II activates protein kinase C and limits myocardial infarction in isolated rabbit hearts.

J Mol Cell Cardiol. 1995;27:883-892.

Liu Y, Ytrehus K, Downey JM.

Evidence that translocation of protein kinase C is a key event during ischemic preconditioning of rabbit myocardium.

J Mol Cell Cardiol. 1994;26:661-668.

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.

Matsuda M, Catena TG, Vander Heide RS, Jennings RB, Reimer KA.

Cardiac protection by ischaemic preconditioning is not mediated by myocardial stunning.

Cardiovasc Res. 1993;27:585-592.

Maulik N, Watanabe M, Zu YL, et al.

Ischemic preconditioning triggers the activation of MAP kinases and MAPKAP kinase 2 in rat hearts.

FEBS Lett. 1996;396:233-237.

Mitchell MB, Meng X, Ao L, Brown JM, Harken AH, Banerjee A.

Preconditioning of isolated rat heart is mediated by protein kinase C.

Circ Res. 1995;76:73-81.

Miura T, Adachi T, Ogawa T, Iwamoto T, Tsuchida A, Iimura O.

Myocardial infarct size-limiting effect of ischemic preconditioning: its natural decay and the effect of repetitive preconditioning.

Cardiovasc Pathol. 1992;1:147-154.

Miura T, Goto M, Urabe K, Endoh A, Shimamoto K, Iimura O.

Does myocardial stunning contribute to infarct size limitation by ischemic preconditioning?

Circulation. 1991;84:2504-2512.

Miyawaki H, Ashraf M.

Ca^{2+} as a mediator of ischemic preconditioning.

Circ Res. 1997;80:790-799.

Miyawaki H, Zhou X, Ashraf M.

Calcium preconditioning elicits strong protection against ischemic injury via protein kinase C signaling pathway.

Circ Res. 1996;79:137-146.

Moolman JA, Genade S, Tromp E, Lochner A.

A comparison between ischemic preconditioning and anti-adrenergic interventions: cAMP, energy metabolism and functional recovery.
Basic Res Cardiol. 1996;91:219-233.

Morita Y, Murakami T, Iwase T, et al.

K_{ATP} channels contribute to the cardioprotection of preconditioning independent of anaesthetics in rabbit hearts.
J Mol Cell Cardiol. 1997;29:1267-1276.

Murphy E, Glasgow W, Fralix T, Steenbergen C.

Role of lipoxygenase metabolites in ischemic preconditioning.
Circ Res. 1995;76:457-467.

Murry CE, Jennings RB, Reimer KA.

Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium.
Circulation. 1986;74:1124-1136.

Murry CE, Richard VJ, Jennings RB, Reimer KA.

Myocardial protection is lost before contractile function recovers from ischemic preconditioning.
Am J Physiol. 1991;260(3, pt 2):H-796–H-804.

Murry CE, Richard VJ, Reimer KA, Jennings RB.

Ischemic preconditioning slows energy metabolism and delays ultrastructural damage during a sustained ischemic episode.
Circ Res. 1990;66:913-931.

Nakagawa Y, Ito H, Kitakaze M, et al.

Effect of angina pectoris on myocardial protection in patients with reperfused anterior wall myocardial infarction: retrospective clinical evidence of "preconditioning."
J Am Coll Cardiol. 1995;25:1076-1083.

Niroomand F, Weinbrenner C, Weis A, et al.

Impaired function of inhibitory G proteins during acute myocardial ischemia of canine hearts and its reversal during reperfusion and a second period of ischemia: possible implications for the protective mechanism of ischemic preconditioning.
Circ Res. 1995;76:861-870.

Ottani F, Galvani M, Ferrini D, et al.

Prodromal angina limits infarct size: a role for ischemic preconditioning.
Circulation. 1995;91:291-297.

Ovize M, Kloner RA, Przyklenk K.

Stretch preconditions canine myocardium.
Am J Physiol. 1994;266(1, pt 2):H-137–H-146.

Ovize M, Przyklenk K, Hale SL, Kloner RA.

Preconditioning does not attenuate myocardial stunning.
Circulation. 1992;85:2247-2254.

Ping P, Zhang J, Qiu Y, et al.

Ischemic preconditioning induces selective translocation of protein kinase C isoforms ε and η in the heart of conscious rabbits without subcellular redistribution of total protein kinase C activity.
Circ Res. 1997;81:404-414.

Przyklenk K, Bauer B, Ovize M, Kloner RA, Whittaker P.

Regional ischemic "preconditioning" protects remote virgin myocardium from subsequent sustained coronary occlusion.
Circulation. 1993;87:893-899.

Przyklenk K, Sussman MA, Simkhovich BZ, Kloner RA.

Does ischemic preconditioning trigger translocation of protein kinase C in the canine model?
Circulation. 1995;92:1546-1557.

Qiu Y, Tang XL, Park SW, Sun JZ, Kalya A, Bolli R.

The early and late phases of ischemic preconditioning: a comparative analysis of their effects on infarct size, myocardial stunning, and arrhythmias in conscious pigs undergoing a 40-minute coronary occlusion.

Circ Res. 1997;80:730-742.

Ramasamy R, Liu H, Anderson S, Lundmark J, Schaefer S.

Ischemic preconditioning stimulates sodium and proton transport in isolated rat hearts.

J Clin Invest. 1995;96:1464-1472.

Sandhu R, Thomas U, Diaz RJ, Wilson GJ.

Effect of ischemic preconditioning of the myocardium on cAMP.

Circ Res. 1996;78:137-147.

Schott RJ, Rohmann S, Braun ER, Schaper W.

Ischemic preconditioning reduces infarct size in swine myocardium.

Circ Res. 1990;66:1133-1142.

Schultz JEJ, Hsu AK, Gross GJ.

Morphine mimics the cardioprotective effect of ischemic preconditioning via a glibenclamide-sensitive mechanism in the rat heart.

Circ Res. 1996;78:1100-1104.

Schultz JEJ, Hsu AK, Gross GJ.

Ischemic preconditioning is mediated by a peripheral opioid receptor mechanism in the intact rat heart.

J Mol Cell Cardiol. 1997;29:1355-1362.

Schultz JEJ, Qian YZ, Gross GJ, Kukreja RC.

The ischemia-selective K_{ATP} channel antagonist, 5-hydroxydecanoate, blocks ischemic preconditioning in the rat heart.

J Mol Cell Cardiol. 1997;29:1055-1060.

Schulz R, Rose J, Heusch G.

Involvement of activation of ATP-dependent potassium channels in ischemic preconditioning in swine.

Am J Physiol. 1994;267(4, pt 2):H-1341–H-1352.

Schulz R, Rose J, Post H, Heusch G.

Involvement of endogenous adenosine in ischaemic preconditioning in swine.

Pflügers Arch. 1995;430:273-282.

Shattock MJ, Lawson CS, Hearse DJ, Downey JM.

Electrophysiological characteristics of repetitive ischemic preconditioning in the pig heart.

J Mol Cell Cardiol. 1996;28:1339-1347.

Shiki K, Hearse DJ.

Preconditioning of ischemic myocardium: reperfusion-induced arrhythmias.

Am J Physiol. 1987;253(6, pt 2):H-1470–H-1476.

Silva PH, Dillon D, Van Wylen DGL.

Adenosine deaminase inhibition augments interstitial adenosine but does not attenuate myocardial infarction.

Cardiovasc Res. 1995;29:616-623.

Simkhovich BZ, Przyklenk K, Hale SL, Patterson M, Kloner RA.

Direct evidence that ischemic preconditioning does not cause protein kinase C translocation in rabbit heart.

Cardiovasc Res. 1996;32:1064-1070.

Speechly-Dick ME, Grover GJ, Yellon DM.

Does ischemic preconditioning in the human involve protein kinase C and the ATP-dependent K^+ channel? Studies of contractile function after simulated ischemia in an atrial in vitro model.

Circ Res. 1995;77:1030-1035.

Speechly-Dick ME, Mocanu MM, Yellon DM.

Protein kinase C: its role in ischemic preconditioning in the rat.
Circ Res. 1994;75:586-590.

Tanaka M, Fujiwara H, Yamasaki K, et al.

Ischemic preconditioning elevates cardiac stress protein but does not limit infarct size 24 or 48 h later in rabbits.
Am J Physiol. 1994;267(4, pt 2):H-1476–H-1482.

Tang XL, Qiu Y, Park SW, Sun JZ, Kalya A, Bolli R.

Time course of late preconditioning against myocardial stunning in conscious pigs.
Circ Res. 1996;79:424-434.

Thornton J, Striplin S, Liu GS, et al.

Inhibition of protein synthesis does not block myocardial protection afforded by preconditioning.
Am J Physiol. 1990;259(6, pt 2):H-1822–H-1825.

Thornton JD, Thornton CS, Downey JM.

Effect of adenosine receptor blockade: preventing protective preconditioning depends on time of initiation.
Am J Physiol. 1993;265(2, pt 2):H-504–H-508.

Tomai F, Crea F, Gaspardone A, et al.

Ischemic preconditioning during coronary angioplasty is prevented by glibenclamide, a selective ATP-sensitive K^+ channel blocker.
Circulation. 1994;90:700-705.

Tomai F, Crea F, Gaspardone A, et al.

Effects of A_1 adenosine receptor blockade by bamiphylline on ischaemic preconditioning during coronary angioplasty.
Eur Heart J. 1996;17:846-853.

Tritto I, D'Andrea D, Eramo N, et al.

Oxygen radicals can induce preconditioning in rabbit hearts.
Circ Res. 1997;80:743-748.

Tsuchida A, Liu Y, Liu GS, Cohen MV, Downey JM.

α_1-Adrenergic agonists precondition rabbit ischemic myocardium independent of adenosine by direct activation of protein kinase C.
Circ Res. 1994;75:576-585.

Tsuchida A, Thompson R, Olsson RA, Downey JM.

The anti-infarct effect of an adenosine A_1-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.

Turrens JF, Thornton J, Barnard ML, Snyder S, Liu G, Downey JM.

Protection from reperfusion injury by preconditioning hearts does not involve increased antioxidant defenses.
Am J Physiol. 1992;262(2, pt 2):H-585–H-589.

Vahlhaus C, Schulz R, Post H, Onallah R, Heusch G.

No prevention of ischemic preconditioning by the protein kinase C inhibitor staurosporine in swine.
Circ Res. 1996;79:407-414.

Van Winkle DM, Thornton JD, Downey DM, Downey JM.

The natural history of preconditioning: cardioprotection depends on duration of transient ischemia and time to subsequent ischemia.
Cor Art Dis. 1991;2:613-619.

Van Wylen DGL.

Effect of ischemic preconditioning on interstitial purine metabolite and lactate accumulation during myocardial ischemia.
Circulation. 1994;89:2283-2289.

Vegh A, Papp JG, Parratt J.

Attenuation of the antiarrhythmic effects of ischaemic preconditioning by blockade of bradykinin B_2 receptors.

Br J Pharmacol. 1994;113:1167-1172.

Vegh A, Papp JG, Szekeres L, Parratt JR.

Are ATP sensitive potassium channels involved in the pronounced antiarrhythmic effects of preconditioning?

Cardiovasc Res. 1993;27:638-643.

Vogt AM, Htun P, Arras M, Podzuweit T, Schaper W.

Intramyocardial infusion of tool drugs for the study of molecular mechanisms in ischemic preconditioning.

Basic Res Cardiol. 1996;91:389-400.

Wang P, Gallagher KP, Downey JM, Cohen MV.

Pretreatment with endothelin-1 mimics ischemic preconditioning against infarction in isolated rabbit heart.

J Mol Cell Cardiol. 1996;28:579-588.

Weinbrenner C, Liu GS, Cohen MV, Downey JM.

Phosphorylation of tyrosine 182 of p38 mitogen-activated protein kinase correlates with the protection of preconditioning in the rabbit heart.

J Mol Cell Cardiol. 1997;29:2383-2391.

Weinbrenner C, Wang P, Downey JM.

Loss of glycogen during preconditioning is not a prerequisite for protection of the rabbit heart.

Basic Res Cardiol. 1996;91:374-381.

Weselcouch EO, Baird AJ, Sleph P, Grover GJ.

Inhibition of nitric oxide synthesis does not affect ischemic preconditioning in isolated perfused rat hearts.

Am J Physiol. 1995;268(1, pt 2):H-242–H-249.

Wolfe CL, Sievers RE, Visseren FLJ, Donnelly TJ.

Loss of myocardial protection after preconditioning correlates with the time course of glycogen recovery within the preconditioned segment.

Circulation. 1993;87:881-892.

Yang XM, Baxter GF, Heads RJ, Yellon DM, Downey JM, Cohen MV.

Infarct limitation of the second window of protection in a conscious rabbit model.

Cardiovasc Res. 1996;31:777-783.

Yang XM, Sato H, Downey JM, Cohen MV.

Protection of ischemic preconditioning is dependent upon a critical timing sequence of protein kinase C activation.

J Mol Cell Cardiol. 1997;29:991-999.

Yellon DM, Alkhulaifi AM, Pugsley WB.

Preconditioning the human myocardium.

Lancet. 1993;342:276-277.

Ytrehus K, Liu Y, Downey JM.

Preconditioning protects ischemic rabbit heart by protein kinase C activation.

Am J Physiol. 1994;266(3, pt 2):H-1145–H-1152.

Zu YL, Ai Y, Gilchrist A, et al.

High expression and activation of MAP kinase–activated protein kinase 2 in cardiac muscle cells.

J Mol Cell Cardiol. 1997;29:2159-2168.

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