

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 A₁ adenosine receptors in rabbit heart

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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 A₁ agonist, R-PIA (*N*⁶-1-[*p*-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 A₁ 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 A₁ 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 $38\pm3\%$ of the region at risk. Infarct size in nonpreconditioned staurosporine and polymyxin B-treated rabbits was $41\pm3\%$ and $42\pm7\%$, 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 $7\pm3\%$. 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 $36\pm3\%$ and $41\pm3\%$, 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 $28\pm5\%$ of the region at risk in controls. The PKC activators 4 β -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 $6\pm1\%$ and $12\pm3\%$, respectively, similar to preconditioning ($12\pm2\%$). Polymyxin B was also used in the isolated heart model and, consistent with the PKC hypothesis, it eliminated preconditioning-induced protection, producing infarcts of $33\pm5\%$.

This was one of 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 A₁ 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. Imura

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 $44\pm 4\%$ 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 $21\pm 3\%$, 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 ($27\pm 4\%$), 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 ($30\pm 6\%$ and $36\pm 4\%$, 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, $16\pm 4\%$; four cycles, $14\pm 3\%$), but there was no significant difference with those seen after a single preconditioning occlusion ($21\pm 3\%$). 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 $64\pm 9\%$ 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 $73\pm 7\%$ 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

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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 μ mol/L) to abolish the protective effects of preconditioning. The adenosine receptor agonist *R*(-)-*N*⁶-(2-phenylisopropyl)adenosine (PIA, 100 μ mol/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 μ mol/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 4 β -phorbol-12-myristate-13-acetate (PMA, 1 μ mol/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 μ mol/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

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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"