

# Differential role of $K_{ATP}$ channels in late preconditioning against myocardial stunning and infarction in rabbits

HITOSHI TAKANO, XIAN-LIANG TANG, AND ROBERTO BOLLI

Experimental Research Laboratory, Division of Cardiology, University of Louisville,  
and the Jewish Hospital Heart and Lung Institute, Louisville, Kentucky 40292

Received 22 February 2000; accepted in final form 2 June 2000

**Takano, Hitoshi, Xian-Liang Tang, and Roberto Bolli.** Differential role of  $K_{ATP}$  channels in late preconditioning against myocardial stunning and infarction in rabbits. *Am J Physiol Heart Circ Physiol* 279: H2350–H2359, 2000.—The role of ATP-sensitive potassium ( $K_{ATP}$ ) channels in the late phase of ischemic preconditioning (PC) remains unclear. Furthermore, it is unknown whether  $K_{ATP}$  channels serve as end effectors both for late PC against infarction and against stunning. Thus, in *phase I* of this study, conscious rabbits underwent a 30-min coronary occlusion (O) followed by 72 h of reperfusion (R) with or without ischemic PC (6 4-min O/4-min R cycles) 24 h earlier. Late PC reduced infarct size ~46% versus controls. The  $K_{ATP}$  channel blocker 5-hydroxydecanoic acid (5-HD), given 5 min before the 30-min O, abrogated the infarct-sparing effect of late PC but did not alter infarct size in non-PC rabbits. In *phase II*, rabbits underwent six 4-min O/4-min R cycles for 3 consecutive days (*days 1, 2, and 3*). In controls, the total deficit of systolic wall thickening (WTh) after the sixth reperfusion was reduced by 46% on *day 2* and 54% on *day 3* compared with *day 1*, indicating a late PC effect against myocardial stunning. Neither 5-HD nor glibenclamide, given on *day 2*, abrogated late PC. The  $K_{ATP}$  channel opener diazoxide, given on *day 1*, attenuated stunning, and this effect was completely blocked by 5-HD. Thus the same dose of 5-HD that blocked the antistunning effect of diazoxide failed to block the antistunning effects of late PC. Furthermore, when diazoxide was administered in PC rabbits on *day 2*, myocardial stunning was further attenuated, indicating that diazoxide and late PC have additive anti-stunning effects. We conclude that  $K_{ATP}$  channels play an essential role in late PC against infarction but not in late PC against stunning, revealing an important pathogenetic difference between these two forms of cardioprotection.

myocardial ischemia; myocardial reperfusion; 5-hydroxydecanoic acid; glibenclamide; diazoxide

ISCHEMIC PRECONDITIONING (PC) is the phenomenon whereby brief episodes of ischemia render the heart more resistant to subsequent ischemia (6, 11, 20, 26). Ischemic PC consists of two phases of protection: an early phase, which appears immediately after the PC ischemia and wanes in 2–4 h (11, 12, 14, 26), and a late phase, which appears 12–24 h after the PC ischemia and lasts for 3–4 days (3, 38). In addition to an infarct-

sparing effect, late PC is also characterized by an antistunning effect (7, 8, 13, 28, 29, 32, 35, 37, 38, 41), which is not observed during the early phase of ischemic PC (6, 24, 27).

Recent studies (8, 19, 36) have shown that the inducible isoform of nitric oxide (NO) synthase (iNOS) is the mediator of late PC against both infarction and stunning in conscious rabbits, but the mechanism whereby NO protects against ischemic injury has not been elucidated. One possible mechanism is the recruitment of ATP-sensitive potassium ( $K_{ATP}$ ) channels, since NO has been shown to open these channels (9, 25, 33, 34). Although numerous reports have suggested that opening of  $K_{ATP}$  channels is essential for the infarct-sparing actions of the early phase of PC (1, 10, 15, 17), little is known regarding the role of  $K_{ATP}$  channels in the late phase of ischemic PC. Mei et al. (23) were the first to suggest a role of  $K_{ATP}$  channels in late PC. They showed in dogs that the delayed protection against infarction induced pharmacologically with monophosphoryl lipid A was abolished by  $K_{ATP}$  channel antagonists. A recent study (5) in open-chest rabbits indicated that blockade of  $K_{ATP}$  channels blocks ischemia-induced late PC against infarction. However, no data are available in conscious animals. Furthermore, it is unknown whether  $K_{ATP}$  channels serve as effectors of late PC against both stunning and infarction. Accordingly, in the present study, we explored the role of  $K_{ATP}$  channels in the protective effect of late PC against myocardial infarction and stunning in chronically instrumented animals. Specifically, the goals of this investigation were to determine, in conscious rabbits, 1) whether the  $K_{ATP}$  channel blocker 5-hydroxydecanoic acid (5-HD), given before a 30-min coronary occlusion, abrogates the protective effects of late PC against myocardial infarction and 2) whether the  $K_{ATP}$  channel blockers 5-HD and glibenclamide, given before the second ischemic challenge (*day 2*) (i.e., after a PC state has developed), abrogate the protective effects of late PC against myocardial stunning. In addition, to verify that the dose of 5-HD employed in this investigation was effective in inhibiting  $K_{ATP}$  channels, we determined whether the  $K_{ATP}$  channel opener diazoxide

Address for reprint requests and other correspondence: R. Bolli, Div. of Cardiology, Univ. of Louisville, Louisville, KY 40292 (E-mail: rbolli@louisville.edu).

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attenuates myocardial stunning and, if so, whether this effect can be abrogated by simultaneous administration of 5-HD.

## MATERIALS AND METHODS

### Experimental Preparation and Protocol

The experimental preparation has been described in detail previously (7, 8, 13, 28–30, 32, 36, 37, 41). Briefly, pentobarbital sodium-anesthetized New Zealand White male rabbits (weight, 2.0–2.5 kg; age, 3–4 mo) were instrumented under sterile conditions with a balloon occluder around a major branch of the left coronary artery, a 10-MHz pulsed Doppler ultrasonic crystal in the center of the region to be rendered ischemic, and bipolar electrocardiogram (ECG) leads on the chest wall. The animals were allowed to recover for a minimum of 10 days after surgery. Throughout the experiments, rabbits were kept in a cage in a quiet, dimly lit room. Left ventricular (LV) systolic wall thickening (WTh), range-gate depth, and the ECG were recorded throughout the experiments on a thermal array chart recorder (Gould TA6000, Valley View, OH). The study consisted of two consecutive phases (*phases I and II*).

**Phase I: studies of myocardial infarction.** To examine the role of K<sub>ATP</sub> channels in the late phase of ischemic PC against myocardial infarction, rabbits were subjected to a 30-min coronary artery occlusion followed by 3 days of reperfusion. The performance of successful coronary occlusions was verified by observing the development of ST-segment elevation and changes in the QRS complex on the ECG and the appearance of paradoxical systolic wall thinning on the ultrasonic crystal recordings. Diazepam was administered 20 min before the onset of ischemia (4 mg/kg ip) to relieve the stress caused by the coronary occlusion. No antiarrhythmic agents were given at any time. Rabbits were assigned to four groups (Fig. 1). *Group I* (control group) underwent the 30-min occlusion with no PC protocol or drug pretreatment. *Group II* (PC group) was preconditioned with a sequence of six 4-min coronary occlusion/4-min reperfusion cycles 24 h before the 30-min coronary occlusion. In *group III* (PC + 5-HD group), preconditioned rabbits were given the selective

mitochondrial K<sub>ATP</sub> channel blocker 5-HD (5 mg/kg iv) 5 min before the 30-min coronary occlusion. In *group IV* (5-HD group), nonpreconditioned rabbits were given the same dose of 5-HD as in *group III*. The dose of 5-HD was chosen on the basis of the previous studies in which 5 mg/kg of 5-HD effectively blocked the infarct-sparing effects of the early phase of ischemic PC (2, 18) and of the late phase of pharmacologically induced PC in rabbits (4).

**Phase II: studies of myocardial stunning.** The experimental protocol consisted of 3 consecutive days of coronary artery occlusions (*days 1, 2, and 3, respectively*). On each day, the rabbits were subjected to a sequence of six 4-min coronary occlusion/4-min reperfusion cycles (Fig. 2). No sedative or antiarrhythmic agents were given at any time.

Rabbits were assigned to six groups (Fig. 2). *Group V* (control group) underwent the coronary artery occlusion/reperfusion protocol on *days 1, 2, and 3* without any treatment. In *group VI* (5-HD group), rabbits received 5-HD (a 5 mg/kg bolus iv followed by a 0.15 mg·kg<sup>-1</sup>·min<sup>-1</sup> iv infusion for 60 min) starting 5 min before the sequence of six 4-min occlusion/4-min reperfusion cycles on *day 2*. In *group VII* (glibenclamide group), rabbits received glibenclamide (a blocker of both sarcolemmal and mitochondrial K<sub>ATP</sub> channels) (0.3 mg/kg ip) 30 min before the sequence of six 4-min occlusion/4-min reperfusion cycles on *day 2*. In *group VIII* (diazoxide *day 1* group), rabbits received an intravenous infusion of the K<sub>ATP</sub> channel opener diazoxide (10 μg·kg<sup>-1</sup>·min<sup>-1</sup> for 84 min) starting 30 min before the sequence of six 4-min occlusion/4-min reperfusion cycles on *day 1*. In *group IX*, on *day 1*, rabbits received an intravenous infusion of diazoxide (10 μg·kg<sup>-1</sup>·min<sup>-1</sup> for 84 min) starting 30 min before the sequence of six 4-min occlusion/4-min reperfusion cycles) in conjunction with 5-HD (5 mg/kg bolus iv followed by a 0.15 mg·kg<sup>-1</sup>·min<sup>-1</sup> iv infusion for 60 min starting 5 min before the sequence of six 4-min occlusion/4-min reperfusion cycles). In *group X*, preconditioned rabbits received an intravenous infusion of diazoxide (10 μg·kg<sup>-1</sup>·min<sup>-1</sup> for 84 min starting 30 min before the sequence of six 4-min occlusion/4-min reperfusion cycles on *day 2*).

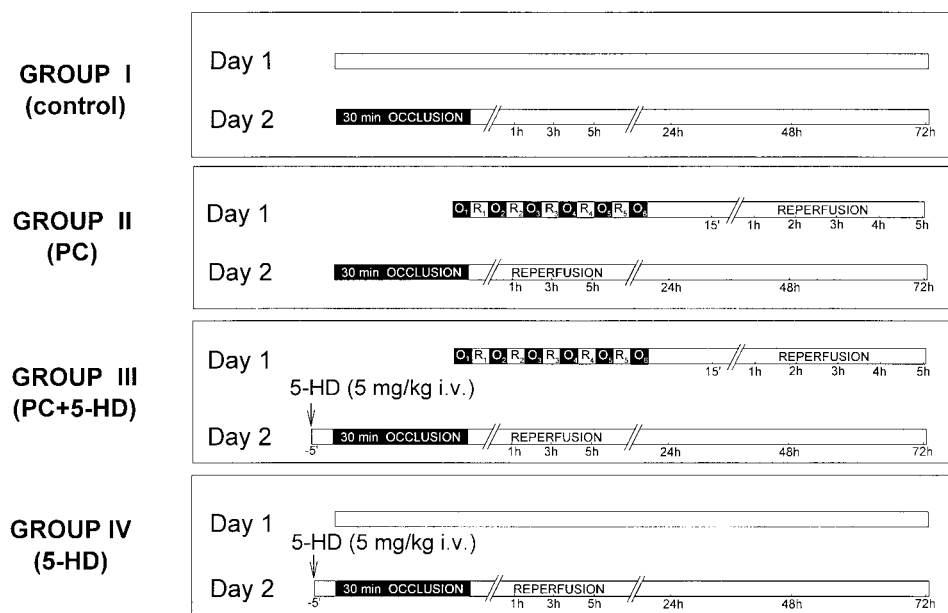


Fig. 1. Experimental protocol for the studies of myocardial infarction (*phase I*). Four groups of rabbits were studied. On *day 2*, all groups underwent a 30-min coronary occlusion followed by 3 days of reperfusion. On *day 2*, rabbits in *groups I* ( $n = 7$ , control group) and *II* ( $n = 7$ , preconditioned (PC) group) received no treatment. On *day 1*, rabbits in *group II* underwent a sequence of 6 4-min coronary occlusion/4-min reperfusion cycles. Rabbits in *group III* ( $n = 7$ , PC + 5-hydroxydecanoic acid (5-HD) group) underwent the same protocol as *group II* on *day 1*; on *day 2*, they received an iv bolus of 5-HD (5 mg/kg) 5 min before the 30-min coronary occlusion. Rabbits in *group IV* ( $n = 7$ , 5-HD group) underwent the same protocol as *group III* except that they were not preconditioned on *day 1*.

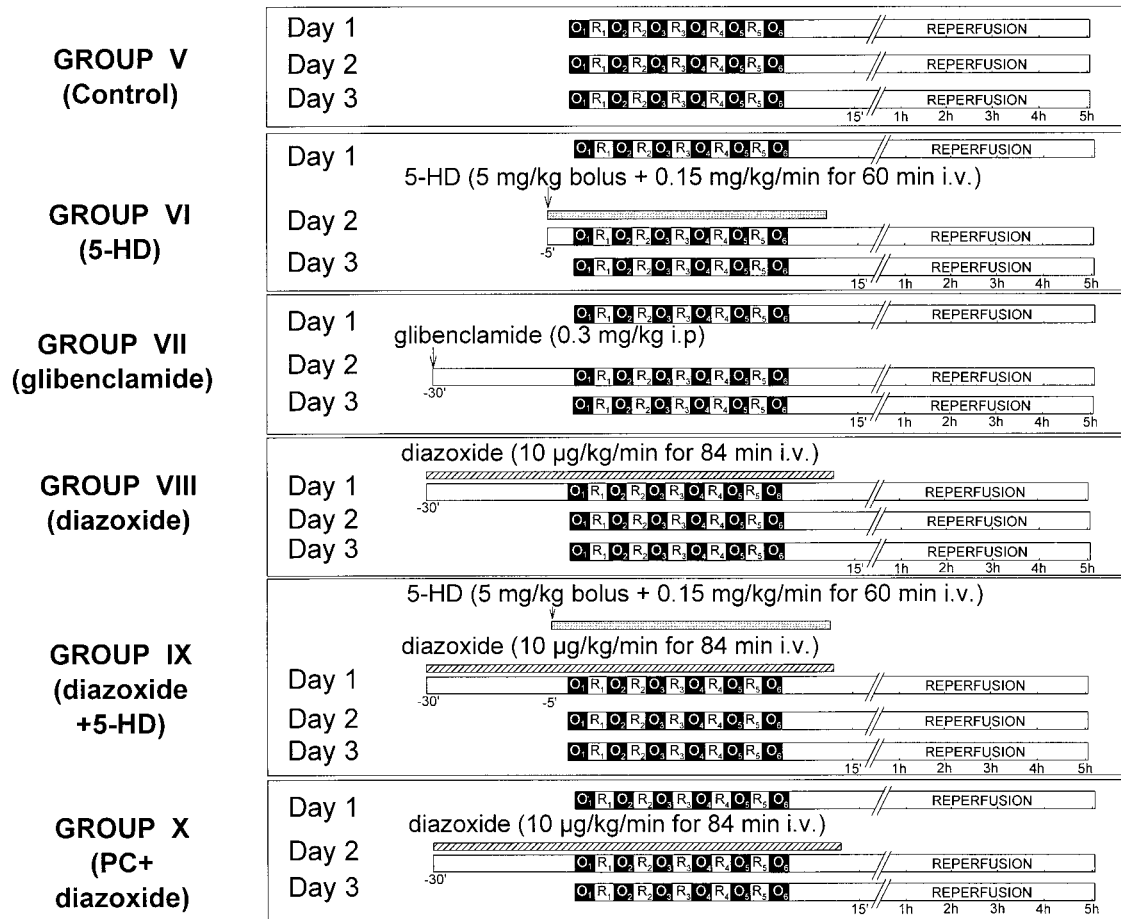


Fig. 2. Experimental protocol for the studies of myocardial stunning (*phase II*). Six groups of rabbits underwent a sequence of six 4-min coronary occlusion/4-min reperfusion cycles followed by a 5-h observation period for 3 consecutive days (*days 1, 2, and 3*). Rabbits in *group V* (control group) did not receive any treatment. On *day 2*, rabbits in *group VI* received an intravenous bolus of 5 mg/kg of 5-HD followed by a continuous intravenous infusion of 5-HD at a rate of  $0.15 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for 60 min (total 14 mg/kg) starting 5 min before the sequence of six 4-min occlusion/4-min reperfusion cycles. Rabbits in *group VII* received an intraperitoneal bolus of glibenclamide (0.3 mg/kg) 30 min before the sequence of six 4-min occlusion/4-min reperfusion cycles on *day 2*. Rabbits in *group VIII* received a continuous intravenous infusion of diazoxide at a rate of  $10 \text{ } \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for 90 min (total 0.9 mg/kg) starting 30 min before the sequence of six 4-min occlusion/4-min reperfusion cycles on *day 1*. Rabbits in *group IX* underwent the same protocol as *group VIII*; in addition, on *day 1*, the animals received the same dose of 5-HD used in *group VI* on *day 2*. Rabbits in *group X* received on *day 2* the same dose of diazoxide used in *groups VIII and IX* on *day 1*.

In the studies of myocardial stunning, 5-HD was given as an initial intravenous bolus (same dose as in the studies of myocardial infarction) followed by a continuous intravenous infusion. In an effort to maintain tissue concentrations of 5-HD as constant as possible, the drug was infused at a rate of  $0.15 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  following the 5 mg/kg bolus iv, which was the fastest infusion rate that did not worsen the severity of myocardial stunning in our pilot studies (see RESULTS). The dose of glibenclamide (0.3 mg/kg) was chosen according to previous studies in which it effectively abolished the infarct-sparing effect of the early phase of PC in rabbits (39) and dogs (43). The dose of diazoxide was selected on the basis of pilot studies in which this dose effectively attenuated myocardial stunning without causing any hemodynamic changes (see RESULTS). 5-HD was dissolved in normal saline (5 mg/ml, total volume  $\sim 2.5 \text{ ml}$  in the studies of infarction and  $\sim 7 \text{ ml}$  in the studies of stunning), glibenclamide in 1 ml of DMSO plus 1 ml of normal saline, and diazoxide in 1.5 ml of DMSO plus 1.5 ml of normal saline. All solutions were filtered through a  $0.2\text{-}\mu\text{m}$  Millipore filter to ensure sterility.

#### Measurement of Regional Myocardial Function

Regional myocardial function was assessed as systolic thickening fraction using the pulsed Doppler probe, as previously described (7, 8, 13, 28–30, 36, 37, 41). In the studies of myocardial stunning, the total deficit of systolic WTh (an integrative assessment of the overall severity of myocardial stunning) was calculated by measuring the area comprised between the systolic WTh-versus-time line and the baseline (100% line) during the 5-h recovery phase after the sixth reperfusion (7, 8, 13, 28, 29, 37, 41). In all animals, measurements from at least 10 beats were averaged at baseline and from at least 5 beats at all subsequent time points.

#### Measurement of Region at Risk and Infarct Size

At the conclusion of the study, the rabbits were given heparin (1,000 U iv), after which they were anesthetized with pentobarbital sodium (50 mg/kg iv) and euthanized with KCl. The heart was excised, and the size of the ischemic-reperfusion region (region at risk) was determined by tying the

coronary artery at the site of the previous occlusion and by perfusing the aortic root for 2 min with a 5% solution of phthalo blue dye in normal saline at a pressure of 70 mmHg using a Langendorff apparatus, as previously described (7, 8, 13, 28–30, 36, 37, 41). The heart was then cut into six to seven transverse slices, which were incubated for 10 min at 37°C in a 1% solution of triphenyltetrazolium chloride in phosphate buffer (pH 7.4). All atrial and right ventricular tissues were excised. In the studies of myocardial infarction (*phase I*), the slices were weighed, fixed in a 10% neutral-buffered formaldehyde solution, and photographed (Nikon AF N6006). Transparencies were projected onto a paper screen at a 10-fold magnification, and the borders of the infarcted, ischemic-reperfused, and nonischemic regions were traced. The corresponding areas were measured by computerized planimetry (Adobe Photoshop, version 4.0), and from these measurements, infarct size was calculated as a percentage of the region at risk (28, 30, 36, 37, 41). In the studies of myocardial stunning (*phase II*), the region at risk (which was identified by the absence of blue dye) was separated from the rest of the left ventricle, and both components were weighed.

#### Statistical Analysis

Data are reported as means  $\pm$  SE. For intragroup comparisons, hemodynamic variables and WTh were analyzed by a two-way repeated-measures ANOVA (time and day) followed by Student's *t*-tests for paired data with the Bonferroni correction (40). For intergroup comparisons, data were analyzed by either a one-way or a two-way repeated-measures (time and group) ANOVA, as appropriate, followed by unpaired Student's *t*-tests with the Bonferroni correction (40). The relationship between infarct size and risk region size was compared among groups with an ANCOVA using the size of the risk region as the covariate (28, 30, 36, 37, 41). The correlation between infarct size and risk region size was assessed by linear regression analysis using the least-squares method. Statistical analyses were performed using SPSS for Windows version 7.0 and SigmaStat for Windows version 2.0.

## RESULTS

A total of 88 rabbits were used in this study (14 for the pilot studies, 35 for the studies of myocardial infarction, and 39 for the studies of myocardial stunning).

#### Pilot Studies

Pilot studies were conducted in 14 rabbits to determine appropriate doses of 5-HD and diazoxide. We sought to identify the highest dose of 5-HD that does not alter hemodynamics or the severity of myocardial stunning in nonpreconditioned hearts but does block the antistunning effects of both PC and diazoxide. In addition, it was necessary to identify a dose of diazoxide that can effectively attenuate myocardial stunning without causing any changes in arterial pressure, heart rate, or WTh. Arterial pressure was measured by cannulating the ear dorsal artery with a 22-gauge angiocatheter under local anesthesia (benzocaine), as previously described (7, 8, 28, 29, 37).

In two rabbits, an intravenous bolus of 5 mg/kg of 5-HD did not cause any changes in arterial pressure,

heart rate, or WTh. In three rabbits, when 5-HD was infused at a rate of 0.33 mg·kg<sup>-1</sup>·min<sup>-1</sup> for 60 min after an intravenous bolus of 5 mg/kg of 5-HD (total dose 25 mg/kg), arterial pressure and WTh did not change significantly. However, this dose increased heart rate by ~20% and worsened the severity of myocardial stunning. Therefore, we reduced the dose to 14 mg/kg (5 mg/kg bolus + 0.15 mg·kg<sup>-1</sup>·min<sup>-1</sup> for 60 min), which did not alter arterial pressure, heart rate, or WTh significantly in three rabbits. In these animals, the total deficit of WTh after the sequence of coronary occlusion/reperfusion cycles on *day 1* was similar to that measured when the same rabbits underwent the same protocol 2 wk later without 5-HD on *day 1* (143  $\pm$  13 vs. 150  $\pm$  14, respectively); furthermore, this dose effectively blocked the antistunning effect of diazoxide.

In one rabbit, an intravenous infusion of diazoxide at a rate of 100  $\mu$ g·kg<sup>-1</sup>·min<sup>-1</sup> for 60 min (total 6 mg/kg) caused a significant decrease in mean arterial pressure (from 86 to 68 mmHg) and WTh (from 32.4 to 26.8%) and an increase in heart rate (from 240 to 288 beats/min) 30 min after the end of the infusion. When 2.4 mg/kg of diazoxide (40  $\mu$ g·kg<sup>-1</sup>·min<sup>-1</sup> for 60 min) was administered in three rabbits, one out of three rabbits showed a decrease in mean arterial pressure (from 80 to 65 mmHg) and WTh (from 34.6 to 24.8%) and an increase in heart rate (from 232 to 276 beats/min) 30 min after the end of the infusion, although the other two rabbits exhibited no hemodynamic changes. Therefore, we reduced the dose to 0.9 mg/kg (10  $\mu$ g·kg<sup>-1</sup>·min<sup>-1</sup> for 90 min), which did not cause any changes in arterial pressure, heart rate, or WTh in three rabbits.

#### Phase I: Studies of Myocardial Infarction

**Exclusions and arrhythmias.** Of the 35 rabbits instrumented for the studies of myocardial infarction, 8 were assigned to *group I* (control group), 8 to *group II* (PC group), 9 to *group III* (PC + 5-HD group), and 10 to *group IV* (5-HD group). Seven rabbits died of ventricular fibrillation during coronary occlusion (1 in *group I*, 1 in *group II*, 2 in *group III*, and 3 in *group IV*). The incidence of ventricular fibrillation during the 30-min occlusion did not differ significantly among groups. Therefore, a total of seven rabbits completed the experimental protocol in each of *groups I, II, III, and IV*. No rabbit included in the final analysis was subjected to defibrillation.

**Hemodynamic variables.** There were no appreciable differences in heart rate among *groups I, II, III, and IV* either during the 30-min coronary occlusion or during the 72-h reperfusion period (data not shown for the sake of brevity). Baseline systolic thickening fraction was also similar among the six groups (32.7  $\pm$  6.4, 31.8  $\pm$  2.9, 29.3  $\pm$  4.4, and 33.6  $\pm$  3.3% in *groups I, II, III, and IV*, respectively).

**Region at risk and infarct size.** There were no significant differences among *groups I, II, III, and IV* with respect to the weight of the region at risk: 0.76  $\pm$  0.15 g (16.3  $\pm$  2.6% of LV weight), 0.76  $\pm$  0.10 g (16.4  $\pm$  1.9%

of LV weight),  $0.74 \pm 0.08$  g ( $17.2 \pm 1.4\%$  of LV weight), and  $0.76 \pm 0.10$  g ( $17.5 \pm 1.9\%$  of LV weight), respectively. The average infarct size was 46% smaller in *group II* (PC group) compared with *group I* (control group) ( $31.9 \pm 3.0\%$  vs.  $59.1 \pm 5.9\%$  of the region at risk, respectively,  $P < 0.05$ ; Fig. 3), indicating a late PC effect against myocardial infarction. In *group III* (PC + 5-HD group), however, infarct size ( $55.5 \pm 3.9\%$  of the region at risk) was significantly greater than in the PC group ( $P < 0.05$ ) and essentially indistinguishable from controls (Fig. 3), indicating that 5-HD abrogated the late PC effect against infarction. In *group IV* (5-HD group), infarct size ( $59.1 \pm 4.1\%$  of the region at risk) did not differ significantly from that in controls (Fig. 3), indicating that administration of 5-HD did not affect the extent of cell death in nonpreconditioned myocardium.

In all groups, the size of the infarction was positively and linearly related to the size of the region at risk ( $r = 0.89$  in *group I*,  $0.81$  in *group II*,  $0.92$  in *group III*, and  $0.90$  in *group IV*; Fig. 4). As expected (28, 30, 36, 37), the regression line was shifted to the right in *group II* (PC group) compared with *group I* (control group) ( $P < 0.05$  by ANCOVA; Fig. 4). In *group III* (PC + 5-HD group), the regression line was virtually indistinguishable from that of *group I* (control group) and was significantly shifted to the left compared with *group II* (PC group) ( $P < 0.05$  by ANCOVA; Fig. 4), indicating that for any given size of the region at risk the resulting infarction was greater in preconditioned rabbits treated with 5-HD than in untreated preconditioned rabbits (Fig. 4). In *group IV* (5-HD group), the regression line did not differ from that observed in *group I* (control group) (Fig. 4).

### Phase II: Studies of Myocardial Stunning

**Exclusions and postmortem analysis.** Of the 39 rabbits instrumented for the studies of myocardial stunning, 7 were assigned to *group V* (control group), 7 to *group VI* (5-HD group), 6 to *group VII* (glibenclamide

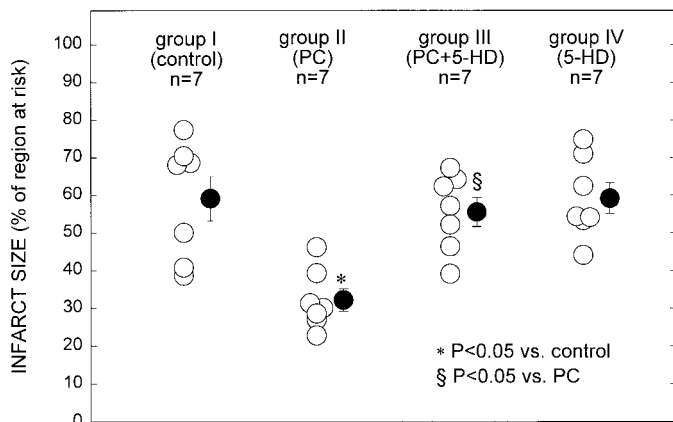


Fig. 3. Myocardial infarct size in *groups I* ( $n = 7$ , control group), *II* ( $n = 7$ , PC group), *III* ( $n = 7$ , PC + 5-HD group), and *IV* ( $n = 7$ , 5-HD group). Infarct size is expressed as a percentage of the region at risk of infarction. ○, Individual rabbits; ●, means  $\pm$  SE. \* $P < 0.05$  vs. *group I* (control group); § $P < 0.05$  vs. *group II* (PC group).

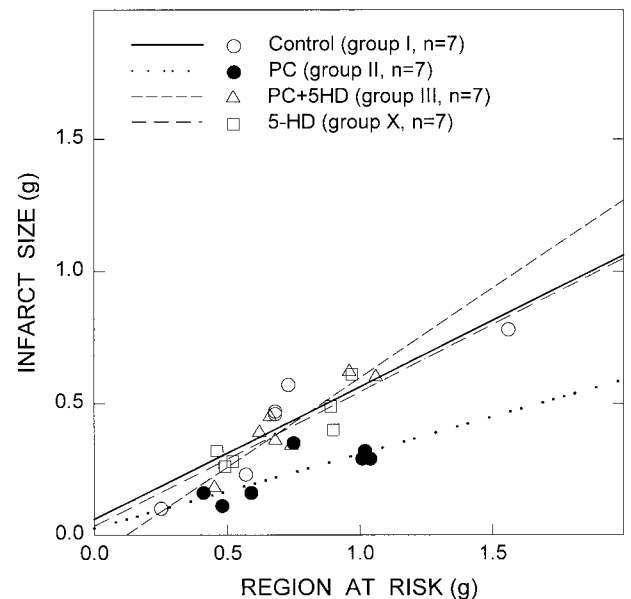


Fig. 4. Relationship between size of the region at risk and size of myocardial infarction. Both individual values and the regression lines obtained by linear regression analysis for *groups I* (control,  $n = 7$ ), *II* (PC group,  $n = 7$ ), *III* (PC + 5-HD,  $n = 7$ ), and *IV* (5-HD group,  $n = 7$ ) are illustrated. In all groups, infarct size was positively and linearly related to risk region size. The linear regression equations were as follows: *group I*,  $y = 0.51x - 0.07$  ( $r = 0.89$ ); *group II*,  $y = 0.28x + 0.02$  ( $r = 0.81$ ); *group III*,  $y = 0.68x - 0.08$  ( $r = 0.92$ ); *group IV*,  $y = 0.70x - 0.08$  ( $r = 0.90$ ). ANCOVA demonstrated that the regression line for *group II* was significantly different from that for *group I* ( $P < 0.05$  for each comparison), indicating that for any given risk region size, infarct size was smaller in the preconditioned rabbits compared with the control rabbits; in contrast, the lines for *groups III* and *IV* were similar to that for *group I*.

group), 7 to *group VIII* (diazoxide group), 6 to *group IX* (5-HD + diazoxide), and 6 to *group X* (PC + diazoxide group). One rabbit in *group VI* died of ventricular fibrillation during the third occlusion on *day 2*, and one rabbit in *group VII* died during the second occlusion on *day 2*; therefore, six rabbits in *group V* and five rabbits in *group VI* completed the whole protocol and were included for the analysis. One rabbit assigned to *group VIII* died of ventricular fibrillation during the fourth occlusion on *day 3*, and one rabbit died in *group IX* during the third occlusion on *day 3*; therefore, seven rabbits in *group VII* and six rabbits in *group IX* completed *days 1* and *2*, and six rabbits in *group VII* and five rabbits in *group IX* completed *day 3*. In *groups IV*, *VIII*, and *X*, all rabbits completed the protocol and were included in the analysis. Postmortem analysis showed that the size of the occluded-reperfused vascular bed was similar in the six groups:  $0.89 \pm 0.10$  g ( $19.8 \pm 1.7\%$  of LV weight) in *group V*,  $0.95 \pm 0.11$  g ( $21.2 \pm 2.3\%$  of LV weight) in *group VI*,  $0.96 \pm 0.14$  g ( $19.8 \pm 3.0\%$  of LV weight) in *group VII*,  $0.93 \pm 0.09$  g ( $19.3 \pm 2.6\%$  of LV weight) in *group VIII*,  $0.91 \pm 0.22$  g ( $20.0 \pm 4.3\%$  of LV weight) in *group IX*, and  $0.94 \pm 0.17$  g ( $20.4 \pm 3.7\%$  of LV weight) in *group X*. Tissue staining with triphenyltetrazolium confirmed the absence of infarction in all animals. In all rabbits, the

ultrasonic crystal was found to be at least 3 mm from the boundaries of the ischemic-reperfused region.

**Hemodynamic variables.** On days 1, 2, and 3, there were no appreciable differences in heart rate among the six groups, either during the sequence of coronary occlusion/reperfusion cycles or during the 5-h reperfusion period (data not shown for the sake of brevity).

**Blood glucose levels.** Blood glucose levels were measured before and after the administration of glibenclamide on day 2 in group VII. Blood samples were obtained before treatment, immediately before the first coronary occlusion, immediately after the third and sixth reperfusion, and after 30 min and 1, 2, and 3 h after the sixth reperfusion. In three out of five rabbits, glibenclamide induced hypoglycemia (65, 70, and 76 mg/dl) immediately after the sixth reperfusion. When glucose levels fell below 80 mg/dl, 1 ml of 50% glucose solution was injected as a bolus iv. The efficacy of this maneuver in correcting hypoglycemia was confirmed in all three rabbits by measurements of blood glucose 30 min after the sixth reperfusion. Blood glucose levels were measured using the Glucometer Elite blood glucose meter (Bayer).

**Regional myocardial function.** The measurements of regional function are summarized in Figs. 5–8. Baseline systolic thickening fraction on days 1, 2, and 3 averaged  $32.9 \pm 2.0$ ,  $33.2 \pm 1.7$ , and  $32.3 \pm 1.9\%$ , respectively, in group V;  $27.4 \pm 2.7$ ,  $27.7 \pm 2.6$ , and  $27.8 \pm 2.4\%$  in group VI;  $29.2 \pm 2.9$ ,  $32.1 \pm 2.4$ , and  $31.3 \pm 2.1\%$  in group VII;  $29.2 \pm 2.9$ ,  $28.9 \pm 2.2$ , and  $28.3 \pm 2.4\%$  in group VIII;  $29.5 \pm 3.1$ ,  $27.7 \pm 3.4$ , and  $27.9 \pm 3.3\%$  in group IX; and  $31.5 \pm 3.2$ ,  $30.6 \pm 2.9$ , and  $30.9 \pm 2.8\%$  in group X. There were no significant differences among groups V, VI, VII, VIII, IX, and X on the same day or among different days within the same group. Furthermore, within the same group, there

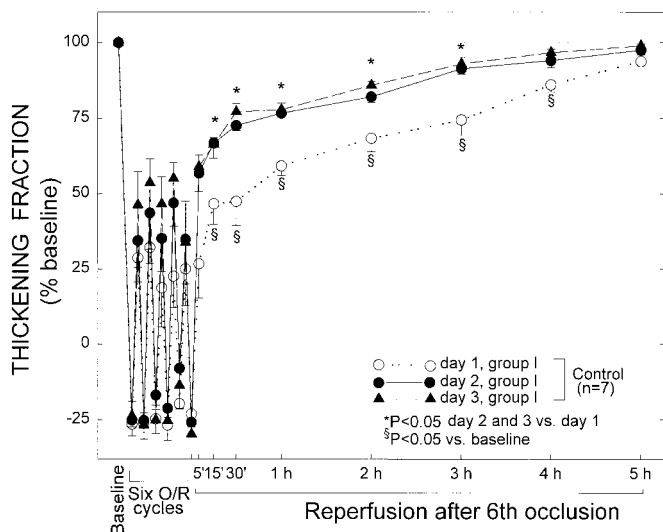


Fig. 5. Systolic thickening fraction in the ischemic-reperfused region in group I (control group,  $n = 7$ ) 5 min before the first occlusion (baseline), 3 min into each coronary occlusion (O), 3 min into each reperfusion (R), and at selected times during the 5-h reperfusion interval after the 6th occlusion. Thickening fraction is expressed as a percentage of baseline values. Data are means  $\pm$  SE.

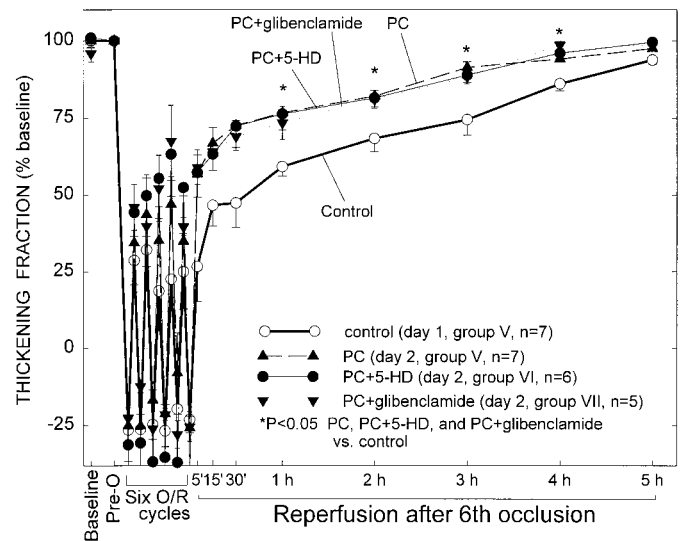


Fig. 6. Systolic thickening fraction in the ischemic-reperfused region 5 min before the first occlusion (baseline), 3 min into each coronary occlusion, 3 min into each reperfusion, and at selected times during the 5-h reperfusion interval after the 6th occlusion in nonpreconditioned control rabbits (day 1, group V,  $n = 7$ ), in preconditioned control rabbits (day 2, group V,  $n = 7$ ), in 5-HD-treated preconditioned rabbits (day 2, group VI,  $n = 6$ ), and in glibenclamide-treated preconditioned rabbits (day 2, group VII,  $n = 5$ ). Thickening fraction is expressed as a percentage of baseline values. Data are means  $\pm$  SE.

were no significant differences among days 1, 2, and 3 with respect to the extent of paradoxical systolic thinning during the six occlusions.

**GROUP V (CONTROL GROUP).** On day 1, thickening fraction remained significantly ( $P < 0.05$ ) depressed for 4 h after the sixth reperfusion and recovered by 5 h (Fig. 5), indicating that the sequence of six 4-min occlusion/4-min reperfusion cycles resulted in severe myocardial stunning that lasted, on average, 4 h. On days 2 and 3, however, the recovery of WTh after the six 4-min occlusion/4-min reperfusion cycles was markedly improved compared with day 1 (Fig. 5). The total deficit of WTh after the sixth reperfusion was 54% less on both days 2 and 3 compared with day 1 ( $P < 0.01$ ) (Fig. 8). Thus, as expected (6–8), myocardial stunning was attenuated markedly, and to a similar extent, on days 2 and 3 compared with day 1.

**GROUPS VI (5-HD GROUP) AND VII (GLIBENCLAMIDE GROUP).** These groups were studied to determine whether inhibition of K<sub>ATP</sub> channels on day 2 abrogates the antistunning effect of late PC. Surprisingly, when the K<sub>ATP</sub> channel blockers 5-HD and glibenclamide were administered on day 2, the recovery of WTh after the six 4-min occlusion/4-min reperfusion cycles was still improved (Fig. 6), and the total deficit of WTh was still decreased (Fig. 8) compared with day 1. Both the recovery of WTh (Fig. 6) and the deficit of WTh (Fig. 8) were similar to those observed on day 3. Thus both 5-HD and glibenclamide failed to abrogate the cardioprotective effects of late PC against stunning. Furthermore, in three additional rabbits, we found that the high dose of 5-HD (5 mg/kg bolus +  $0.33 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for 60 min, total 25 mg/kg), which aggravated myocardial stunning in our pilot stud-

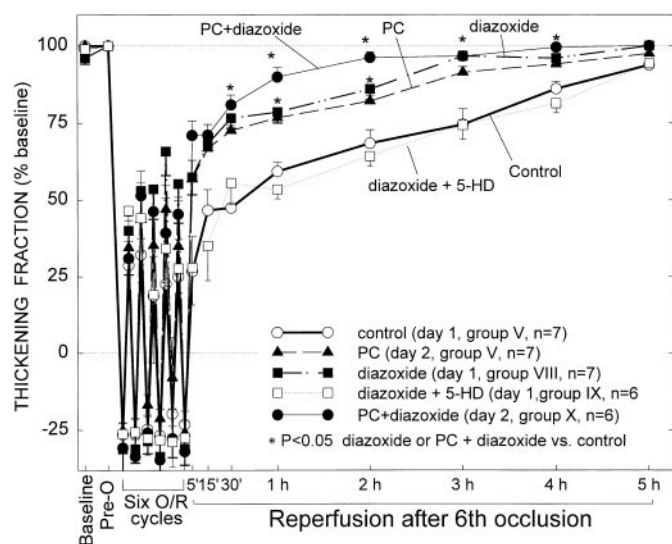


Fig. 7. Systolic thickening fraction in the ischemic-reperfused region 5 min before the first occlusion (baseline), 3 min into each coronary occlusion, 3 min into each reperfusion, and at selected times during the 5-h reperfusion interval following the sixth occlusion in nonpreconditioned control rabbits (day 1, group V,  $n = 7$ ), in preconditioned control rabbits (day 2, group V,  $n = 7$ ), in diazoxide-treated nonpreconditioned rabbits (day 1, group VIII,  $n = 7$ ), in diazoxide + 5-HD-treated nonpreconditioned rabbits (day 1, group IX,  $n = 6$ ) and in diazoxide-treated preconditioned rabbits (day 2, group X,  $n = 6$ ). Thickening fraction is expressed as a percentage of baseline values. Data are means  $\pm$  SE.

ies, also failed to block the antistunning effect of late PC. In these three rabbits, there was a 48% decrease in the deficit of WTh when 5-HD was given on day 2 compared with the deficit of WTh measured in the same rabbits when 5-HD was given on day 1 ( $185 \pm 4$  vs.  $97 \pm 17$ , respectively).

**GROUP VIII (DIAZOXIDE GROUP).** This group was studied to determine whether pharmacologic opening of K<sub>ATP</sub> channels has an antistunning effect in this model. Although on day 1 the extent of paradoxical wall thinning in these rabbits was similar to that noted in group

V (control group), the recovery of WTh after the sixth reperfusion was faster than in group V, and this improvement was sustained throughout the entire reperfusion interval (Fig. 7). The total deficit of WTh in this group was 60% less than that observed in group V on day 1 ( $P < 0.05$ ) and similar to that observed in group V on days 2 and 3 (Fig. 8). Thus infusion of diazoxide on day 1 produced an attenuation of myocardial stunning that was indistinguishable from that observed during the late phase of ischemic PC.

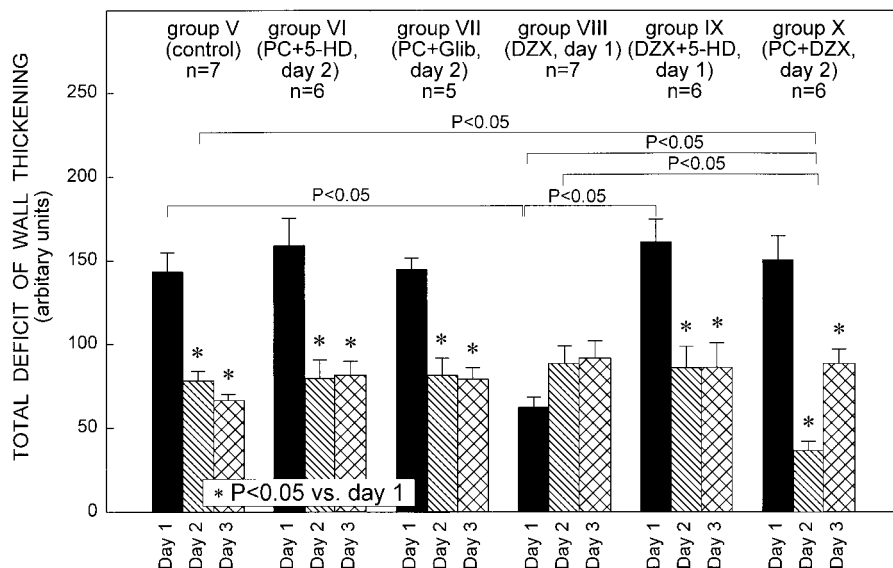
**GROUP IX (DIAZOXIDE + 5-HD GROUP).** The combination of diazoxide and 5-HD was studied to determine whether the dosage of 5-HD that we used in group VI was sufficient to inhibit K<sub>ATP</sub> channels in this model. When 5-HD and diazoxide were coadministered on day 1, the recovery of WTh after the six 4-min occlusion/4-min reperfusion cycles was not improved (Fig. 7), and the total deficit of WTh was not decreased compared with those observed on day 1 in group V (control group) (Fig. 8). Thus the same dose of 5-HD that failed to block the antistunning effect of late PC (Fig. 6) completely blocked the antistunning effect of diazoxide (Fig. 7), indicating that this dose of 5-HD was sufficient to inhibit pharmacologically induced opening of K<sub>ATP</sub> channels in this model.

**GROUP X (PC + DIAZOXIDE GROUP).** Diazoxide was administered on day 2 to determine whether opening of K<sub>ATP</sub> channels and late PC have additive effects in this model. When preconditioned rabbits were given diazoxide on day 2, the recovery of WTh after the six 4-min occlusion/4-min reperfusion cycles was further improved (Fig. 7), and the deficit of WTh was further decreased (Fig. 8) compared with those observed on day 2 in untreated preconditioned rabbits (group V) (Fig. 8) or on day 1 in diazoxide-treated nonpreconditioned rabbits (group VIII) (Fig. 8). Thus diazoxide and late PC exerted additive antistunning effects.

## DISCUSSION

Little information is currently available regarding the role of K<sub>ATP</sub> channels in late PC against myocardial

Fig. 8. Total deficit of wall thickening (WTh) after the sixth reperfusion on days 1, 2, and 3 in groups V (control group,  $n = 7$ ), VI (5-HD,  $n = 6$ ), VII (glibenclamide,  $n = 5$ ), VIII [diazoxide (DZX),  $n = 7$  on days 1 and 2 and  $n = 6$  on day 3], IX (DZX + 5-HD,  $n = 6$  on days 1 and 2 and  $n = 5$  on day 3), and X (DZX,  $n = 6$ ). Data are means  $\pm$  SE. The total deficit of WTh was measured in arbitrary units.



infarction, and nothing is known regarding their role in late PC against myocardial stunning. The present study provides significant new information regarding these issues. Our data demonstrate that, in conscious rabbits, the K<sub>ATP</sub> channel blocker 5-HD abrogates the protective effects of late PC against infarction, whereas neither 5-HD nor glibenclamide abrogates the protective effects of late PC against stunning, indicating that K<sub>ATP</sub> channel opening is necessary for the former but not the latter. The same dose of 5-HD that failed to block the antistunning effect of late PC did block the antistunning effect of diazoxide, suggesting that the mechanism of the protection induced by the late phase of ischemic PC includes phenomena other than K<sub>ATP</sub> channel opening. The conclusion that the antistunning effects of the late phase of ischemic PC are not mediated via opening of the K<sub>ATP</sub> channels is further corroborated by the finding that pharmacological opening of K<sub>ATP</sub> channels with diazoxide produces attenuation of myocardial stunning above and beyond that produced by the late phase of ischemic PC, suggesting two different modes of action for these two protective interventions.

Previous studies have implicated K<sub>ATP</sub> channels as mediators of the infarct-sparing effects of pharmacologically induced late PC in dogs (23) and of ischemically induced late PC in open-chest rabbits (5). To our knowledge, this is the first report to indicate a differential role of K<sub>ATP</sub> channels in the late phase of ischemic PC against myocardial stunning vis-a-vis infarction. Our findings imply an important difference in the mechanism of these two forms of cardioprotection. Furthermore, this is the first study to examine the role of K<sub>ATP</sub> channels in ischemia-induced late PC against infarction in a conscious animal preparation devoid of the potentially confounding effects of anesthesia and other abnormal conditions associated with open-chest models (21).

In contrast to the early phase of ischemic PC, which protects against infarction but not against stunning (6, 24, 27), the late phase of ischemic PC protects against both infarction (30, 42) and stunning (7, 8, 13, 28, 29, 35, 37, 38, 41). One important issue that has not been addressed thus far is whether the mechanism of late PC-induced protection against stunning is the same as that of late PC-induced protection against infarction. Myocardial stunning and infarction are two very different phenomena, and conclusions regarding the pathophysiology of one cannot necessarily be extrapolated to the other (6, 24, 31). Accordingly, in the present investigation, we studied the role of K<sub>ATP</sub> channels in both of these settings. In accordance with previous studies of early (2, 18) and late (5, 23) PC, we found that 5-HD completely blocked the infarct-sparing effect of the late phase of ischemic PC in our conscious rabbit model. Because 5-HD did not alter infarct size in nonpreconditioned rabbits, the abrogation of late PC cannot be ascribed to a detrimental effect of this agent. Therefore, K<sub>ATP</sub> channels appear to be common effectors of the infarct-sparing actions of both the early and the late phases of ischemic PC.

In contrast, both of the K<sub>ATP</sub> channel blockers tested, 5-HD and glibenclamide, failed to block the antistunning effect of late PC. This result cannot be ascribed to insufficient dosage of 5-HD, because the same dose of 5-HD blocked the protection induced by the K<sub>ATP</sub> channel opener diazoxide (the magnitude of diazoxide-induced protection was equivalent to that induced by the late phase of ischemic PC). On the basis of these results, we propose that late PC confers cardioprotection through at least two distinct mechanisms, one involving opening of K<sub>ATP</sub> channels, which is operative against cell death, and the other involving K<sub>ATP</sub> channel-independent mechanisms, which is operative against reversible postischemic dysfunction.

Our conclusion that late PC against stunning does not require opening of K<sub>ATP</sub> channels is not in conflict with the finding that pharmacological opening of K<sub>ATP</sub> channels by diazoxide alleviated myocardial stunning in *group VIII*. These results could be reconciled if late PC activates multiple cardioprotective mechanisms, and at least one of them (besides opening of K<sub>ATP</sub> channels) is sufficient to alleviate stunning. Because myocardial stunning reflects a milder degree of injury than myocardial infarction, it is not implausible that other beneficial changes brought about by late PC, even though not sufficient to prevent cell death, may be sufficient to alleviate stunning. Alternatively, opening of K<sub>ATP</sub> channels might occur earlier and to a greater extent during a sustained 30-min coronary occlusion than during brief 4-min occlusions interspersed with reperfusion, so that blocking K<sub>ATP</sub> channels would have a greater impact on the former. Regardless of these conjectures, our data indicate that K<sub>ATP</sub> channel opening is sufficient but not necessary for late PC against stunning, whereas it is necessary for late PC against infarction.

Recent studies (8, 16, 36) support the concept that late PC against both stunning and infarction is mediated by increased synthesis of NO by iNOS. The mechanism whereby NO protects during late PC remains speculative. Studies from Marbán's group (33) have demonstrated in isolated myocytes that administration of the NO donor *S*-nitroso-*N*-acetylpenicillamine selectively opens the mitochondrial K<sub>ATP</sub> channels and also potentiates the opening of mitochondrial K<sub>ATP</sub> channels by diazoxide. In addition, NO has been found to open the sarcolemmal K<sub>ATP</sub> channel (9, 25, 34). Because 5-HD is a selective blocker of mitochondrial K<sub>ATP</sub> channels (22), the present results are consistent with the concept that NO-dependent late PC against infarction (but not late PC against stunning) is mediated, at least in part, via opening of the mitochondrial K<sub>ATP</sub> channel. Another interesting finding of this investigation is the demonstration that diazoxide, in the absence of hemodynamic alterations, exerts a powerful antistunning effect, equivalent to that elicited by the late phase of ischemic PC. Thus opening of K<sub>ATP</sub> channels, in itself, can alleviate myocardial stunning, although this mechanism does not appear to be necessary for the protection afforded by late PC.

In conclusion, this study demonstrates that in conscious, chronically instrumented rabbits, opening of K<sub>ATP</sub> channels is necessary for the infarct-sparing effects of late PC to become manifest. In contrast, opening of K<sub>ATP</sub> channels does not appear to be necessary for the antistunning effect of late PC, because 1) the same dose of 5-HD that blocked the antistunning effect of diazoxide failed to block the antistunning effect of late PC, and 2) glibenclamide also failed to abrogate late PC against stunning. The fact that diazoxide alleviated myocardial stunning indicates that opening of K<sub>ATP</sub> channels does protect against this type of dysfunction; however, the fact that diazoxide and late PC had additive antistunning effects indicates that K<sub>ATP</sub> channels opening is not the sole or indispensable mechanism whereby late PC protects against stunning. The differential involvement of K<sub>ATP</sub> channels in late PC against infarction and late PC against stunning reveals an important pathogenetic difference between these two forms of cardioprotection and warrants further investigation into the K<sub>ATP</sub> channel-independent mechanisms that alleviate stunning during late PC.

We gratefully acknowledge Gregg Shirk and Larisa Hodge for expert technical assistance.

This study was supported in part by National Institutes of Health Grants R01 HL-43151 and HL-55757 (to R. Bolli), by American Heart Association Ohio Valley Affiliate Grant 9951533V (to X.-L. Tang), by the American Heart Association Ohio Valley Affiliate Fellowship Award 9804558 (to H. Takano), and by the Medical Research Grant Program of the Jewish Hospital Foundation, Louisville, Kentucky. H. Takano was an International Research Fellow from Nippon Medical School, Tokyo.

## REFERENCES

1. **Auchampach JA, Grover GJ, and Gross GJ.** Blockade of ischaemic preconditioning in dogs by the novel ATP-dependent potassium channel antagonist sodium 5-hydroxydecanoate. *Cardiovasc Res* 26: 1054–1062, 1992.
2. **Baines CP, Liu GS, Birincioglu M, Critz SD, Cohen MV, and Downey JM.** Ischemic preconditioning depends on interaction between mitochondrial K<sub>ATP</sub> channels and actin cytoskeleton. *Am J Physiol Heart Circ Physiol* 276: H1361–H1368, 1999.
3. **Baxter GF, Goma FM, and Yellon DM.** Characterisation of the infarct-limiting effect of delayed preconditioning: timecourse and dose-dependency studies in rabbit myocardium. *Basic Res Cardiol* 92: 159–167, 1997.
4. **Baxter GF and Yellon DM.** ATP-sensitive K<sup>+</sup> channels mediate the delayed cardioprotective effect of adenosine A1 receptor activation. *J Mol Cell Cardiol* 31: 981–989, 1999.
5. **Bernardo NL, D'Angelo M, Okubo S, Joy A, and Kukreja RC.** Delayed ischemic preconditioning is mediated by opening of ATP-sensitive potassium channels in the rabbit heart. *Am J Physiol Heart Circ Physiol* 276: H1323–H1330, 1999.
6. **Bolli R.** The early and late phases of preconditioning against myocardial stunning and the essential role of oxyradicals in the late phase: an overview. *Basic Res Cardiol* 91: 57–63, 1996.
7. **Bolli R, Bhatti ZA, Tang XL, Qiu Y, Zhang Q, Guo Y, and Jadoon AK.** Evidence that late preconditioning against myocardial stunning in conscious rabbits is triggered by the generation of nitric oxide. *Circ Res* 81: 42–52, 1997.
8. **Bolli R, Manchikalapudi S, Tang XL, Takano H, Qiu Y, Guo Y, Zhang Q, and Jadoon AK.** The protective effect of late preconditioning against myocardial stunning in conscious rabbits is mediated by nitric oxide synthase. Evidence that nitric oxide acts both as a trigger and as a mediator of the late phase of ischemic preconditioning. *Circ Res* 81: 1094–1107, 1997.
9. **Cameron JS, Kibler KKA, Berry H, Barron DN, and Soderder VH.** Nitric oxide activates ATP-sensitive potassium channels in hypertrophied ventricular myocytes (Abstract). *FASEB J* 10: A65, 1996.
10. **Carr CS and Yellon DM.** Ischaemic preconditioning may abolish the protection afforded by ATP-sensitive potassium channel openers in isolated human atrial muscle. *Basic Res Cardiol* 92: 252–260, 1997.
11. **Cohen MV and Downey JM.** Preconditioning during ischemia; basic mechanisms and potential clinical applications. *Cardiol Rev* 3: 998–1004, 1995.
12. **Cohen MV, Yang XM, and Downey JM.** Conscious rabbits become tolerant to multiple episodes of ischemic preconditioning. *Circ Res* 74: 998–1004, 1994.
13. **Dawn B, Xuan YT, Qiu Y, Takano H, Tang XL, Ping P, Banerjee S, Hill M, and Bolli R.** Bifunctional role of protein tyrosine kinases in late preconditioning against myocardial stunning in conscious rabbits. *Circ Res* 85: 1154–1163, 1999.
14. **Downey JM.** Ischemic preconditioning: nature's own cardioprotective intervention. *Trends Cardiovasc Med* 2: 170–176, 1992.
15. **Gross GJ and Auchampach JA.** Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. *Circ Res* 70: 223–233, 1992.
16. **Guo Y, Jones WK, Xuan YT, Tang XL, Bao W, Wu WJ, Han H, Laubach VE, Ping P, Yang Z, Qiu Y, and Bolli R.** The late phase of ischemic preconditioning is abrogated by targeted disruption of the inducible NO synthase gene. *Proc Natl Acad Sci USA* 96: 11507–11512, 1999.
17. **Haruna T, Horie M, Kouchi I, Nawada R, Tsuchiya K, Akao M, Otani H, Murakami T, and Sasayama S.** Coordinate interaction between ATP-sensitive K<sup>+</sup> channel and Na<sup>+</sup>, K<sup>+</sup>-ATPase modulates ischemic preconditioning. *Circulation* 98: 2905–2910, 1998.
18. **Hide EJ and Thiernemann C.** Limitation of myocardial infarct size in the rabbit by ischaemic preconditioning is abolished by sodium 5-hydroxydecanoate. *Cardiovasc Res* 31: 941–946, 1996.
19. **Jones WK, Flaherty MP, Tang XL, Takano H, Qiu Y, Banerjee S, Smith T, and Bolli R.** Ischemic preconditioning increases iNOS transcript levels in conscious rabbits via a nitric oxide-dependent mechanism. *J Mol Cell Cardiol* 31: 1469–1481, 1999.
20. **Kuzuya T, Hoshida S, Yamashita N, Fuji H, Oe H, Hori M, Kamada T, and Tada M.** Delayed effects of sublethal ischemia on the acquisition of tolerance to ischemia. *Circ Res* 72: 1293–1299, 1993.
21. **Li XY, McCay PB, Zughuib M, Jeroudi MO, Triana JF, and Bolli R.** Demonstration of free radical generation in the "stunned" myocardium in the conscious dog and identification of major differences between conscious and open-chest dogs. *J Clin Invest* 92: 1025–1041, 1993.
22. **Liu Y, Sato T, Seharaseyon J, Szewczyk A, O'Rourke B, and Marban E.** Mitochondrial ATP-dependent potassium channels. Viable candidate effectors of ischemic preconditioning. *Ann NY Acad Sci* 874: 27–37, 1999.
23. **Mei DA, Elliott GT, and Gross GJ.** K<sub>ATP</sub> channels mediate late preconditioning against infarction produced by monophosphoryl lipid A. *Am J Physiol Heart Circ Physiol* 271: H2723–H2729, 1996.
24. **Miyamae M, Fujiwara H, Kida M, Yokota R, Tanaka M, Katsuragawa M, Hasegawa K, Ohura M, Koga K, and Yabuuchi Y.** Preconditioning improves energy metabolism during reperfusion but does not attenuate myocardial stunning in porcine hearts. *Circulation* 88: 223–234, 1993.
25. **Murphy ME and Brayden JE.** Nitric oxide hyperpolarizes rabbit mesenteric arteries via ATP-sensitive potassium channels. *J Physiol (Lond)* 486: 47–58, 1995.
26. **Murry CE, Jennings RB, and Reimer KA.** Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 74: 1124–1136, 1986.
27. **Ovize M, Przyklenk K, Hale SL, and Kloner RA.** Preconditioning does not attenuate myocardial stunning. *Circulation* 85: 2247–2254, 1992.
28. **Ping P, Takano H, Zhang J, Tang XL, Qiu Y, Li RC, Banerjee S, Dawn B, Balafonova Z, and Bolli R.** Isoform-selective activation of protein kinase C by nitric oxide in the heart of

- conscious rabbits: a signaling mechanism for both nitric oxide-induced and ischemia-induced preconditioning. *Circ Res* 84: 587–604, 1999.
29. **Qiu Y, Ping P, Tang XL, Manchikalapudi S, Rizvi A, Zhang J, Takano H, Wu WJ, Teschner S, and Bolli R.** Direct evidence that protein kinase C plays an essential role in the development of late preconditioning against myocardial stunning in conscious rabbits and that epsilon is the isoform involved. *J Clin Invest* 101: 2182–2198, 1998.
  30. **Qiu Y, Rizvi A, Tang XL, Manchikalapudi S, Takano H, Jadoon AK, Wu WJ, and Bolli R.** Nitric oxide triggers late preconditioning against myocardial infarction in conscious rabbits. *Am J Physiol Heart Circ Physiol* 273: H2931–H2936, 1997.
  31. **Qiu Y, Tang XL, Park SW, Sun JZ, Kalya A, and Bolli R.** The early and late phases of ischemic preconditioning: a comparative analysis of their effects on infarct size, myocardial stunning, and arrhythmias in conscious pigs undergoing a 40-minute coronary occlusion. *Circ Res* 80: 730–742, 1997.
  32. **Rizvi A, Tang XL, Qiu Y, Xuan YT, Takano H, Jadoon AK, and Bolli R.** Increased protein synthesis is necessary for the development of late preconditioning against myocardial stunning. *Am J Physiol Heart Circ Physiol* 277: H874–H884, 1999.
  33. **Sasaki N, Sato T, Ohler A, O'Rourke B, and Marban E.** Activation of mitochondrial ATP-dependent potassium channels by nitric oxide. *Circulation* 101: 439–445, 2000.
  34. **Shinbo A and Iijima T.** Potentiation by nitric oxide of the ATP-sensitive K<sup>+</sup> current induced by K<sup>+</sup> channel openers in guinea-pig ventricular cells. *Br J Pharmacol* 120: 1568–1574, 1997.
  35. **Sun JZ, Tang XL, Knowlton AA, Park SW, Qiu Y, and Bolli R.** Late preconditioning against myocardial stunning. An endogenous protective mechanism that confers resistance to postischemic dysfunction 24 h after brief ischemia in conscious pigs. *J Clin Invest* 95: 388–403, 1995.
  36. **Takano H, Manchikalapudi S, Tang XL, Qiu Y, Rizvi A, Jadoon AK, Zhang Q, and Bolli R.** Nitric oxide synthase is the mediator of late preconditioning against myocardial infarction in conscious rabbits. *Circulation* 98: 441–449, 1998.
  37. **Takano H, Tang XL, Qiu Y, Guo Y, French BA, and Bolli R.** Nitric oxide donors induce late preconditioning against myocardial stunning and infarction in conscious rabbits via an antioxidant-sensitive mechanism. *Circ Res* 83: 73–84, 1998.
  38. **Tang XL, Qiu Y, Park SW, Sun JZ, Kalya A, and Bolli R.** Time course of late preconditioning against myocardial stunning in conscious pigs. *Circ Res* 79: 424–434, 1996.
  39. **Toombs CF, Moore TL, and Shebuski RJ.** Limitation of infarct size in the rabbit by ischaemic preconditioning is reversible with glibenclamide. *Cardiovasc Res* 27: 617–622, 1993.
  40. **Wallenstein S, Zucker CL, and Fleiss JL.** Some statistical methods useful in circulation research. *Circ Res* 47: 1–9, 1980.
  41. **Xuan YT, Tang XL, Banerjee S, Takano H, Li RC, Han H, Qiu Y, Li JJ, and Bolli R.** Nuclear factor- $\kappa$ B plays an essential role in the late phase of ischemic preconditioning in conscious rabbits. *Circ Res* 84: 1095–1109, 1999.
  42. **Yamashita N, Hoshida S, Taniguchi N, Kuzuya T, and Hori M.** A “second window of protection” occurs 24 h after ischemic preconditioning in the rat heart. *J Mol Cell Cardiol* 30: 1181–1189, 1998.
  43. **Yao Z, Mizumura T, Mei DA, and Gross GJ.** K<sub>ATP</sub> channels and memory of ischemic preconditioning in dogs: synergism between adenosine and K<sub>ATP</sub> channels. *Am J Physiol Heart Circ Physiol* 272: H334–H342, 1997.

