Differential effects of postconditioning on myocardial stunning and infarction: a study in conscious dogs and anesthetized rabbits

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IN 2003, Zhao et al. (28) reported for the first time the phenomenon of ischemic postconditioning (PCD) in which a series of repetitive cycles of brief reperfusion and reclosure of the coronary artery applied immediately at the onset of reperfusion after prolonged ischemia reduced infarct size. Among the different mechanisms associated with PCD are a reduction of oxidative injury and neutrophil accumulation (10, 28), a blunted endothelial dysfunction (28), and an attenuation of both apoptotic cell death and mitochondrial calcium accumulation (21). In addition, PCD activates the phosphatidylinositol trisphosphate (PI3K)-Akt pathway and p70S6K (25) as well as endothelial nitric oxide synthase, extracellular signal-regulated kinase (ERK) 1/2 and mitochondrial ATP-dependent K+ (KATP) channels (5, 25, 27). It also inhibits the opening of mitochondrial permeability transition pore (2) and prevents both mitochondria peroxide production and glutathione depletion (17).

The protection afforded by PCD against myocardial infarction has been extended to the occurrence of arrhythmias (7). However, it remains unknown whether PCD is able to reduce another major consequence of myocardial ischemia, i.e., myocardial stunning. Because PCD has been demonstrated to reduce lipid peroxidation and the production of superoxide anions (10, 28) as well as the damage to cardiomyocytes by inhibiting intracellular calcium overload (21), we might expect some protection, since these mechanisms play a crucial role in the pathogenesis of myocardial stunning (3, 9, 13, 14, 16, 20). Therefore, the first goal of this study was to evaluate the consequences of PCD against myocardial stunning in chronically instrumented conscious dogs. Because duration of PCD cycle seems more important than their number (26), three protocols of PCD were tested, i.e., the number of cycles was similar (n = 4) but the duration of each cycle was different (15 s, 30 s, and 1 min). We also repeated these myocardial stunning experiments in a model of myocardial stunning in anesthetized rabbits (devoid of native collaterals) and verified that, in these experimental conditions, PCD also reduces myocardial infarction.

METHODS

The animal instrumentation and the experiments were performed in accordance with the official regulations of the French Ministry of Agriculture.

Chronically instrumented conscious dogs. As previously described (6), dogs (17–22 kg) were subjected to a left thoracotomy. Fluid-filled Tygon catheters were placed in the descending thoracic aorta and the left atrium for measurement of blood pressure. Silastic catheters were implanted in the pulmonary artery. A solid-state pressure transducer (P7A; Konigsberg Instruments, Pasadena, CA) was introduced in the apex of the left ventricle (LV). Flow probes (Transonic Systems, Ithaca, NY) and pneumatic occluders were placed on the left circumflex and the left anterior descending coronary arteries. Two pairs of ultrasonic crystals were used for measurement of LV wall thickening in the distribution of the left circumflex and the left anterior descending coronary arteries. One crystal was implanted in the endocardium,
and the other was sutured to the epicardium. All catheters and wires were exteriorized between the scapulae, and the pneumothorax was administered before and during the first week after surgery. Postoperative analgesia was provided with morphine during 4 days.

All hemodynamic data were recorded and analyzed using the data acquisition software HEM v3.5 (Notocord Systems, Croissy sur Seine, France). Aortic and left atrial pressures were measured with a Statham P23 ID strain-gauge transducer (Gould-Nicolet, Courtaboeuf, France). Coronary blood flow was measured using a T206 blood flowmeter (Transonic Systems) to assess proper coronary artery occlusion (CAO), i.e., coronary blood flow was equal to 0 ml/min during ischemia. LV pressure was measured using the Konigsberg gauge, and the change in LV pressure over time (LV dP/dt) was computed from the LV pressure signal. LV pressure was calibrated in vitro with a mercury manometer and in vivo with the left atrial and aortic pressures. Wall thicknesses were obtained by using an ultrasonic transit-time dimension gauge (Module 201, System 6; Triton Technology, San Diego, CA). To determine wall thickening, end-diastolic wall thickness was measured at the initiation of the upstroke of LV pressure tracing, and the end-systolic wall thickness was measured 20 ms before peak negative LV dP/dt. Percent wall thickening was defined as end-systolic minus end-diastolic thicknesses times 100 and divided by end-diastolic thickness.

Open-chest anesthetized rabbits. As previously described (1, 24), male New Zealand rabbits (2–2.5 kg) were anesthetized with pentobarbital sodium (20–30 mg/kg iv). They were intubated and mechanically ventilated with 100% oxygen (ventilation rate: 25 breaths/min; tidal volume: 25 ml). The animals were placed on a temperature-controlled heating pad. A catheter was positioned in the rabbit’s ear marginal artery for arterial pressure measurement (Statham P23 ID strain gauge; Statham Instruments, Oxnard, CA). An external electrocardiogram (ECG) was also recorded. A left thoracotomy was performed at the fourth intercostal space, and the pericardium was opened. A 4/0 Prolene suture was passed beneath a major branch of the left coronary artery. The ends of the ligature were passed through a short segment of propylene tubing to form a snare to perform episodes of CAO and reperfusion. Proper CAO was confirmed by observing cyanosis of the myocardium as well as ST-segment deviation of the ECG during ischemia. A pair of 1-mm ultrasonic piezoelectric crystals was inserted in the ventricular wall to measure left ventricular segment shortening using sonomicrometry (Module 201, System 6; Triton Technology). Percent segment shortening was calculated from the segment length recordings and defined as end-diastolic minus end-systolic segment lengths divided by end-diastolic segment length times 100. In additional rabbits, the same procedure was used without implanting the ultrasonic crystals to investigate the cardioprotective effect of PCD against myocardial infarction after prolonged ischemia.

At the end of the protocol, animals received heparin (1,000 units) and pentobarbital sodium (50 mg/kg iv). Potassium chloride was then administered intravenously to induce cardiac arrest. The hearts were excised. The ascending aorta was cannulated and perfused (120 mmHg) retrogradely with saline followed by Evans blue (1%) after ligation of the previously occluded artery. The LV was cut into 8–10 slices. These slices were weighed and incubated with 1% triphenyltetrazolium chloride (TTC; Sigma, Poole, UK) in a pH 7.4 buffer at 37°C. Slices were fixed overnight in 10% formaldehyde and then photographed with a digital camera. With the use of a computerized planimetric program (Scion Image; Scion, Frederick, MD), the area at risk and the infarcted zones were quantified. The area at risk was identified as the nonblue region and was expressed as a percentage of the LV weight. Infarcted area was identified as the TTC-negative zone and was expressed as a percentage of the area at risk.

Experimental protocols. All protocols are illustrated in Fig. 1.

To determine the effect of PCD on myocardial stunning in conscious dogs, the experiments were conducted 3–4 wk after surgery when the dogs were healthy and with normal body temperature. Each animal was submitted to control and different PCD sequences 1 wk apart in a random order using the same site of CAO. In the “control sequence,” dogs were subjected to a 10-min period of CAO (CAO). In the PCD sequences, a series of four cycles of 15 s CAO/15 s coronary artery reperfusion (CAR), 30 s CAO, or 1 min CAO/1 min CAR was performed immediately at the onset of reperfusion after the 10 min CAO for “PCD 15 s,” “PCD 30 s,” and “PCD 1 min,” respectively. In preliminary experiments, we verified that the repetition of 10 min CAO with CAR episodes performed 1 wk apart produces reproducible myocardial stunning.

To determine the effect of PCD on myocardial stunning in anesthetized rabbits, animals instrumented with ultrasonic crystals underwent either a control sequence (10 min CAO followed by 3 h CAR; n = 7) or a PCD sequence (4 cycles of 30 s CAR/30 s CAO performed at completion of 10 min CAO followed by 3 h CAR; n = 6). Four sham-operated animals were also studied.

To determine the effect of PCD on myocardial infarction in anesthetized rabbits, additional animals without ultrasonic crystals underwent either a control sequence (30 min CAO followed by 3 h CAR; n = 7) or a PCD sequence (4 cycles of 30 s CAR/30 s CAO performed at completion of 30 min CAO followed by 3 h CAR; n = 6).
between the control and PCD sequences at baseline, during reperfusion (CAR) followed by 15 s, 30 s, or 1 min CAO; HR, heart rate; MAP, mean arterial pressure; LV dP/dt, change in left ventricular pressure over time.

Values are means ± SE, n, no. of animals. CAO, coronary artery occlusion; PCD, postconditioning with 4 cycles of 15 s, 30 s, or 1 min coronary artery reperfusion (CAR) followed by 15 s, 30 s, or 1 min CAO; HR, heart rate; MAP, mean arterial pressure; LV dP/dt, change in left ventricular pressure over time.

Animals were excluded from the study if the quality of ultrasonic or hemodynamic signals was not suitable or if ventricular fibrillation occurred.

Statistical analysis. Data are reported as means ± SE. For all hemodynamic parameters, comparisons were performed using two-way ANOVA for repeated measures. If needed, individual comparisons were then conducted using a paired Student’s t-test with Bonferroni correction. In the rabbit study, the areas at risk and infarct sizes were compared by a Student’s t-test. A value of P < 0.05 was considered significant.

RESULTS

PCD and myocardial stunning in conscious dogs. As shown in Table 1, all the investigated hemodynamic parameters, i.e., heart rate, mean arterial pressure, and LV dP/dt, were similar between the control and PCD sequences at baseline, during CAO, and throughout CAR.

As shown in Table 2, LV wall thickening measured at baseline was similar between the control and PCD sequences. During CAO, LV wall thickening was similarly reduced in PCD 15 s, PCD 30 s, PCD 1 min compared with their respective control sequence. This decrease in LV wall thickening was constant throughout the 10 min CAO.

As shown in Fig. 2, LV wall thickening remained depressed in the ischemic zone in all sequences, indicating myocardial stunning. Depressions in LV wall thickening measured with PCD 15 s, PCD 30 s, PCD 1 min were similar to those observed with their respective control sequences, demonstrating that these PCD protocols do not protect against myocardial stunning.

PCD and myocardial stunning in anesthetized rabbits. As shown in Table 3, heart rate and mean arterial pressure were not significantly different between control and PCD rabbits throughout the protocol.

Table 2. LV wall thickening measured in conscious dogs with control and postconditioning sequences

<table>
<thead>
<tr>
<th>n</th>
<th>Baseline</th>
<th>CAO</th>
<th>10 min</th>
<th>15 min</th>
<th>30 min</th>
<th>1 h</th>
<th>3 h</th>
<th>6 h</th>
<th>24 h</th>
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<tbody>
<tr>
<td>NIZ Wth, %</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>37±6</td>
<td>34±7</td>
<td>36±8</td>
<td>36±8</td>
<td>37±8</td>
<td>38±8</td>
<td>38±8</td>
<td>36±7</td>
</tr>
<tr>
<td>PCD 15 s</td>
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<td>35±8</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>36±5</td>
<td>33±5</td>
<td>36±5</td>
<td>36±5</td>
<td>35±7</td>
<td>37±5</td>
<td>37±5</td>
<td>35±5</td>
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<tr>
<td>PCD 30 s</td>
<td>7</td>
<td>35±6</td>
<td>31±5</td>
<td>33±5</td>
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<td>34±5</td>
<td>35±6</td>
<td>35±6</td>
<td>36±6</td>
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<tr>
<td>Control</td>
<td>6</td>
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<td>38±6</td>
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<td>39±6</td>
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<tr>
<td>IZ Wth, %</td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>42±5</td>
<td>−1±1</td>
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<td>5</td>
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<td>−2±1</td>
<td>20±4</td>
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<td>Control</td>
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<td>40±4</td>
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<td>22±3</td>
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<td>27±5</td>
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<td>37±4</td>
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<tr>
<td>PCD 1 min</td>
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<td>26±3</td>
<td>30±4</td>
<td>33±4</td>
<td>37±3</td>
<td>37±2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. NIZ Wth, nonischemic zone wall thickening; IZ Wth, ischemic zone wall thickening.
At baseline, segment length shortening was similar between the control and PCD 30 s animals. During CAR, this parameter was depressed, indicating myocardial stunning. As shown in Fig. 3, right, segment length shortening was similarly reduced during CAO and CAR among control and PCD, therefore confirming the results obtained in conscious dogs.

**PCD and myocardial infarction in anesthetized rabbits.** Left ventricular weights and areas at risk were not significantly different between control (n = 7) and PCD 30 s (n = 6) rabbits (4.0 ± 0.2 vs. 4.1 ± 0.2 g and 31 ± 7 vs. 28 ± 7%, respectively). As shown in Fig. 3, left, infarct size was significantly reduced by PCD 30 s compared with control (39 ± 7 vs. 56 ± 3% of the area at risk, respectively, P < 0.05).

**DISCUSSION**

This study demonstrates that PCD does not induce any protection against myocardial stunning in either chronically instrumented conscious dogs or open-chest anesthetized rabbits. We challenged PCD against a 10-min period of myocardial ischemia that is well known to induce myocardial stunning without any infarction (18). The results of this study could appear on the one hand as negative results but, on the other hand, they demonstrate that additional episodes of ischemia-reperfusion do not compromise postischemic myocardial performance. Importantly, we also confirmed that PCD is able to significantly reduce infarct size in anesthetized rabbits.

The fact that in our experimental conditions, PCD does not limit stunning cannot be related to differences in animal species or experimental conditions, since we observed the same results in both large and small animals and in both the anesthetized and conscious states. Concerning the PCD stimulus, we clearly observed in anesthetized rabbits that myocardial infarct size was reduced significantly by PCD although it failed to reduce myocardial stunning. Inducing myocardial infarction in conscious dogs would require the use of intensive premedications for evident ethical reasons, and this would render the interpretation of the results difficult, since proper pharmacological protective or preconditioning-like effects of many of these substances are well known (8). Moreover, reduction in infarct size with quite similar PCD stimulus has already been demonstrated in anesthetized dogs (28). Nevertheless, as a limitation of this study, we cannot conclude with certainty that PCD is not able to protect against myocardial stunning in conscious dogs, since we did not directly verify that it can reduce infarct size in our experimental conditions. Another

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### Table 3. Hemodynamic parameters and LV segment length shortening measured in anesthetized rabbits with control and postconditioning sequences

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<thead>
<tr>
<th></th>
<th>Recovery</th>
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<tr>
<td>HR, beats/min</td>
<td>n</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
</tr>
<tr>
<td>PCD 30 s</td>
<td>6</td>
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<tr>
<td>MAP, mmHg</td>
<td>n</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
</tr>
<tr>
<td>PCD 30 s</td>
<td>6</td>
</tr>
<tr>
<td>SL, %</td>
<td>n</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
</tr>
<tr>
<td>PCD 30 s</td>
<td>6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. SL, LV segment length shortening.
aspect should be discussed, i.e., it has been demonstrated that, when the beginning of PCD was delayed (10, 27): this was not the case in our study, since PCD was started immediately at completion of the 10 min CAO period. Concerning the PCD algorithm, we used a unique number of cycles (n = 4) according to previous studies demonstrating that four cycles were as effective as six cycles in anesthetized rabbits (27). Because it appears that the duration of each cycle is more important to induce cardioprotection than the number of cycles (26), we investigated PCD protocols that have been demonstrated to reduce infarct size (2, 26, 27). Finally, the lack of regional myocardial blood flow measurement during CAO in conscious dogs is a limitation of this study. Differences in collateral blood flows between the control and PCD sequences might have biased some of our dog results. This is, however, unlikely, since we observed similar results during stunning experiments performed in rabbits, which are devoid of native collateral circulation.

Such differential effect between cardioprotection against myocardial infarction and stunning has already been reported with other strategies. For example, KATP channels play an obligatory role for protection against myocardial infarction but not against stunning (23), and adenosine A1 receptor agonists induce delayed preconditioning against myocardial infarction (22) but not against myocardial stunning (12). In addition, early classical preconditioning is universally known to reduce infarct size but not to protect against myocardial stunning (15).

Regarding the mechanisms of PCD, we initially expected that this strategy would reduce myocardial stunning by attenuating free radical production and calcium overload. However, it should be underlined that the effects of PCD have usually been gained using open-chest models that are known to potentiate the production of free radicals compared with the conscious state (11) and in the setting of myocardial infarction, i.e., long period of ischemia (30 min) as opposed to brief ischemia (10 min). In addition, the production of free radicals is also known to start within a few minutes of CAO and to persist up to 3 h after reflow (4). Concerning calcium overload, it is again surprising to observe that PCD does not protect against myocardial stunning, since Sun et al. (21) reported that it reduces the damage to cardiomyocytes by inhibiting intracellular calcium overload. However, calcium overload is not only limited to reperfusion, but it also occurs during ischemia (13). Finally, PCD has been mainly demonstrated to activate the reperfusion injury salvage kinase pathway (PI3K-Akt, p70S6K, and ERK; see Refs. 5, 25, 27) and to limit the opening of the mitochondrial permeability transition pore (2). These mechanisms cannot be involved in myocardial stunning, per se, since there is no myocardial cell death.

In conclusion and contrasting to its beneficial effects on myocardial infarction, we demonstrate that PCD does not protect against myocardial stunning in dogs and rabbits. Conversely, additional episodes of CAO and reperfusion do not alter the functional recovery of stunned myocardium. One could therefore speculate that PCD is not deleterious and is safe for surrounding viable tissue contractility in the management of myocardial infarction in humans (19).

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REFERENCES

6. De Curzon OP, Ghaleh B, Tissier R, Giudicelli JF, Hittinger I, and Berdeau A. Myocardial stunning in exercise-induced ischemia in dogs: