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

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Abstract

Objectives: Myocardial protection associated with ischemic preconditioning (PC) wanes within an hour or two. It has recently been observed, however, that a delayed phase of protection appears about 24 h after ischemic PC in anesthetized rabbits and dogs which might be related to synthesis of cytoprotective proteins. We tested whether a second window of protection could be induced in conscious rabbits. Methods: Rabbits chronically instrumented with a coronary artery occluder and ECG electrodes experienced a 30-min coronary occlusion followed by 3 h reperfusion. Infarct size was measured with triphenyltetrazolium chloride. Results: 35.7 ± 2.3% of the risk zone infarcted in control animals. PC with 4 cycles of 5-min coronary occlusion/10-min reperfusion 24 h prior to the 30-min ischemia decreased infarction to 24.1 ± 1.4% of the risk zone (P < 0.01). During the 30-min occlusion 3 of 7 non-PC rabbits developed ventricular fibrillation, while this arrhythmia did not occur in the 7 PC animals (P < 0.1). Myocardial hsp70 content in PC rabbits was twice that in controls. Collateral blood flow was not different in the two groups. Conclusions: A second window of protection exists in conscious rabbits which minimizes both infarction and arrhythmias, and cytoprotective protein content is increased in the myocardium of protected animals.

Keywords: Collateral vessels; Heat shock protein; Myocardial infarction; Myocardial ischemia; Preconditioning; Arrhythmias; Myocardial protection; Myocardial infarct size; Rabbit

1. Introduction

Myocardial protection associated with ischemic preconditioning as classically described is known to last approximately 1–2 h in a variety of species [1–4]. It has recently been observed, however, that a delayed phase of protection appears 24 h after ischemic preconditioning in both rabbits [5] and dogs [6]. This phenomenon has been termed the 'second window of protection' [7].

Myocardial protection has also been induced with heat stress [5,8,9]. Because both ischemia and heat stress elevate a 70 kDa heat stress protein (hsp70i) to a similar extent in the heart and are associated with a similar reduction of infarct size [5], it has been tempting to postulate that this enhanced resistance to infarction occurs as a direct consequence of induction of hsp70i. The actual mechanism whereby hsp70i might limit infarct size is still not understood.

Prior evaluations of this second window have exclusively used ischemic preconditioning immediately after surgical exposure of the heart, thus making it difficult to exclude the possibility that the surgery itself or the anesthesia might have influenced the production of hsp70i in those hearts. We have recently developed a chronically instrumented, awake rabbit model [10] in which the coronary artery can be occluded at will without need to anesthetize the animal. This model is ideal for studying this second window of protection. By eliminating the confounding effects of anesthesia [11], we can better simulate the clinical setting and test whether myocardial ischemia...
per se is responsible for this protection. Accordingly, rabbits which had completely recovered from the stress of surgery were examined in the conscious state to determine whether their hearts were indeed protected from infarction 24 h after an ischemic preconditioning protocol. In addition, we measured collateral flow in these animals to test whether induction of collateral vessels might explain some of the previously documented protection.

2. Methods

2.1. Animal surgery

All procedures were approved by the institutional animal care and use committee, and the study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1985). New Zealand White rabbits weighing between 2 and 3 kg were anesthetized with intravenous sodium pentobarbital (30 mg/kg), intubated with an endotracheal tube, and mechanically ventilated with a positive pressure respirator (MD Industries, Mobile, AL) and 100% oxygen. After intravenous administration of 100 mg sodium cefazolin, a left thoracotomy was performed in the third intercostal space. The technique for placement of the pneumatic occluder around the coronary artery has previously been described [10]. Briefly, a balloon occluder fashioned from 18-gauge Tygon tubing was wedged onto the blunt end of a curved taper needle which was passed beneath a prominent branch of the left coronary artery. The balloon occluder was pulled through the myocardium, and the end was doubled over the artery and anchored to the myocardium with 4-0 suture. Proper functioning of the occluder was confirmed by noting cyanosis of the distal myocardium and cessation of effective contraction after inflation of the balloon and hyperemia and resumption of contraction after deflation.

In some rabbits an 18-gauge Tygon catheter was inserted into the left atrium (LA) through the appendage and secured for injection of radioactive microspheres. The chest wound was closed in layers, and air was aspirated from the thorax. Before approximation of the skin layer, ECG wires were anchored in the subcutaneous tissue with one lead at each end of the incision. Balloon occluder, ECG leads, and LA catheter were brought through the chest wall and tunneled subcutaneously to exit between the scapulae. The LA catheter was filled with a dilute solution of heparin. Following surgery and again 2 days later rabbits were treated with intramuscular injections of 600,000 U of benzathine penicillin.

2.2. Protocol

Seven days after surgery when rabbits appeared to be fully recovered, they were brought to the laboratory and randomly divided into two experimental groups based on the day of the week. In any given week, equal numbers of rabbits were selected for each group. The ECG leads were attached to an ECG amplifier and signals were recorded on a multichannel oscillograph.

In the ischemic preconditioning group 4 cycles of 5-min regional ischemia/10-min reperfusion were produced by manual inflation and deflation of the balloon occluder. Appearance of electrocardiographic S-T segment elevations in addition to changes in the QRS complex confirmed onset of myocardial ischemia. After each 5-min occlusion the balloon was deflated by aspiration of air. Normalization of the ECG documented successful reperfusion of the coronary artery. All rabbits were subsequently returned to their cages.

The next day, approximately 24 h after the ischemic preconditioning protocol, the rabbits were again brought to the laboratory. All settings for recording of the ECG were identical to those used on the previous day. In these animals the balloon occluder was reinflated for 30 min. Because these 30-min occlusions did not appear to cause distress or any behavioral changes in the rabbits, no analgesic agents were administered. The hearts were then reperfused for 180 min, after which they were removed for determination of risk zone and infarct sizes.

A sham group of rabbits also studied 1 week following instrumentation was brought to the laboratory for 1 h during the first day without any other pretreatment. Twenty-four hours later these animals underwent a 30-min coronary occlusion followed by 3 h of reflow before they were sacrificed for infarct size assessment.

No antiarrhythmic agents were administered to the rabbits at any time. If ventricular fibrillation occurred, external defibrillation was attempted at an energy level of 100 W. If the animal fibrillated during a coronary occlusion, resuscitation was tried without defibrilating the balloon.

2.3. Coronary collateral blood flow

A measurement of regional myocardial blood flow was made in 3 rabbits of each group which had implanted left atrial catheters. $1 \times 10^5$ microspheres (NEN, Boston, MA) labeled with $^{85}$Sr and suspended in 1 ml of saline were mechanically agitated and ultrasonicated for 10–15 min. Five minutes after the onset of the 30-min occlusion they were injected into the left atrium over a 5-s period followed by a saline flush. For simplification of the animal preparation no indwelling intra-arterial line had been inserted at the time of surgery and, therefore, no arterial reference sample was withdrawn at the time of the microsphere injection.

2.4. Risk zone and infarct size assessment

After completion of the 180-min period of reperfusion, rabbits were re-anesthetized with sodium pentobarbital (30
were powdered in liquid nitrogen and suspended and ho-
cardium. Left ventricular specimens were then rapidly
(risk zone) was separated from stained (perfused) my-
ventricular free wall were removed. The area without dye
moved from the perfusion rig, and atria, fat and right
(Sigma Chemical Company, St. Louis, MO) was intro-
fused with saline to remove blood. Monastral blue dye
ing the coronary artery branch. The aortic root was per-
apparatus. The silk suture was tied tightly, thus reocclud-
removed and hung by the aortic root from a Langendorff
they were anesthetized as described above and their hearts
not undergo a terminal 30-min coronary occlusion. Rather
animals were returned to the laboratory 24 h later, but did
rabbits preconditioned with 4 cycles of 5-min
2.6. Data analysis
Collateral flow was defined as flow to the non-fluo-
rescent myocardium, whereas flow to fluorescent tissue
was assumed to be normal. Raw radioactive counts/minute
were summed for all fluorescent and non-fluorescent pieces
was assumed to be normal. Raw radioactive counts/minute
were summed for all fluorescent and non-fluorescent pieces
and normalized for the respective total tissue mass. To
avoid contamination of ischemic myocardium by inadver-
tent inclusion of some tissue with normal flow, non-fluo-
rescent areas were quantitated by planimetry with the aid of a
digitizer (SAC, Norwalk, CT) interfaced to a computer,
and volumes were calculated by multiplying areas by slice
thickness. In those hearts in which collateral flow was
measured, the non-fluorescent tissue was carefully sepa-
rated from the fluorescing myocardium. Both fluorescent
and non-fluorescent pieces were cut into wedges, weighed,
and put into glass tubes. The radioactivity of these tissues
was counted with a gamma spectrometer (LKB Wallac
1282 Compugamma, Turku, Finland).

2.5. Stress protein estimation
Stress proteins were assayed in myocardium from 3
rabbits preconditioned with 4 cycles of 5-min
ischemia/10-min reperfusion and in 4 sham rabbits. These
animals were returned to the laboratory 24 h later, but did
not undergo a terminal 30-min coronary occlusion. Rather
they were anesthetized as described above and their hearts
removed and hung by the aortic root from a Langendorff
apparatus. The silk suture was tied tightly, thus reocclud-
ing the coronary artery branch. The aortic root was per-
fused with saline to remove blood. Monastral blue dye
(Sigma Chemical Company, St. Louis, MO) was intro-
duced into the coronary perfusate. Hearts were then re-
moved from the perfusion rig, and atria, fat and right
ventricular free wall were removed. The area without dye
(risk zone) was separated from stained (perfused) my-
ocardium. Left ventricular specimens were then rapidly
frozen in liquid nitrogen.

At a later date myocardial risk zone specimens (0.1 g)
were powdered in liquid nitrogen and suspended and ho-
mogenized in 1 ml sodium dodecyl sulfate (SDS) sample
buffer (20% glycerol and 6% SDS in 0.12M Tris at pH
6.8). After homogenization 2-mercaptopethanol (10% v/v)
was added. The samples were then heated over boiling
water for 5 min. The cooled solution was centrifuged at
10 000 X g for 5 min, and the supernatant transferred to a
clean tube with the addition of 10 μl/ml of 8% bro-
 Stephano-phenol blue in ethanol.

Proteins were separated by SDS-PAGE on 0.8-mm-
thick, 10% polyacrylamide gels according to Laemmli
[12]. Equivalence of loading and adequacy of sample
preparation were determined by visualization of proteins
with C0omassie Brilliant blue R250 stain. For Western
blotting following electrophoresis, proteins from the gels
were transferred electrophoretically onto nitrocellulose
membranes (Hybond C, Amersham, Bucks, UK) at 4°C
overnight using a current of 180 mA.

The nitrocellulose membrane was washed in phosphate-
buffered saline (pH 7.2) with 0.1% dried skimmed milk
powder (0.1% 'Marvel' and 0.05% Tween-20) to block
nonspecific binding sites. After washing, the membrane
was incubated at room temperature for 1 h with mouse
monoclonal IgG raised to hsp70i (C92F3A-5; Stressgen,
Victoria, B.C., Canada) at 1 : 1000 dilution in milk powder
solution. After repeated washing in buffer (0.05% Tween-
20 in phosphate-buffered saline), the membrane was incu-
bated with horseradish-peroxidase-conjugated rabbit anti-
mouse IgG (DAKO, Denmark) at 1 : 2500 dilution in the
same buffer at room temperature for 1 h. The filter was
then washed as above, developed by use of enhanced
chemiluminescence detection (Amersham, Bucks, UK) and
exposed to Kodak X-Omat film for 30 s to 3 min after
allowing at least 3 min for maximum light emission to
develop.

Western blots were scanned using a Biorad model
GS-670 imaging densitometer, and peak area integration
was performed with Biorad Molecular Analyst Software.
Calibration curves were constructed to determine the lin-
earity of the chemiluminescence detection system using
serial loadings of total ventricular protein's anti-hsp70i/hsc
70 monoclonal antibody. Light emission was proportional
to protein loading in the range used. Sample loadings were
equalized relative to the optical density of the actin band
(38 kDa). Optical densities of the hsp70i immunoreactivity
band were normalized to the actin band to adjust for slight
variations in protein loading between samples.

2.6. Data analysis
Collateral flow was defined as flow to the non-fluo-
rescent myocardium, whereas flow to fluorescent tissue
was assumed to be normal. Raw radioactive counts/minute
were summed for all fluorescent and non-fluorescent pieces
and normalized for the respective total tissue mass. To
avoid contamination of ischemic myocardium by inadver-
tent inclusion of some tissue with normal flow, non-fluo-
resistant risk zone pieces bordering normally perfused, fluorescent myocardium were omitted. Collateral flow was expressed as a percentage of the normal flow to the same heart. Volumes of ischemic and risk zone tissues were calculated by multiplying planimetered areas by slice thickness. Infarct size was calculated as a percentage of the risk zone.

2.7. Statistics

Data are presented as mean ± s.e.m. Statistical significance of comparisons between groups was assessed by unpaired Student's *t*-test and *χ²*-test (Fisher's exact method for small numbers). A *P*-value of < 0.05 was considered to be significant.

3. Results

Twenty-one rabbits, 11 sham and 10 ischemically preconditioned animals, were entered into the present study. No rabbits died during the initial surgery. Fourteen rabbits (7 sham and 7 preconditioned) were entered into the infarct study, while the hearts of the remaining 7 (4 sham and 3 preconditioned) were used for analysis of cytoprotective proteins. In the sham pretreatment group 7 rabbits underwent 30-min ischemia, and 3 of them developed ECG-documented ventricular fibrillation (VF) during the coronary occlusion and were resuscitated. No VF occurred in the 7 preconditioned animals during the 30-min coronary occlusion. The difference in incidence of VF between the two groups was not quite significant (*P* < 0.1).

3.1. Infarct size

Table 1 compares the heart weights, risk zones, infarct sizes, and infarct-to-risk zone ratios between the two groups. The heart weights and risk zone sizes were comparable. Table 1 and Fig. 1 present the average as well as individual animal infarct size data. In the sham pretreatment group 35.7 ± 2.3% of the risk zone infarcted. Infarct sizes in the 3 animals developing VF were 46.6, 40.8, and 31.9% of the respective risk zones. Although average infarct size was modestly greater in animals requiring defibrillation (39.8 ± 4.3 vs. 32.7 ± 2.1%), the difference was not significant. Four cycles of 5-min ischemia 24 h prior to the 30-min ischemia decreased infarction to 24.1 ± 1.4% (*P* < 0.01). For purposes of comparison, historical data from a group of conscious rabbits in which ischemic preconditioning (5-min coronary occlusion/10-min reperfusion) had occurred immediately prior to the 30-min coronary occlusion (13) are also included in Fig. 1. In this group infarct size averaged 5.6 ± 1.1% of the risk zone.

3.2. Collateral blood flow

Myocardial flow was measured in 3 of the 7 animals of each group. When expressed as a percentage of flow to normal tissue, collateral flow averaged 5.1 ± 1.2% in the sham animals and 4.5 ± 0.8% in the preconditioned group. There was no difference between the 2 groups.

3.3. Stress protein assay

Risk zone myocardial samples from 4 sham and 3 preconditioned animals were harvested 24 h after the initial laboratory visit or preconditioning protocol, respectively. As demonstrated in Fig. 2, the normalized optical density of the hsp70i band in the preconditioned myocardium averaged 2 times higher than that in the normally

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Table 1

<table>
<thead>
<tr>
<th>Infarct size data</th>
<th>n</th>
<th>Heart weight (g)</th>
<th>Risk zone (cm³)</th>
<th>Infarct zone (cm³)</th>
<th>Infarction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>7</td>
<td>8.3 ± 0.3</td>
<td>0.894 ± 0.081</td>
<td>0.375 ± 0.041</td>
<td>35.7 ± 2.3</td>
</tr>
<tr>
<td>Ischemic preconditioning</td>
<td>7</td>
<td>7.9 ± 0.3</td>
<td>0.922 ± 0.099</td>
<td>0.222 ± 0.024</td>
<td>24.1 ± 1.4 *</td>
</tr>
</tbody>
</table>

Mean ± s.e.m. Infarction (%) = percent infarction of the risk zone; *n* = number of rabbits in each group.

* *P* < 0.01 vs. sham.
Fig. 2. Upper panel: Representative Western blot probed with mAb for hsp70i (hsp72) of myocardial samples from 4 sham nonpreconditioned and 3 preconditioned (PC) hearts 24 h after either simple observation for 1 h or preconditioning with four 5-min coronary artery occlusions, respectively. These bands are compared to a neuronal cell line (ND7) overexpressing a transfected human hsp70 cDNA (pAPr2). Blots were developed using peroxidase-conjugated secondary antibody and enhanced chemiluminescence. The actin band from a Coomassie-Blue-stained gel run in parallel is shown for comparison of total protein loading. Lower panel: Results of computerized densitometry analysis of the Western blots of all rabbits. Immunoreactivity was normalized for total protein loading using the actin band. Data are, therefore, expressed as the hsp70i/actin ratio for sham and PC groups. Myocardial risk zone content of hsp70i in preconditioned rabbits is double that measured in sham rabbits (P < 0.1). Bars represent s.e.m.

4. Discussion

The present study clearly demonstrates that preconditioning of myocardium in conscious rabbits induces delayed protection 24 h later. This protection was manifested as a modest but significant reduction in infarct size and the apparent abolition of ischemia-induced ventricular fibrillation. All previously reported studies of the second window of protection have used anesthetized animal protocols in which both preconditioning and subsequent infarction have been effected under anesthesia. The present experiment reveals that protection is a direct result of transient coronary occlusion and that it does not involve induction of collateral vessels. Furthermore, these hearts were not only protected against infarction but against life-threatening arrhythmias as well.

4.1. Infarct reduction

The 32% reduction in infarct size in this study was slightly less than that observed in studies of the second window of protection in anesthetized rabbits. Following the same experimental protocol (four 5 min-coronary artery occlusions 24 h before a 30-min coronary occlusion), reductions in I/R of 45% [5], 39% [14] and 35% [15] have been reported in the anesthetized rabbit. In an anesthetized canine model, Kuzuya et al. [6] reported a 49% reduction of the infarct/risk zone ratio 24 h after preconditioning. It is noteworthy that the variance observed in the present study was markedly lower than that seen in previous studies and may be a reflection of better control of physiological parameters such as myocardial temperature during ischemia [16], myocardial contractility, heart rate and arterial pressure. It is equally important to note that the appearance of VF in 3 of the control animals did not by itself cause the largest infarcts. Although average infarct size in these 3 rabbits was modestly greater than that in the other 4 animals, the difference was not statistically significant. This is not surprising since the arrhythmia was converted to a sinus mechanism in less than 10 s and in previous investigations in which brief VF was successfully terminated, arterial pressure monitoring demonstrated that the period of hypotension following defibrillation was always less than 10 s (the time required to re-attach and flush the pressure gauge). In these prior two studies infarct size was also not consistently greater in animals developing VF. Furthermore, if the 3 animals requiring defibrillation are eliminated from the control group, the difference in magnitude of infarction between the remaining control rabbits and those having been preconditioned continues to be highly significant (P < 0.01).

It is also obvious from Fig. 1 that the protection realized when ischemic preconditioning had occurred 24 h prior to the prolonged coronary occlusion was not as marked as that observed with classical preconditioning in which the brief ischemic episode immediately preceded the longer coronary occlusion. Receptor-mediated protection, whether induced by endogenous adenosine released during brief ischemia or administration of exogenous agonist coupling to protein kinase C [17], reproducibly reduces infarct size from 35–45% of the risk zone to 10–15%. In this context of immediate protection the effects of cytoprotective proteins, of which production by the stressed myocyte is delayed for several hours, are obviously minor and
suggest that the early and late phases of protection have different mechanisms and end-effectors. Nonetheless, one must be impressed with the ability of the myocyte to adapt and initiate multiple reactions serving to attenuate the stress for at least several days.

Not all investigators have observed a second window of protection. Tanaka et al. [18] studied rabbits anesthetized with sodium pentobarbital and ventilated with air supplemented with oxygen. After a left thoracotomy preconditioning was effected by four 5-min occlusions of an anterolateral branch of the circumflex artery. Animals were then allowed to recover for 24 or 48 h. Sustained ischemia was again induced for 30 min followed by 180 min reperfusion and infarct size assessment with TTC. Although these workers showed that this preconditioning protocol induced early class protection (72% reduction in infarct size), no protection was observed 24 or 48 h later. Despite the lack of protection in the preconditioned hearts an increase in hsp70i immunoreactivity documented by a histochemical technique was observed at the late time-points. Reasons for this absence of protection and difference from the observations of Marber et al. [5] are not immediately clear. There were several differences in experimental technique, including anesthetic used during the preconditioning procedure (sodium pentobarbital vs. fentanyl/fluanisone/diazepam) and ventilation gas (air supplemented with oxygen vs. 100% oxygen). We do not know which, if any, accounts for the contradictory findings.

4.2. VF reduction

Recently, Vegh et al. [19] described delayed protection against ischemia-induced ventricular arrhythmias in the canine heart “preconditioned” with four 5-min periods of rapid ventricular pacing 20 h before a 25-min coronary occlusion. Prior pacing reduced the occurrence of VF during coronary occlusion from 45% in control dogs to 10% in pre-paced dogs. Reperfusion-induced VF was also attenuated by pre-pacing (100% incidence in controls vs. 40% in pre-paced dogs). Whether the rapid pacing protocol employed by these workers (70 beats/min above resting heart rate) is severe enough to induce demand ischemia and hence ischemic preconditioning stricto sensu is not clear.

In contrast to the anesthetized dog, coronary occlusion in the anesthetized rabbit causes a relatively low incidence of VF, although arrhythmias such as isolated ventricular premature beats, couplets and salvos are common. Retrospective analysis of experimental records from two published studies of the second window of protection in pentobarbital-anesthetized rabbits [14,15] has shown that VF occurred during the 30-min coronary occlusion in 2/20 control animals and in 2/21 animals preconditioned 24 h earlier (unpublished data). Because there is a much higher incidence of VF during ischemia in conscious rabbits (43%) as demonstrated in the present study than in pentobarbital-anesthetized rabbits (10%), the former model is well suited for evaluating a treatment’s antiarrhythmic effect. Although the primary end-point in this study was infarct size, the difference in incidence of potentially fatal arrhythmias between the control and preconditioned groups was noteworthy. The difference is not statistically significant (P < 0.1), but the trend is intriguing. A similar, but significant antiarrhythmic effect was observed with classical preconditioning in chronically instrumented, conscious rabbits in which only 10 min–2 h of reperfusion separated the 5- and 30-min occlusions [3,13]. If the data from the latter two reports are pooled, 10 of 16 control rabbits developed either ventricular fibrillation, non-sustained ventricular tachycardia, or prolonged periods of ventricular bigeminy, in contrast to only 2 of 21 of the ischemically preconditioned groups (P < 0.001). The higher incidence of occlusion-induced VF in the conscious rabbit compared with the pentobarbital-anesthetized rabbit would suggest an arrhythmia-inhibiting effect of the anesthetic agent in this species.

The mechanism of the possible antiarrhythmic effect which accompanies the second window is unknown. Preconditioning could involve the induction of nitric oxide synthase (iNOS) since the antiarrhythmic protection observed by Vegh et al. [19] could be abolished by pretreatment with dexamethasone. However, the effects of repetitive brief coronary artery occlusions on iNOS activity in myocardium have not, to our knowledge, been reported. A similar antiarrhythmic effect was observed by Steare and Yellon [20] in the rat 24 h after transient whole-body hyperthermia. They observed a reduction in susceptibility to reperfusion-induced VT and VF 24 h after heat shock, a procedure that, like repetitive brief periods of ischemia, is associated with increases in myocardial hsp70i content. It is possible that the late antiarrhythmic activity of both ischemic preconditioning and heat shock is a manifestation of induced hsp70i or perhaps some other protective protein. Whether the mechanism involves a direct antiarrhythmic effect or whether the reduction of arrhythmias is secondary to a generalized anti-ischemic effect on the myocardium is not known.

4.3. Hsp70i expression

In addition to its induction by hyperthermia [5,8,9] hsp70i is known to be induced in myocardium by several other stimuli including ischemia [5,21,22] and is a marker of a generalized stress response in tissues. There is now some evidence that the protein is directly cytoprotective and is able to confer resistance to myocardial ischemia. Isolated myocytes and myocyte-derived cells transfected with the gene coding for hsp70i have enhanced tolerance to hypoxic injury [23,24]. Hearts from transgenic mice which constitutively over-express the hsp70i gene are also more resistant to ischemia-reperfusion injury [25–27]. The approximately two-fold increase in hsp70i in the at-risk
myocardium from preconditioned hearts compared with sham-operated hearts in the present study is similar to that seen in rabbits preconditioned under anesthesia [5]. In the latter investigation myocardial heat shock protein content was increased from 1.0 ± 0.3 to 2.5 ± 0.2 units 24 h after four 5-min episodes of coronary ligation (P < 0.01). The increase in the present study represented a strong trend, but was not quite statistically significant (P < 0.1).

5. Conclusion

The second window of protection has been demonstrated in a conscious animal model free of the immediate effects of surgery and anesthesia. Therefore, this is a biologically significant phenomenon, and not a model-dependent artifact. The association between the delayed protection and rise in intracellular content of cytoprotective proteins is both intriguing and suggestive, but a direct etiologic link cannot be established by this study. Further investigation is required to achieve a better understanding of the mechanism and possibly fulfill the hope of a clinical application.

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References


