Dipyridamole Potentiates the Myocardial Infarct Size-Limiting Effect of Ischemic Preconditioning

Tetsuji Miura, MD, PhD; Takashi Ogawa, MD; Toshihiro Iwamoto, MD; Kazuaki Shimamoto, MD, PhD; and Osamu Imura, MD, PhD

Background. Recent studies implicated a key role for adenosine (ADO) receptor activation in the enhancement of ischemic tolerance by ischemic preconditioning. In this study, we aimed to test the hypothesis that dipyridamole, an ADO transport inhibitor, enhances the preconditioning effect.

Methods and Results. Six groups of rabbits underwent 30-minute coronary occlusion and 72-hour reperfusion. Infarct size (IS) and the area-at-risk (AR) were determined by histology and by use of fluorescent particles, respectively. IS expressed as the percentage of AR (%IS/AR) was 46.5±3.4% (n=13) in control rabbits. Preconditioning with 2-minute ischemia tended to limit %IS/AR (%IS/AR, 35.5±3.5%, n=9), and that possible protection was abolished by pretreatment with 10 mg/kg 8-phenylthiotheophylline (8-PT), an ADO receptor antagonist (%IS/AR, 43.9±5.8%, n=9). Administration of dipyridamole (0.25 mg/kg) before the 2-minute preconditioning markedly limited %IS/AR to 13.8±2.6% (n=12), indicating the potentiation of the preconditioning effect by this agent. Furthermore, this enhancement of preconditioning effect by dipyridamole treatment was significantly attenuated by 8-PT (%IS/AR, 27.6±2.1%, n=11). Dipyridamole given before the 30-minute ischemia, without preconditioning, did not reduce %IS/AR (55.3±5.2%, n=7), and a previous study from this laboratory had demonstrated that the present dose of 8-PT alone did not modify IS in the rabbit.

Conclusions. Dipyridamole significantly potentiated the IS-limiting effect of preconditioning. This finding strongly supports the hypothesis that stimulation of ADO receptors by endogenous ADO, which builds up during preconditioning ischemia, mediates the increased ischemic tolerance afforded by preconditioning. (Circulation 1992;86:979–985)

KEY WORDS • myocardial infarction • adenosine • preconditioning • dipyridamole

The phenomenon that myocardium exposed to brief transient ischemia obtains enhanced ischemic tolerance to subsequent ischemia has been recognized in several models of ischemic myocardial injury.1–5 This phenomenon is called "preconditioning," and its cardioprotective mechanism is currently the subject of intensive investigation.1–8 Most recently, studies from Downey's laboratory have reported that adenosine receptor antagonists blocked the infarct size-limiting effect of preconditioning and that pretreatment with adenosine-A1 agonists could limit myocardial infarct size in the rabbit.6,7 Based on these findings, they proposed the hypothesis that the protective effect of preconditioning is mediated by adenosine-A1 receptor stimulation.6,7 However, infarct size limitation by exogenous A1 agonists does not necessarily demonstrate that the preconditioning effect is attributable to endogenous adenosine.

If endogenous adenosine, which builds up in the interstitial space during preconditioning, indeed triggers the mechanism of preconditioning, administration of dipyridamole may potentiate the preconditioning effect because this nucleoside transport blocker reportedly enhances ischemia-induced elevation of interstitial adenosine.9,10 To test this hypothesis, we assessed the effect of a combination of dipyridamole and preconditioning on infarct size in the rabbit heart and compared it with the effects of dipyridamole or preconditioning alone. In addition, with the adenosine receptor antagonist 8-phenylthiotheophylline (8-PT),11,12 we analyzed the function of adenosine receptors in the effect of the combination of dipyridamole and preconditioning.

Methods

Surgical Preparation

The surgical preparation was essentially the same as in our previous studies.3,8,13,14 In brief, male rabbits (Japanese White) weighing 2.2–2.7 kg were anesthetized with pentobarbital (40 mg/kg i.v.) and ventilated with a respirator (model 681, Harvard Apparatus, South Natick, Mass.). Systemic blood pressure was monitored by a Nihon-Kohden SCK-580 pressure transducer.
connected to the catheter in the carotid artery. Precordial ECG was monitored by bipolar leads across the chest. The heart was exposed via left thoracotomy, and a 4-0 silk tie was passed around a marginal branch of the left coronary artery with a taper needle. The ends of the silk were threaded through a small vinyl tube to make a coronary snare.

Rabbits were assigned to six experimental groups that received pharmacological treatments and/or preconditioning (see “Experimental Groups”). After the preconditioning protocol, the coronary artery was occluded by tightening the snare. Occlusion of the coronary artery caused cyanosis in the ischemic region and marked ST segment elevation in the ECG. After 30 minutes of coronary occlusion, the snare was released, and the coronary artery was reperfused. Reperfusion was indicated by color change (cyanosis to hyperemia) over the ventricular surface. The silk thread passed around the coronary branch was left in place. The surgical wounds were repaired, and the rabbit was returned to its cage for recovery.

These surgical procedures were performed under sterile conditions, and a combination of 50 mg ampicillin and 50 mg cloxacillin was injected intramuscularly for the prophylaxis of infection. Seventy-two hours after surgery, the rabbit was heparinized intravenously with 2,000 units heparin and then given a pentobarbital overdose. The heart was removed for postmortem analysis.

**Analysis of Infarct Size and Area-at-Risk**

Excised hearts were handled by the same procedure as in our previous studies. In brief, the heart was mounted on a Langendorff apparatus and perfused with saline at 80 mm Hg to wash out blood. The coronary branch was reclosed by ligating the silk tie left around the branch. Fluorescent particles (3–30-μm diameter; Duke Scientific, Palo Alto, Calif.) were then injected into the perfusion line to subsequently determine the area-at-risk. After the atria were removed, the heart was weighed, fixed in 20% buffered formalin for 24 hours, and then stored in 10% formalin for at least 3 days.

The fixed heart was sectioned into 3-mm slices from the apex to the base using a tissue slicer, and two histological slides from each heart slice (one stained with hematoxylin and eosin and the other with Mallory’s connective tissue stain modified by Heidenhain) were prepared by standard histological technique. By illuminating the paraffin block with ultraviolet light, area-at-risk (i.e., the myocardial region of the occluded coronary branch) was visualized as the area deficient of fluorescent particles and traced. Infarct size was traced from the histology slides under ×7 magnification, and the areas of the infarct and the risk region traces were determined using PIAS II, a computer-assisted image-analysis system (PIAS Co., Osaka, Japan). As described previously, the heart slices shrank during the process of fixation and embedding in the paraffin, and the mean ratio of the area after embedding to that before fixation in our model was 0.724. To correct this volume change during tissue preparation and to calculate the original volumes of infarct and area-at-risk, we divided the areas, which were obtained by tracing, by 0.724 and then multiplied by the sample thickness (i.e., 3 mm).

**Experimental Groups**

After the coronary snare was placed and hemodynamic parameters had been stabilized for 5 minutes, the rabbits were divided into six groups and treated as shown in Figure 1. The control group was subjected to simple 30-minute coronary artery occlusion and 72-hour reperfusion. The dipyridamole group was given an intravenous injection of 0.25 mg/kg dipyridamole (Pentarixin injection; Behring-Ingelheim, Japan) at 22 minutes before the 30-minute coronary occlusion. The 2-minute–preconditioning group was preconditioned with 2-minute ischemia and 5-minute reperfusion before the 30-minute coronary occlusion. The dipyridamole–2-minute–preconditioning group and the 8-PT–2-minute–preconditioning groups were treated, at 15 minutes before the 2-minute ischemic preconditioning, with dipyridamole (0.25 mg/kg) and 10 mg/kg 8-PT, respectively. The 8-PT–8-phenyltheophylline–2-minute–preconditioning group was treated with 8-PT before dipyridamole injection and 2-minute preconditioning. The 8-PT solution was prepared according to Jeremy et al,11 sterilized by passing it through a Millex-GS filter.

**TABLE 1. Mortality for Experimental Groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Total</th>
<th>VF*</th>
<th>Survived</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>1</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>DIP</td>
<td>7</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>2’PC</td>
<td>10</td>
<td>1</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>DIP-2’PC</td>
<td>13</td>
<td>1</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>PT-2’PC</td>
<td>10</td>
<td>1</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>PT/DIP-2’PC</td>
<td>12</td>
<td>1</td>
<td>11</td>
<td>8</td>
</tr>
</tbody>
</table>

DIP, dipyridamole; 2’, 2-minute; PC, preconditioning; PT, 8-phenyltheophylline.

*Death: ventricular fibrillation during coronary occlusion.
TABLE 2. Multiple Regression Analysis of Infarct Size: \( \beta \)-Coefficient Table

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
<th>SEM</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-3.130</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area-at-risk</td>
<td>0.736</td>
<td>0.074</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart rate</td>
<td>0.01</td>
<td>6.48/10^3</td>
<td>0.134</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0.029</td>
<td>0.015</td>
<td>0.077</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>-5.61/10^3</td>
<td>4.2/10^3</td>
<td>0.210</td>
</tr>
<tr>
<td>RPP</td>
<td>-8.86/10^5</td>
<td>6.11/10^5</td>
<td>0.178</td>
</tr>
</tbody>
</table>

BP, blood pressure; RPP, rate–pressure product. \( n=16 \).

(pore size, 0.22 \( \mu \)m, Japan Millipore Ltd.), and intravenously injected at 5 minutes before the dipyridamole administration. The dose of 8-PT was chosen as one that attenuated the hypotension to adenosine infusion (0.30 mg/kg/min i.v.) by 80%. A previous study from this laboratory\(^{15} \) had shown that this dosage of 8-PT does not modify the infarct size in the rabbit.

**Statistical Analysis**

Data presented in this study are expressed as mean±SEM. Difference in mortality was tested by \( \chi^2 \) test. Multiple regression equation and coefficients were calculated by using STATVIEW 512+ (Brain Power Inc., Calabasas, Calif.), a statistics program package. Multiple comparison of parameters among the experimental groups was performed by one-way ANOVA with a Student-Newman-Keuls post hoc test.\(^{16} \) Difference in regression lines was tested by ANCOVA. Difference was considered significant at a value of \( p<0.05 \). This study was conducted in strict accordance with the Guidelines of Sapporo Medical College on Animal Use and the position of the American Heart Association on research animal use.

**Results**

**Mortality**

Table 1 presents the total number of rabbits that entered the protocol and the mortality for each group. None of the rabbits died before the onset of 30-minute ischemia, and five rabbits died from ventricular fibrillation during the sustained coronary occlusion. The mortality rate was not significantly different among the experimental groups.

**Determinants of Infarct Size Variability Among Untreated Rabbits**

Before assessing the effect of preconditioning and/or the drugs, we examined the possible determinants of infarct size in the present rabbit model. To enlarge the sample size, we pooled the present control data (\( n=13 \)) with recent historical control rabbit data (\( n=3 \)). The results were essentially the same when only the present control data were analyzed. When the multiple regression model was estimated for infarct size, the regression equation (\( n=16 \)) was

\[
\text{IS} = 0.736 \text{AR} + 0.01 \text{HR} + 0.029 \text{BP} - 5.61 \text{DBP}/10^3 - 8.86 \text{RPP}/10^5 - 3.13
\]

where IS is infarct size (cm\(^3 \)), AR is area-at-risk (cm\(^2 \)), HR is heart rate (beats per minute), SBP is systolic blood pressure (mm Hg), DBP is diastolic blood pressure (mm Hg), and RPP is rate–pressure product. The hemodynamic data used in that equation were those obtained at 2 minutes after the coronary artery occlusion. The \( r \) value of the multiple regression was 0.967 and statistically significant. However, as summarized in Table 2, the \( \beta \)-coefficients of those parameters were not statistically significant except for the coefficient of the risk area size. Furthermore, the \( r \) value of the simple regression model using only risk area size (IS=0.653AR–0.146) was 0.932 (\( p<0.01 \)), which was almost as high as that of the multiple regression model. In the univariate analysis, there was no significant correlation between infarct size as percentage of risk area size (%IS/AR) with rate–pressure products (\( r=0.22 \)), heart rate (\( r=0.25 \)), systolic blood pressure (\( r=0.12 \)), or diastolic blood pressure (\( r=0.09 \)). These findings indicate that neither heart rate, blood pressure, nor rate–pressure products are major determinants of infarct size and that the variation of the risk area size explains the major part of infarct size variation in the present rabbit model. Thus, we used two approaches in the present study to assess the infarct size–limiting effect of preconditioning and the drugs. One was a comparison of the infarct size normalized as percentage of risk area size between the experimental groups. The other was an analysis of the alteration of the infarct size–risk area size relation by the interventions.

**Hemodynamic Parameters**

Heart rate and systemic blood pressure during the 30-minute coronary occlusion are summarized in Table 3. There were no significant differences in the hemodynamic parameters in comparison to the control group. However, the blood pressure of the dipyridamole–2-minute–preconditioning group was slightly lower than that in the dipyridamole and 8-PT–2-minute–preconditioning groups.

**TABLE 3. Summary of Hemodynamic and Infarct Size Data**

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>Heart rate (bpm)</th>
<th>Systolic BP/diastolic BP (mm Hg)</th>
<th>Heart weight (g)</th>
<th>Area-at-risk (cm(^2 ))</th>
<th>Infarct (cm(^3 ))</th>
<th>IS/AR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13</td>
<td>266±8</td>
<td>102±4/81±4</td>
<td>8.2±0.3</td>
<td>0.93±0.09</td>
<td>0.33±0.05</td>
<td>46.5±3.4</td>
</tr>
<tr>
<td>DIP</td>
<td>7</td>
<td>276±15</td>
<td>119±47/95±5†</td>
<td>8.2±0.2</td>
<td>0.90±0.06</td>
<td>0.49±0.05</td>
<td>55.3±5.2</td>
</tr>
<tr>
<td>2'PC</td>
<td>9</td>
<td>265±8</td>
<td>113±5/91±4</td>
<td>9.0±0.5</td>
<td>1.00±0.14</td>
<td>0.38±0.08</td>
<td>35.5±3.5</td>
</tr>
<tr>
<td>DIP-2'PC</td>
<td>12</td>
<td>244±6</td>
<td>100±4/78±3†</td>
<td>7.6±0.3</td>
<td>0.81±0.08</td>
<td>0.12±0.02*</td>
<td>13.8±2.6*</td>
</tr>
<tr>
<td>PT-2'PC</td>
<td>9</td>
<td>274±16</td>
<td>117±5/95±4†</td>
<td>7.9±0.3</td>
<td>1.04±0.08</td>
<td>0.45±0.06</td>
<td>43.9±5.8</td>
</tr>
<tr>
<td>PT/DIP-2'PC</td>
<td>11</td>
<td>237±7</td>
<td>105±3/86±3†</td>
<td>7.5±0.2</td>
<td>0.89±0.10</td>
<td>0.26±0.04</td>
<td>27.6±2.1*†</td>
</tr>
</tbody>
</table>

bpm, Beats per minute; BP, blood pressure; IS/AR, infarct size normalized as a percentage of area-at-risk; DIP, dipyridamole; 2', 2-minute; PC, preconditioning; PT, 8-phenyltheophylline.

*\( p<0.05 \) vs. control group; †\( p<0.05 \) vs. DIP-2'PC group.
Myocardial Infarct Size

Data of heart weight, the size of area-at-risk, and infarct size volume are also summarized in Table 3. All experimental groups were comparable for heart weight and the size of area-at-risk. Infarct size expressed as a percentage of the area-at-risk (%IS/AR) was 46.5±3.4% in the control group and 55.3±5.2% in the dipyridamole group (p=NS). The %IS/AR was smaller in the 2-minute–preconditioning group (%IS/AR, 35.5±3.5%) compared with the control group, suggesting a slight protection by 2-minute preconditioning. However, the difference did not reach statistical significance. On the other hand, the dipyridamole–2-minute–preconditioning group had a markedly smaller %IS/AR (13.8±2.6%), which was significantly smaller than the infarct size in the control and the 2-minute–preconditioning groups. These findings clearly indicate that dipyridamole potentiated the infarct size–limiting effect of 2-minute preconditioning.

Although the slight protection by the 2-minute preconditioning was suggested by smaller %IS/AR in the 2-minute–preconditioning group, it was not observed in the 8-PT–2-minute–preconditioning group (%IS/AR, 43.9±5.8%), which was treated with 8-PT before the preconditioning. In the 8-PT–dipyridamole–2-minute–preconditioning group, which received 8-PT before dipyridamole plus 2-minute preconditioning, %IS/AR was 27.6±2.1%. This value was significantly larger compared with %IS/AR in the dipyridamole–2-minute–preconditioning group (i.e., 13.8±2.6%), although it is still smaller than the control %IS/AR value. This difference in the infarct size between the 8-PT–dipyridamole–2-minute–preconditioning group and the dipyridamole–2-minute–preconditioning group suggests that 8-PT partially blocked the dipyridamole-induced potentiation of the preconditioning effect because 8-PT alone at the present dosage did not alter infarct size in the rabbit heart.15

Figure 2 illustrates the alteration of the infarct size–risk area size relation by preconditioning and the drugs. Figure 2A shows the comparison between the control and 2-minute–preconditioning groups. There are overlaps in the scatter of the points and the regression lines were marginally different in the intercepts, but the difference in the slope did not reach statistical significance. This finding and the small difference in %IS/AR between the control and 2-minute–preconditioning groups (Table 3) indicate that the infarct size–limiting effect of 2-minute preconditioning, if any, is very modest. The dipyridamole group is presented in Figure 2B, in which the data of the dipyridamole group are almost superimposable on the control group points. On the other hand, as shown in Figure 2C, combination of dipyridamole and 2-minute preconditioning in the dipyridamole–2-minute–preconditioning group shifted the infarct size–risk area size relation significantly downward. In the 8-PT–2-minute–preconditioning group (Figure 2D), all data, except for one having a small infarct, scattered close to the control points. The infarct size–risk area size relation in the 8-PT–dipyridamole–2-minute–preconditioning group (Figure 2E) was in the middle between those of the control and dipyridamole–2-minute–preconditioning groups. The slope of the regression line in the 8-PT–dipyridamole–2-minute–preconditioning group was significantly smaller compared with control but significantly larger than that of the dipyridamole–2-minute–preconditioning group.

Although heart rate and systemic blood pressure are unlikely to influence the infarct size in untreated rabbits (Table 2), there remained the possibility that slightly lower heart rate and systemic blood pressure in the dipyridamole–2-minute–preconditioning group was somewhat responsible for the infarct size limitation observed in that group. To exclude this possibility, we plotted %IS/AR against the rate–pressure product at the time of coronary occlusion. As shown in Figure 3, %IS/AR did not correlate with rate–pressure products in any of the control, 2-minute–preconditioning or the dipyridamole–2-minute–preconditioning groups, and the points of %IS/AR in the dipyridamole–2-minute–preconditioning group fell below the control points regardless of the rate–pressure products. This finding indicates that the infarct size limitation observed in the dipyridamole–2-minute–preconditioning group cannot be explained by the difference in the hemodynamic parameters.

Discussion

In the present study, dipyridamole administered before 2-minute preconditioning significantly enhanced the infarct size–limiting effect of the preconditioning. Furthermore, that potentiation of the preconditioning effect was attenuated by 8-PT, a nonselective adenosine receptor blocker. These results strongly support the hypothesis that endogenous adenosine building up during ischemic preconditioning triggers the cardioprotective mechanism of preconditioning via adenosine receptor activation.

Critical assessment of any interventions for limiting infarct size, including preconditioning, requires an understanding of the determinants of infarct size variation. We previously reported that the infarct size in the rabbit heart is primarily determined by duration of the coronary occlusion and the size of the area-at-risk.14 In the present study, we reassessed the possible determinants of infarct size. The results confirmed that risk area size is a major determinant and also indicate that heart rate, blood pressure, and rate–pressure products do not contribute to the infarct size variation. These findings in the rabbit are consistent with those in the dog models studied by the Animal Models for Protecting Ischemic Myocardium (AMPIM) project.17 With multiple regression analysis, the AMPIM study17 found that in the unconscious dog model, which received coronary artery occlusion under anesthesia, infarct size variation was mainly attributed to the size of area-at-risk and collateral blood flow level. The rate–pressure product did not correlate with the infarct size (as percentage of risk area size) in the unconscious dog model, although it made a very modest contribution to the infarct size variation in a conscious dog model.17 Accordingly, the present rabbit model appears quite similar to the unconscious dog model of the AMPIM study17 regarding the baseline determinants of infarct size, except that collateral blood flow is not important in the rabbit because of poorly developed coronary collaterals.14

A methodological limitation in the present study is that interstitial fluid adenosine concentration was not...
FIGURE 2. Scatterplots of relations between infarct size and size of area-at-risk. Panel A: Control group (○) versus 2-minute preconditioning (2'PC) group (●). There was a significant linear correlation between infarct size and risk area size in both the control group \((y=0.70x-2.51, \tau=0.94)\) and the 2'PC group \((y=0.49x-1.30, \tau=0.89)\). The difference in the slope did not reach statistical significance. Panel B: Control group (○) versus dipyridamole (DIP) group (●). Panel C: Control group (○) versus DIP-2'PC group (●). The regression line of the DIP-2'PC group \((y=0.19x-0.04, \tau=0.74, p<0.01)\) was less steep than the control regression line \((p<0.01\) for slope and intercept). Panel D: Control group (○) versus PT-2'PC group (●). Panel E: Control group (○) versus PT/DIP-2'PC (●) group. The slope of the regression line in the PT/DIP-2'PC group \((y=0.43x-0.13, \tau=0.94, p<0.01)\) was smaller than in the control group but larger than in the DIP-2'PC group \((p<0.01\) for either comparison).

determined in the heart. Thus, the effect of dipyridamole on interstitial adenosine level and its relation with the preconditioning effect could not be directly demonstrated. However, several lines of circumstantial evidence suggest that dipyridamole enhances the elevation of interstitial adenosine level during myocardial ischemia and that it delays washout of adenosine during reperfusion. First, several studies\(^{16-20}\) have reported that dipyridamole enhances reactive hyperemia after transient ischemia. Second, a study by Knabb et al\(^9\) indicated that myocardial hyperemia by dipyridamole was associated with elevation of interstitial fluid adenosine concentration. Third, although dipyridamole suppresses not only the uptake but also the release of adenosine,\(^{21-23}\) a steady-state mathematical model analysis predicts that extracellular adenosine level is increased by dipyridamole provided that washout of the interstitial compartment is restricted.\(^{10}\) Accordingly, it is quite probable that dipyridamole enhanced the interstitial adenosine accumulation during the preconditioning and delayed the washout of adenosine during the reperfusion period between the preconditioning ischemia and the subsequent longer ischemia.

FIGURE 3. Scatterplots of the relation between rate–pressure product and infarct size normalized as a percentage of the area-at-risk (AAR). There was no significant correlation between the rate–pressure product and infarct size in the control group (○), the 2-minute preconditioning group (△), or the dipyridamole–2-minute–preconditioning group (▲). In the dipyridamole–2-minute–preconditioning group, infarct size (percent of AAR) was smaller than in the control and 2-minute–preconditioning groups regardless of the rate–pressure products.
Administration of dipyridamole alone, without preconditioning, did not limit myocardial infarct size in the rabbit heart (Figure 2B). This observation is consistent with earlier reports. Matsuoka et al infused intravenous dipyridamole in the dog model, but the various dosages of dipyridamole failed to limit infarct size. Although another study suggested the possibility that dipyridamole may salvage ischemic myocardium through increasing coronary collateral flow in the dog heart, this protective mechanism would not be expected in the rabbit heart because the native coronary collaterals are very poorly developed in this species. Nevertheless, Figure 2B suggests that the effect of dipyridamole per se cannot explain the marked limitation of infarct size in rabbits treated with dipyridamole and 2-minute preconditioning (Figure 2C).

The failure of dipyridamole to limit infarct size (Figure 2B) might appear contradictory to the adenosine hypothesis for the preconditioning because dipyridamole is known to elevate the interstitial adenosine level in nonischemic myocardium. However, one explanation for the apparent contradiction is that dipyridamole failed to increase interstitial adenosine concentration up to a "threshold level" for triggering the preconditioning mechanism. That threshold for interstitial adenosine level might exist during the preconditioning ischemia or during the reperfusion period between preconditioning ischemia and the subsequent ischemic insult. The presence of such a threshold is suggested by an earlier observation that the duration of preconditioning ischemia is one of the major determinants of the protective effect of preconditioning. In the present study, the preconditioning with 2-minute ischemia was marginally protective (Table 3), but the preconditioning with 5-minute ischemia limited infarct size to approximately 45% of the control value, indicating again that the length of the preconditioning ischemia is critical.

The present results showed that dipyridamole pretreatment potentiates the preconditioning effect. Our interpretation of this finding is that the effect of dipyridamole and the preconditioning per se share a common mechanism, i.e., adenosine receptor activation. This interpretation appears to be supported by two lines of evidence obtained by the present and previous experiments in this laboratory. First, the infarct size–risk size relation in rabbits that received dipyridamole plus 2-minute preconditioning (Figure 2C) is quite similar to that observed in rabbits preconditioned with four cycles of 5-minute ischemia, suggesting a common mechanism. Second, rabbits that received 8-PT before the combination of dipyridamole and preconditioning (the 8-PT–dipyridamole–2-minute–preconditioning group) had significantly larger infarct size than the group given dipyridamole and preconditioning (the dipyridamole–2-minute–preconditioning group). The same dose of 8-PT alone did not modify the infarct size of unpreconditioned rabbit hearts (%IS/AR, 42.4±5.1%) in a recent study, which we conducted in parallel to the present study. Accordingly, the infarct size difference between the dipyridamole–2-minute–preconditioning group and the 8-PT–dipyridamole–2-minute–preconditioning group can be most reasonably interpreted as being 8-PT attenuation of the preconditioning effect potentiated by dipyridamole. This observation provides additional supportive evidence for the key role of adenosine in the enhancement of the preconditioning effect by dipyridamole. The failure of 8-PT to completely block that effect of dipyridamole might have been due to an insufficient dose of 8-PT, a competitive antagonist, although we cannot exclude the possibility that some mechanism unrelated to adenosine also plays a role in the potentiation of the preconditioning by dipyridamole.

The present study strongly supports the concept that adenosine receptor activation by preconditioning ischemia mediates the increased ischemic tolerance afforded by preconditioning. However, it remains unknown how the adenosine receptor stimulation increases the resistance of myocytes against ischemic injury. In a recent extensive review, Olsson and Pearson listed the effectors that are coupled to adenosine receptors in several tissues (myocardium, brain, vascular smooth muscle, neurons, and fat cells), i.e., adenylyl cyclase, potassium channel, phospholipase A2, phospholipase C, guanylate cyclase, calcium channel, and glucose transporter. It is unknown which effectors, if any, are coupled with adenosine receptors activated by preconditioning and thereby contribute to affording the myocardium ischemic tolerance. Fortunately, some speculation appears possible based on earlier studies on pharmacological interventions for infarct size limitation. β-Adrenergic blockers and calcium channel blockers are two of the agents most extensively studied for their infarct size–limiting effects. However, β-blockers did not modify infarct size, and verapamil also failed to limit infarct size in the rabbit model, although the results of studies using dog models for verapamil and other calcium antagonists are conflicting. These findings suggest that adenosine receptor activation by preconditioning does not protect myocardium by inhibiting calcium channel opening or suppressing the stimulated adenylyl cyclase.

In a recent preliminary study, Auchampach and Gross reported that a blocker of the ATP-sensitive potassium channel, glibenclamide, abolished the infarct size limitation of preconditioning in the dog. This report suggests that this channel may be a primary effector of preconditioning-activated adenosine receptors. However, Thornton and Downey found that glibenclamide did not block the preconditioning effect in the rabbit heart. The reason for the discrepancy between those two studies is currently unknown. The possible involvement of other effectors of the adenosine receptors in preconditioning might also warrant further investigation.

The finding that dipyridamole potentiates the preconditioning effect may have an important clinical implication. The existence of preconditioning and its clinical significance in the human heart have not been established. However, recent studies suggest that preconditioning may occur in humans as well, at least when myocardium is subjected to repetitive regional ischemia during percutaneous transluminal coronary angioplasty. If that is the case, dipyridamole might be useful to enhance the preconditioning effect in patients undergoing angioplasty and in those with unstable angina. Obviously, further investigation is needed to characterize the preconditioning phenomenon in humans as well as the effect of dipyridamole.

In conclusion, we found that dipyridamole potentiates the infarct size–limiting effect of preconditioning...
and that the effect of dipyridamole was attenuated by an
adenosine receptor antagonist, 8-PT. These data sup-
port the hypothesis that the increase in the ischemic
myocardial tolerance by preconditioning is mediated by
endogenous adenosine build-up during the preconditioning.

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