Preconditioning Does Not Attenuate Myocardial Stunning

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Background. Despite numerous reports that one or more episodes of brief coronary artery occlusion preconditioning the myocardium and dramatically reduces myocardial infarct size produced by a subsequent prolonged ischemia, we recently demonstrated that preconditioning does not attenuate contractile dysfunction in the peri-infarct tissue. However, the specific effects of preconditioning on myocardium in which wall motion has not been compromised by the preconditioning regimen per se and is further submitted to a short ischemic insult (that is, not confounded by necrosis) remain unknown.

Methods and Results. We addressed these issues in the canine model of myocardial stunning. Eighteen anesthetized dogs underwent 15 minutes of coronary occlusion followed by 3 hours of reperfusion. Before the 15-minute coronary occlusion, each dog received one of three treatments: no intervention (control group, n=6), one episode of 5-minute coronary occlusion/5-minute reperfusion (PC5 group, n=6), or one episode of 2.5-minute coronary occlusion/5-minute reperfusion (PC2.5 group, n=6). Segment shortening (SS) in the ischemic/reperfused midmyocardium was monitored by sonomicrometry, and myocardial blood flow was assessed by injection of radiolabeled microspheres. All three groups were equally ischemic during the 15-minute coronary occlusion: Midmyocardial blood flow averaged 0.05±0.02, 0.07±0.04, and 0.08±0.03 ml/min/g in control, PC2.5, and PC5 groups, respectively. Before the 15-minute coronary occlusion, PC5 dogs exhibited significant stunning (SS=55% baseline; p<0.01 versus control), whereas PC2.5 dogs did not (SS=91% baseline; p=NS versus control). However, segment shortening during the subsequent 15-minute coronary occlusion was equally depressed at -25% to -42% of baseline values among the three groups. Furthermore, all three groups demonstrated a similar degree of stunning after reperfusion: SS at 3 hours after reflow averaged 24±12%, 34±16%, and 48±12% of baseline in control, PC2.5, and PC5 groups, respectively (p=NS). The degree of recovery of function after reperfusion correlated with the amount of midmyocardial blood flow during coronary artery occlusion. However, this relation was not different among the three groups: Specifically, for any given collateral flow during ischemia, preconditioning did not reduce the degree of stunning.

Conclusions. Preconditioning neither preserves contractile function during a reversible ischemic insult nor prevents myocardial stunning during the initial hours of reflow. (Circulation 1992;85:2247–2254)

KEY WORDS • contractile function • myocardial ischemia • reperfusion

Recent myocardial ischemia is commonly observed in patients with coronary artery disease who suffer from frequent anginal attacks. Several studies have recently demonstrated that repeated brief episodes of coronary occlusion precondition the heart and reduce infarct size produced by subsequent sustained ischemia.1–4 In contrast, little is known about the effects of preconditioning on regional contractile function.

We recently observed that, although ischemic preconditioning in part preserves myocardial viability, it neither improves contractile function during a sustained 60-minute coronary occlusion nor attenuates postischemic stunning of the salvaged subepicardium during the initial hours of reperfusion.5 However, analysis of the effect of the preconditioning phenomenon on contractile function after prolonged coronary occlusion may be confounded by two important factors: 1) the presence of subendocardial necrosis, which influences wall motion in the surrounding viable myocardium, and 2) the preconditioning regimen (i.e., repeated brief episodes of ischemia/reperfusion) results in contractile dysfunction before sustained ischemia and may thereby limit or mask a beneficial effect of preconditioning on wall motion.

To address these issues, we assessed the effect of preconditioning on acute recovery of contractile function in canine myocardium stunned by a brief 15-minute ischemic insult. We compared two different preconditioning regimens aimed at inducing tolerance to ischemia: one resulting in significant stunning before the 15-minute ischemia and the other causing virtually no deterioration of contractile function. Specifically, we sought to determine whether preconditioning improves regional systolic function during coronary occlusion and/or after reperfusion in a model of brief, transient ischemia not associated with myocardial necrosis.
Methods

This study conformed with the principles in “Position of the American Heart Association on Research Animal Use” (Circulation 1985;71:849), followed the University of Southern California Code of Ethics for the Humane Treatment of Animals, and was approved by the Institutional Animal Care and Use Committee of the Hospital of the Good Samaritan.

Surgical Preparation

Forty mongrel dogs of either sex weighing 15–30 kg were sedated with morphine sulfate (15 mg s.c.), anesthetized with sodium pentobarbital (30 mg/kg i.v.), intubated, and ventilated with room air. Cannulas were inserted into the left jugular vein (for administration of drugs and fluids) and the left carotid artery (for measurement of heart rate and blood pressure and for withdrawal of reference samples for measurement of myocardial blood flow). A thoracotomy was performed in the fourth left intercostal space, and the heart was suspended in a pericardial cradle. A 5F microtipped pressure transducer (Millar) was positioned within the left ventricular (LV) cavity via the left atrial appendage for measurement of the LV pressure and its first derivative dP/dt. Through the same incision, a cannula was inserted in the left atrium for later injection of radiolabeled microspheres for measurement of regional myocardial blood flow (RMBF).

One pair of ultrasonic crystals used to assess regional contractile function was positioned in the center of the soon-to-be ischemic left anterior descending coronary artery (LAD) bed, as previously described.8 Crystals were inserted via small scalp incisions into the myocardium at a separation of 6–12 mm and oriented parallel to the minor axis of the heart. Regional contractile function, LV dP/dt, and arterial and LV pressures were monitored continuously throughout the experiment on a Gould recorder (Gould Inc., Cleveland, Ohio).

After the crystals were positioned, a segment of the LAD coronary artery was isolated, usually just distal to its major diagonal branch. After these surgical procedures, the animals were allowed 15 minutes to reach steady state.

Preliminary Study

It has been demonstrated that one episode of 5-minute coronary occlusion/10-minute reperfusion precedes the canine ischemic myocardium.2 There is indirect evidence that an even shorter coronary occlusion is sufficient to induce preconditioning: Deutsch et al7 demonstrated in humans that one 90-second coronary occlusion with an angioplasty balloon can precondition the heart. In addition, Warner et al8 reported that in the canine model, one 3-minute coronary occlusion blunted the accumulation of H+ ions during a subsequent episode of ischemia, a metabolic response that has been related to the preconditioning phenomenon.9

We performed a preliminary study to assess whether one episode of 2.5-minute coronary occlusion followed by 5 minutes of reperfusion was sufficient to protect myocardium from sustained ischemia as defined by Murry et al.3 That is, we sought to ensure that 2.5 minutes of ischemia/5 minutes of reperfusion was a valid preconditioning regimen. Twelve dogs underwent one episode of 2.5-minute coronary occlusion/5-minute reperfusion followed by 60 minutes of coronary occlusion and 4.5 hours of reperfusion. These dogs were compared with 10 control dogs (from two concurrent studies in our laboratory) that were submitted to 60 minutes of coronary occlusion and 4.5 hours of reperfusion. In all dogs, myocardial blood flow was measured 30 minutes after occlusion. Area at risk and infarct size were measured as described below. Although the preconditioned dogs in this preliminary study were not randomized, both studies were conducted at the same time by two investigators using the same surgical procedures and techniques.

Experimental Protocol

The remaining 28 dogs were assigned into one of three groups in a two-step randomization. At the beginning of the study, 16 dogs were randomized into either the control or the PC2.5 group. Later, 12 dogs were randomized into the PC5 or the control group. In all groups, dogs underwent 15 minutes of LAD coronary artery occlusion followed by 3 hours of reperfusion. Before this, a treatment period was completed, which consisted of either no intervention for 10 minutes (control group), one episode of 2.5-minute LAD coronary artery occlusion followed by 5 minutes of reperfusion (PC2.5 group), or one episode of 5-minute LAD coronary artery occlusion followed by 5 minutes of reperfusion (PC5 group).

At the end of each experiment, the LAD was reoccluded and 0.5 mg/kg Unisperse Blue Pigment (CIBA-GEIGY, Hawthorne, N.Y.) was injected into the coronary vasculature via the left atrial appendage to delineate the in vivo area at risk. With this technique, the nonischemic myocardium appears blue, whereas the previously ischemic myocardium (area at risk) remains unstained. Under deep anesthesia, the hearts were stopped by intracardiac injection of potassium chloride (40 mEq) and excised for further analysis.

Hemodynamics and Contractile Function

In all groups, measurements of heart rate, systolic, diastolic, and mean arterial pressures, and segment shortening were made at baseline (i.e., before treatment), just before the 15-minute coronary occlusion, and were then monitored throughout the 15-minute LAD coronary artery occlusion and the subsequent 3 hours of reperfusion. In the preconditioned groups, hemodynamics and segment shortening were also measured at 2 minutes into the brief occlusion.

Measurement of Regional Myocardial Blood Flow

RMBF was assessed by injection of microspheres labeled with 113m-cerium, 95m-niobium, or 103m-ruthenium (New England Nuclear). In all dogs, RMBF was measured at 10 minutes into the 15-minute occlusion. In the preconditioned groups, RMBF was also assessed during the brief occlusion of the treatment period.

Myocardial High-Energy Phosphate Content

Transmural biopsies were obtained in both nonischemic and ischemic zones at the end of the experiment (before the LAD was reoccluded for determination of
the area at risk) with a disposable biopsy needle (Travellon, Deerfield, Ill.). Tissue samples were frozen in acetone cooled with dry ice within seconds of their removal from the heart and divided into endocardial and epicardial halves. Adenosine triphosphate (ATP) and creatine phosphate (CP) were measured by the method of Lowry and Passoneau\(^{10}\) and protein by the method of Lowry et al.\(^{11}\)

**Analysis**

**Area at risk and area of necrosis.** After excision, all hearts were cut into five to seven transverse slices parallel to the atroventricular groove. The correct position of the two crystals inside the area at risk and the midmyocardium was confirmed. After right ventricular tissue had been removed, the heart slices were weighed. The basal surface of each slice was photographed for later measurement of the area at risk. Each slice was then incubated for 10 minutes in a 1% solution of triphenyltetrazolium chloride at 37°C. This method has been shown to reliably identify necrotic myocardium (which appears pale) from viable myocardium, which stains brick red.\(^{12}\) The slices were then rephotographed. Enlarged projections of these slices were traced for determination of the boundaries of the area at risk and area of necrosis. Extent of the area at risk (and the area of necrosis in the preliminary study) was quantified by computerized planimetry and corrected for the weight of the tissue slice. Total weight of the area at risk and the area of necrosis was then calculated and expressed as a percentage of the total left ventricular weight.

**Regional myocardial blood flow.** Tissue blocks were cut from the center of the LAD bed and the circumflex bed and subdivided into endocardial, midmyocardial, and epicardial segments. RMBF was then determined by the technique of Domenech et al.\(^{13}\)

**Hemodynamics and segment shortening.** Heart rate and arterial blood pressure were measured and averaged over five continuous cardiac cycles in sinus rhythm for each sample period. LV dP/dt was used to define the timing of the cardiac cycle for segment length measurements with ultrasonic crystals; end-diastolic lengths (EDL) were measured at the onset of the rapid increase in LV dP/dt, whereas end-systolic lengths (ESL) were measured at peak negative LV dP/dt. EDL and ESL were obtained from three well-separated cardiac cycles in each sample period, averaged, and used to compute segment shortening (SS), an index of regional systolic contractile function defined as SS=[(mean EDL−mean ESL)/mean EDL]×100%.\(^{14}\) SS during each sample period was normalized and expressed as percentage of the respective baseline values.

**Exclusion criteria.** Dogs with high collateral blood flow during coronary occlusion and/or a small area at risk were excluded from the final analysis. Specifically, our standard exclusion criteria, established before the onset of the protocol, were values of RMBF in the ischemic endocardium >0.20 ml/min/g during sustained LAD occlusion and/or an area at risk occupying <10% of the left ventricle. In addition, no attempt was made to resuscitate dogs that developed ventricular fibrillation at any time of the experiment.

**Statistics**

All measurements are expressed as group mean±SEM. Comparisons of RMBF, high-energy phosphate content, and area at risk among groups were performed by ANOVA. If significant F ratios were obtained, comparisons between groups were then made by Tukey’s test.\(^{15}\) Comparison of high-energy phosphate content within each group was made by paired t test. ANOVA and Tukey’s test with Bonferroni’s correction for multiple comparisons were used to compare hemodynamics and SS measurements among the three groups. ANCOVA, with recovery of SS at 3 hours after reflow as dependent variable and midmyocardial blood flow as covariate, was used to analyze differences among regression lines of control, PC\(_{2.5}\), and PC\(_{5}\) groups. ANCOVA was also used in the preliminary study, with infarct size (as percentage of the area at risk) as dependent variable and inner two thirds of myocardial blood flow as covariate to analyze differences between control and PC\(_{2.5}\) group regression lines.

**Results**

**Preliminary Protocol**

Of the 12 dogs that entered the preliminary study, three died from ventricular fibrillation (20–25 minutes into the 60-minute ischemia), and two were not ischemic (Figure 1). Thus, data are reported from the remaining seven PC\(_{2.5}\) dogs (compared with 10 control dogs).
Area at risk was not significantly different between the two groups, averaging 23.2±2.7% and 20.1±2.1% of the LV weight in control and preconditioned groups, respectively. During coronary artery occlusion, myocardial blood flow in the inner two thirds of the LV wall was similar between groups, averaging 0.05±0.01 ml/min/g in the control group versus 0.03±0.01 ml/min/g in the preconditioned group. Infarct size (expressed as percentage of the area at risk) was significantly reduced in the PC25 group: 12.2±2.9% versus 25.0±5.4% in the control group (p<0.05 by t test). This beneficial effect was further confirmed when we plotted infarct size versus collateral blood flow during coronary artery occlusion (Figure 1): The regression line for the PC25 group was shifted downward with respect to the control line (p<0.05 by ANCOVA). However, the reduction in infarct size with only 2.5 minutes of preconditioning ischemia is clearly less dramatic than that usually observed in this model after longer (i.e., >5 minutes) brief coronary artery occlusions.1,2,4

Therefore, one episode of 2.5 minutes of coronary occlusion was sufficient to precondition the ischemic myocardium in the canine model of ischemia/reperfusion and may consequently be used to assess the effect of preconditioning on contractile function after a 15-minute coronary occlusion.

Experimental Protocol

Mortality and exclusions. Of the 28 dogs entered into the study, three dogs were excluded because they had subendocardial blood flow >0.20 ml/min/g during the sustained occlusion (two in the PC25 and one in the PC5 groups). See Table 1.

Seven dogs developed ventricular fibrillation and were not resuscitated: One died during the brief occlusion in the PC5 group, one died during the 15-minute occlusion in the PC25 group, and five died at the onset of reperfusion in the control group.

Data are thus presented for the 18 dogs (six in each group) that successfully completed this portion of the study.

Area at risk. Area at risk (as percentage of the LV weight) was not different among groups: 20.5±1.4% in control, 18.4±1.2% in PC25, and 22.5±1.5% in PC5 dogs. As expected, there was no evidence of infarction in any of the dogs subjected to the 15-minute ischemia.

Hemodynamics. Heart rate did not differ among groups at any time point of the experiment (see Table 2). At baseline, arterial pressure (systolic, diastolic, and mean) was significantly lower in the control group compared with the PC25 group (p<0.05) but did not differ from the PC5 group. This pattern persisted throughout the course of the experiment.

Regional myocardial blood flow. All three groups were equally ischemic during the 5-minute LAD occlusion: Midmyocardial blood flow averaged 0.05±0.02, 0.07±0.04, and 0.08±0.03 ml/min/g in control, PC2.5, and PC5 groups, respectively (p=NS). Myocardial blood flow did not differ among groups in the circumflex bed either during the brief or the 15-minute LAD occlusion. See Table 3.

Myocardial high-energy phosphate content. ATP and CP data were available in five dogs in the control group, five dogs in the PC2.5 group, and six dogs in the PC5 group. See Table 4.

ATP content in the normally perfused circumflex bed averaged 25–28 nmol/mg cardiac protein and did not differ among the three groups. As expected, ATP levels were depleted in the previously ischemic LAD territory; i.e., in the subendocardium, ATP stores were reduced to 18.2±1.3, 16.2±1.2, and 18.8±1.8 nmol/mg cardiac protein in control, PC2.5, and PC5 groups, respectively. There was no difference, however, in either subendocardial or subepicardial ATP content among the three groups.

CP content in the nonischemic circumflex bed averaged 42–58 nmol/mg cardiac protein in the three groups. All dogs exhibited CP overshoot in the previously ischemic LAD bed: CP values in the subendocardium were increased to 86.0±8.3, 77.3±5.4, and 60.5±2.9 nmol/mg cardiac protein in control, PC2.5, and PC5 groups, respectively. CP overshoot in the subendocardium was higher in control than in PC2.5 (but not PC5) dogs (p<0.05), probably because control animals were slightly more ischemic during the 15-minute coronary artery occlusion.

Regional contractile function. All preconditioned dogs exhibited similar dyskinesis in the LAD bed during the brief episode of coronary occlusion: SS averaged −21.6±4.2% of baseline in the PC2.5 group and −29.0±7.8% of baseline in the PC5 group (p=NS). (See Figures 2 and 3.) As expected, only dogs from the PC5 group exhibited myocardial stunning after the brief episode of ischemia: Immediately before the 15-minute occlusion, SS averaged 101±2% of baseline in the control group, 90.8±4.1% in the PC2.5 group (p=NS versus control), and 55.3±12.0% in the PC5 group (p<0.01 versus both control and PC2.5).

All groups were equally dysskinetic throughout the 15-minute coronary occlusion, with SS averaging −25% to −42% of baseline values among the three groups. Furthermore, PC2.5 and PC5 animals demonstrated the
same degree of contractile dysfunction both during the brief ischemic episode and during the 15-minute occlusion. In both preconditioned groups, there was no change in SS between 2 and 10 minutes after the 15-minute period of ischemia. In addition, all groups were similarly stunned throughout reperfusion: At 3 hours after reflow, SS averaged 24.0±11.8%, 34.0±15.9%, and 48.3±11.9% of baseline values in the control, PC2.5, and PC5 groups, respectively (p=NS versus control for both preconditioned groups). Recovery of contractile function was also expressed as a function of collateral flow during coronary artery occlusion (Figure 3). ANCOVA revealed no statistically significant difference among groups in the recovery of function at 3 hours after reperfusion. Thus, for any level of regional myocardial blood flow during the ischemic event, preconditioning did not improve the degree of recovery of the stunned myocardium during the early hours of reperfusion.

Discussion

The present study demonstrates that preconditioning neither preserves contractile function during a short coronary occlusion nor attenuates myocardial stunning during the first hours after reperfusion. Thus, although ischemic preconditioning can preserve myocyte viability, this phenomenon does not enhance the acute recovery of postischemic contractile function.

High-Energy Phosphates

After 3 hours of reperfusion, all groups exhibited a similar ATP depletion in the previously ischemic region. The persistence of low levels of myocardial ATP after a few hours of reperfusion is in agreement with previous studies that have shown that ATP resynthesis requires days after a brief ischemic insult.16,17 If we therefore assume that ATP levels after 3 hours of reflow reflect ATP levels at the end of the 15-minute ischemia, our

### Table 2. Hemodynamics

<table>
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<th>Baseline</th>
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Values are mean±SEM. HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; PC2.5 and PC5, groups receiving 2.5-minute coronary occlusion/5-minute reperfusion and 5-minute coronary occlusion/5-minute reperfusion, respectively.

### Table 3. Regional Myocardial Blood Flow

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<th></th>
<th>Brief occlusion (ml/min/g)</th>
<th>15-Minute occlusion (ml/min/g)</th>
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<td></td>
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<td>Mid</td>
<td>Epi</td>
<td>Endo</td>
<td>Mid</td>
<td>Epi</td>
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</table>

Values are mean±SEM. No significant difference among groups.

Endo, subendocardial blood flow; Mid, midmyocardial blood flow; Epi, subepicardial blood flow; LAD, left anterior descending; PC2.5 and PC5, groups receiving 2.5-minute coronary occlusion/5-minute reperfusion and 5-minute coronary occlusion/5-minute reperfusion, respectively; Cx, circumflex.
TABLE 4. Myocardial High-Energy Phosphates Expressed as nmol/mg Cardiac Protein and Percent of Normal, Nonischemic Values

| ATP | Ischemic | Nonischemic | | | Ischemic | Nonischemic |
|-----|----------|-------------| | | Ischemic | Nonischemic |
| Endo | Epi | Endo | Epi | | Endo | Epi | Endo | Epi |
| Control | 18.2±1.3* | 19.8±1.6* | 25.2±1.1 | 26.3±0.6 | | 86.0±8.3* | 74.2±9.5* | 49.2±3.7 | 46.2±2.2 |
| (72±5%) | (75±6%) | (100%) | (100%) | | (175±17%) | (161±21%) | (100%) | (100%) |
| PC_{2.5} | 16.2±1.2* | 19.6±0.5* | 28.2±1.8 | 26.7±1.3 | | 77.3±5.4* | 68.2±2.8* | 58.1±6.3 | 55.4±4.4 |
| (57±4%) | (73±2%) | (100%) | (100%) | | (133±9%) | (123±5%) | (100%) | (100%) |
| PC_{1} | 18.8±1.8* | 20.1±0.5* | 25.3±0.8 | 24.6±0.8 | | 60.5±2.9* | 60.2±5.1* | 48.1±3.9 | 41.8±2.4 |
| (74±7%) | (82±2%) | (100%) | (100%) | | (126±6%) | (144±12%) | (100%) | (100%) |

Values are mean±SEM.
ATP, adenosine triphosphate; CP, creatine phosphate; Endo and Epi, subendocardial and subepicardial halves of transmural biopsy sample; PC_{2.5} and PC_{1}, groups receiving 2.5-minute coronary occlusion/5-minute reperfusion and 5-minute coronary occlusion/5-minute reperfusion, respectively.
*p<0.05 vs. nonischemic.
†p<0.05 vs. control.

Results imply that all three groups had similar ATP depletion after 15 minutes of coronary artery occlusion. This is concordant with the study of Murr et al, who demonstrated that the reduced ATP depletion observed at 10 minutes after occlusion was no longer present after 20 minutes of coronary occlusion. Our data further imply that the time frame of the slowed rate of ATP depletion in the preconditioned groups is narrow.

Effect of Preconditioning on Contractile Function After Reversible Ischemic Insult
Cohen et al recently described the effect of preconditioning on contractile function in the rabbit model. They found that preconditioning attenuated stunning after 30 minutes of coronary occlusion, a period of ischemia associated with myocyte necrosis in this model; however, this improvement in postischemic contractile function was probably a consequence of infarct size reduction and proximity of the ultrasonic crystals relative to the infarct rather than direct preservation of contractility afforded by preconditioning. In contrast, we recently demonstrated in the canine model that preconditioning does not attenuate myocardial stunning of the peri-infarct tissue after 60 minutes of coronary artery occlusion. The reasons for the discrepancy be-

FIGURE 2. Graph shows effect of preconditioning on contractile function: segment shortening (SS) in the ischemic left anterior descending (LAD) coronary bed. Data were obtained at baseline (Bas), during the brief occlusion (CO) at the end of the treatment period (Pre), and at multiple time points throughout the 15-minute CO and after reperfusion. PC_{2.5} and PC_{2.5}, groups receiving 5-minute CO/5-minute reperfusion and 2.5-minute CO/5-minute reperfusion, respectively. *p<0.05 vs. control.

FIGURE 3. Graph shows recovery of contractile function as a function of collateral flow during ischemia: The difference (delta recovery SS%) between segment shortening (SS) at 3 hours after reflow and 10 minutes into coronary artery occlusion (CO) is plotted against collateral flow during CO. In all three groups, there was a proportional relation between functional recovery and collateral flow. PC_{2.5} and PC_{2.5}, groups receiving 5-minute CO/5-minute reperfusion and 2.5-minute CO/5-minute reperfusion, respectively. PC_{2.5} and PC_{2.5} symbols lie both above and below the control line, indicating that preconditioning did not enhance recovery of SS when compared with controls. This was confirmed by ANCOVA: There was no difference between the regression lines of the control and the preconditioned groups (not shown).
between these two studies remain unclear but may be partly due to the use of different models.

In the present study, we used the classic model of myocardial stunning to specifically assess the effect of preconditioning on contractile function in the absence of myocardial necrosis. It is known that "tethering" of the subepicardium to the necrotic subendocardium influences the wall motion of the viable tissue. Moreover, the pathophysiology of stunning may not be the same in peri-infarct tissue submitted to a prolonged lethal episode of ischemia as in myocardium subjected to a completely reversible 15-minute ischemic insult. Contractile function during coronary occlusion was not improved by preconditioning. Preconditioned dogs exhibited the same degree of dyskinesis during the initial brief coronary occlusion and the subsequent 15-minute ischemia. Although the lack of further deterioration of contractile function during the second coronary occlusion could be interpreted as a protective effect, it may simply reflect the fact that the contractile status had reached a nadir during the first coronary occlusion. Indeed, previous studies have reported a similar lack of deterioration in function in myocardium submitted to repeated episodes of brief coronary occlusion. Preconditioning did not attenuate myocardial stunning during the first hours after reperfusion. As expected, all three groups were significantly stunned after the 15-minute coronary occlusion. The slight difference among the three groups at 3 hours after reflow was not significant and is probably due to a moderately (although not significant) higher myocardial blood flow in the preconditioned groups during the 15-minute coronary occlusion. To test this hypothesis, we expressed the degree of recovery of contractile function at 3 hours after reperfusion as a function of collateral flow during ischemia (Figure 3). If preconditioning improved recovery of function, data points for the preconditioned groups would be expected to lie above the points for the control group. This was not, however, the case: Points for the preconditioned groups were equally distributed above and below the regression line for the control group. That is, when collateral flow was taken into account, preconditioned dogs failed to demonstrate a better recovery of function 3 hours after reflow.

However, the fact that preconditioning does not attenuate myocardial stunning during the initial 3 hours of reperfusion does not preclude the possibility that ultimate recovery of normal contractile function may be accelerated during the initial days after reflow. This concept awaits further investigation in a chronic protocol.

Confounding Effect of the Preconditioning Regimen on Contractile Function

In previous preconditioning studies, the analysis of the effect of preconditioning on contractile function was confounded by the fact that repeated brief episodes of ischemia/reperfusion resulted in myocardial stunning before the sustained ischemia. Thus, we sought to determine whether any protection afforded by preconditioning on myocardial function may have been overwhelmed by this deterioration of contractile function caused by the initial brief episodes of ischemia/reperfusion.

In agreement with previous investigations in the canine model, we observed that one 5-minute coronary occlusion resulted in myocardial stunning, whereas one coronary occlusion shorter than 3 minutes did not. However, although systolic function was not significantly impaired before the 15-minute coronary occlusion in the PC group, it did not differ from those in the PC group at any later time point of the experiment. Thus, our results suggest that the initial contractile abnormalities caused by the preconditioning regimen in the PC group did not mask any beneficial effect of preconditioning on contractile function.

Another important observation was made in the preliminary study. Reduction of infarct size as a consequence of preconditioning occurred in the absence of myocardial stunning; segment shortening was 92±8% of baseline before the 60-minute coronary occlusion in the preconditioned group versus 97±4% in the control group (p=NS). This confirms our evidence and that of others that preconditioning is not a consequence of stunning.

Conclusions

This study demonstrates that preconditioning neither preserves contractile function during a 15-minute coronary occlusion nor attenuates myocardial stunning during the initial hours of reperfusion. It further confirms previous investigations indicating that the mechanisms of preconditioning and stunning are probably not related.

References

Preconditioning does not attenuate myocardial stunning.
M Ovize, K Przyklenk, S L Hale and R A Kloner

Circulation. 1992;85:2247-2254
doi: 10.1161/01.CIR.85.6.2247

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