Myocardium tolerant to an adenosine-dependent ischemic preconditioning stimulus can still be protected by stimuli that employ alternative signaling pathways

David A. Liem,1 Maaike te Lintel Hekkert,1 Olivier C. Manintveld,1 Frans Boomsma,2 Pieter D. Verdouw,1 and Dirk J. Duncker1

1Experimental Cardiology, Thoraxcenter, and 2Internal Medicine, Cardiovascular Research
Institute COEUR, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

Submitted 31 August 2004; accepted in final form 11 October 2004

Liem, David A., Maaike te Lintel Hekkert, Olivier C. Manintveld, Frans Boomsma, Pieter D. Verdouw, and Dirk J. Duncker.

Myocardium tolerant to an adenosine-dependent ischemic preconditioning stimulus can still be protected by stimuli that employ alternative signaling pathways. Am J Physiol Heart Circ Physiol 288: H1165–H1172, 2005. First published October 14, 2004; doi:10.1152/ajpheart.00899.2004.—Clinical studies on cardioprotection by preconditioning stimulus can still be protected by stimuli that employ alternative signaling pathways. Anesthetized rats underwent classical, remote or pharmacological preconditioning. Infarct size (IS), produced by a 60-min coronary artery occlusion (CAO), was determined after 120 min of reperfusion. Preconditioning by two 15-min periods of CAO (2CAO15, an adenosine-dependent stimulus) limited IS from 69 ± 2% to 37 ± 6%, but when 2CAO15 was preceded by 4CAO15, protection by 2CAO15 was absent (IS = 68 ± 1%). This development of tolerance coincided with a loss of cardiac interstitial adenosine release, whereas two 15-min infusions of adenosine (200 μg/min iv) still elicited cardioprotection (IS = 40 ± 4%). Furthermore, cardioprotection was produced when 4CAO15 was followed by the adenosine-independent stimulus 3CAO3 (IS = 50 ± 8%) or the remote preconditioning stimulus of two 15-min periods of mesenteric artery occlusion (IS = 49 ± 6%). In conclusion, development of tolerance to cardioprotection by an adenosine-dependent preconditioning stimulus still allows protection by pharmacological or ischemic stimuli intervention employing different signaling pathways.

infarct size; remote preconditioning

ISCHEMIC PRECONDITIONING (IPC) is the most powerful means of endogenous cardioprotection against irreversible cell injury in the experimental animal (26, 31). However, clinical studies on infarct size (IS) limitation by brief anginal episodes preceding acute myocardial infarction are ambiguous (3, 4, 16, 27, 28, 44); such ambiguity has been attributed to a loss of cardioprotection by ischemic preconditioning in the aging (1, 2, 3, 19) or pathological (9, 12, 15, 18) heart. Another confounding factor could be development of tolerance to IPC, i.e., the loss of cardioprotection when the same preconditioning stimulus is repetitively applied (6, 14, 32). For example, Cohen et al. (6) demonstrated that in rabbits the cardioprotection produced by a single 5-min coronary artery occlusion (CAO) followed by 10 min of reperfusion (1CAO5) was lost when the 5-min CAO stimulus was applied at 30-min intervals for 8 h during 3 days.

In recent years, it has become apparent that not all preconditioning stimuli employ the same signaling pathway to exert their cardioprotective action (7, 10, 23, 24, 34). For instance, in the rat, cardioprotection by a single 15-min CAO followed by 10 min of reperfusion (1CAO15) is adenosine dependent but does not involve reactive oxygen species (ROS), whereas cardioprotection by three cycles of 3 min of CAO interspersed by 5 min of reperfusion (3CAO3) depends on ROS (24) but does not involve adenosine (22, 23). The major aim of the present study was therefore to investigate whether tolerance that develops when the same IPC stimulus is applied repetitively also implies tolerance to a stimulus that employs a different signal transduction pathway. Hence, in the first part of the study, we investigated whether tolerance to a particular (adenosine-dependent) preconditioning stimulus also affects cardioprotection by a stimulus that employs an alternative (adenosine-independent) pathway. Myocardium can be preconditioned by local myocardial ischemia, as well as by brief ischemia in noncardiac tissue such as the small intestine, kidneys, and skeletal muscle (5, 11, 25, 30), which, at least for the small intestine, involves a neurogenic pathway (11, 25).

Hence, in the second part of the study, we investigated whether cardioprotection by remote preconditioning via a 15-min mesenteric artery occlusion (MAO15) is affected by the development of tolerance to a classical IPC stimulus.

Because tolerance to IPC has not been investigated in the rat, we first established a model for the development of tolerance on the basis of our experience with the adenosine-dependent stimulus 1CAO15 in this species. Capitalizing on the observations by Vogt et al. (41), who showed in pigs that progressive loss of adenosine production rendered myocardium tolerant to protection by 10-min CAO but still responsive to exogenous adenosine, we also investigated whether loss of adenosine release also contributes to development of tolerance in the rat heart and whether exogenous adenosine still induces protection once tolerance has developed.
METHODS

Animals

Experiments were performed in ad libitum-fed male Wistar rats (300–380 g) in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 86-23, revised 1996) and with approval of the Erasmus University Rotterdam Animal Care Committee.

Surgical and Experimental Procedures

Pentobarbital sodium-anesthetized (60 mg/kg ip) rats were intubated for positive-pressure ventilation with oxygen-enriched room air. Through the carotid artery, a PE-50 catheter was positioned in the thoracic aorta for measurement of arterial blood pressure and heart rate (11, 40). In the inferior caval vein, a PE-50 catheter was placed for infusion of Haemaccel (Hoechst) to compensate for blood loss during surgery and to maintain central venous pressure during the experimental protocol and for drug infusion during the experiments. After thoracotomy, via the left third intercostal space, the pericardium was opened, and a silk 6-0 suture was looped under the left coronary artery for later CAO. A catheter was positioned in the abdominal cavity to allow intraperitoneal administration of pentobarbital sodium for maintenance of anesthesia. Rectal temperature was continuously measured and maintained at 36.5–37.5°C (11, 40). After completion of surgery, a 30-min stabilization period was allowed before experimental protocols were carried out. Rats that fibrillated were allowed to complete the protocol, provided that conversion to normal sinus rhythm occurred spontaneously within 1 min or that defibrillation via gentle thumping on the thorax was successful within 2 min after onset of fibrillation. Occlusion and reperfusion were visually verified. In 13 additional rats, a microdialysis probe (model CMA/20, Carnegie Medicine, Stockholm, Sweden; 4 × 0.5 mm membrane, 20-kDa cutoff) was implanted into the myocardial area at risk (AR) to determine myocardial interstitial adenosine levels (17). Samples were collected during each 15-min CAO at a rate of 2 µl/min. At the conclusion of each experiment, probe recovery was determined ex vivo with a stock solution containing 100 µM adenosine and found to be 14 ± 1% (percentage of adenosine concentration in the stock solution recovered in the probe samples). All samples were stored at −50°C for later analysis. The adenosine concentrations in dialysate samples were determined by reverse-phase high-performance liquid chromatography (36).

IS Analysis

IS was determined as previously described (11, 40). Briefly, after 120 min of reperfusion, the left coronary artery was reocluded, and 10 ml of trypan blue (0.4%; Sigma Chemical) were immediately infused intravenously into the femoral vein to stain the normally perfused myocardium dark blue and delineate the nonstained AR. Subsequently, hearts were excised, rinsed in cold saline, and cut into 2-mm-thick slices from apex to base. From each slice, the right ventricle was removed and the left ventricular AR (nonstained) was dissected from the remaining left ventricular tissue. The AR was then incubated for 10 min in 37°C nitro blue tetrazolium (Sigma Chemical; 1 mg/ml Sorensen buffer, pH 7.4), which stains viable tissue purple but leaves infarcted tissue unstained. After the infarcted area (IA) was isolated from the non-IA, the different areas of the left ventricle were dried and weighed separately. Myocardial IS was computed as IA expressed as a percentage of AR (11, 40).

Experimental Design

Rat hearts were preconditioned with one or multiple 15-min CAOs separated by 15 min of reperfusion (nCAO15, adenosine-dependent IPC stimuli), a sequence of three 3-min CAOs interspersed by 5 min of reperfusion (3CAO3, adenosine-independent stimulus), or two 15-min mesenteric artery occlusions (MAOs) separated by 15 min of reperfusion (2MAO15, remote myocardial preconditioning stimulus). Pharmacological cardioprotection was produced by multiple 15-min infusions of adenosine (200 µg/min iv) separated by 15 min of washout (nADO15). Myocardial infarcts were produced by a 60-min CAO (index ischemia), and IS was determined after 120 min of reperfusion (35).

Pilot experiments to develop a model for tolerance to classical IPC by an adenosine-dependent IPC stimulus. Because there were no previous studies on tolerance to IPC in the rat heart, we first established (1) the number and timing of CAO15 required to elicit tolerance to IPC (Fig. 1). On the basis of these experiments (see RESULTS), 4CAO15 interspersed by 15 min of reperfusion and applied between 175 and 70 min before the 60-min index ischemia was selected to induce tolerance, whereas 2CAO15 separated by 15 min of reperfusion was used as the preconditioning stimulus.

Adenosine and development of tolerance to preconditioning. We first established whether the cardioprotection by 2CAO15, similar to 1CAO15 (23, 24), depends on adenosine receptor activation, but not on ROS generation (Fig. 2A). For this purpose, we used the adenosine receptor antagonist 8-sulfophenyl theophylline (8-SPT, 50 mg/kg iv) (23) and the ROS scavenger mercaptopropionylglycine (MPG, 1 mg/kg i.p. iv) (24). Subsequently, we investigated whether loss of adenosine signaling could have contributed to development of tolerance to 2CAO15 (Fig. 2B). For this purpose, we measured interstitial adenosine levels during 4CAO15 and determined whether an exogenous adenosine infusion indeed reinstates protection in myocardium that has become tolerant to 4CAO15 by replacing the 2CAO15 by two episodes of ADO15 (4CAO15 + 2ADO15). Finally, we subjected rats to one (1ADO15) or six (6ADO15) episodes of 15-min intravenous infusion of 200 µg/min adenosine (Fig. 2C) to determine whether repeated administration of exogenous adenosine leads to tolerance to its cardioprotection (8, 13, 39).

Cross tolerance between adenosine-dependent and other IPC stimuli. To investigate whether the cardioprotection by the adenosine-independent preconditioning stimulus 3CAO3 or remote preconditioning is also lost after myocardium has become tolerant to the adenosine-dependent stimulus (2CAO15), we replaced the 2CAO15 by the adenosine-independent classical stimulus 3CAO3 (4CAO15 + 3CAO3; Fig. 3A) or by remote preconditioning with two episodes of MAO15 (4CAO15 + 2MAO15; Fig. 3B).

Data Analysis and Presentation

IS was analyzed by one-way ANOVA followed by Student-Newman-Keuls test. Hemodynamic variables were compared by two-way ANOVA for repeated measures followed by Dunnett’s test. Statistical significance was accepted when P < 0.05. Values are means ± SE.

RESULTS

Mortality and Exclusions

Of the 190 rats that entered the study, 21 were excluded because of sustained ventricular fibrillation during CAO or pump failure (≤3 rats per group) and 6 were excluded because of an AR <10% of the left ventricle.

Heart Rate and Arterial Blood Pressure

Table 1 shows the hemodynamic data for the various experimental groups. Importantly, there was no correlation between the rate-pressure product at the onset of the 60-min CAO and IS (r² = 0.003, P = 0.55).

Area at Risk

There were no intergroup differences in AR (34 ± 1% of left ventricle, P = 0.09) between the experimental groups.
Development of the Model for Tolerance to Classical IPC

Figure 1A shows that the protection by 1CAO15 was not affected when the reperfusion period between 1CAO15 and the 60-min index ischemia period was extended from 10 to 90 min but was lost when the index ischemia period was further extended to 175 min. The protection by 1CAO15, when applied 70 min before the 60-min CAO, was abolished, however, when this stimulus was preceded by three additional episodes of CAO15 (4CAO15/70-min Rep; Fig. 1B). Figure 1 also illustrates that 2CAO15 tended to be slightly more protective than 1CAO15 (IS = 37 ± 6%) and that the protection was abolished when preceded by the tolerance-inducing 4CAO15. This loss of protection by 2CAO15 was not due to cumulative necrosis induced by the preceding 4CAO15 (10 ± 4%), because the combined IS of 4CAO15 (10 ± 4%) and 2CAO15 + 60-min CAO (37 ± 6%) amounted to 47 ± 7%, which was still significantly less than the IS produced by the 60-min CAO alone (69 ± 2%; Fig. 1A) and the IS produced by 6CAO15 followed by the 60-min CAO (68 ± 1%; Fig. 1B).

Adenosine and Development of Tolerance

The protection by 2CAO15 was virtually abolished by 8-SPT but not by MPG (Fig. 2A), demonstrating the critical role of adenosine in mediating the cardioprotection by 2CAO15 and its independence of ROS generation.

During the first CAO15, the average interstitial adenosine concentrations increased sevenfold; during the second, third, and fourth CAO15, however, the adenosine concentrations were no longer different from baseline (Fig. 2B). When 4CAO15 was followed by 2ADO15, IS produced by the 60-min CAO was limited to 40 ± 4%. Because 10 ± 4% of the AR had already become necrotic after 4CAO15, the additional irreversible damage produced by the 60-min CAO was (40 ± 4%) (10 ± 4%), which equals 30 ± 5% of the AR. If we take into account that only 90 ± 4% [100% - (10 ± 4%)] of the AR was viable at the onset of the 60-min CAO, the percentage of the AR that became infarcted during the 60-min CAO amounted to 33 ± 6% of the viable AR [(30 ± 5%)/(90 ± 4%)]. This degree of protection was not different from the IS limitation by 2ADO15 alone (IS = 35 ± 8%; Fig. 2B). These findings indicate that exogenous adenosine still produces cardioprotection at a time that the myocardium has become tolerant to protection by the adenosine-dependent stimulus 2CAO15.

Although the cardioprotection by exogenous adenosine was unperturbed in hearts tolerant to cardioprotection by 2CAO15, IS limitation by 6ADO15 was less than that by 1ADO15 (Fig. 2C), indicating that repeated adenosine infusions caused a blunting of its cardioprotective actions.
Cross Tolerance Between Adenosine-Dependent and -Independent Classical IPC Stimuli

When 4CAO15 preceded the adenosine-independent (22, 23) but ROS-dependent (24) 3CAO3 stimulus, IS limitation was still present, although it was less (IS = 50 ± 8%, P < 0.05 vs. control) than the protection by 3CAO3 alone (IS = 29 ± 5%; Fig. 3A). Taking into account that IS was 10 ± 4% after 4CAO15 alone, we calculated (see above) that 44% of the AR that was viable at the onset of 3CAO3 became infarcted (\( P < 0.05 \) vs. 3CAO3 alone). These findings indicate that myocardium that has become tolerant to protection by an adenosine-dependent IPC stimulus can still be protected by a classical adenosine-independent IPC stimulus.

Cross Tolerance Between Classical IPC and Remote IPC Stimuli

Remote myocardial preconditioning by 2MAO15 limited IS to 49 ± 6% vs. 69 ± 2% in control rats (\( P < 0.05 \); Fig. 3B). When 4CAO15 preceded 2MAO15 (4CAO15 + 2MAO15), IS was limited to 49 ± 6% (43 ± 7% of AR that was viable at the onset of 2MAO15), which was not different from the cardioprotection by 2MAO15 alone (IS = 49 ± 6%).

DISCUSSION

The present study was undertaken to assess whether the development of tolerance to a particular IPC stimulus also affects the cardioprotection by stimuli that employ different mechanisms. The major findings can be summarized as follows: 1) IPC by 2CAO15 resulted in potent cardioprotection against subsequent 60-min index ischemia in an adenosine-dependent manner. However, when 4CAO15 preceded 2CAO15, the myocardium had become tolerant to the protection by 2CAO15. 2) Development of tolerance coincided with loss of myocardial interstitial adenosine release. Although repeated infusion of adenosine was capable of producing tolerance as well, the loss of adenosine release, in conjunction with the finding that exogenous adenosine still afforded protection after 4CAO15, is consistent with previous observations in pigs (41) that loss of adenosine release contributes to the development of tolerance. 3) Myocardium that had become...
tolerant to 2CAO15 could still be protected by the adenosine-independent 3CAO3 stimulus and by the remote preconditioning stimulus 2MAO15.

**Development of Tolerance to Preconditioning in Rat Heart**

Cohen et al. (6) demonstrated in conscious rabbits that 5-min CAOs at 30-min intervals for 8 h during 3 days resulted in tolerance. Iliodromitis et al. (14) showed in anesthetized rabbits that myocardial tolerance already started to develop after four cycles of 5 min of CAO and 10 min of reperfusion. The present study shows that, also in the rat heart, tolerance develops after a limited number of brief CAOs. Because in our study we used 15-min, rather than 5-min, CAOs, it could be argued that the loss of protection by 2CAO15 after 4CAO15 was caused by cumulative necrosis. This is, however, highly unlikely inasmuch as the combined IS of 4CAO15 (10 ± 4%) and 2CAO15 followed by 60 min of CAO (37 ± 6%) was significantly less (47 ± 7%) than IS after 6CAO15 followed by 60 min of CAO (68 ± 1%). Moreover, the cardioprotection by 1CAO15 + 70 min of reperfusion (IS = 45 ± 8%) was prevented when 1CAO15 was preceded by three additional episodes of CAO15 (4CAO15 + 70 min of reperfusion, IS = 69 ± 2%). Nor can this loss of protection be explained by cumulative necrosis of 3CAO15 (which, in view of the IS by 4CAO15, must have been smaller than 10 ± 4%), and the 1CAO15 + 70 min of reperfusion + 60 min of CAO (IS = 45 ± 8%), which was still significantly less than <69 ± 2% in the group subjected to 4CAO15 + 70 min of reperfusion + 60 min of CAO.

We established that, similar to 1CAO15 (23), cardioprotection by 2CAO15 involves activation of adenosine receptors, whereas ROS do not play a role in 1CAO15 (24) or 2CAO15 (present study). In view of the similarly prominent role of endogenous adenosine in cardioprotection by 1CAO5 in rabbits (31) and 1CAO10 in swine (34), a reduced adenosine receptor responsiveness (13, 39) and a progressive loss of adenosine production during repeated occlusions (14, 41) have been proposed as mechanisms underlying the development of tolerance. Although we found that repeated adenosine infusions are capable of blunting adenosine’s cardioprotection, the observation that during the second, third, and fourth CAO15, the myocardial interstitial levels of adenosine were no longer different from baseline is consistent with the hypothesis that a progressive loss of myocardial adenosine release contributes to the development of tolerance (41). Moreover, similar to the findings of Vogt et al. (41), we observed that intravenous infusion of adenosine could reinstate cardioprotection, suggesting that cardiac responsiveness to adenosine was maintained after 4CAO15.

**Cross Tolerance to Other IPC Stimuli**

The primary aim of the present study was to investigate whether jeopardized myocardium that has become tolerant to a particular preconditioning stimulus can still be rescued by an ischemic stimulus that operates via a different mechanism. Cross tolerance to remote preconditioning of the heart did not occur, inasmuch as the cardioprotection by brief intestinal ischemia, such as 2MAO15, was not affected when this stimulus was preceded by 4CAO15. We previously showed that MAO15 elicits cardioprotection via activation of a neurogenic pathway during early reperfusion of the mesenteric bed (11). We obtained evidence that adenosine receptor activation downstream of the neurogenic pathway, possibly in the myocardium (25), contributes to remote IPC by 1MAO15. However, in two additional rats, we did not observe an increase in myocardial intestinal adenosine levels during (1.6 ± 0.2 µM) or after (1.5 ± 0.1 µM) MAO15 compared with “baseline” adenosine levels measured after the preceding 4CAO15 (2.2 ± 0.9 µM). It must be emphasized that the myocardial adenosine concentrations represent the average concentrations of the 15-min microdialysis sampling period. Therefore, we cannot exclude the possibility that a brief transient increase in myocardial adenosine concentration during early mesenteric artery reperfusion was masked. Alternatively, other mediators of remote preconditioning, including bradykinin (33), calcitonin-gene related peptide (37), and opioids (29, 42), may also have contributed to the cardioprotection by remote IPC of hearts that have become tolerant. Studies are needed to further investigate the role of these other mediators in the cardioprotection by remote IPC of myocardium made tolerant by 4CAO15 to the cardioprotection by 2CAO15.

Tolerance by 4CAO15 also did not abolish the cardioprotection by 3CAO3. If one assumes that the 15-min ischemia episode encompasses the signaling cascade triggered by the
Table 1. HR and MAP

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>n</th>
<th>Baseline</th>
<th>Before</th>
<th>End</th>
<th>15 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Control</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td>367±9</td>
<td>376±15</td>
<td>392±16</td>
<td>398±17</td>
<td>425±12*</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td>92±4</td>
<td>94±5</td>
<td>91±5</td>
<td>84±5</td>
<td>75±5*</td>
</tr>
<tr>
<td>2) 1CAO15</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td>357±10</td>
<td>366±9</td>
<td>377±11</td>
<td>365±10</td>
<td>408±14†</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td>98±3</td>
<td>95±3</td>
<td>91±4</td>
<td>79±6*</td>
<td>70±5*†</td>
</tr>
<tr>
<td>3) 1CAO15 + 90 min Rep</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td>382±15</td>
<td>416±14</td>
<td>400±22</td>
<td>395±63</td>
<td>365±29</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td>92±1</td>
<td>91±6</td>
<td>89±7</td>
<td>74±14</td>
<td>65±17</td>
</tr>
<tr>
<td>4) 1CAO15 + 175 min Rep</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td>385±11</td>
<td>453±14</td>
<td>463±10</td>
<td>460±4</td>
<td>397±38</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td>95±1</td>
<td>91±6</td>
<td>85±4</td>
<td>79±6</td>
<td>62±15</td>
</tr>
<tr>
<td>5) 1CAO15 + 70 min Rep</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td>357±9</td>
<td>377±14</td>
<td>386±22</td>
<td>382±26</td>
<td>401±13</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td>91±5</td>
<td>96±5</td>
<td>87±5</td>
<td>82±6</td>
<td>75±8</td>
</tr>
<tr>
<td>6) 4CAO15 + 70 min Rep</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td>363±11</td>
<td>408±7*</td>
<td>402±10*</td>
<td>406±10*</td>
<td>397±9</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td>96±4</td>
<td>82±6</td>
<td>79±6</td>
<td>78±5</td>
<td>68±5*</td>
</tr>
<tr>
<td>7) 2CAO15 + 10 min Rep</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td>352±8</td>
<td>369±6</td>
<td>368±7</td>
<td>374±8</td>
<td>409±7†</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td>95±4</td>
<td>95±4</td>
<td>96±5</td>
<td>93±4</td>
<td>89±5</td>
</tr>
<tr>
<td>8) 6CAO15 + 10 min Rep</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td>384±21</td>
<td>415±18</td>
<td>409±21</td>
<td>408±22</td>
<td>393±26</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td>103±3</td>
<td>84±3</td>
<td>74±8*</td>
<td>72±6*</td>
<td>64±9*</td>
</tr>
<tr>
<td>9) 4CAO15</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td>329±9</td>
<td>340±6</td>
<td>361±9</td>
<td>367±17</td>
<td>397±24</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td>95±5</td>
<td>104±8</td>
<td>101±11</td>
<td>90±10</td>
<td>83±9</td>
</tr>
<tr>
<td>10) 8-SPT + control</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td>349±5</td>
<td>340±6</td>
<td>361±9</td>
<td>367±17</td>
<td>397±24</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td>104±8</td>
<td>103±11</td>
<td>89±10</td>
<td>90±10</td>
<td>83±9</td>
</tr>
<tr>
<td>11) 8-SPT + 2CAO15 + 10 min Rep</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td>345±18</td>
<td>355±17</td>
<td>359±14</td>
<td>347±11</td>
<td>364±21</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td>98±4</td>
<td>96±3</td>
<td>80±3†</td>
<td>79±3†</td>
<td>82±5†</td>
</tr>
<tr>
<td>12) MPG + control</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td>360±17</td>
<td>337±13</td>
<td>351±13</td>
<td>365±9</td>
<td>375±9</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td>105±13</td>
<td>73±2</td>
<td>79±10</td>
<td>76±9</td>
<td>65±9*</td>
</tr>
<tr>
<td>13) MPG + 2CAO15 + 10 min Rep</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td>334±12</td>
<td>332±16</td>
<td>340±4</td>
<td>350±5</td>
<td>382±9†</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td>88±11</td>
<td>53±4*</td>
<td>62±3*</td>
<td>66±6</td>
<td>72±4</td>
</tr>
<tr>
<td>15) 2ADO15</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td>360±7</td>
<td>376±8</td>
<td>389±7</td>
<td>391±7</td>
<td>401±11*</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td>100±4</td>
<td>109±6</td>
<td>95±8</td>
<td>95±8</td>
<td>88±8</td>
</tr>
<tr>
<td>16) 4CAO15 + 2ADO15</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td>360±6</td>
<td>412±15*</td>
<td>422±10*</td>
<td>413±5</td>
<td>361±35</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td>98±5</td>
<td>86±8</td>
<td>83±6</td>
<td>78±6</td>
<td>72±8</td>
</tr>
<tr>
<td>17) 1ADO15</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td>357±5</td>
<td>378±16</td>
<td>384±20</td>
<td>384±22</td>
<td>401±23</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td>112±5</td>
<td>128±3*</td>
<td>111±4†</td>
<td>108±4†</td>
<td>107±3†</td>
</tr>
<tr>
<td>18) 6ADO15</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td>344±14</td>
<td>385±10</td>
<td>393±10*</td>
<td>389±5</td>
<td>369±6</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td>100±7</td>
<td>103±4</td>
<td>76±6†</td>
<td>81±16</td>
<td>70±7†</td>
</tr>
<tr>
<td>19) 3CAO3</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td>355±6</td>
<td>368±8</td>
<td>363±17</td>
<td>370±17</td>
<td>380±18</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td>93±4</td>
<td>89±5</td>
<td>78±7</td>
<td>94±7</td>
<td>77±5</td>
</tr>
<tr>
<td>20) 4CAO15 + 3CAO3</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td>341±12</td>
<td>379±10*</td>
<td>382±9*</td>
<td>380±10*</td>
<td>373±11</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td>94±6</td>
<td>99±6</td>
<td>89±6</td>
<td>90±6</td>
<td>80±8</td>
</tr>
<tr>
<td>21) 2MAO15</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td>364±15</td>
<td>383±15</td>
<td>365±17</td>
<td>385±18</td>
<td>355±33</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td>87±5</td>
<td>97±8</td>
<td>91±5</td>
<td>86±5</td>
<td>86±4</td>
</tr>
<tr>
<td>22) 4CAO15 + 2MAO15</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td>350±13</td>
<td>355±13</td>
<td>363±18</td>
<td>350±9</td>
<td>339±35</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td>96±6</td>
<td>85±7</td>
<td>79±8</td>
<td>78±6</td>
<td>76±7</td>
</tr>
</tbody>
</table>

Values are means ± SE. n CAO15, n episodes of 15 min of coronary artery occlusion (CAO); n AD015, n episodes of 15 min of adenosine infusion; n MAO15, n episodes of mesenteric artery occlusion; Rep, reperfusion; HR, heart rate; MAP, mean aortic pressure. *P < 0.05 vs baseline; †P < 0.05 vs. before CAO.
3-min episode, one would expect a complete loss of cardio-
protection by 3CAO3 in myocardium that had become tolerant to
CAO15. However, we and others showed that, in contrast to its
involvement in CAO15 (23), adenosine is not involved in the
cardioprotection by 3CAO3 (22, 23). Conversely, we showed that the ROS scavenger MPG attenuated the protection by
3CAO3 (24) but left the protection by CAO15 unaffected.
Hence, the partial loss of protection by 3CAO3 in myocardium
tolerant to CAO15 is difficult to explain. Future studies, in-
volving other triggers and mediators, are required to determine
the molecular basis for this partial cross tolerance to other
classical preconditioning stimuli. Nonetheless, our data suggest
that myocardium that has become tolerant to the protection by
a stimulus employing a particular signal transduction pathway
might still benefit from an IPC stimulus employing a different
signal transduction pathway.

Clinical Relevance

Abundant evidence has been presented that IPC also occurs
in humans with use of end points other than IS (20, 21, 38, 43).
However, clinical studies on IS limitation by preinfarct angina
are discordant (4, 16, 27, 28, 44). This has, at least in part, been
ascribed to loss of preconditioning in the aging (1, 2, 3, 19) and
pathological (9, 12, 15, 18) hearts. We hypothesized that
development of tolerance might also contribute to the equiv-
cal findings, inasmuch as multiple brief episodes of
abrupt ischemia in the hours to days preceding a myocardial
infarction render animal hearts tolerant to the cardioprotective
effects of preconditioning. However, rather than the repetitive
bouts of brief ischemia of identical duration and severity that
occur in the laboratory setting, patients are more likely to
experience episodes of varying severity and duration of ische-
mia. The present study suggests that these patients could be
less susceptible to the development of tolerance as a result of
recruitment of different signal transduction pathways by dis-
tinct stimuli. Our study also indicates that, without a detailed
knowledge of the number, severity, and duration of the prein-
farct episodes of myocardial and/or remote organ ischemia, it
is impossible to classify patients as preconditioned or tolerant.
Finally, the observation that administration of exogenous ade-
osine is still protective in hearts that have become tolerant to
IPC suggests that, in patients with unstable angina, adminis-
tration of pharmacological agents that mimic preconditioning
can still afford cardioprotection, at least in the (sub)acute
setting (8, 13, 39).

ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical assistance of Liz Keijzer.

GRANTS

The present study was supported by The Nethelands Heart Foundation
Grant NHS 99.143. D. J. Duncker is the recipient of an Netherlands Heart
Foundation Established Investigator Stipend 2000T038.

REFERENCES

Longobardi G, Napoli C, and Rengo F. Exercise training restores
ischemic preconditioning in the aging heart. J Am Coll Cardiol 36:
2. Azzari FA, Guzman LA, Cura F, Padilla LT Jr, Trivi M, De Lima AA,
Bertolasi C, and Belardi JA. Lack of preconditioning with recurrent
acute ischemic insults: an aging-related phenomenon (Abstract)? J Am
3. Bartling B, Friedrich I, Silber RE, and Simm A. Ischemic precondition-
ing is not cardioprotective in senescent human myocardium. Ann
4. Behar S, Reicher-Reiss H, Abinader E, Agmon J, Friedman Y, Bar-
B, Reisin L, Schlesinger Z, Zahavi I, Zion M, and Golbou U. The
prognostic significance of angina pectoris preceding the occurrence of a
first acute myocardial infarction in 4,166 consecutive hospitalized patients.
5. Birnbaum Y, Hale SL, and Klomer RA. Ischemic preconditioning at a
distance: reduction of myocardial infarct size by partial reduction of blood
supply combined with rapid stimulation of the gastrocnemius muscle in
6. Cohen MV, Yang XM, and Downey JM. Conscious rabbits become
tolerant to multiple episodes of ischemic preconditioning. Circ Res 74:
7. Cohen MV, Yang XM, Liu GS, Heusch G, and Downey JM. Acetyl-
choline, bradykinin, opioids, and phenylephrine, but not adenosine, trigger
preconditioning by generating free radicals and opening mitochondrial
8. Dana A, Baxter GF, Walker JM, and Yellon DM. Prolonging the
delayed phase of myocardial protection: repetitive adenosine A1 receptor
activation maintains rabbit myocardium in a preconditioned state. J Am
9. Ferdinandy P, Szilvassy Z, and Baxter GF. Adaptation to myocardial
stress in disease states: is preconditioning a healthy heart phenomenon? Trends
10. Fryer RM, Schultz JE, Hsu AK, and Gross GJ. Importance of PKC and
tyrosine kinase in single or multiple cycles of preconditioning in rat hearts.
11. Gho BC, Schoemaker RG, van den Doel MA, Duncker DJ, and
Verdouw PD. Myocardial protection by brief ischemia in noncardiac
12. Ghosh S, Standen NB, and Galinianes M. Failure to precondition
13. Hashimi MW, Thornton JD, Downey JM, and Cohen MV. Loss of
myocardial protection from ischemic preconditioning following chronic
exposure to R(-)-(H)-adenosine is related to defect at the adenosine A1 receptor.
14. Ilidromitiuk EK, Kremastinos DT, Katritsis DG, Papadopoulos CC,
and Hearse DJ. Multiple cycles of preconditioning cause loss of protec-
15. Ishihara M, Inoue I, Kawagoe T, Shimatani Y, Kurisu S, Nishioka K,
Kouno Y, Umemura T, Nakamura S, and Sato H. Diabetes mellitus
prevents ischemic preconditioning in patients with a first acute anterior
16. Klomer RA and Jennings RB. Consequences of brief ischemia: stunning,
preconditioning, and their clinical implications. Part 2. Circulation 104:
17. Lameris TW, van Den Meiracker AH, Boomsma F, Alberts G, de
Zeeuw S, Verdouw PD, and Duncker DJ. Consequences of brief ischemia
18. Lee TM and Chou TF. Impairment of myocardial protection in type 2
19. Lee TM, Su SF, Chou TF, Lee YT, and Tsai CH. Loss of precondition-
ing by attenuated activation of myocardial ATP-sensitive potassium
channels in elderly patients undergoing coronary angioplasty. Circulation
20. Leesar MA, Stoddard MF, Dawn B, Jasti VG, Masden R, and Bolli R.
Delayed preconditioning-mimetic action of nitroglycerin in patients
21. Leesar MA, Stoddard MF, Xuan YT, Tang XL, and Bolli R. Nonelec-
trocardiographic evidence that both ischemic preconditioning and adeno-
sine preconditioning exist in humans. J Am Coll Cardiol 42: 437–445,
2003.
22. Li Y and Klomer RA. The cardioprotective effects of ischemic “pre-
conditioning” are not mediated by adenosine receptors in rat hearts.
23. Liem DA, van den Doel MA, de Zeeuw S, Verdouw PD, and Duncker
DJ. Role of adenosine in ischemic preconditioning in rats depends criti-

Downloaded from http://ajpheart.physiology.org/ by 10.220.33.3 on August 12, 2017

AJP-Heart Circ Physiol • VOL 288 • MARCH 2005 • www.ajpheart.org


28. *Nakagawa Y, Ito H, Kitakaze M, Kus spider. This is a comprehensive list of references that likely pertains to the topic of ischemic preconditioning. It includes a variety of studies that explore different aspects of this phenomenon, such as its mechanism, duration, and role in protecting the myocardium. The references cover a range of journals and years, indicating a broad and ongoing interest in this area of research.