Adenosine A1/A2a receptor agonist AMP-579 induces acute and delayed preconditioning against in vivo myocardial stunning

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Kristo, Gentian, Yukihiro Yoshimura, Byron J. Keith, Randy M. Stevens, Salik A. Jahania, Robert M. Mentzer, Jr., and Robert D. Lasley. Adenosine A1/A2a receptor agonist AMP-579 induces acute and delayed preconditioning against in vivo myocardial stunning. Am J Physiol Heart Circ Physiol 287: H2746–H2753, 2004. First published July 22, 2004; doi:10.1152/ajpheart.00493.2004.—The purpose of this study was to determine whether the adenosine A1/A2a receptor agonist AMP-579 induces acute and delayed preconditioning against in vivo myocardial stunning. Regional stunning was produced by 15 min of coronary artery occlusion and 3 h of reperfusion (RP) in anesthetized open-chest pigs. In acute protection studies, animals were pretreated with saline, low-dose AMP-579 (15 μg/kg iv bolus 10 min before ischemia), or high-dose AMP-579 (50 μg/kg iv bolus + 1.2 μg·kg·min−1 for 30 min before coronary occlusion). The delayed preconditioning effects of AMP-579 were evaluated 24 h after administration of saline vehicle or high-dose AMP-579 (50 μg/kg iv). Load-insensitive contractility was assessed by measuring regional preload recruitable stroke work (PRSW) and PRSW area. Acute preconditioning with AMP-579 dose dependently improved regional PRSW: 129 ± 5 and 100 ± 2% in high- and low-dose AMP-579 groups, respectively, and 78 ± 5% in the control group at 3 h of RP. Administration of the adenosine A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (0.7 mg/kg) blocked the acute protective effect of high-dose AMP-579, indicating that these effects are mediated through A1 receptor activation. Delayed preconditioning with AMP-579 significantly increased recovery of PRSW area: 64 ± 5 vs. 33 ± 5% in control at 3 h of RP. In isolated perfused rat heart studies, kinetics of the onset and washout of AMP-579 A1 and A2a receptor-mediated effects were distinct compared with those of other adenosine receptor agonists. The unique nature of the adenosine agonist AMP-579 may play a role in its ability to induce delayed preconditioning against in vivo myocardial stunning.

Adenosine receptors; ischemia-reperfusion

THE CARDIOPROTECTIVE EFFECTS of adenosine are well recognized. Adenosine administered before ischemia and/or during reperfusion has been shown to attenuate reversible posts ischemic contractile dysfunction (myocardial stunning) and reduce infarct size in isolated hearts, isolated myocytes, and in vivo preparations (14, 29). These effects are mediated primarily via the activation of cardiac adenosine A1 receptors before ischemia and A2a receptor subtypes during reperfusion. More recent studies have documented a second aspect of adenosine receptor-mediated myocardial protection. Bolus injections of adenosine A1 receptor agonists have been shown to induce delayed protection, referred to as delayed or late-phase preconditioning, 24 h later (2, 13, 27). This delayed protection may persist for up to 72 h (2). Adenosine A1 receptor delayed preconditioning has been shown to decrease infarct size in in vivo and isolated heart preparations in multiple species (2, 13, 27).

Although acute and delayed preconditioning with adenosine A1 receptor agonists improves posts ischemic function in globally ischemic isolated heart preparations (4, 16), these models utilize ischemic periods (≥20 min) that are associated with irreversible injury. Thus the improved function is likely to be due in part to less cell death. There have been very few studies documenting the acute beneficial effects of adenosine receptor agonists in in vivo stunned myocardium (1, 10, 15, 34). A limiting factor with acute in vivo studies has been the lack of selectivity of available adenosine A1 receptor agonists. Therapeutic doses of adenosine A1 receptor agonists, such as 2-chloro-N6-cyclopropyl adenosine (CCPA) and N6-phenylisopropyl adenosine (PIA), which decrease in vivo myocardial infarct size, are generally associated with significant and sustained hypotension and bradycardia (6, 23, 26, 28). These hemodynamic effects preclude their use in stunning studies, because changes in preload and afterload may alter posts ischemic function independent of any direct A1 signaling mechanisms (5a). There are even fewer studies examining A1 receptor delayed preconditioning against in vivo myocardial stunning. In the only published study, Maldonado et al. (19) reported that the adenosine A1 receptor agonist CCPA failed to induce delayed preconditioning against myocardial stunning in conscious rabbits.

The purpose of this study was to address these deficiencies in the literature by using a unique adenosine receptor agonist, AMP-579 ([15-[1a,2b,3b,4a(S*)]]-4-[7-[2-(3-chloro-2-thienyl)-1-methylpropyl][amino]-3H-imidazo[4,5-b]pyridyl]-3-yl)cyclopentane carboxamide). This agonist has high affinity for A1 (Ki = 5 nM) and A2a (Ki = 56 nM) receptor subtypes (22). Consistent with this ability to activate both receptor subtypes, AMP-579 can reduce infarct size in different species (rabbithogs, dogs, and pigs) when administered before ischemia or during reperfusion (9, 20, 21, 24, 30–33). This is one of the unique characteristics of AMP-579 that distinguishes it from other adenosine receptor agonists, because A1 agonists, such as CCPA, are cardioprotective only when administered before ischemia (6, 16, 26), and A2a agonists, such as CGS-21680, are beneficial only during reperfusion (15). Another unique aspect of AMP-579 is its ability to induce protection with little or no hemodynamic effects (20, 21, 24, 30–33).

Despite the apparent distinctive nature of this adenosine receptor agonist, there have been no studies comparing the A1...
and A$_{2a}$ cardiac effects of AMP-579 with those of other adenosine receptor agonists. In the present study, acute and delayed adenosine receptor preconditioning protocols were used to test the ability of AMP-579 to reduce in vivo myocardial stunning in a porcine model of regional myocardial ischemia. Additional studies were conducted in isolated perfused rat hearts to compare the adenosine A$_1$ and A$_{2a}$ effects of AMP-579 with the effects of nonselective and selective adenosine receptor agonists.

**METHODS**

All animals received humane care according to the guidelines set forth in the *Principles of Laboratory Animal Care*, formulated by the National Society for Medical Research, and the *Guide for the Care and Use of Laboratory Animals*, prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, Revised 1996). In addition, animals were used in accordance with the guidelines of the University of Kentucky Institutional Animal Care and Use Protocol.

**Porcine Regional Ischemia**

Domestic pigs of either gender, weighing 20–30 kg, were used. Anesthesia was induced with ketamine (20 mg/kg im), followed by pentobarbital sodium (15–18 mg/kg iv), through an ear vein. Anesthesia was maintained with additional pentobarbital sodium (1.5–2 mg/kg iv) every 15 min. Core body temperature was monitored with an esophageal temperature probe and maintained at 37–37.5°C with a heating pad. A transit time perivascular flow probe (Transonic Systems, Ithaca, NY) was placed around a distal segment of the left anterior descending coronary artery (LAD) to measure coronary blood flow. The remainder of the surgical procedure and cardiac instrumentation were identical to our previously published methods (12, 15).

**Experimental Protocols**

The preparation was allowed to stabilize for 30 min after all instrumentation was complete before the experimental protocol was initiated. Regional myocardial stunning was induced with 15 min of LAD occlusion followed by 3 h of reperfusion. All animals received heparin (100 U/kg iv bolus) before the coronary occlusion. Lidocaine (2% solution, 2 mg/kg iv bolus) was administered before reperfusion. AMP-579 was made as a 50 μg/ml solution in 10% DMSO (balance phosphate-buffered saline). The specific treatment protocols were as follows.

- **AMP-579 acute cardioprotection.** Animals were pretreated with saline (n = 8), low-dose AMP-579 (15 μg/kg iv bolus 10 min before ischemia, n = 7), or high-dose AMP-579 (50 μg/kg iv at 14 μg/kg bolus + 1.2 μg·kg$^{-1}$·min$^{-1}$ for 30 min before coronary occlusion, n = 7). Additional pigs were given 1.3-dipropyl-8-cyclopentylxanthine (DPCPX, 0.7 mg/kg iv bolus 30 min before the high dose of AMP-579, n = 4). The adenosine A$_1$ receptor antagonist DPCPX was administered as a 10% DMSO-10% 0.2 N NaOH solution in saline.
- **AMP-579 delayed preconditioning against myocardial stunning.** Pigs (n = 8/group) were sedated with ketamine (20 mg/kg im) and received a 40-min intravenous infusion of AMP-579 (total dose 50 μg/kg) or vehicle 24 h before the myocardial stunning protocol. On the next day, anesthetized open-chest pigs underwent 15 min of LAD occlusion and 3 h of reperfusion.

**Assessment of Regional Myocardial Function and Contractility**

Pairs of piezoelectric segment-shortening crystals (Crystal Biotech, Houston, TX) were placed in the LAD and left circumflex coronary artery perfused beds to measure segment shortening (SS) by sonomicroscopy. Crystals were placed in the midmyocardium (4–6 mm deep) 5–15 mm apart and aligned such that the intercrystal axis was parallel to the direction of myocardial fiber shortening. SS was defined as end-diastolic length (EDL) − end-systolic length (ESL), and percent SS was calculated as [(EDL − ESL)/EDL] × 100%.

Measurements of load-insensitive parameters of regional cardiac contractility, preload recruitable stroke work (PRSW), and PRSW area (PRSWA), were generated from the segment length, and left ventricular (LV) pressure data were collected during brief (7-s) vena cava occlusions using the methods of Glower et al. (7, 8), as previously described (12, 15). The inferior vena cava was occluded by gradual tightening of a snare formed of umbilical tape around its supradiaphragmatic portion. During data acquisition, ventilation was held at end expiration to avoid effects of varying venous return on preload. Baseline and caval occlusion data were saved at specific times in the protocol for offline analysis. An average of 9–11 beats was used in each calculation. All hemodynamic and sonomicroscopy signals were fed through a 32-bit analog-to-digital converter into an online data acquisition computer with customized software (Augury, Coyote Bay Instruments, Manchester, NH). All hemodynamic data were continuously displayed on a computer monitor.

**Area at Risk and Necrosis Measurements**

After 3 h of reperfusion, the ischemic area at risk was determined by reocclusion of the LAD and infusion of a 0.5% Evans blue solution into the LV during occlusion of the aorta. The area at risk was devoid of the Evans blue stain. The animals were then euthanized, the hearts were excised, and the LV were divided into four slices of equal thickness in a plane parallel to the atroventricular groove. The slices were incubated in a 1% triphenyltetrazolium chloride (TTC) solution in phosphate-buffered saline solution at 37°C for 15 min for determination of the presence of any TTC-negative or infarcted tissue within the area at risk.

**Isolated Perfused Rat Heart Preparation**

Experiments were conducted in adult male Sprague-Dawley rats (300–350 g). Animals were heparinized and then anesthetized with pentobarbital sodium (65 mg/kg ip). The heart was rapidly excised and immediately placed into ice-cold Krebs-Henseleit buffer (KHB) to produce cardiac arrest. After cannulation of the aorta, hearts were initially perfused at a coronary perfusion pressure of 70 mmHg with KHB consisting of (in mM) 118 NaCl, 4.7 KCl, 1.2 MgSO$_4$, 1.2 KH$_2$PO$_4$, 1.5 CaCl$_2$, 25.0 NaHCO$_3$, 11.0 glucose, 1.0 pyruvate, and 0.005 EDTA. The perfusate also contained 15 g/l dextran (68,800 avg mol wt). The perfusate was maintained at 37°C in a constant-temperature reservoir and bubbled with 95% O$_2$-5% CO$_2$, resulting in pH 7.4. Myocardial temperature was maintained at 37°C by partial submersion of the heart in a water-jacketed chamber filled with KHB. A fluid-filled latex balloon was inserted into the LV to monitor LV function and heart rate.

Isolated hearts were allowed 15 min of equilibration; then coronary flow was maintained constant during the generation of dose-response curves with AMP-579 (n = 6) or the A$_1$/A$_2$ agonist 5’-(N-ethylcarboxamido)adenosine (NECA, n = 5). After exposure to the final dose (100 nM) of each agent, the hearts were monitored for an additional 20 min for assessment of the kinetics of washout. An additional series of hearts (2–3 per group) were exposed to one of the following agonists at 5 or 50 nM: 1) AMP-579, 2) NECA, 3) the A$_1$ agonist CCPA, or 4) the A$_{2a}$ agonist CGS-21680. Coronary flow and heart rate responses were recorded. Each heart was exposed to only one agonist.

**Statistical Analysis**

Myocardial stunning results are means ± SE and were analyzed by two-way ANOVA for treatment and time followed by posttest analysis with Bonferroni’s test. Intragroup differences were analyzed by one-way ANOVA with repeated measures. Isolated heart data were
Table 1. Systemic hemodynamics and coronary blood flow in acute AMP-579 preconditioning protocol

<table>
<thead>
<tr>
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<th>Baseline</th>
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<td>HR, beats/min</td>
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<tr>
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<td>88±3‡†</td>
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<tr>
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<td>Control CBF, ml/min</td>
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Values are means ± SE; *n = 8 (control), n = 7 (low- and high-dose AMP-579), and n = 4 [1,3-dipropyl-8-cyclopentylxanthine (DPCPX) + high-dose AMP-579]. Baseline values were measured before onset of 15-min left anterior descending coronary artery occlusion; ischemia values were measured at 15 min of occlusion. RP, reperfusion; HR, heart rate; MAP, mean arterial pressure; CBF, coronary blood flow. †P < 0.05 vs. control. ‡P < 0.05 vs. baseline. *P < 0.05 vs. DPCPX + high-dose AMP-579.

analyzed by one-way ANOVA. P < 0.05 was considered statistically significant.

RESULTS

AMP-579 Acute Preconditioning Against Myocardial Stunning

Table 1 summarizes systemic hemodynamic and coronary blood flow data in the acute preconditioning protocol. At baseline and at 1 h of reperfusion, the LAD coronary blood flow was significantly higher in the low-dose AMP-579 group than in the control group. In the DPCPX + high-dose AMP-579 group, the heart rate was higher than in the control group during ischemia and higher than in the high-dose AMP-579 group during drug administration and ischemia. There were no other significant differences in hemodynamics among the groups. Administration of high-dose AMP-579 was associated with a significant decrease in heart rate and mean arterial pressure (MAP). The reduction in heart rate persisted through the 1st h of reperfusion. The ischemic areas at risk were similar in all groups (25 ± 2% in control, 21 ± 2% in low-dose AMP-579, 23 ± 2% in high-dose AMP-579, and 28 ± 1% in DPCPX + high-dose AMP-579). There was no evidence of infarction (based on TTC-negative staining) in any animals. Regional ventricular function and contractility in the left circumflex coronary bed in all groups remained stable throughout the experiment (data not shown).

Figure 1 summarizes the recovery of SS in the acute preconditioning protocol. There were no significant differences in baseline SS values between groups. Although recovery of SS in the animals treated with low and high doses of AMP-579 tended to be higher than in controls, these effects were not statistically different. Similarly, SS in the DPCPX + AMP-579 group was not statistically different from control.

Figure 2 illustrates the effects of acute AMP-579 treatments on LAD PRSW. The preischemic PRSW values were similar in all four groups. Preischemic administration of low and high doses of AMP-579 significantly improved recovery of PRSW throughout reperfusion compared with the control group. At 3 h of reperfusion, recovery of PRSW was greater in the high-dose AMP-579 group. Treatment with the adenosine A1 antagonist DPCPX blocked the effects of high-dose AMP-579 on PRSW recovery. Recovery of PRSW at 1, 2, and 3 h of reperfusion was 48 ± 5, 62 ± 6, and 78 ± 5%, respectively, in the control group; 79 ± 7, 86 ± 3, and 100 ± 2%, respectively, in the low-dose AMP-579 group; 74 ± 5,
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102 ± 5, and 129 ± 5%, respectively, in the high-dose AMP-579 group; and 55 ± 9, 66 ± 9, and 76 ± 12%, respectively, in the DPCPX + high-dose AMP-579 group.

The effects of AMP-579 treatment on LAD PRSWA are shown in Fig. 3. Baseline PRSWA values were similar between groups. There were no differences in PRSWA recovery during reperfusion between the control and low-dose AMP-579 groups. Treatment with high-dose AMP-579 enhanced PRSWA throughout reperfusion, although the improvement at 1 h of reperfusion was not statistically significant. Recovery of PRSWA with high-dose AMP-579 was blocked by pretreatment with DPCPX. Recovery of PRSWA at 2 and 3 h of reperfusion was 29 ± 3 and 41 ± 3%, respectively, in the control group; 36 ± 6 and 39 ± 4%, respectively, in the low-dose AMP-579 group; 48 ± 3 and 60 ± 3%, respectively, in the high-dose AMP-579 group; and 27 ± 3 and 39 ± 9%, respectively, in the DPCPX + high-dose AMP-579 group.

AMP-579 Delayed Preconditioning Against Myocardial Stunning

There were no significant differences in heart rate, MAP, or LAD coronary blood flow between the groups at any time during the experimental protocol, except at 2 h of reperfusion, when MAP was greater in the AMP-579 than in the control group (Table 2). The ischemic areas at risk were similar in both groups: 24 ± 1 and 24 ± 2% in the control and AMP-579 groups, respectively. There was no TTC evidence of infarction in either group.

There was no difference in preischemic SS values between the two groups (Fig. 4). Animals that were preconditioned with AMP-579 24 h before LAD occlusion achieved a faster rate of SS recovery throughout reperfusion. The differences in SS between the two groups were statistically significant after the

Fig. 2. Recovery of preload recruitable stroke work (PRSW) in control (n = 8), low-dose AMP-579 (n = 7), high-dose AMP-579 (n = 7), and DPCPX + high-dose AMP-579 (n = 4) groups after 15 min of LAD occlusion. PRSW was based on linear regression of stroke work-end-diastolic segment length relation, according to the methods of Glower et al. (7, 8). Values (means ± SE) are expressed as percent recovery of baseline PRSW. *P < 0.05 vs. control. †P < 0.05 vs. low-dose AMP-579. ‡P < 0.05 vs. DPCPX + high-dose AMP-579.

Fig. 3. Effects of preischemic treatments with low- and high-dose AMP-579 on recovery of load-independent PRSW area (PRSWA) after 15 min of LAD occlusion. PRSWA was calculated from PRSW and segment length according to the methods of Glower et al. (7, 8). Values (means ± SE) are expressed as percent recovery of baseline PRSWA; n = 8 in control group, n = 7 in low- and high-dose AMP-579 groups, and n = 4 in DPCPX + high-dose AMP-579 group. *P < 0.05 vs. control; †P < 0.05 vs. low-dose AMP-579; ‡P < 0.05 vs. DPCPX + high-dose AMP-579.

Fig. 4. Baseline and reperfusion segment shortening in control and 24-h AMP-579-preconditioned groups. Values are means ± SE; n = 8 animals per group. *P < 0.05 vs. control.

Table 2. Systemic hemodynamics and coronary blood flow in AMP-579 delayed preconditioning protocol

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<td>CBF, ml/min</td>
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<tr>
<td>Control</td>
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</table>

Values are means ± SE; n = 8. Baseline values were measured before onset of vehicle/AMP-579 infusion; ischemia values were measured at 15 min of occlusion. *P < 0.05 vs. control.
1st h of reperfusion. In the control group, SS at 2 and 3 h of reperfusion was 5.9 ± 1.3 and 7.8 ± 1.7%, respectively. In the AMP-579 group, SS at 2 and 3 h of reperfusion was 9.9 ± 0.8 and 12.1 ± 0.7%, respectively.

Figure 5 represents the effects of 24-h delayed preconditioning with AMP-579 on LAD PRSW. AMP-579 significantly increased PRSW recovery throughout reperfusion. Recovery of PRSW in the control group at 1, 2, and 3 h of reperfusion was 50 ± 9, 68 ± 5, and 69 ± 6%, respectively. In the AMP-579-preconditioned group, recovery of PRSW at the same time points was 74 ± 6, 100 ± 5, and 119 ± 7%, respectively.

Figure 6 summarizes the effects of AMP-579 late-phase preconditioning on LAD PRSWA. There were no differences in preischemic PRSWA values between groups. Delayed preconditioning with AMP-579 significantly improved PRSWA recovery throughout reperfusion. After 3 h of reperfusion, recovery of PRSWA was 64 ± 5% in AMP-579-treated animals and only 33 ± 5% in the control group.

**Isolated Perfused Heart Results**

The dose-dependent effects of the A<sub>1</sub> and A<sub>2</sub> agonists AMP-579 and NECA on heart rate and coronary perfusion pressure are shown in Fig. 7. Both agonists induced similar reductions in heart rate and coronary perfusion pressure, but recovery of spontaneous heart rate and coronary perfusion pressure was significantly delayed in AMP-579-treated hearts. To generate accurate dose-response curves, the AMP-579 infusions had to be extended to 6–8 min to reach a steady state, whereas NECA effects were attained after 3–4 min.

The kinetic differences among different adenosine receptor agonists are summarized in Fig. 8, in which hearts were perfused with each agonist at 50 or 5 nM. NECA and CCPA reduced heart rate below 200 beats/min within 3 min, whereas AMP-579 had essentially no effect until after 8 min of infusion (Fig. 8A). The maximal effect of AMP-579 on heart rate was not reached until after the infusion was terminated. A similar pattern is shown in Fig. 8B with respect to the delayed effects of AMP-579 on coronary perfusion pressure. The coronary dilating effects of AMP-579 were slower in onset than those of NECA and CGS-21680, and NECA exhibited a much faster washout than AMP-579.

**DISCUSSION**

The primary results of this study indicate that 1) acute pretreatment with the adenosine A<sub>1</sub>/A<sub>2</sub> receptor agonist AMP-
In vivo models of myocardial stunning, there appear to be only two reports documenting the antistunning effects of adenine receptor agonists in models of irreversible injury. The overwhelming majority of these studies have been conducted in small animal models, with only minor, transient reductions in heart rate and blood pressure with the high dose of AMP-579. Although several reports document the in vivo cardioprotective effects of AMP-579 (9, 20, 21, 24, 30–33), our present findings are the first to document the ability of acute AMP-579 pretreatment or preconditioning to attenuate in vivo myocardial stunning. Acute pretreatment with a low dose of AMP-579 significantly enhanced the recovery of regional PRSW, whereas the high dose of AMP-579 significantly increased the recovery of PRSW and PRSWA. However, low and high doses of AMP-579 failed to improve the recovery of SS. The difference between these measurements is that SS is a load-dependent parameter of regional ventricular function, whereas PRSW and PRSWA are load-independent contractility parameters.

Although the vast majority of previous cardioprotection studies with AMP-579 have used reperfusion treatments (9, 30–33), there are two reports that AMP-579 infusion before ischemia reduced myocardial infarct size (20, 24). We also recently reported that preischemic administration of AMP-579 dose dependently reduced infarct size in in vivo porcine myocardium (11). In the present study, pretreatment with the adenosine A1 receptor antagonist DPCPX blocked the high-dose AMP-579-induced decrease in heart rate and protection against myocardial stunning, indicating the involvement of the adenosine A1 receptor. When given alone, DPCPX did not exert any effects on myocardial stunning (data not shown). Although AMP-579 has been reported to decrease infarct size via A2a receptor activation (9, 30), A2a receptor agonists are beneficial only when administered during reperfusion (15, 16, 24, 26, 29–34). In contrast, adenosine A1 agonists are cardioprotective only when administered before ischemia (3, 9, 14, 16, 17, 23, 24, 26, 29). Given these differences in the timing of A1 compared with A2a agonist-mediated protection and the fact that DPCPX blocked the antistunning effect of AMP-579 preconditioning, it appears that the acute protective effects of AMP-579 in stunned myocardium are mediated by A1 receptor activation.

In addition to its acute cardioprotective effects, adenosine A1 receptor activation has also been shown to induce delayed protection against myocardial infarction (2, 13, 27). Although the majority of these studies have been conducted in small animal models, AMP-579 has been shown to induce late-phase preconditioning against infarction in in vivo porcine myocardium (21). The present results are the first to show that an adenosine receptor agonist can induce late-phase preconditioning against myocardial stunning. Delayed preconditioning with AMP-579 improved not only the recovery of regional SS, which is load dependent, but also the recovery of load-insensitive PRSW and PRSWA. The results of additional experiments (n = 3, data not shown) indicated that AMP-579-induced delayed cardioprotection did not persist at 48 h.

There is only one previous study examining the ability of adenosine receptor agonists to induce late-phase preconditioning against myocardial stunning. Maldonado et al. (19) showed that, in conscious rabbits, the development of delayed precon-
tioning against myocardial stunning was not induced by activation of adenosine A1 receptors with CCPA, nor did the nonselective adenosine receptor antagonist 8-p-sulphophenyl theophylline block ischemic preconditioning-induced delayed protection against stunning. The same laboratory previously reported that 8-p-sulphophenyl theophylline failed to block ischemic preconditioning-induced delayed protection against stunning in pigs (25). These observations have been used to support the hypothesis that adenosine receptors are not involved in delayed preconditioning against stunning (5).

The discrepancy between our results and those cited above (19, 25) could be due to species differences and/or methodology. Maldonado et al. (19) used multiple brief occlusions to induce myocardial stunning, which was measured by the deficit in wall thickening. We used a single 15-min coronary occlusion and assessed stunning by not only regional SS but also by the load-insensitive parameters PRSW and PRSWA. Another possible explanation for these differences may be our use of AMP-579, rather than CCPA, to induce A1 receptor late-phase preconditioning. As our isolated heart results indicate, AMP-579 dose-response curves for A1 receptor-induced bradycardia and A2a receptor-induced coronary dilation were similar to those of the nonselective agonist NECA, but the kinetics of the responses were quite different. The times to peak A1 and A2a responses were slower with AMP-579 than with NECA. The time to maximal bradycardia with AMP-579 was slower than with the A1 agonist CCPA, and the time to maximal coronary dilation was slower than with the A2a agonist CGS-21680. Likewise, the washout of AMP-579 was delayed compared with other adenosine receptor agonists. Because these differences in direct A1 and A2a receptor-mediated actions were independent of plasma protein binding and kidney or liver metabolism, there may be fundamental chemical differences between AMP-579 and other adenosine receptor agonists. Despite these differences, the fact that DPCPX blocked the antistunning effects of AMP-579 preconditioning in porcine myocardium suggests that this effect is mediated via A1 receptor activation.

The results of two cardioprotection studies also support the conclusion that AMP-579 is a unique adenosine receptor agonist (9, 30). In both cases, although the infarct-reducing effects of AMP-579 administration during reperfusion were attributed to A2a receptor activation, they were not mimicked by the A2a receptor-selective agonist CGS-21680 (9, 30). In the study by Kis et al. (9), neither A1- nor A2a-selective agonists, alone or in combination, mimicked the protective effect of AMP-579.

In conclusion, in the present study, we demonstrated that the A1/A2a adenosine receptor agonist AMP-579 induces acute and delayed cardioprotection against in vivo myocardial stunning. Enhancing restoration of posts ischemic myocardial contractility with AMP-579 may represent an effective therapeutic approach for reducing morbidity and mortality associated with myocardial ischemia-reperfusion injury.

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