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FORUM REVIEW ARTICLE

Ischemic Postconditioning: From Receptor to End-Effector

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Abstract

Ischemic preconditioning, a robust cardioprotective intervention, has limited clinical efficacy because it must be initiated before myocardial ischemia. Conversely, ischemic postconditioning, repeated brief reocclusions of a coronary artery after release of prolonged coronary occlusion, provides cardioprotection in clinically feasible settings, that is, coronary angioplasty. Ischemic postconditioning's signaling is being investigated to identify pharmacological triggers that could be used without angioplasty. In initial minutes of reperfusion H^+ washes out of previously ischemic cells. pH rises enabling mitochondrial permeability transition pores (MPTPs) to form leading to cessation of ATP production and cell necrosis. Coronary reocclusions maintain sufficient acidosis to keep MPTP closed while signaling is initiated that can generate endogenous antagonists of MPTP formation even after cellular pH normalizes. Reintroduction of oxygen generates reactive oxygen species that activate protein kinase C to increase sensitivity of adenosine A_{2b} receptors allowing adenosine released from ischemic cells to bind leading to activation of phosphatidylinositol 3-kinase and extracellular signal-regulated kinase 1/2. Phosphatidylinositol 3-kinase activation results in phosphorylation of Akt promoting activation of nitric oxide synthase and nitric oxide production, which inhibits glycogen synthase kinase-3β, perhaps the final cytosolic signaling step before inhibition of MPTP formation. Interference with MPTP may be the final step that determines cell salvage. *Antioxid. Redox Signal.* 14, 821–831.

Introduction

BEFORE THE DESCRIPTION of ischemic preconditioning (IP) in 1986 by Murry *et al.* (41), cardioprotection was no more than a dream. Although the concept was given a boost by the seminal studies of Maroko and colleagues (38, 39), subsequent reports were equivocal. Often the success claimed by one investigator could not be reproduced by another. Some critics even argued that infarct size modification was, in fact, theoretically impossible. Hence, Murry's report in 1986 was initially treated with skepticism, but as IP was studied in a variety of animal models by different research groups, it became clear that this intervention, no matter how improbable it was felt to be, was a robust cardioprotective strategy that greatly diminished myocardial infarction after a prolonged period of coronary occlusion followed by reperfusion. Murry *et al.* (41) reported that four cycles of 5-min occlusion/5-min reperfusion before a 40-min coronary occlusion in dogs decreased infarction by 75% from that seen in dogs with only a 40-min coronary occlusion. Thus, the brief periods of ischemia had prepared or preconditioned the myocardium to somehow make it more resistant to the stress of a longer ischemic interval.

The Mechanism of IP

After acceptance of IP as a bona fide cardioprotective intervention, investigators focused on identification of its mechanism with the intention of applying IP clinically. Our lab was the first to report that IP was triggered by adenosine released by ischemic myocardium revealing that IP's protection resulted from signal transduction pathways (36). After this early report we and other investigators have identified many of IP's signaling steps. The complex pathway can be divided into a preischemic trigger phase and a mediator phase in early reperfusion. The trigger phase involves release of several receptor agonists from ischemic tissue, including adenosine, bradykinin, and opioids (12, 65). All eventually target protein kinase C (PKC), adenosine quite directly through activation of phospholipase C, and the latter 2 through a more involved pathway that includes serial activation of phosphatidylinositol 3-kinase (PI3K), Akt, nitric oxide synthase (NOS), guanylyl cyclase (GC), and protein kinase G (PKG), opening of mitochondrial ATP-dependent potassium (K_{ATP}) channels, and finally production and release of reactive oxygen species (ROS) that target PKC (65) (Fig. 1). Once this pathway is activated

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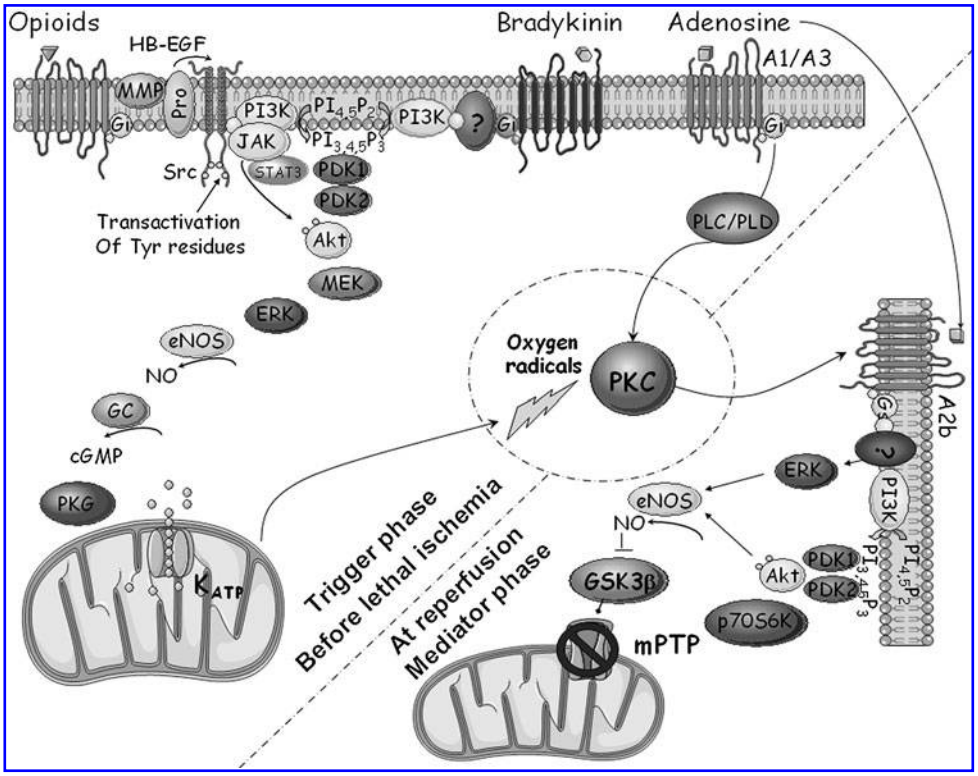

FIG. 1. Proposed signaling scheme for ischemic preconditioning. Note that in the figure eNOS signals through PKG upstream in the trigger pathway but acts in a PKG-independent manner downstream in the mediator pathway. The interaction between NO and GSK-3β, if any, is unclear. MMP, matrix metalloproteinases; HB-EGF, heparin-binding epidermal growth factor-like growth factor; Pro, pro-HB-EGF; Src, sarcoma tyrosine kinase; PI3K, phosphatidylinositol 3-kinase; JAK, janus kinase; STAT, signal transducer and activator of transcription; $PI_{4,5}P_2$, phosphatidylinositol bisphosphate; $PI_{3,4,5}P_3$, phosphatidylinositol trisphosphate; PDK1/2, 3′-phosphoinositide-dependent kinase-1/-2; MEK, mitogen activated protein kinase; ERK, extracellular signal-regulated kinase; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; GC, guanylyl cyclase; cGMP, cyclic guanosine monophosphate; PKG, protein kinase G; PLC/PLD, phospholipase C/D; PKC, protein kinase C; K_{ATP}, ATP-dependent potassium channel; p70S6K, p70S6 kinase; GSK-3β, glycogen synthase kinase-3β; MPTP, mitochondrial permeability transition pore. Modified from Ref. (65).

the heart enters a protected phenotype that persists for an hour or more even after the triggering agonists have been washed away. Hence, this part of the signaling cascade is termed the "trigger pathway." PKC then modifies an unusual adenosine receptor (AR) subtype, the A_{2b} receptor (33, 34, 50), so that endogenous adenosine can bind to it early in the reperfusion period. Many of the A_{2b} receptor's downstream signaling events recapitulate those in the trigger pathway. This signaling is thought to ultimately prevent formation of mitochondrial permeability transition pores (MPTPs) (77). If the latter are allowed to form, electrochemical equilibrium between the cytoplasm and mitochondrial matrix abolishes the critical electrochemical gradients, resulting in cessation of ATP production and cell necrosis. Thus, a series of signaling steps before and after ischemia leading to blockade of MPTP formation accounts for cardioprotection in IP.

Ischemic Postconditioning Can Be Administered After Reperfusion

IP is a powerful cardioprotective intervention with but one drawback: it must be applied before ischemia. Therefore, IP would be useless in the patient presenting to the hospital with acute myocardial infarction (AMI), since the ischemic process has already been initiated. However, Vinten-Johanssen's group made another improbable observation in 2003 (76). Instead of applying brief cycles of coronary occlusion/reperfusion before the index ischemia in dogs, they performed those cycles during the early minutes of reperfusion after the prolonged coronary occlusion. They termed this procedure ischemic postconditioning (IPoC) and infarct reduction was equivalent to that seen after IP. We indeed reproduced IPoC's protection in rabbits (73) (Fig. 2) as did others in a variety of species (58). IPoC was an intervention that could indeed be applied clinically to treat AMI. In AMI patients undergoing angioplasty to effect reperfusion of an occluded coronary artery, it would be possible to successively reocclude and reperfuse the latter for brief periods with the balloon, and indeed IPoC was done in these patients with resulting myocardial salvage (61, 63). However, not all patients with AMI are reperfused with catheter-based interventions. Thrombolytic agents are used in many countries and IPoC could not be administered in that setting. Hence, it was still necessary to understand the mechanism of this phenomenon with the hope that a pharmacologic approach could be developed that would not be limited to use in a catheterization laboratory.

Why Does IPoC Protect?

We initially observed that IPoC was equally effective in isolated (72) and *in situ* (73) cardiac models of ischemia/reperfusion. This observation excluded the critical importance of circulating blood elements or neurologic reflexes, and, as in IP, suggested that some intracellular biochemical or biophysical modification was leading to protection. Further, we observed that IPoC was very time dependent (51, 73). In the rabbit if the four cycles of 30-s reperfusion/30-s reocclusion did not occur immediately upon release of the index coronary occlusion, protection was lost (Fig. 2). Therefore, whatever was happening had to occur in the first minute of reperfusion.

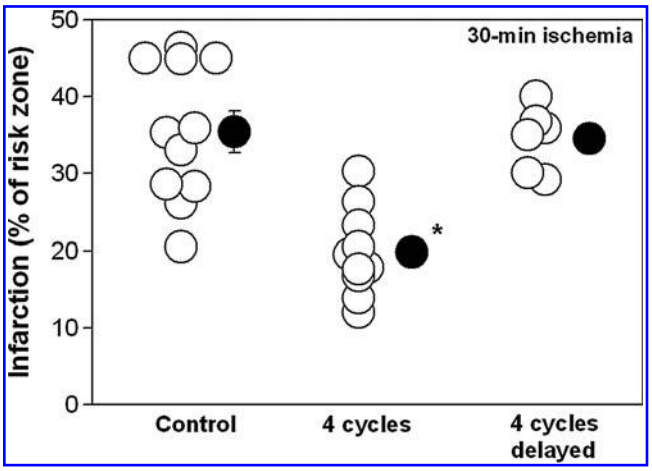

FIG. 2. Infarct size in *in situ* rabbit hearts. All animals had a 30-min coronary occlusion and 3-h reperfusion. *Open circles* represent individual experiments, while *closed circles* depict group means with SEM. Four cycles of IPoC in which the reperfusion and reocclusion phases each lasted for 30 s protected ischemic hearts, whereas delayed postconditioning elicited no protection. In the latter experiments depicted in this figure a 10-min interval between the onset of reperfusion and initiation of postconditioning was imposed. Similar loss of protection was seen if the delay was for only 1 min. $^*p < 0.05$ *versus* control. Modified from Ref. (73). IPoC, ischemic postconditioning; SEM, standard error of the mean.

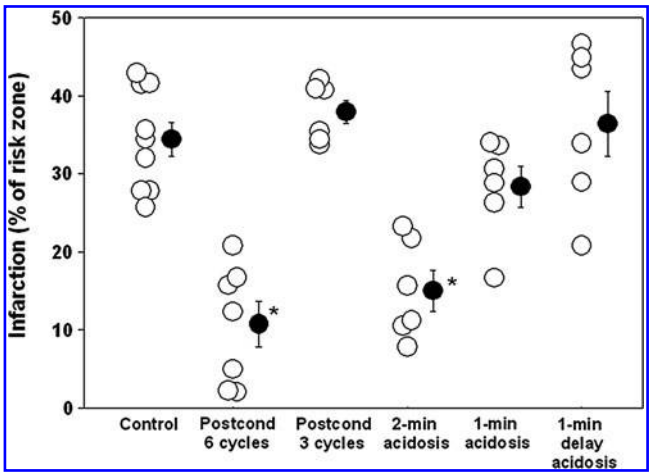

FIG. 3. Infarct size as a percentage of risk zone in individual isolated hearts with 30-min coronary occlusion and 2-h reperfusion (*open circles*) and groups (*closed circles* with SEM). IPoC (Postcond) with six cycles of 10-s reperfusion/10-s reocclusion was quite protective and similar to that seen when the hearts were instead perfused with acidic buffer during the initial 2 min after release of the 30-min coronary occlusion. Neither postconditioning with only 3 cycles (total 1 min) nor perfusion of acidic buffer for only 1 min protected, and protection was lost if perfusion of the heart with acidic buffer for 2 min were delayed for 1 min. $^*p < 0.001$ *versus* control. Reproduced with permission from Ref. (5).

The pH Hypothesis

So what was so different about the first minute of reperfusion that dictated the critical timing requirements of the IPoC cycles? Certainly, myocardial pH is rapidly changing during these early minutes. During ischemia anaerobic glycolysis leads to production of hydrogen ions that accumulate in the tissue resulting in a pH of ~7.0. Interestingly, acidosis inhibits MPTP formation by blocking Ca^{++} binding to adenine nucleotide translocase (ANT) and displacing cyclophilin, a necessary component of MPTP formation (44, 45), but resumption of antegrade coronary flow quickly washes out H^+ restoring pH to 7.4, thus enabling MPTP to form in the ATP-depleted tissue leading to irreversible mitochondrial damage and necrosis. We tested whether IPoC might protect by maintaining an acidic tissue pH during early reoxygenation. If our hypothesis were correct, then maintaining a low pH by reperfusing with acidic buffer should mimic IPoC's protection in isolated rabbit hearts (5). Whereas control hearts subjected to a 30-min coronary artery occlusion and subsequent reperfusion with buffer at pH 7.4 resulted in infarction of 34% of the risk zone (Fig. 3), IPoC with six cycles of 10-s reperfusion/10-s reocclusion (total 2 min) immediately after release of the coronary occlusion decreased infarction to 11%. IPoC with only three cycles (total 1 min of IPoC) was not protective, and, more importantly, a 1-min delay of 2 min of IPoC was also ineffective (see above). We then lowered the pH of the perfusion buffer by equilibrating it with 80% O_2/20% CO_2. We chose high CO_2 to acidify the buffer because it would diffuse through cell membranes and quickly lower the myocyte's cytosolic pH. Hypercapnic buffer (pH 6.9) for the first 2 min of reperfusion in lieu of IPoC was equally protective as IPoC (15% infarction) (Fig. 3), but only 1 min of acidosis or delaying 2-min of reperfusion with acidic buffer for 1 min abolished protection as it had done in IPoC. On the other hand, reperfusion with alkaline buffer (pH 7.7) blocked IPoC's protection, but the hearts could be rescued if cyclosporin A, an inhibitor of MPTP formation, were added to the perfusate (Fig. 4). The salutary effect of prolonging acidosis in

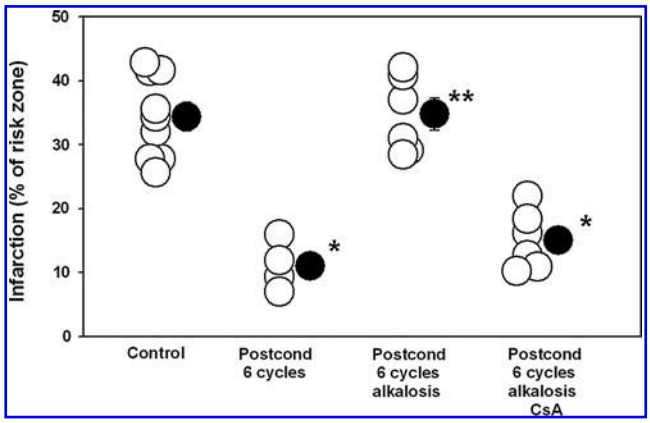

FIG. 4. Infarct size as a percentage of risk zone in individual isolated hearts with 30-min coronary occlusion and 2-h reperfusion (*open circles*) and groups (*closed circles* with SEM). Alkalotic buffer perfused only during reflow phases of IPoC (Postcond) aborted the protection of six cycles of postconditioning, but these hearts could be rescued if cyclosporin A (CsA) were added to the alkaline perfusate. $^*p < 0.001$ *versus* Control $^{**}p < 0.001$ *versus* Postcond-6 cycles and Postcond-6 cycles + alkalosis + CsA. Modified from Ref. (5).

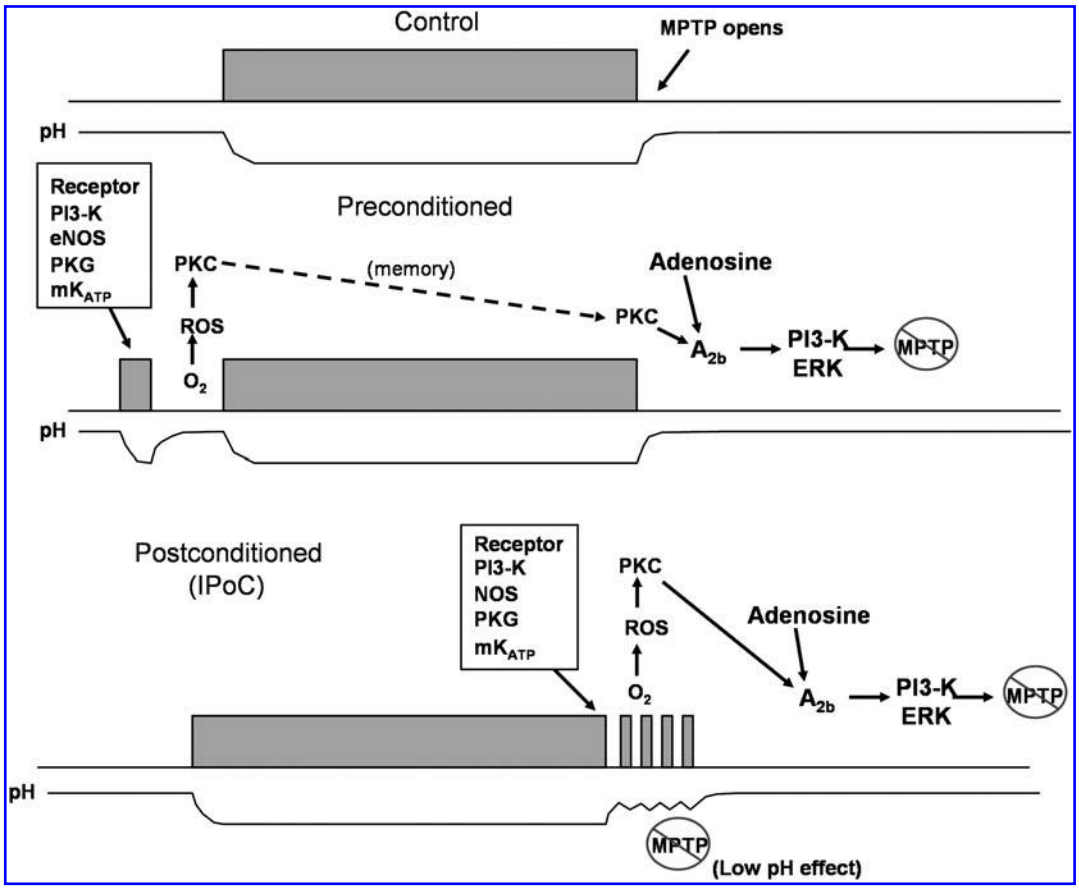

FIG. 5. Cartoon depicting the pH hypothesis and its effect on IPoC. In the control heart pH falls to 7.0 or less during myocardial ischemia (*gray boxes*), but abruptly rises with reperfusion as H^+ is washed out of the previously ischemic cells by restored blood flow. Formation of MPTP had been blocked during ischemia by the low pH despite increased cellular levels of Ca^{++} and ROS, but as pH returns to its baseline level MPTP form, mitochondrial electrochemical gradients are disrupted, ATP production is halted, and the cell is targeted for necrosis. In the preconditioned heart the same pH changes are hypothesized. However, elaborate signaling during the brief preconditioning ischemia and reperfusion has poised the cell for completion of the signaling as soon as flow and oxygen are restored to the myocardium to produce ROS and endogenous antagonists of MPTP formation are generated to keep the pores from forming. In IPoC the pH at the onset of reperfusion would also rise quickly except that the repeated brief coronary reocclusions keep the pH low enough to inhibit MPTP formation. This provides time for the signaling to generate those endogenous inhibitors of MPTP formation, which keep the pores from forming even after return of pH to the baseline value. A_{2b}, adenosine A_{2b} receptor; mK_{ATP}, mitochondrial ATP-sensitive potassium channel; ROS, reactive oxygen species.

early reperfusion has been confirmed in both dog (15) and pig (53). Thus, as depicted in Figure 5, we concluded that the reocclusions of the IPoC cycles acted to slow the return of tissue pH to 7.4 for >1 min. Why should reoxygenation with lowered pH for only several minutes protect? We hypothesized that the prolonged acidosis prevented MPTP formation long enough to allow the traditional IP signaling pathway an opportunity to produce endogenous inhibitors of MPTP formation that would continue to function after tissue pH was restored. The alternation between ischemia and reperfusion would bring some oxygen to the tissue but not enough flow to normalize pH. This effect of IPoC on the rise of pH during early reperfusion is critical and will be amplified by other contributions to this focused issue (29).

Oxygen is Required to Fuel Redox Signaling

We assumed that O_2 resupply would be the critical element. To determine the importance of reintroduction of O_2, we altered the O_2 content of the reperfusate by equilibrating the latter with 80% N_2/20% CO_2 (4). As predicted, protection was lost when the acidic perfusate was also hypoxic. However, hearts experiencing hypoxic and acidic reperfusion could be rescued by addition to the perfusate of phorbol 12-myristate 13-acetate (PMA), a PKC activator. This suggested that oxygen was somehow involved in activation of PKC, a key kinase in both IP and IPoC (40, 50, 74, 75). Penna and colleagues (49) were the first to show that the ROS scavenger *N*-acetylcysteine can abort IPoC's protection, and we confirmed their observation using the scavenger *N*-2-mercaptopropionyl glycine (11). We also found that the same ROS scavenger will block the protection of acidotic reperfusion (5). Thus, ROS generation is part of IPoC's signaling. We had previously presented evidence that in the IP heart redox signaling by ROS generated from molecular O_2 directly activates PKC, which then protects the reperfused heart (48). This sequence would explain our observation above that PMA could rescue hearts experiencing hypoxic and acidic reperfusion (4).

Many of these observations were extended to IPoC itself (4). IPoC's protective effect was abrogated by either making the oxygenated perfusate alkaline during the reperfusion phases or making the reperfusion buffer hypoxic. Additionally chelerythrine, a nonspecific PKC antagonist, blocks IPoC's protection (49, 50, 75). Hence, reintroduction of O_2 at the beginning of reperfusion permitted generation of signaling ROS that could activate PKC. The next challenge was determination of PKC's target.

A_{2b}ARs Must Be Occupied

As already noted, the first discovery leading to the elucidation of the signaling of IP was the observation that adenosine released by ischemic myocardium could trigger IP's cardioprotection (36). Four AR have been identified: A_1 and A_3, which couple to G_i and decrease cAMP, and A_{2a} and A_{2b}, which couple to G_s and stimulate adenylyl cyclase to increase cAMP production. The A_{2a} and A_{2b} receptors were first identified by their differential ability to stimulate cAMP production in brain slices at low (0.1–1 μM) and high (>10 μM) adenosine concentrations, respectively (54). The low-affinity receptor was called the A_{2b}AR. IP is triggered by both the A_1AR (64) and the A_3AR (35), which were responsible for initiating the signaling cascade upstream of PKC as shown in Figure 1. However, IP was also found to be dependent on AR stimulation in the reperfusion phase after the index ischemia (59). Nonselective AR blockade with 8-p-(sulfophenyl) theophylline aborted IPoC's infarct-sparing effect (72). Moreover, there was the suggestion that activation of the A_{2b}AR subtype at reperfusion was required for IP's protection as well (59).

We also addressed the nature of the AR subtype in isolated rabbit hearts. Neither selective A_1 [8-cyclopentyl-1,3-dipropylxanthine] nor A_{2a} [8-(13-chlorostyryl) caffeine] agonists had any effect on IPoC's infarct-sparing effect (50) (Fig. 6), but the selective A_{2b}AR antagonist MRS1754 aborted the expected protection (50), thus implicating the A_{2b}AR as a signaling element. These data were supplemented by experiments in which the A_{2a}AR agonist CGS 21680 administered during early reperfusion had little effect (70), whereas the nonselective but potent A_{2b} agonist 5′-(*N*-ethylcarboxamido) adenosine (NECA) was protective (37, 50, 70, 71). Additionally, when the highly selective A_{2b}AR agonist BAY 60–6583 became available, its infusion during early reperfusion produced cardioprotection that mimicked that of IPoC (33). Eckle *et al.* (13) attempted to precondition mice genetically modified to lack one of the four AR subtypes. While A_1, A_{2a}, and A_3AR knockout mice could be preconditioned, the A_{2b}AR knockout mice could not, suggesting an absolute requirement for A_{2b}AR somewhere in the signaling pathway, presumably at the onset of reperfusion. A_{2b}AR involvement at reperfusion to protect the heart was unexpected, but appeared to be critical for both IP and IPoC.

PKC Signaling Is Upstream of the A_{2b}AR

The PKC activator PMA administered at reperfusion after a prolonged coronary occlusion in rabbits protected those hearts, and the latter effect was blocked by chelerythrine, confirming that PMA acted through PKC activation (50). In that study NECA's protective effect was not affected by chelerythrine, but rather MRS1754 blocked PMA's salutary effect (50), suggesting that A_{2b}AR's effect is under the control of PKC, which is the opposite of what is usually observed. Typically, PKC activation follows ligand binding to a receptor, but in IPoC PKC appears to be upstream of A_{2b}AR.

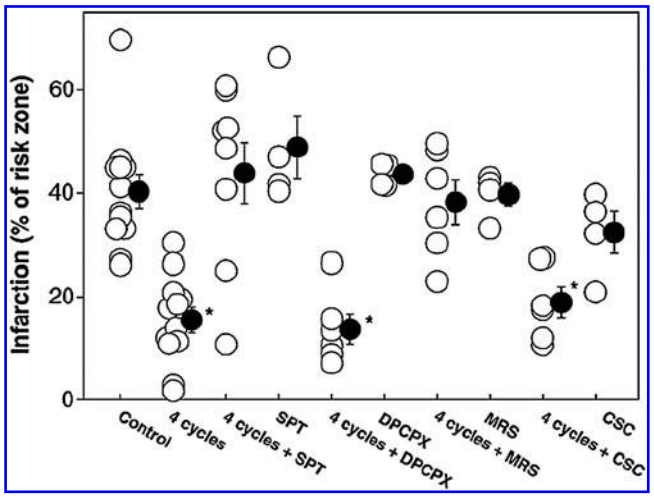

FIG. 6. Infarct size as a percentage of risk zone in *in situ* rabbit hearts undergoing a 30-min coronary occlusion and 3-h reperfusion. *Open circles* represent individual experiments and *closed circles* depict group means with SEM. Four postconditioning cycles of IPoC protected against ischemia/reperfusion. Both SPT, a nonselective adenosine receptor antagonist, and MRS1754 (MRS), a selective adenosine A_{2b} receptor blocker, completely aborted the protection of IPoC. Conversely, neither DPCPX nor CSC, selective adenosine A_1 and A_{2a} receptor antagonists, respectively, had any effect. *$p < 0.001$ *versus* control. Reproduced with permission from Ref. (50). SPT, 8-p-(sulfophenyl) theophylline; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; CSC, 8-(13-chlorostyryl) caffeine.

Although the above data clearly implicated the A_{2b}AR in both IP's and IPoC's signal transduction pathway at reperfusion (Fig. 1), this observation created a troubling conundrum. As already noted, the A_{2b}AR is a low-affinity receptor. Under basal conditions the extracellular concentration of adenosine in the heart ranges from 30 to 300 nM. Although the concentration increases substantially during ischemia to 1–4 μM, it rarely exceeds 10 μM (54). Yet, the K_i of the A_{2b}AR for adenosine may be as high as 24 μM (17). Therefore, how could endogenous adenosine released from ischemic heart muscle significantly bind to A_{2b}AR? And why was this happening only in pre- or postconditioned hearts?

Since PKC was upstream of A_{2b}AR, we wondered if PKC might somehow be modifying A_{2b}AR in hearts subjected to ischemia/reperfusion. It is well known that PKC activity can sensitize A_{2b}AR signaling (43, 47, 67), although the mechanism has never been elucidated nor had, until now, any physiological function been attributed to the sensitization (14). Kuno *et al.* (33) tested whether this sensitization phenomenon might be involved in IP and IPoC. NECA given to a normally perfused rabbit heart increased phosphorylation of the 2 kinases Akt and extracellular signal-regulated kinase (ERK) 1/2 in ventricular biopsies in a dose-dependent manner (Fig. 7). Kuno *et al.* then determined a subthreshold dose of NECA that had no effect on kinase phosphorylation (2.5 nM). When they combined this subthreshold dose of NECA with

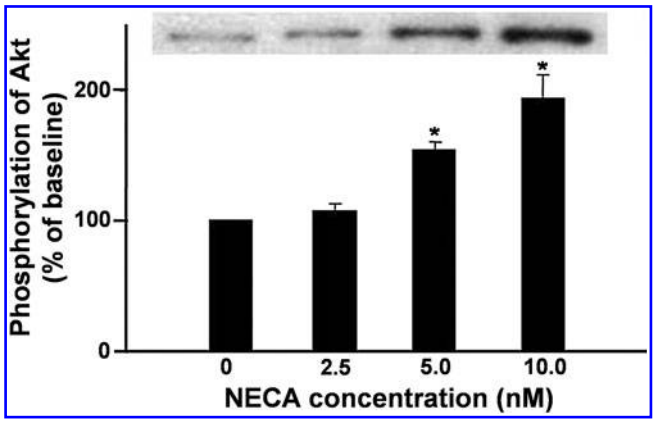

FIG. 7. Akt phosphorylation by NECA in isolated non-ischemic hearts. Akt phosphorylation (Ser473) as a percent of phospho-Akt at baseline after 5 min of 2.5, 5.0, or 10 n*M* NECA infusion in escalating doses in four isolated hearts. A representative Western blot of one of these hearts is seen at the top of the figure. Phospho-Akt significantly increased after infusion of 5.0 and 10 n*M* NECA, whereas 2.5 n*M* NECA did not change phosphorylation status of Akt. $*p < 0.05$ *versus* baseline (0 n*M*). Modified from Ref. (33). NECA, 5′-(*N*-ethylcarboxamido) adenosine.

either PMA or an IP protocol, kinase phosphorylation was increased (Fig. 8). PMA or IP by themselves had no effect on phosphorylation. Because of these observations we proposed that the key event in the protective signaling cascade is the increase in A_{2b}AR sensitivity mediated by PKC, which enables endogenous adenosine to activate A_{2b}-dependent signaling. The nature of the interaction between PKC and A_{2b}AR is unclear, but could likely be phosphorylation of the receptor or one of its coupling proteins. Yet, it is this PKC-A_{2b}AR interaction that cements the principal role of A_{2b}AR.

A_{2a}ARs May Also Be Important

Although the data strongly support a role for A_{2b}AR in cardioprotective signaling in IPoC, A_{2a}AR may also play a role. In the *in vivo* heart IPoC decreases neutrophil accumulation in reperfused myocardium and this anti-inflammatory effect may be the result of adenosine binding to A_{2a}AR (42, 76). This anti-neutrophil effect would appear late after reperfusion (42).

Kin *et al.* (31) also reported that AR were involved in a rat model of IPoC, but they concluded that it was the A_{2a} and A_3 receptor subtypes that were important. Recent work by Xi *et al.* (69) suggests that both A_{2a} and A_{2b} receptors are probably required. A_{2a}AR will be occupied in a reperfused heart whether it is in a protected state or not because of the receptor's high affinity for adenosine. However, because of A_{2b}AR's very low affinity, we believe that they will be occupied only in pre- or postconditioned hearts in which the receptors have been sensitized by PKC. This explains why CGS21680, an A_{2a} agonist, was unable to protect the reperfused heart when administered alone, whereas the selective A_{2b} agonist BAY 60–6583 could. Most recently, we studied *in situ* mouse hearts in which both BAY 60–6583 and IPoC decreased infarction (C. Methner, M.V. Cohen, J.M. Downey, and T. Krieg, unpublished observation). The beneficial effect of either was blocked by the selective A_{2b} antagonist MRS1754. CD73, also known as 5′-ectonucleotidase, breaks down AMP to adenosine and is the major source of adenosine production in ischemic myocardium. As predicted, IPoC could not protect CD73 knockout mice hearts (C. Methner, M.V. Cohen, J.M. Downey, and T. Krieg, unpublished observation) and neither BAY 60–6583 nor CGS21680 when administered alone protected them. However, co-administration of BAY 60–6583 and CGS21680 elicited profound protection, indicating that both A_{2a} and A_{2b}AR must be simultaneously bound by agonists to promote IPoC's protection. It is not known to what the A_{2a} receptors couple to effect their protection. There may be some synergistic relationship between A_{2a} and A_{2b}AR in IPoC. One unanswered question raised by this A_{2a}AR hypothesis is how Eckle *et al.* (13) were able to precondition A_{2a}AR knockout mice.

A_{2b}ARs Control Reperfusion Injury Salvage Kinase

The next question posed was, "Why should binding of A_{2b}AR be protective?" Our earliest investigation of IPoC in *in situ* rabbit hearts revealed that protection was aborted by wortmannin, a PI3K antagonist (73). In a paradigm-shifting report, Hausenloy *et al.* (26, 27) showed that cardioprotection in IP was dependent on phosphorylation of several critical kinases, PI3K and ERK, in the first minutes of reperfusion. The authors coined the phrase "reperfusion injury salvage kinases" or RISK to describe these kinases.

In addition to abrogation of IPoC's protection by wortmannin, we also observed that PD98059, a mitogen-activated protein kinase 1/2 and therefore ERK 1/2 inhibitor, blocked IPoC's salvage of ischemic myocardium (73). RISK are critical signaling elements for IP's protection in at least rabbits and rats and appear to also be central to IPoC's mechanism. It must be recognized that IPoC's protection may not be dependent on RISK in all species since Skyschally *et al.* (57) were unable to find any involvement of RISK in their porcine low-coronary-flow ischemia model of IPoC. It is not known whether this reflects a true species difference or might be related to some unique feature of their low-flow model. It is perhaps noteworthy that Schwartz and Lagranha (55) were able to document increased phosphorylation of ERK and Akt after several cycles of reocclusion/reperfusion in the early minutes of reflow in pigs, but in their model there was no cardioprotection from IPoC. However, in other experimental models mounting evidence indicates that RISK are certainly important in IPoC's cardioprotection.

The Role of NO

Our early investigation into IPoC also revealed that N^{ω}-nitro-L-arginine-methyl ester, an inhibitor of endothelial NOS, blocked protection (73). This observation is consistent with the RISK pathway since PI3K activation results in phosphorylation and activation of Akt, which promotes activation of NOS (10, 16). In IP's trigger pathway (Fig. 1) NOS activation generates NO that triggers GC-cGMP-PKG signaling, and the latter opens mitochondrial K_{ATP} channels (7). In many biologic processes, increased NO leads to activation of PKG through GC that can then phosphorylate a variety of other proteins or channels. It is not known whether NO protects at reperfusion in a similar PKG-dependent manner. Garlid studied isolated rat mitochondria and found that NO inhibited MPTP formation through a PKG-dependent cascade (6). However, PKG-independent S-nitrosylation of proteins

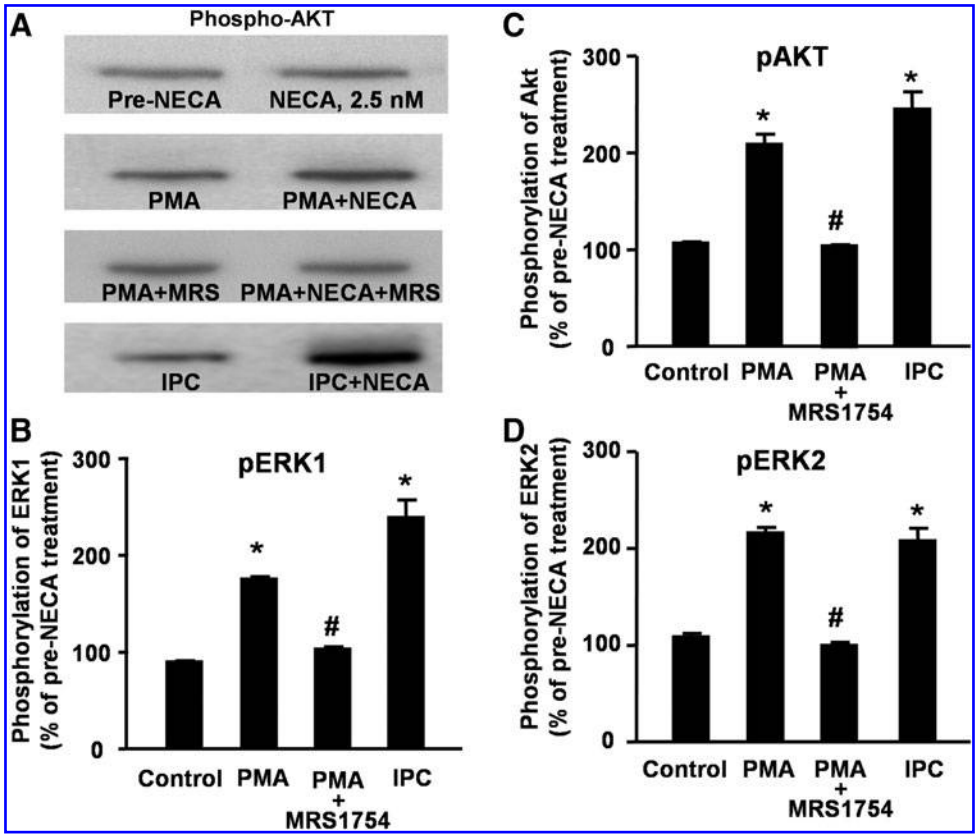

FIG. 8. Akt and ERK phosphorylation before and after A_{2b}AR sensitization by NECA or brief ischemia. (**A**) Representative Western blots of bands for phospho-Akt (Ser473) in control hearts (pre-NECA), hearts treated with PMA (0.05 n*M*) alone, PMA + MRS1754, or hearts exposed to the brief preconditioning ischemia of ischemic preconditioning (IPC). The left-hand column presents phospho-Akt bands before infusion of the subthreshold dose of NECA, while the right-hand column presents bands after NECA treatment. Phosphorylation increased after NECA administration only in hearts pretreated with either PMA or IPC. MRS1754 blocked any increase. (**B**) Phospho-Akt (Ser473) and (**C** and **D**) phospho-ERK 1/2 (Thr202/Thr204) as percent of levels measured just before treatment with NECA. All hearts in panels (**B–D**) were treated for 5 min with the subthreshold dose of 2.5 n*M* NECA. NECA had no effect in untreated hearts. After pretreatment with PMA the subthreshold dose of NECA now caused a robust phosphorylation of Akt and ERK 1/2. NECA had no effect on phosphorylation when co-infused with a selective A_{2b} receptor antagonist MRS1754 in PMA-treated hearts. Brief ischemia (IPC) also enabled the subthreshold dose of NECA to phosphorylate Akt and ERK 1/2. Each bar is mean ± SEM of four observations. $^{*}p < 0.05$ *versus* control, $^{\#}p < 0.05$ *versus* PMA. Modified from Ref. (33). PMA, phorbol 12-myristate 13-acetate.

has also been proposed as a possible mechanism of IP's protection (62). For example, when mitochondrial complex I is S-nitrosylated, it produces fewer free radicals (56), possibly attenuating formation of MPTP. So, NO could conceivably protect the heart through a PKG-independent mechanism.

Glycogen Synthase Kinase-3β Signaling

Glycogen synthase kinase (GSK)-3β is a known downstream target of PI3K and may possibly be the last cytoplasmic signaling element in IPoC's pathway. GSK-3β signaling was implicated in IP's protection by Tong *et al.* (66). In IP this kinase was phosphorylated in a PI3K-dependent manner. Phosphorylation of GSK-3β inhibits, rather than activates, its kinase activity. Pharmacological inhibition of GSK-3β mimicked IP's protection. Because GSK-3β was phosphorylated in IP, they proposed that it was in the cardioprotective signaling pathway. SB216763, a blocker of GSK-3β, decreases infarct size when given to hearts of wild-type mice just before reperfusion, but the protective effect is lost in GSK-3β transgenic mice in which GSK-3β activity cannot be blocked (19). More importantly, IPoC could not protect the transgenic mice either. However, cyclosporin, a direct inhibitor of MPTP, did protect them. Other investigators have placed GSK-3β at the very distal end of the signaling cascade as well (22). Juhaszova *et al.* (30) concluded that although many diverse cardioprotective interventions may trigger protective signaling, all pathways lead to GSK-3β. Despite this impressive evidence leading one to conclude that GSK-3β plays a critical role in IPoC, Nishino *et al.* (46) demonstrated that double knockin mice carrying GSK-3 alleles encoding inactivation-resistant kinases could still be protected by both IP and IPoC.

MPTP Inhibition Is Thought to Be IPoC's End-Effector

Of course, there may be other intermediate signaling steps in this pathway that have not yet been identified, but the current weight of evidence indicates that inhibition of MPTP is the end-effector. This pore, first described in 1976 by Hunter *et al.* (28), is a high conductance channel located in the inner mitochondrial membrane that when formed permits communication between the cytoplasm and mitochondrial matrix. The open probability of this channel is regulated by several factors, including pH, Ca^{++}, and ROS, which are all influenced by myocardial ischemia. Pore formation results in increased mitochondrial permeability to ions and solutes with molecular weights of up to 1.5 kDa, matrix swelling, loss of critical electrochemical gradients, and cessation of ATP production. Many have postulated that MPTP formation is the event that leads to irreversible changes in cellular function

and cell death. Di Lisa *et al.* (9) observed that addition of Ca^{++} to isolated rat heart mitochondria caused mitochondrial swelling and profound decrease in NAD^+ content. These events were prevented by cyclosporin A. Thirty to 90 min of global ischemia in isolated, perfused rat hearts led to 30% depletion of tissue NAD^+, whereas reperfusion further exacerbated NAD^+ loss with remaining tissue NAD^+ ~20% of preischemia levels. Mitochondrial NAD^+ levels after ischemia/reperfusion were only ~10% of the preischemia level. Notably, these changes in tissue and mitochondrial NAD^+ levels after ischemia/reperfusion were largely prevented by cyclosporin A. Therefore, MPTP formation is likely to be a causative event in reperfusion injury. If the MPTP hypothesis is correct, then the observations with IPoC and IP would suggest that a great proportion of cell death after ischemia/reperfusion results from MPTP formation.

What Is the MPTP?

The structure of these pores is not clear. Many reports suggested that the pore consisted of the voltage-dependent anion channel (VDAC) on the outer mitochondrial membrane and the ANT on the inner membrane (8, 24, 32). Additionally, the peptidyl-prolyl *cis-trans* isomerase cyclophilin D to which cyclosporin A binds was in the matrix (18, 23, 32). Cyclophilin D was, therefore, believed to be responsible for modulation of MPTP, and not considered to be a structural component. It was assumed that when ANT and VDAC lined up, the pore would be formed. However, knockout mice lacking either ANT (2) or VDAC (3) still could form MPTP, suggesting that neither ANT nor VDAC is needed to form MPTP. Although its structure is not known, MPTP can still be identified by its functional characteristics.

Argaud *et al.* (1) evaluated MPTP formation in rabbits after ischemia/reperfusion by evaluating the response of isolated mitochondria to successive additions of Ca^{++} boluses to the bathing medium. Early Ca^{++} increments were taken up by mitochondria until the matrix Ca^{++} level rose to the point at which MPTP formed. Then, the mitochondria disgorged all of the Ca^{++} previously taken up, resulting in a dramatic rise in the Ca^{++} level of the bath. In these rabbits IPoC decreased infarct size from 61% of the risk region in controls to 29%. In control mitochondria the average Ca^{++} load required to open MPTP was $16 \pm 4\ \mu M$ $CaCl_2$ per mg of mitochondrial protein, whereas the load was significantly increased to $41 \pm 4\ \mu M$/mg in mitochondria from the risk region of ischemically postconditioned hearts. This Ca^{++} load needed to form MPTP was nearly the same as that seen in mitochondria from IP hearts, and was only modestly less than that in mitochondria from hearts treated with a pharmacologic inhibitor of MPTP.

We have already indicated that cyclosporin A could rescue IPoC hearts exposed to alkaline perfusate in the early minutes of reperfusion, which normally blocks IPoC's protection (5). We (68), as well as many others (20, 21, 25, 60), have documented the salutary effects of cyclosporin A when administered at or shortly before reperfusion in experimental animals. It too decreases infarct size in patients with AMI when administered just before the occluded coronary artery is reperfused (52). Thus, it is likely that MPTP formation is indeed the final event in the signaling cascade of IPoC and many others forms of cardioprotection, and these salutary interventions block their formation.

Conclusions

IPoC preserves ischemic myocardium and has set the stage for other cardioprotective interventions introduced at reperfusion. Identification of the signaling elements between the $A_{2b}AR$ and the MPTP should permit development of pharmacologic agents that can access the pathway at intermediate points with the same goal of prevention of MPTP formation. Cardioprotection is finally a realistic clinical approach and should provide a powerful tool in the treatment of patients with ischemic heart disease.

References

1. Argaud L, Gateau-Roesch O, Raisky O, Loufouat J, Robert D, and Ovize M. Postconditioning inhibits mitochondrial permeability transition. *Circulation* 111: 194–197, 2005.
2. Baines CP. The molecular composition of the mitochondrial permeability transition pore. *J Mol Cell Cardiol* 46: 850–857, 2009.
3. Baines CP, Kaiser RA, Sheiko T, Craigen WJ, and Molkentin JD. Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death. *Nat Cell Biol* 9: 550–555, 2007.
4. Cohen MV, Yang X-M, and Downey JM. Acidosis, oxygen, and interference with mitochondrial permeability transition pore formation in the early minutes of reperfusion are critical to postconditioning's success. *Basic Res Cardiol* 103: 464–471, 2008.
5. Cohen MV, Yang X-M, and Downey JM. The pH hypothesis of postconditioning: staccato reperfusion reintroduces oxygen and perpetuates myocardial acidosis. *Circulation* 115: 1895–1903, 2007.
6. Costa ADT and Garlid KD. Intramitochondrial signaling: interactions among mitoK_{ATP}, PKCE, ROS, and MPT. *Am J Physiol* 295: H874–H882, 2008.
7. Costa ADT, Garlid KD, West IC, Lincoln TM, Downey JM, Cohen MV, and Critz SD. Protein kinase G transmits the cardioprotective signal from cytosol to mitochondria. *Circ Res* 97: 329–336, 2005.
8. Crompton M. The mitochondrial permeability transition pore and its role in cell death. *Biochem J* 341: 233–249, 1999.
9. Di Lisa F, Menabò R, Canton M, Barile M, and Bernardi P. Opening of the mitochondrial permeability transition pore causes depletion of mitochondrial and cytosolic NAD^+ and is a causative event in the death of myocytes in postischemic reperfusion of the heart. *J Biol Chem* 276: 2571–2575, 2001.
10. Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, and Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature* 399: 601–605, 1999.
11. Downey JM and Cohen MV. A really radical observation: a comment on Penna *et al.* in Basic Res Cardiol (2006) 101:180–189. *Basic Res Cardiol* 101: 190–191, 2006.
12. Downey JM, Krieg T, and Cohen MV. Mapping preconditioning's signaling pathways: an engineering approach. *Ann NY Acad Sci* 1123: 187–196, 2008.
13. Eckle T, Krahn T, Grenz A, Köhler D, Mittelbronn M, Ledent C, Jacobson MA, Osswald H, Thompson LF, Unertl K, and Eltzschig HK. Cardioprotection by ecto-5′-nucleotidase (CD73) and A_{2B} adenosine receptors. *Circulation* 115: 1581–1590, 2007.

14. Feoktistov I and Biaggioni I. Adenosine A_{2B} receptors. *Pharmacol Rev* 49: 381–402, 1997.
15. Fujita M, Asanuma H, Hirata A, Wakeno M, Takahama H, Sasaki H, Kim J, Takashima S, Tsukamoto O, Minamino T, Shinozaki Y, Tomoike H, Hori M, and Kitakaze M. Prolonged transient acidosis during early reperfusion contributes to the cardioprotective effects of postconditioning. *Am J Physiol* 292: H2004–H2008, 2007.
16. Gao F, Gao E, Yue T-L, Ohlstein EH, Lopez BL, Christopher TA, and Ma X-L. Nitric oxide mediates the antiapoptotic effect of insulin in myocardial ischemia-reperfusion: the roles of PI3-kinase, Akt, and endothelial nitric oxide synthase phosphorylation. *Circulation* 105: 1497–1502, 2002.
17. Gao Z-G and Jacobson KA. Emerging adenosine receptor agonists. *Expert Opin Emerg Drugs* 12: 479–492, 2007.
18. Giorgio V, Soriano ME, Basso E, Bisetto E, Lippe G, Forte MA, and Bernardi P. Cyclophilin D in mitochondrial pathophysiology. *Biochim Biophys Acta* 1797: 1113–1118, 2010.
19. Gomez L, Paillard M, Thibault H, Derumeaux G, and Ovize M. Inhibition of GSK3β by postconditioning is required to prevent opening of the mitochondrial permeability transition pore during reperfusion. *Circulation* 117: 2761–2768, 2008.
20. Griffiths EJ and Halestrap AP. Mitochondrial non-specific pores remain closed during cardiac ischaemia, but open upon reperfusion. *Biochem J* 307: 93–98, 1995.
21. Griffiths EJ and Halestrap AP. Protection by cyclosporin A of ischemia/reperfusion-induced damage in isolated rat hearts. *J Mol Cell Cardiol* 25: 1461–1469, 1993.
22. Gross ER, Hsu AK, and Gross GJ. Opioid-induced cardioprotection occurs via glycogen synthase kinase β inhibition during reperfusion in intact rat hearts. *Circ Res* 94: 960–966, 2004.
23. Halestrap AP, Clarke SJ, and Javadov SA. Mitochondrial permeability transition pore opening during myocardial reperfusion—a target for cardioprotection. *Cardiovasc Res* 61: 372–385, 2004.
24. Halestrap AP, Kerr PM, Javadov S, and Woodfield K-Y. Elucidating the molecular mechanism of the permeability transition pore and its role in reperfusion injury of the heart. *Biochim Biophys Acta* 1366: 79–94, 1998.
25. Hausenloy DJ, Maddock HL, Baxter GF, and Yellon DM. Inhibiting mitochondrial permeability transition pore opening: a new paradigm for myocardial preconditioning? *Cardiovasc Res* 55: 534–543, 2002.
26. Hausenloy DJ, Tsang A, Mocanu MM, and Yellon DM. Ischemic preconditioning protects by activating prosurvival kinases at reperfusion. *Am J Physiol* 288: H971–H976, 2005.
27. Hausenloy DJ and Yellon DM. New directions for protecting the heart against ischaemia-reperfusion injury: targeting the Reperfusion Injury Salvage Kinase (RISK)-pathway. *Cardiovasc Res* 61: 448–460, 2004.
28. Hunter DR, Haworth RA, and Southard JH. Relationship between configuration, function, and permeability in calcium-treated mitochondria. *J Biol Chem* 251: 5069–5077, 1976.
29. Inserte J, Ruiz-Meana M, Rodriguez-Sinovas A, Barba I, and Garcia-Dorado D. Contribution of delayed intracellular pH recovery to ischemic postconditioning protection. *Antioxid Redox Signal* 14: 923–939, 2011.
30. Juhaszova M, Zorov DB, Kim S-H, Pepe S, Fu Q, Fishbein KW, Ziman BD, Wang S, Ytrehus K, Antos CL, Olson EN, and Sollott SJ. Glycogen synthase kinase-3β mediates convergence of protection signaling to inhibit the mitochondrial permeability transition pore. *J Clin Invest* 113: 1535–1549, 2004.
31. Kin H, Zatta AJ, Lofye MT, Amerson BS, Halkos ME, Kerendi F, Zhao Z-Q, Guyton RA, Headrick JP, and Vinten-Johansen J. Postconditioning reduces infarct size via adenosine receptor activation by endogenous adenosine. *Cardiovasc Res* 67: 124–133, 2005.
32. Kroemer G, Galluzzi L, and Brenner C. Mitochondrial membrane permeabilization in cell death. *Physiol Rev* 87: 99–163, 2007.
33. Kuno A, Critz SD, Cui L, Solodushko V, Yang X-M, Krahn T, Albrecht B, Philipp S, Cohen MV, and Downey JM. Protein kinase C protects preconditioned rabbit hearts by increasing sensitivity of adenosine A_{2b}-dependent signaling during early reperfusion. *J Mol Cell Cardiol* 43: 262–271, 2007.
34. Kuno A, Solenkova NV, Solodushko V, Dost T, Liu Y, Yang X-M, Cohen MV, and Downey JM. Infarct limitation by a protein kinase G activator at reperfusion in rabbit hearts is dependent on sensitizing the heart to A_{2b} agonists by protein kinase C. *Am J Physiol* 295: H1288–H1295, 2008.
35. Liu GS, Richards SC, Olsson RA, Mullane K, Walsh RS, and Downey JM. Evidence that the adenosine A_3 receptor may mediate the protection afforded by preconditioning in the isolated rabbit heart. *Cardiovasc Res* 28: 1057–1061, 1994.
36. Liu GS, Thornton J, Van Winkle DM, Stanley AWH, Olsson RA, and Downey JM. Protection against infarction afforded by preconditioning is mediated by A_1 adenosine receptors in rabbit heart. *Circulation* 84: 350–356, 1991.
37. Liu Y, Yang X, Yang X-M, Walker S, Förster K, Cohen MV, Krieg T, and Downey JM. AMP579 is revealed to be a potent A_{2b}-adenosine receptor agonist in human 293 cells and rabbit hearts. *Basic Res Cardiol* 105: 129–137, 2010.
38. Maroko PR, Kjekshus JK, Sobel BE, Watanabe T, Covell JW, Ross J, Jr., and Braunwald E. Factors influencing infarct size following experimental coronary artery occlusions. *Circulation* 43: 67–82, 1971.
39. Maroko PR, Libby P, Ginks WR, Bloor CM, Shell WE, Sobel BE, and Ross J, Jr. Coronary artery reperfusion. I. Early effects on local myocardial function and the extent of myocardial necrosis. *J Clin Invest* 51: 2710–2716, 1972.
40. Mifune M, Ohtsu H, Suzuki H, Nakashima H, Brailoiu E, Dun NJ, Frank GD, Inagami T, Higashiyama S, Thomas WG, Eckhart AD, Dempsey PJ, and Eguchi S. G protein coupling and second messenger generation are indispensable for metalloprotease-dependent, heparin-binding epidermal growth factor shedding through angiotensin II type-1 receptor. *J Biol Chem* 280: 26592–26599, 2005.
41. Murry CE, Jennings RB, and Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 74: 1124–1136, 1986.
42. Mykytenko J, Kerendi F, Reeves JG, Kin H, Zatta AJ, Jiang R, Guyton RA, Vinten-Johansen J, and Zhao Z-Q. Long-term inhibition of myocardial infarction by postconditioning during reperfusion. *Basic Res Cardiol* 102: 90–100, 2007.
43. Nash MS, Wood JPM, and Osborne NN. Protein kinase C activation by serotonin potentiates agonist-induced stimulation of cAMP production in cultured rat retinal pigment epithelial cells. *Exp Eye Res* 64: 249–255, 1997.
44. Nicolli A, Basso E, Petronilli V, Wenger RM, and Bernardi P. Interactions of cyclophilin with the mitochondrial inner

membrane and regulation of the permeability transition pore, a cyclosporin A-sensitive channel. *J Biol Chem* 271: 2185–2192, 1996.
45. Nicolli A, Petronilli V, and Bernardi P. Modulation of the mitochondrial cyclosporin A-sensitive permeability transition pore by matrix pH. Evidence that the pore open-closed probability is regulated by reversible histidine protonation. *Biochemistry* 32: 4461–4465, 1993.
46. Nishino Y, Webb IG, Davidson SM, Ahmed AI, Clark JE, Jacquet S, Shah AM, Miura T, Yellon DM, Avkiran M, and Marber MS. Glycogen synthase kinase-3 inactivation is not required for ischemic preconditioning or postconditioning in the mouse. *Circ Res* 103: 307–314, 2008.
47. Nordstedt C, Kvanta A, Van der Ploeg I, and Fredholm BB. Dual effects of protein kinase-C on receptor-stimulated cAMP accumulation in a human T-cell leukemia line. *Eur J Pharmacol* 172: 51–60, 1989.
48. Pain T, Yang X-M, Critz SD, Yue Y, Nakano A, Liu GS, Heusch G, Cohen MV, and Downey JM. Opening of mitochondrial K_{ATP} channels triggers the preconditioned state by generating free radicals. *Circ Res* 87: 460–466, 2000.
49. Penna C, Rastaldo R, Mancardi D, Raimondo S, Cappello S, Gattullo D, Losano G, and Pagliaro P. Post-conditioning induced cardioprotection requires signaling through a redox-sensitive mechanism, mitochondrial ATP-sensitive K^+ channel and protein kinase C activation. *Basic Res Cardiol* 101: 180–189, 2006.
50. Philipp S, Yang X-M, Cui L, Davis AM, Downey JM, and Cohen MV. Postconditioning protects rabbit hearts through a protein kinase C-adenosine A_{2b} receptor cascade. *Cardiovasc Res* 70: 308–314, 2006.
51. Philipp S, Yang X-M, Willenbrock R, Downey JM, and Cohen MV. Postconditioning must be initiated in less than 1 minute following reperfusion and is dependent on adenosine 2b receptors and PI3-kinase. *Z Kardiol* 94(Suppl 1): V803, 2005. (Abstract)
52. Piot C, Croisille P, Staat P, Thibault H, Rioufol G, Mewton N, Elbelghiti R, Cung TT, Bonnefoy E, Angoulvant D, Macia C, Raczka F, Sportouch C, Gahide G, Finet G, André-Fouët X, Revel D, Kirkorian G, Monassier J-P, Derumeaux G, and Ovize M. Effect of cyclosporine on reperfusion injury in acute myocardial infarction. *N Engl J Med* 359: 473–481, 2008.
53. Rodríguez-Sinovas A, Cabestrero A, García del Blanco B, Inserte J, García A, and García-Dorado D. Intracoronary acid infusion as an alternative to ischemic postconditioning in pigs. *Basic Res Cardiol* 104: 761–771, 2009.
54. Schulte G and Fredholm BB. Signalling from adenosine receptors to mitogen-activated protein kinases. *Cell Signal* 15: 813–827, 2003.
55. Schwartz LM and Lagranha CJ. Ischemic postconditioning during reperfusion activates Akt and ERK without protecting against lethal myocardial ischemia-reperfusion injury in pigs. *Am J Physiol* 290: H1011–H1018, 2006.
56. Shiva S, Sack MN, Greer JJ, Duranski M, Ringwood LA, Burwell L, Wang X, MacArthur PH, Shoja A, Raghavachari N, Calvert JW, Brookes PS, Lefer DJ, and Gladwin MT. Nitrite augments tolerance to ischemia/reperfusion injury via the modulation of mitochondrial electron transfer. *J Exp Med* 204: 2089–2102, 2007.
57. Skyschally A, van Caster P, Boengler K, Gres P, Musiolik J, Schilawa D, Schulz R, and Heusch G. Ischemic postconditioning in pigs: no causal role for RISK activation. *Circ Res* 104: 15–18, 2009.
58. Skyschally A, van Caster P, Iliodromitis EK, Schulz R, Kremastinos DT, and Heusch G. Ischemic postconditioning: experimental models and protocol algorithms. *Basic Res Cardiol* 104: 469–483, 2009.
59. Solenkova NV, Solodushko V, Cohen MV, and Downey JM. Endogenous adenosine protects preconditioned heart during early minutes of reperfusion by activating Akt. *Am J Physiol* 290: H441–H449, 2006.
60. Squadrito F, Altavilla D, Squadrito G, Saitta A, Campo GM, Arlotta M, Quartarone C, Ferlito M, and Caputi AP. Cyclosporin-A reduces leukocyte accumulation and protects against myocardial ischaemia reperfusion injury in rats. *Eur J Pharmacol* 364: 159–168, 1999.
61. Staat P, Rioufol G, Piot C, Cottin Y, Cung TT, L'Huillier I, Aupetit J-F, Bonnefoy E, Finet G, André-Fouët X, and Ovize M. Postconditioning the human heart. *Circulation* 112: 2143–2148, 2005.
62. Sun J, Morgan M, Shen R-F, Steenbergen C, and Murphy E. Preconditioning results in S-nitrosylation of proteins involved in regulation of mitochondrial energetics and calcium transport. *Circ Res* 101: 1155–1163, 2007.
63. Thibault H, Piot C, Staat P, Bontemps L, Sportouch C, Rioufol G, Cung TT, Bonnefoy E, Angoulvant D, Aupetit J-F, Finet G, André-Fouët X, Macia JC, Raczka F, Rossi R, Itti R, Kirkorian G, Derumeaux G, and Ovize M. Long-term benefit of postconditioning. *Circulation* 117: 1037–1044, 2008.
64. Thornton JD, Liu GS, Olsson RA, and Downey JM. Intravenous pretreatment with A_1-selective adenosine analogues protects the heart against infarction. *Circulation* 85: 659–665, 1992.
65. Tissier R, Cohen MV, and Downey JM. Protecting the acutely ischemic myocardium beyond reperfusion therapies: are we any closer to realizing the dream of infarct size elimination? *Arch Mal Coeur Vaiss* 100: 794–802, 2007.
66. Tong H, Imahashi K, Steenbergen C, and Murphy E. Phosphorylation of glycogen synthase kinase-3β during preconditioning through a phosphatidylinositol-3-kinase-dependent pathway is cardioprotective. *Circ Res* 90: 377–379, 2002.
67. Trincavelli ML, Marroni M, Tuscano D, Ceruti S, Mazzola A, Mitro N, Abbracchio MP, and Martini C. Regulation of A2B adenosine receptor functioning by tumour necrosis factor a in human astroglial cells. *J Neurochem* 91: 1180–1190, 2004.
68. Weinbrenner C, Liu GS, Downey JM, and Cohen MV. Cyclosporine A limits myocardial infarct size even when administered after onset of ischemia. *Cardiovasc Res* 38: 676–684, 1998.
69. Xi J, McIntosh R, Shen X, Lee S, Chanoit G, Criswell H, Zvara DA, and Xu Z. Adenosine A_{2A} and A_{2B} receptors work in concert to induce a strong protection against reperfusion injury in rat hearts. *J Mol Cell Cardiol* 47: 684–690, 2009.
70. Xu Z, Downey JM, and Cohen MV. AMP 579 reduces contracture and limits infarction in rabbit heart by activating adenosine A_2 receptors. *J Cardiovasc Pharmacol* 38: 474–481, 2001.
71. Yang X-M, Krieg T, Cui L, Downey JM, and Cohen MV. NECA and bradykinin at reperfusion reduce infarction in rabbit hearts by signaling through PI3K, ERK, and NO. *J Mol Cell Cardiol* 36: 411–421, 2004.

72. Yang X-M, Philipp S, Downey JM, and Cohen MV. Post-conditioning's protection is not dependent on circulating blood factors or cells but involves adenosine receptors and requires PI3-kinase and guanylyl cyclase activation. *Basic Res Cardiol* 100: 57–63, 2005.
73. Yang X-M, Proctor JB, Cui L, Krieg T, Downey JM, and Cohen MV. Multiple, brief coronary occlusions during early reperfusion protect rabbit hearts by targeting cell signaling pathways. *J Am Coll Cardiol* 44: 1103–1110, 2004.
74. Ytrehus K, Liu Y, and Downey JM. Preconditioning protects ischemic rabbit heart by protein kinase C activation. *Am J Physiol* 266: H1145–H1152, 1994.
75. Zatta AJ, Kin H, Lee G, Wang N, Jiang R, Lust R, Reeves JG, Mykytenko J, Guyton RA, Zhao Z-Q, and Vinten-Johansen J. Infarct-sparing effect of myocardial postconditioning is dependent on protein kinase C signalling. *Cardiovasc Res* 70: 315–324, 2006.
76. Zhao Z-Q, Corvera JS, Halkos ME, Kerendi F, Wang N-P, Guyton RA, and Vinten-Johansen J. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol* 285: H579–H588, 2003.
77. Zorov DB, Juhaszova M, Yaniv Y, Nuss HB, Wang S, and Sollott SJ. Regulation and pharmacology of the mitochondrial permeability transition pore. *Cardiovasc Res* 83: 213–225, 2009.

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Abbreviations Used

AMI = acute myocardial infarction
ANT = adenine nucleotide translocase
AR = adenosine receptor
cGMP = cyclic guanosine monophosphate
CsA = cyclosporin A
CSC = 8-(13-chlorostyryl) caffeine
DPCPX = 8-cyclopentyl-1,3-dipropylxanthine
eNOS = endothelial nitric oxide synthase
ERK = extracellular signal-regulated kinase
GC = guanylyl cyclase
GSK = glycogen synthase kinase
HB-EGF = heparin-binding epidermal growth factor-like growth factor
IP = ischemic preconditioning
IPoC = ischemic postconditioning
JAK = janus kinase
K_{ATP} = ATP-dependent potassium channel
MEK = mitogen activated protein kinase
mK_{ATP} = mitochondrial ATP-sensitive potassium channel
MMP = matrix metalloproteinases
MPTP = mitochondrial permeability transition pore
NECA = 5′-(*N*-ethylcarboxamido) adenosine
NO = nitric oxide
PDK1/2 = 3′-phosphoinositide-dependent kinase-1/-2
$PI_{3,4,5}P_3$ = phosphatidylinositol trisphosphate
$PI_{4,5}P_2$ = phosphatidylinositol bisphosphate
PI3K = phosphatidylinositol 3-kinase
PKC = protein kinase C
PKG = protein kinase G
PLC/PLD = phospholipase C/D
PMA = phorbol 12-myristate 13-acetate
RISK = reperfusion injury salvage kinases
ROS = reactive oxygen species
SEM = standard error of the mean
SPT = 8-p-(sulfophenyl) theophylline
Src = sarcoma tyrosine kinase
STAT = signal transducer and activator of transcription
VDAC = voltage-dependent anion channel

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2. Antonio Rodríguez-Sinovas, Jose A. Sánchez, Celia Fernandez-Sanz, Marisol Ruiz-Meana, David Garcia-Dorado. 2012. Connexin and pannexin as modulators of myocardial injury. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1818**:8, 1962-1970. [CrossRef]
3. Claudia Penna , Maria-Giulia Perrelli , Pasquale Pagliaro . Mitochondrial Pathways, Permeability Transition Pore, and Redox Signaling in Cardioprotection: Therapeutic Implications. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
4. R. Hermann, M. G. Marina Prendes, M. E. Torresin, D. Vélez, E. A. Savino, A. Varela. 2012. Effects of the AMP-activated protein kinase inhibitor compound C on the postconditioned rat heart. *The Journal of Physiological Sciences* **62**:4, 333-341. [CrossRef]
5. Robert M. Bell, Derek M. Yellon. 2012. Conditioning the whole heart—not just the cardiomyocyte. *Journal of Molecular and Cellular Cardiology* **53**:1, 24-32. [CrossRef]
6. Danina M. Muntean, Valentin Ordodi, René Ferrera, Denis Angoulvant. 2012. Volatile anaesthetics and cardioprotection - lessons from animal studies. *Fundamental & Clinical Pharmacology* n/a-n/a. [CrossRef]
7. Claudia Penna, Teresa Pasqua, Maria-Giulia Perrelli, Pasquale Pagliaro, Maria Carmela Cerra, Tommaso Angelone. 2012. Postconditioning with glucagon like peptide-2 reduces ischemia/reperfusion injury in isolated rat hearts: role of survival kinases and mitochondrial KATP channels. *Basic Research in Cardiology* **107**:4. . [CrossRef]
8. D. Garcia-Dorado, M. Ruiz-Meana, J. Inserte, A. Rodriguez-Sinovas, H. M. Piper. 2012. Calcium-mediated cell death during myocardial reperfusion. *Cardiovascular Research* . [CrossRef]
9. Karin Przyklenk. 2011. Efficacy of Cardioprotective 'Conditioning' Strategies in Aging and Diabetic Cohorts. *Drugs & Aging* **28**:5, 331-343. [CrossRef]
10. Zhoupeng Zheng, Meijuan Yang, Fengjiang Zhang, Jing Yu, Junkuan Wang, Leilei Ma, Yanbo Zhong, Lingbo Qian, Gang Chen, Lina Yu, Min Yan. 2011. Gender-Related Difference of Sevoflurane Postconditioning in Isolated Rat Hearts: Focus on Phosphatidylinositol-3-Kinase/Akt Signaling. *Journal of Surgical Research* . [CrossRef]
11. Pasquale Pagliaro , Francesca Moro , Francesca Tullio , Maria-Giulia Perrelli , Claudia Penna . 2011. Cardioprotective Pathways During Reperfusion: Focus on Redox Signaling and Other Modalities of Cell Signaling. *Antioxidants & Redox Signaling* **14**:5, 833-850. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
12. Pasquale Pagliaro , Claudia Penna . 2011. Cardiac Postconditioning. *Antioxidants & Redox Signaling* **14**:5, 777-779. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]