

## Long-Term Stimulation of Adenosine A2b Receptors Begun After Myocardial Infarction Prevents Cardiac Remodeling in Rats

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**Background**—Adenosine inhibits proliferation of cardiac fibroblasts and hypertrophy of cardiomyocytes, both of which may play crucial roles in cardiac remodeling. In the present study, we investigated whether chronic stimulation of adenosine receptors begun after myocardial infarction (MI) prevents cardiac remodeling.

**Methods and Results**—MI was produced in Wistar rats by permanent ligation of the left anterior descending coronary artery. One week after the onset of MI, animals were randomized into 8 groups: vehicle, dipyridamole (DIP; the adenosine uptake inhibitor, 50 mg/kg), 2-chloroadenosine (CADO; the stable analogue of adenosine, 2 mg/kg), and CADO in the presence of the nonselective adenosine receptor antagonist 8-sulfophenyltheophylline (8-SPT) or the selective antagonist for adenosine A1, A2a, A2b, or A3 receptor. Three weeks after treatment, hemodynamic and echocardiographic parameters in the DIP and CADO groups were significantly improved compared with the vehicle group. These hemodynamic and echocardiographic improvements were blunted by either 8-SPT or the selective adenosine A2b antagonist MRS1754 but not by the selective antagonists for other subtypes of adenosine receptors. The collagen volume fraction was smaller, and gene expression of the molecules associated with cardiac remodeling such as matrix metalloproteinase in noninfarcted areas was reduced in the DIP and CADO groups compared with the vehicle group, both of which were attenuated by either 8-SPT or MRS1754.

**Conclusions**—Long-term stimulation of adenosine A2b receptors begun after MI attenuates cardiac fibrosis in the noninfarcted myocardium and improves cardiac function. Drugs that stimulate adenosine A2b receptors or increase adenosine levels are new candidates for preventing cardiac remodeling after MI. (*Circulation*. 2006;114:1923-1932.)

**Key Words:** adenosine ■ heart failure ■ myocardial infarction ■ remodeling

Chronic heart failure (CHF) is a major complication of myocardial infarction (MI) that substantially worsens its prognosis.<sup>1,2</sup> Although several major therapeutic advances have been made in the management of MI, postinfarction CHF remains a common cause of high morbidity, hospitalization, and cardiac death.<sup>3,4</sup> Worsening of cardiac functions after MI is followed by a complex sequence of structural changes of the left ventricle (LV), referred to as postinfarction remodeling.<sup>3,4</sup> These changes include progressive chamber dilatation, cardiac hypertrophy, and fibrosis.<sup>4,5</sup> Recent studies have highlighted the importance of fibrosis in noninfarcted areas remote from the site of infarction for the pathogenesis of postinfarction cardiac dysfunction.<sup>3-7</sup>

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Adenosine is a nucleoside that exerts multiple functions through specific subtypes of adenosine receptors.<sup>8,9</sup> Four

subtypes of adenosine receptors (A1, A2a, A2b, and A3) have been cloned and pharmacologically characterized.<sup>10,11</sup> Adenosine triggers/mediates cardioprotection against short-term ischemia/reperfusion injury mainly through adenosine A1, A2a, and A3 receptors.<sup>12-14</sup> In addition, adenosine inhibits cardiac hypertrophy as a result of pressure overload through adenosine A1 receptors.<sup>15</sup> Adenosine also inhibits proliferation of cultured myocardial fibroblast through adenosine A2b receptors,<sup>16</sup> suggesting that adenosine may play an important role in cardiac remodeling. Recently, we found that plasma adenosine levels increase in patients with CHF<sup>17</sup> and that a further elevation of plasma adenosine levels resulting from either dipyridamole (DIP) or dilazep reduces the severity of CHF.<sup>18</sup> However, the long-term effects of adenosine that start after the completion of the necrotic process following MI on cardiac remodeling are unclear. Furthermore, it has

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not been determined which subtypes of adenosine receptors are involved in cardiac remodeling. In the present study, we investigated whether the long-term stimulation of adenosine receptors prevents cardiac remodeling in rat MI model and, if so, which subtype of adenosine receptors are involved in this condition.

## Methods

### Animals

All procedures were performed in conformity with the *Guide for the Care and Use of Laboratory Animals* (NIH publication No. 85-23, 1996 revision) and were approved by the Osaka University Committee for Laboratory Animal Use.

### Materials

DIP, the adenosine uptake inhibitor, was kindly provided by Boehringer-Ingelheim (Ingelheim, Germany). 2-Chroloadenosine (CADO; the stable analogue of adenosine), 8-sulphophenyltheophylline (8-SPT; the nonselective antagonist of adenosine receptors), 1,3-diethyl-8-phenylxanthine (DPCPX; the selective adenosine A1 antagonist), 5-amino-7-(phenylethyl)-2-(2-furyl)-pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c] pyrimidine (SCH58261; the selective adenosine A2a antagonist), 8-[4-[(4-cyanophenyl)carbamoylmethyl]oxy]phenyl]-1, 3-di(n-propyl)xanthine (MRS1754; the selective adenosine A2b receptor antagonist), and (5-propyl-2-ethyl-4-propyl-3-(ethylsulfanylcarbonyl)-6-phenylpyridine-5-carboxylate) (MRS1523; the selective adenosine A3 receptor antagonist) were purchased from Sigma Chemical Co (St Louis, Mo). DIP, CADO, and 8-SPT were dissolved in saline. DPCPX, MRS1754, and MRS1523 were dissolved in a solution of 50% dimethyl sulfoxide in distilled water and diluted immediately before use in saline. SCH58261 was dissolved in Tween 80 aqueous suspension (5 mL/kg). Antibodies for matrix metalloproteinase (MMP)-2 and MMP-9 were purchased from Santa Cruz Biotechnology (Santa Cruz, Calif).

### Experimental Protocols

Male Wistar rats (age, 8 weeks; weight, 240 to 260 g; Japan Animals, Osaka, Japan) were used in these experiments. MI was induced by permanent ligation of the left anterior descending coronary artery as previously described.<sup>19</sup> Briefly, the rats were anesthetized with sodium pentobarbital (30 mg/kg IP), the thorax was opened, the heart was exteriorized, and a ligature was placed around the proximal left anterior descending coronary artery. The heart was returned to its normal position, and the thorax was closed. Mortality was 47% within the first 24 hours. The same surgical procedure was performed in a sham group of rats (n=8) except that the suture around the coronary artery was not tied.

Starting on the seventh postoperative day, sham rats received 2% dimethyl sulfoxide intraperitoneally, and surviving MI rats were randomly allocated to 1 of the following 8 treatment groups: vehicle (2% dimethyl sulfoxide), DIP (50 mg·kg<sup>-1</sup>·d<sup>-1</sup> PO), CADO (2 mg·kg<sup>-1</sup>·d<sup>-1</sup>), CADO (2 mg·kg<sup>-1</sup>·d<sup>-1</sup>) plus 8-SPT (10 mg·kg<sup>-1</sup>·d<sup>-1</sup>), CADO (2 mg·kg<sup>-1</sup>·d<sup>-1</sup>) plus DPCPX (0.1 mg·kg<sup>-1</sup>·d<sup>-1</sup>), CADO (2 mg·kg<sup>-1</sup>·d<sup>-1</sup>) plus SCH58261 (1 mg·kg<sup>-1</sup>·d<sup>-1</sup>), CADO (2 mg·kg<sup>-1</sup>·d<sup>-1</sup>) plus MRS1754 (1 mg·kg<sup>-1</sup>·d<sup>-1</sup>), or CADO (2 mg·kg<sup>-1</sup>·d<sup>-1</sup>) plus MRS1523 (1 mg·kg<sup>-1</sup>·d<sup>-1</sup>); these treatments were given to MI rats (n=8, respectively) for 3 weeks. DIP was given by gastric gavage twice daily. CADO and all adenosine receptor antagonists were given intraperitoneally twice daily. The administration of CADO at the dose used in the present study (1 mg/kg IP) into sham or MI rats (n=5 for both) caused maximal decrease 5 minutes after its administration in pulse rate (24±4% and 22±6% reduction from the baseline, respectively) and systolic blood pressure (26±5% and 27±3% reduction from the baseline, respectively), both of which returned to baseline 90 minutes after the administration was discontinued. Administration of 8-SPT (5 mg/kg IP) blocked the CADO-induced hemodynamic changes. Doses of antagonists for subtypes of adenosine receptors used in the in vivo

study were chosen on the basis of previous studies of their efficacy.<sup>15,20–25</sup>

### Noninvasive Blood Pressure and Pulse Rate

Both blood pressure and pulse rate were measured before MI and 1 and 4 weeks after MI by the tail-cuff method without the use of anesthesia (MK-2000, Muromachi, Tokyo, Japan).

### Echocardiographic Studies

Rats were lightly anesthetized with sodium pentobarbital anesthesia (30 mg/kg IP).<sup>26</sup> Echocardiography was performed using a commercially available echocardiographic system equipped with a 15-MHz phased-array transducer (SONOS 5500, Hewlett Packard, Andover, Mass). A 2-dimensional short-axis view of the LV was obtained at the level of the papillary muscles. These studies were performed at both 1 and 4 weeks after MI.

### Hemodynamic Studies

LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), maximal rate of pressure rise and decline (LV dP/dt<sub>max</sub> and LV dP/dt<sub>min</sub>, respectively), and heart rate were measured with a 3.5F Millar pressure catheter 4 weeks after MI under sodium pentobarbital anesthesia (30 mg/kg IP).

### Histology

The right ventricle and LV were separated in ice-cold saline and weighed. The LV was cut into 3 transverse sections: apex, mid ventricle, and base. For the light microscopic study, the specimens were fixed in 10% formaldehyde, embedded in paraffin, and cut into 4-μm-thick sections, which were stained with hematoxylin and eosin and Masson's trichrome stains (n=8 for both).

Infarct size was determined as previously described.<sup>27</sup> Briefly, infarct size was calculated as the average of all slices stained with Masson's trichrome and expressed as a percentage of the fibrotic length to the mean LV circumference. Rats with an infarct size <30% were excluded from analysis because they did not show typical LV remodeling.

The cross-sectional area of cardiomyocytes was measured as previously described.<sup>26</sup> One hundred cardiomyocytes per heart stained with hematoxylin and eosin were counted, and the average area was determined.

The collagen volume fraction was measured while omitting fibrosis of the perivascular, epicardial, and endocardial areas.<sup>26</sup> The collagen volume fraction was expressed as the average of connective tissue to the total tissue area of all slices stained with Masson's trichrome.

### Quantitative Real-Time Reverse-Transcriptase Polymer Chain Reaction

Quantitative real-time reverse-transcriptase polymer chain reaction was performed as described previously.<sup>28</sup> Total RNA from the noninfarcted and infarcted LV was extracted with RNA-Bee-RNA Isolation Reagent (Tel-Test, Friendswood, Tex). Then, 200 ng total RNA was reverse transcribed and amplified with an Omniscript RT Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Oligonucleotide primers and TaqMan probes for rat collagen type I, rat collagen type III, rat transforming growth factor-β1 (TGF-β1), rat MMP-2, rat MMP-9, rat tissue inhibitor of metalloproteinase (TIMP)-1, TIMP-2, rat atrial natriuretic factor (ANF), rat brain natriuretic peptide (BNP), and rodent glyceraldehyde phosphate dehydrogenase were purchased from Applied Biosystems (Foster City, Calif).

### Immunoblotting

Immunoblotting was performed as described previously.<sup>28</sup> Immunoreactive bands were quantified by densitometry (Molecular Dynamics).

### Collagen Synthesis in Cultured Cardiac Fibroblasts

Adult cardiac fibroblasts were prepared as described previously.<sup>16</sup> The effects of either DIP or CADO on collagen synthesis in cardiac fibroblasts were evaluated on confluent cultures by incorporating [<sup>3</sup>H]proline into cells as previously described.<sup>29</sup> Then, either DIP or CADO with or without antagonists for subtypes of adenosine A1, A2a, A2b, or A3 receptors was added; [<sup>3</sup>H]proline (0.5  $\mu$ Ci/mL) also was added. After the cells were incubated for 24 hours, the radioactivity of aliquots of the trichloroacetic acid-insoluble material was determined with a liquid scintillation counter. Cardiac fibroblasts in the first or second passage were used for all experiments. Doses of antagonists for subtypes of adenosine receptors used in the *in vitro* study were chosen on the basis of previous studies of their efficacy.<sup>11,16</sup>

### Statistical Analysis

All data are expressed as mean  $\pm$  SEM. Comparisons of the time course changes between groups were performed by use of 2-way repeated-measures ANOVA. Comparisons of other data between groups were performed through the use of 1-way fractional ANOVA. The Bonferroni-Holm procedure was used to correct multiple comparisons. A value of  $P < 0.05$  was considered statistically significant.

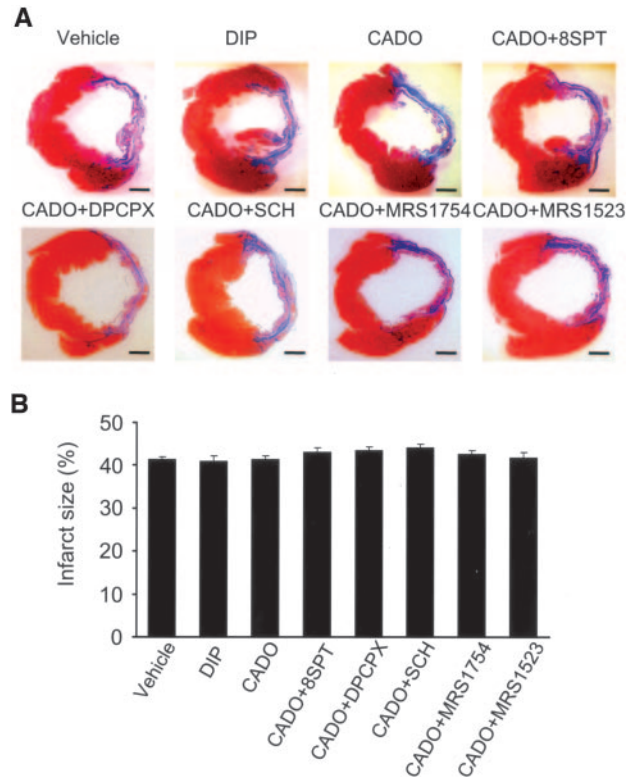
The authors had full access to the data and take full responsibility for their integrity. All authors have read and agree to the manuscript as written.

## Results

### Effects of Adenosine and Antagonists for Subtypes of Adenosine Receptors on Infarct Size and Hemodynamic Parameters

Both blood pressure ( $110 \pm 8$  mm Hg) and pulse rate ( $405 \pm 11$  bpm) under baseline conditions in the sham group did not change throughout the protocol and were not different at any time points compared with the MI groups. In addition, body weight at baseline ( $250 \pm 5$  g) and at the end of the protocol ( $424 \pm 3$  g) did not differ from other groups. No difference in infarct size among groups tested was found (Figure 1).

Four weeks after the onset of MI, LVEDP in all MI groups was higher than in the sham group (Figure 2A). Both LV  $dP/dt_{max}$  and LV  $dP/dt_{min}$  in all MI groups were smaller than in the sham group (Figure 2B and 2C). Long-term adenosine receptor stimulation by either DIP or CADO decreased LVEDP and increased both LV  $dP/dt_{max}$  and LV  $dP/dt_{min}$ . The improvement in hemodynamic parameters as a result of CADO treatment was blunted by 8-SPT or MRS1754 but not by other antagonists for subtypes of adenosine receptors (Figure 2A through 2C). No statistical difference existed in LVSP (Figure 2D) or heart rate (sham,  $372 \pm 8$  bpm) among all groups when hemodynamic parameters such as LVEDP, LV  $dP/dt_{max}$ , and LV  $dP/dt_{min}$  were measured. The ratios of heart weight to body weight, ventricular weight to body weight, and lung weight to body weight increased in all MI groups compared with the sham group. Long-term stimulation of adenosine receptors by CADO decreased all 3 ratios compared with other MI groups (the Table). Either 8-SPT or MRS1754, but not the antagonist for other subtypes of adenosine receptors, blunted the decrease in all 3 ratios by CADO.



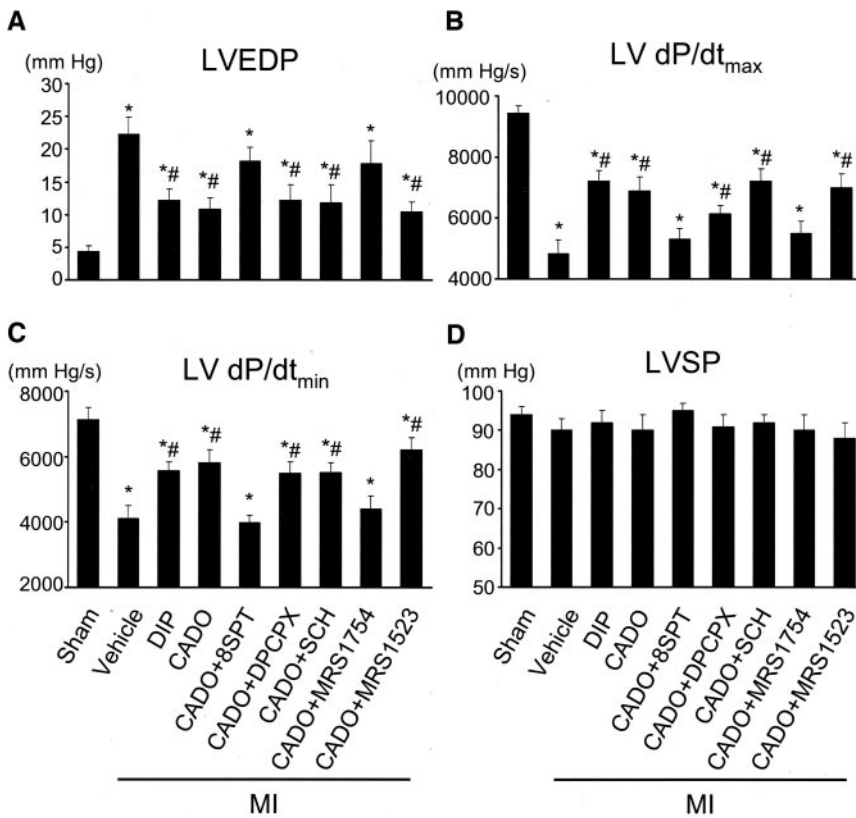
**Figure 1.** Effects of adenosine and antagonists for subtypes of adenosine receptors on infarct size. A, Representative LV cross sections. Scale bar=1 mm. B, Quantitative analysis of myocardial infarct size. Values are mean  $\pm$  SEM. No statistical difference was found among all groups (n=8).

### Effects of Adenosine and the Antagonists for Subtypes of Adenosine Receptors on Echocardiographic Parameters

Figure 3A shows the M-mode view of all groups. Four weeks after the onset of MI, both LV end-diastolic dimension and LV end-systolic dimension in all MI groups were larger than in the sham group. Although no statistical difference existed in LV end-diastolic dimension among MI groups, both DIP and CADO reduced LV end-systolic dimension and increased fractional shortening. The improvement in echocardiographic parameters by CADO was blunted by either 8-SPT or MRS1754 but not by the antagonist for other subtypes of adenosine receptors (Figure 3B).

### Effects of Adenosine and Antagonists for Subtypes of Adenosine Receptors on Cardiac Collagen Volume in Noninfarcted Areas

To clarify the pathophysiological mechanism of the improved cardiac performance caused by the long-term stimulation of adenosine receptors by either DIP or CADO, we examined the collagen volume fraction in noninfarcted areas that may affect cardiac remodeling. The collagen volume fraction in all MI groups increased more than in the sham group, and administration of either DIP or CADO attenuated an increase in morphometric collagen volume fraction in noninfarcted areas (Figure 4). Either 8-SPT or



**Figure 2.** Effects of adenosine and antagonists for subtypes of adenosine receptors on hemodynamic parameters. A, LVEDP; B, LV dP/dt<sub>max</sub>; C, LV dP/dt<sub>min</sub>; D, LVSP. Values are mean±SEM. \**P*<0.05 vs sham; #*P*<0.05 vs vehicle (n=8).

MRS1754, but not antagonists for other subtypes of adenosine receptors, abolished the effects of CADO on the collagen volume fraction in the noninfarcted areas. CADO (93±4%) or DIP (90±5%) did not change the collagen volume fraction in infarcted areas compared with vehicle (88±4%).

### Effects of Adenosine and Antagonists for Subtypes of Adenosine Receptors on Cardiac Hypertrophy in Noninfarcted Areas

The cross-sectional area of cardiomyocytes in all MI groups increased more than in the sham group, and either DIP or CADO inhibited hypertrophy of cardiomyocytes in noninfarcted areas (Figure 5). Either 8-SPT or DPCPX, but

not other adenosine receptor antagonists, abolished the effects of CADO on hypertrophy of cardiomyocytes.

### Effects of Antagonists for Subtypes of Adenosine Receptors on Molecules Associated With Cardiac Remodeling in Noninfarcted Areas

To examine the molecular mechanisms by which adenosine attenuates cardiac fibrosis in the noninfarcted myocardium, we examined the mRNA levels of molecules associated with fibrosis and hypertrophy in noninfarcted areas after MI (Figure 6). The increased mRNA expressions of collagen type I, TGF-β1, MMP-2, and TIMP-1 after MI were suppressed by treatment with either DIP or CADO. Interestingly, either 8-SPT or MRS1754, but not the antagonist for other subtypes

### Heart Weight and Lung Weight 4 Weeks After MI

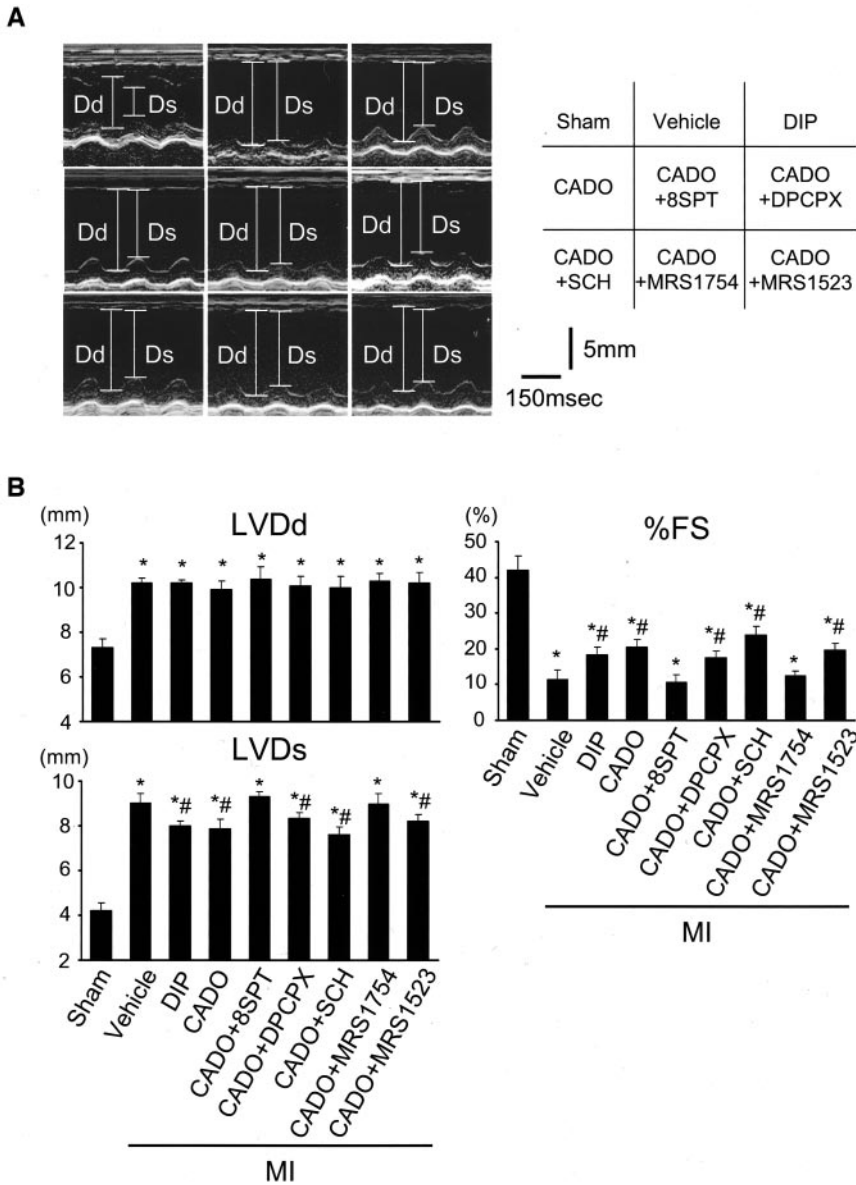
|              | MI        |            |             |             |            |             |               |              |              |
|--------------|-----------|------------|-------------|-------------|------------|-------------|---------------|--------------|--------------|
|              | Sham      | Vehicle    | DIP         | CADO        | CADO+8SPT  | CADO+DPCPX  | CADO+SCH58261 | CADO+MRS1754 | CADO+MRS1523 |
| HW/BW, mg/g  | 2.93±0.06 | 4.05±0.11* | 3.52±0.15*† | 3.60±0.11*† | 3.95±0.12* | 3.58±0.09*† | 3.51±0.12*†   | 3.89±0.06*   | 3.45±0.19*†  |
| VW/BW, mg/g  | 2.54±0.06 | 3.65±0.11* | 3.25±0.09*† | 3.21±0.10*† | 3.54±0.11* | 3.17±0.08*† | 3.11±0.12*†   | 3.48±0.07*   | 3.05±0.19*†  |
| LWV/BW, mg/g | 1.93±0.07 | 2.57±0.07* | 2.62±0.12*  | 2.60±0.10*  | 2.60±0.17* | 2.56±0.10*  | 2.50±0.11*    | 2.48±0.09*   | 2.47±0.19*   |
| LW/BW, mg/g  | 3.3±0.10  | 8.2±1.0*   | 4.8±0.4*†   | 4.4±0.2*†   | 8.8±0.7*   | 5.1±0.4*†   | 4.4±0.6*†     | 7.7±0.7*     | 4.2±0.3*†    |

HW indicates heart weight; BW, body weight; VW, ventricular weight; LWV, LV weight; and LW, lung weight. Values are mean±SEM (n=8).

\**P*<0.05 vs sham.

†*P*<0.05 vs vehicle.





**Figure 3.** Effects of adenosine and antagonists for subtypes of adenosine receptors on echocardiographic parameters. A, Representative 2D echocardiograms. B, Quantitative analysis of echocardiographic parameters. Values are mean  $\pm$  SEM. LVDd indicates LV end-diastolic dimension; LVDs, LV end-systolic dimension; and %FS, fractional shortening. \* $P < 0.05$  vs sham; # $P < 0.05$  vs vehicle ( $n = 8$ ).

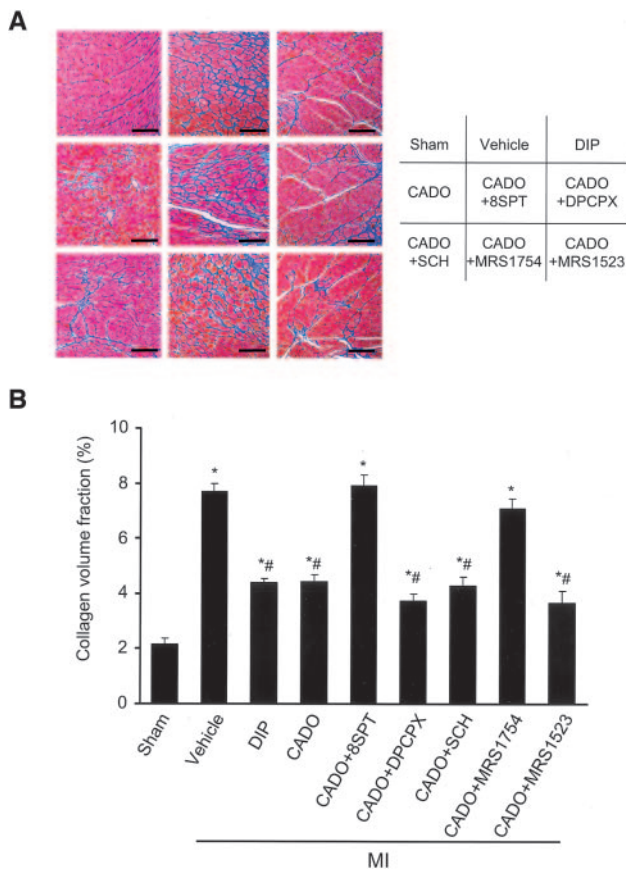
of adenosine receptors, blunted the suppression of mRNA levels of collagen type I, TGF- $\beta$ 1, MMP-2, or TIMP-1 by CADO. However, neither DIP nor CADO changed the mRNA levels of MMP-9 or TIMP-2, each of which may also link with cardiac remodeling after MI.<sup>30</sup> Consistent with the mRNA levels of MMP-2 and MMP-9, CADO decreased MMP-2, but not MMP-9, protein levels in noninfarcted areas (Figure 7). Either DIP or CADO treatment resulted in suppression of the ANF and BNP mRNA levels, both of which are useful markers of cardiac hypertrophy, in noninfarcted areas (Figure 6G and 6H). 8-SPT or DPCPX, but not the antagonist for other subtypes of adenosine receptors, blunted the suppression of mRNA levels of ANF by CADO. 8-SPT, DPCPX, or MRS1754, but not the antagonist for other subtype of adenosine receptors, blunted the suppression of mRNA levels of BNP by CADO. In infarcted areas, all of the evaluated molecules increased in all MI groups compared with the sham group, but no difference existed in expression levels among MI groups (data not shown).

### Cellular Mechanisms of the Antifibrosis Action of Adenosine

Both DIP and CADO decreased the incorporation of [<sup>3</sup>H]proline into cardiac fibroblasts from rat adult rats in a dose-dependent manner, either of which was blocked by 8-SPT (Figure 8A). The decrease in [<sup>3</sup>H]proline incorporation by CADO was completely blocked by MRS1754, a selective adenosine A2b receptor antagonist, at a concentration of  $10^{-7}$  mol/L (Figure 8B). DPCPX, SCH58261, or MRS1523 at a dose from  $10^{-8}$  to  $10^{-6}$  mol/L did not affect the reduction in [<sup>3</sup>H]proline incorporation by CADO ( $10^{-6}$  mol/L) (data not shown).

### Discussion

Adenosine or adenosine receptor agonist administration before the onset of ischemia or during early reperfusion has been documented by several investigators not only to reduce infarct size but also to improve functional recovery after MI.<sup>31-33</sup> In the present study, we demonstrated that

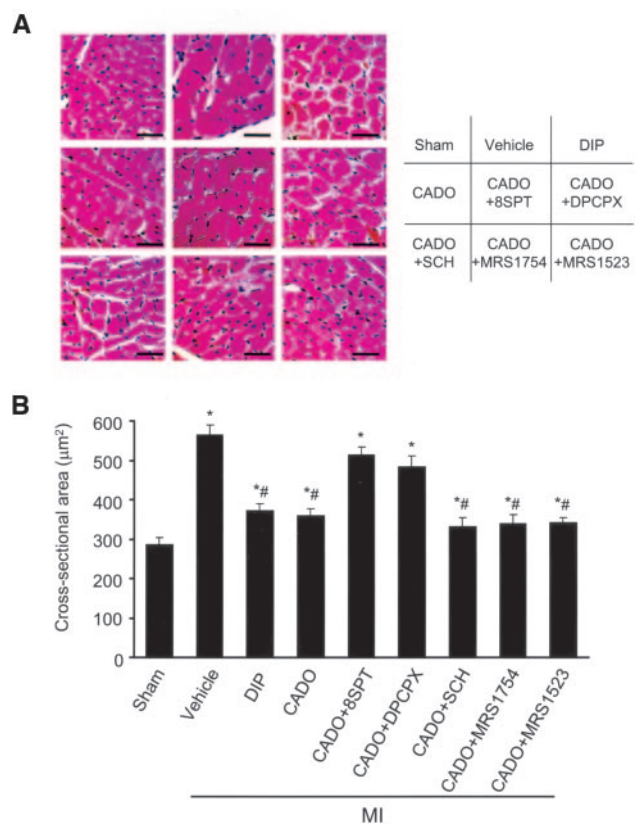


**Figure 4.** Effects of adenosine and antagonists for subtypes of adenosine receptors on cardiac collagen volume fraction in noninfarcted areas. A, Representative histological findings stained with Masson's trichrome in noninfarcted areas. Scale bar=200  $\mu$ m. B, Quantitative analysis of collagen volume fraction in noninfarcted areas. Values are mean $\pm$ SEM. \* $P$ <0.05 vs sham; # $P$ <0.05 vs vehicle (n=8).

the long-term administration of either DIP or CADO that starts 1 week after the onset of MI, during which time the necrotic process may be completed,<sup>34</sup> improves cardiac performance in MI rats, as indicated by hemodynamic and echocardiographic parameters. To the best of our knowledge, this study is the first to document that adenosine administered after the completion of the necrotic process exerts cardioprotective effects.

We also demonstrated that the attenuation of cardiac remodeling by adenosine was blunted by 8-SPT, indicating that the activation of adenosine receptors is responsible for the attenuation of cardiac remodeling after MI. Importantly, we further examined which subtype of adenosine receptor was involved in cardiac remodeling after MI. Because 4 subtypes of adenosine receptors have been cloned in the rat,<sup>9,10</sup> we used specific antagonists for subtypes of adenosine receptors: DPCPX for A1 adenosine receptors, A2a for SCH58161, A2b for MRS1754, and A3 for MRS1523.<sup>20,22–25,35</sup>

We found that the improvement in cardiac performance by long-term stimulation by CADO was blunted by MRS1754 but not by the antagonist of other subtypes of adenosine receptors. To the best of our knowledge, this study was the

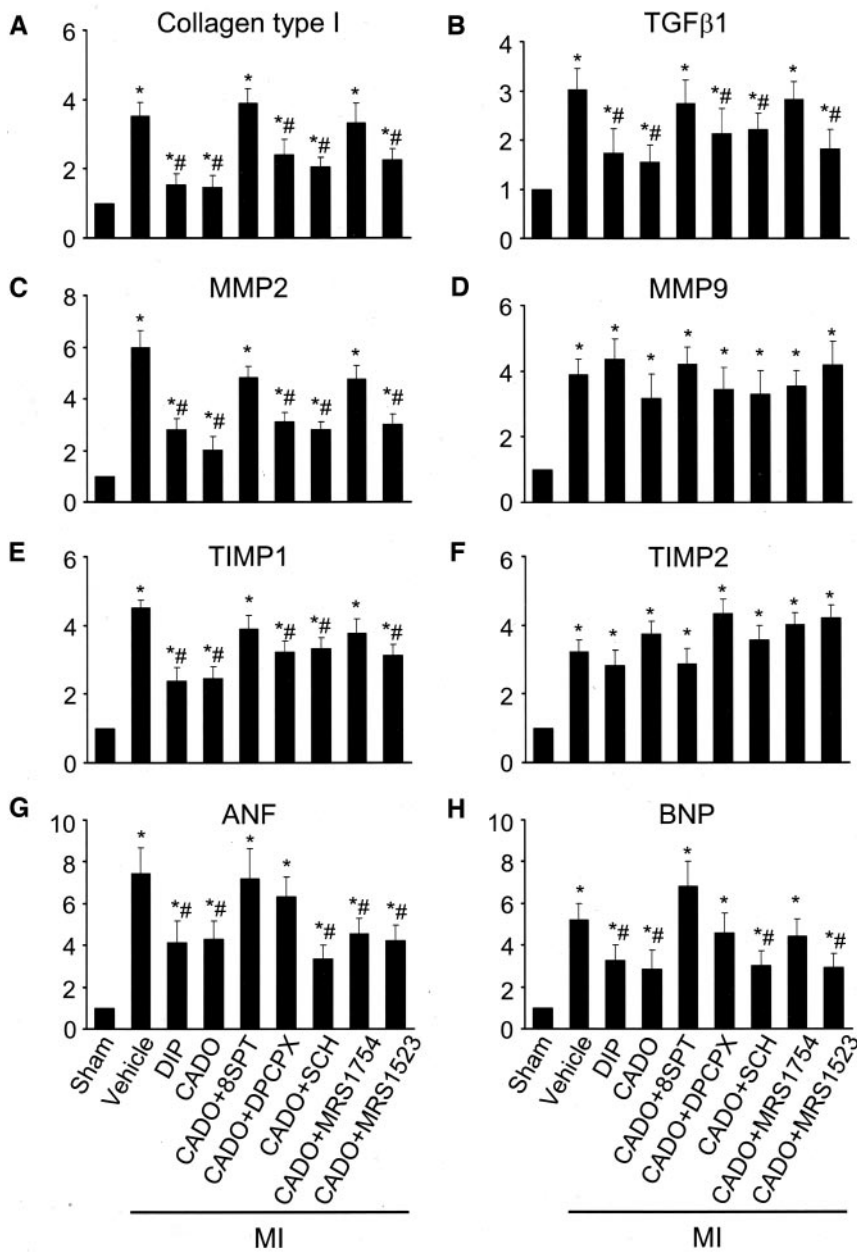


**Figure 5.** Effects of adenosine and antagonists for subtypes of adenosine receptors on cardiac hypertrophy in noninfarcted areas. A, Representative histological findings of cardiomyocytes stained with hematoxylin and eosin in noninfarcted areas. Scale bar=20  $\mu$ m. B, Quantitative analysis of cross-sectional areas of cardiomyocyte. Values are mean $\pm$ SEM. \* $P$ <0.05 vs sham; # $P$ <0.05 vs vehicle (n=8).

first to demonstrate the involvement of adenosine A2b receptor in cardiac remodeling.

Because the excess deposition of extracellular matrix proteins in noninfarcted areas has gained recognition as an important contributor to adverse remodeling and ventricular dysfunction after MI,<sup>36,37</sup> we have examined the in vivo effects of either DIP or CADO on the extent of fibrosis in noninfarcted areas. We found that either DIP or CADO significantly attenuates an increase in morphometric collagen volume fraction in noninfarcted areas. The attenuation in collagen volume fraction in noninfarcted areas by either DIP or CADO was blunted by 8-SPT or MRS1754 but not by the antagonist of other subtypes of adenosine receptors. The amount of myocardial collagen deposition after MI in infarcted and noninfarcted areas during the healing process was reported to influence and to be integral to the process of ventricular remodeling.<sup>38</sup> In addition, it has been shown that excessive accumulation of myocardial collagen may result in rigidity of the myocardium and severely impaired relaxation.<sup>39</sup> Our data strongly suggest that the reduction in collagen volume in noninfarcted areas through adenosine A2b receptors may contribute to the improvement in cardiac function after MI.

Multiple subtypes of adenosine receptors have been reported to contribute to the antihypertrophic effects of

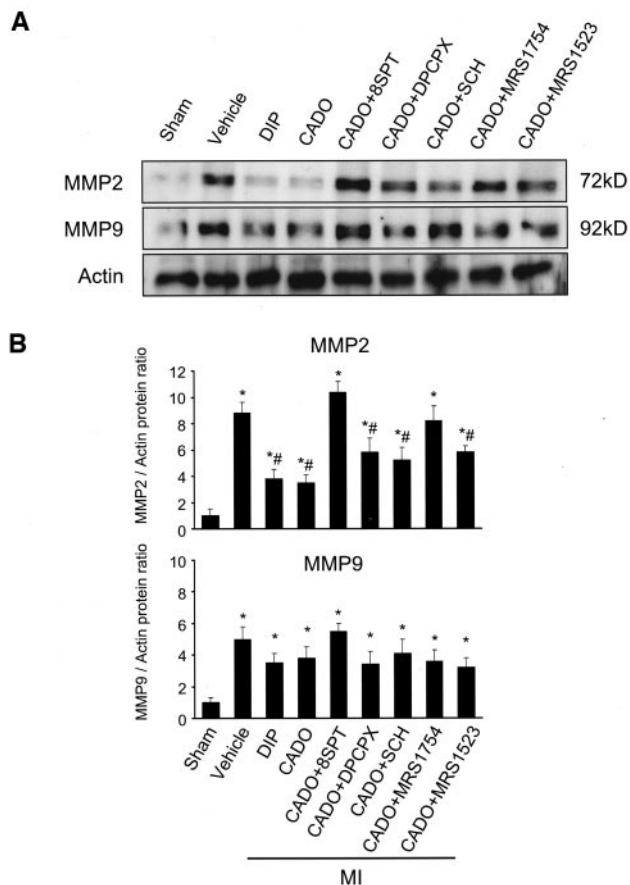


**Figure 6.** Effects of adenosine and antagonists for subtypes of adenosine receptors on molecules associated with cardiac remodeling in noninfarcted areas. Quantitative real-time reverse-transcriptase polymerase chain reaction analysis of collagen type I (A), TGF-β1 (B), MMP-2 (C), MMP-9 (D), TIMP-1 (E), TIMP-2 (F), ANF (G), and BNP (H). Each mRNA value was corrected for glyceraldehyde phosphate dehydrogenase mRNA value. Levels in sham group were arbitrarily assigned a value of 1.0. Values are mean ± SEM. \*P < 0.05 vs sham; #P < 0.05 vs vehicle. Results represent analysis of 3 independent experiments.

adenosine in cardiomyocytes.<sup>40</sup> We have recently demonstrated that adenosine inhibits cardiac hypertrophy through adenosine A1 receptor in pressure-overloaded hearts.<sup>15</sup> Interestingly, we found that either DIP or CADO attenuated hypertrophic changes in cardiomyocytes and mRNA levels of ANF and BNP in the noninfarcted LV, both of which were blunted by DPCPX, the antagonist of adenosine A1 receptors. However, DPCPX did not blunt the improvement in cardiac performance after MI by long-term stimulation of adenosine receptors during the experimental period, suggesting that long-term stimulation of the adenosine A1 receptor may not play an important role in cardiac remodeling after MI. One potential explanation for the discrepancy in the effects of adenosine A1 and A2 receptors on cardiac function is the difference in the pathophysiology in hearts, ie, hypertrophy versus MI. We

need to consider the possibility that the long-term stimulation of adenosine A1 receptor may attenuate cardiac remodeling through its effects on cardiomyocytes.

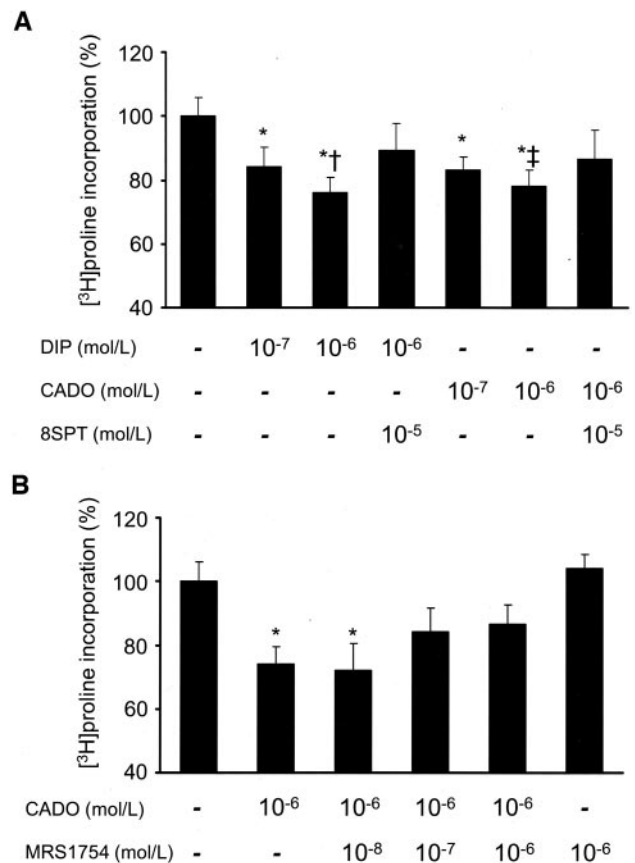
To examine the molecular mechanism by which adenosine attenuates cardiac fibrosis in noninfarcted areas, we examined the gene expression of molecules associated with cardiac remodeling such as TGF-β1, collagen, MMPs, and TIMPs. CADO attenuated the expression of collagen type I, TGF-β1, MMP-2, and TIMP-1 in noninfarcted areas at 4 weeks after MI. In addition, the effect of CADO was blunted by either 8-SPT or MRS1754 but not by the antagonist of other subtypes of adenosine receptors. These results provide *in vivo* evidence that adenosine is a potent “fibrosis-inhibitory agent” after MI. Interestingly, either DIP or CADO failed to attenuate the mRNA levels of MMP-9 or TIMP-2. However, because the extracellular



**Figure 7.** Effects of adenosine and antagonists for subtypes of adenosine receptors on MMP protein levels in noninfarcted areas. A, Representative immunoblot analysis of MMP-2 and MMP-9 proteins. B, Quantitative analysis of MMP-2 and MMP-9 proteins by densitometry. Each protein value was corrected for actin protein value. Levels in sham group were arbitrarily assigned a value of 1.0. Values are mean±SEM. \**P*<0.05 vs sham; #*P*<0.05 vs vehicle. Results represent analysis of 3 independent experiments.

matrix dramatically changes during the time course after MI,<sup>41</sup> we need to carefully consider the effects of adenosine on the molecules associated with cardiac remodeling. Further investigation is needed to clarify the effect of adenosine on the regulation of extracellular matrix after MI.

The activation of adenosine A2b receptors directly inhibits collagen production<sup>42</sup> and mitogenesis in cardiac fibroblasts from adult rats.<sup>43,44</sup> Consistent with the previous study,<sup>42</sup> our in vitro study using rat adult cardiac fibrosis showed that the decrease in [<sup>3</sup>H]proline incorporation by either DIP or CADO was completely blocked by MRS1754, the selective A2b receptor antagonist, but not the antagonist of other subtypes of adenosine receptors. These findings support the idea that the activation of adenosine A2b receptors decreases the severity of myocardial fibrosis in the noninfarcted LV. Although we chose the specific antagonist for the each subtype of adenosine receptors, we must notice that these antagonists still have capacity to antagonize other subtype of adenosine receptors.<sup>22,45</sup> Future studies using genetically engineered ani-



**Figure 8.** Cellular mechanism of the antifibrosis action of adenosine. A, The effect of DIP or CADO on [<sup>3</sup>H]proline incorporation in adult cardiac fibroblasts with or without 8-SPT (10<sup>-5</sup> mol/L). Results represent analysis of 3 independent experiments. B, [<sup>3</sup>H]proline incorporation into adult cardiac fibroblasts with or without MSR1754, the selective A2b receptor antagonist, in the presence of CADO (10<sup>-6</sup> mol/L). Values are mean±SEM. \**P*<0.05 vs no treatment; †*P*<0.05 vs DIP (10<sup>-7</sup> mol/L); ‡*P*<0.05 vs CADO (10<sup>-7</sup> mol/L).

mals are needed to clarify the exact role of each subtype of adenosine receptors.

The cause of CHF may not be unique, and several neurohumoral factors contribute largely to the pathophysiology of CHF.<sup>3,4</sup> Therefore, it is important in the treatment of CHF to attenuate these neurohumoral factors. Because adenosine is reported to attenuate the sympathetic nervous system, renin-angiotensin system, and cytokine system,<sup>46–49</sup> elevation of adenosine levels may contribute largely to the beneficial treatment of CHF by modulating neurohumoral factors. Recently, Yang et al<sup>50</sup> demonstrated the augmentation of proinflammatory cytokines such as TNFα in adenosine A2b receptor knockout mice. Further studies are needed to determine the contribution ratio of direct and indirect effects of adenosine A2b receptor on cardiac remodeling.

A number of therapeutic approaches to limiting ventricular remodeling in MI have been reported.<sup>3,4</sup> These agents include angiotensin-converting enzyme inhibitors, angiotensin II type 1 receptor blockers, β-adrenergic blockers, aldosterone antagonists, and MMP inhibitors.<sup>3–5</sup> Our findings suggest that the increased adenosine levels in CHF



may be compensatory against CHF, leading to the idea that further elevation of adenosine levels or long-term stimulation of adenosine A2b receptors may be a new strategy for treating CHF.

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### Disclosures

None.

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### CLINICAL PERSPECTIVE

Chronic heart failure is a major complication of myocardial infarction (MI) that substantially worsens its prognosis. Postinfarction remodeling includes progressive chamber dilatation, cardiac hypertrophy, and fibrosis. Fibrosis in the noninfarcted myocardium contributes to the pathogenesis of postinfarction cardiac dysfunction. Adenosine mediates cardioprotection against acute ischemia/reperfusion injury, mainly through adenosine A1, A2a, and A3 receptors. Adenosine also inhibits proliferation of cardiac fibroblasts and hypertrophy of cardiomyocytes, both of which may play crucial roles in cardiac remodeling. The present study demonstrated that long-term stimulation of adenosine receptors, begun 1 week after the onset of MI, improved hemodynamic and echocardiographic parameters. Long-term adenosine receptor stimulation reduced the collagen volume fraction and gene expression of the molecules associated with cardiac remodeling in noninfarcted myocardium. These improvements were blunted by the selective adenosine A2b antagonist. These data suggest that the long-term stimulation of adenosine A2b receptors begun after MI attenuates cardiac fibrosis in the noninfarcted regions and improves cardiac function. Drugs that stimulate adenosine A2b receptors or increase adenosine levels may be new candidates to attenuate post-MI cardiac remodeling.

## Long-Term Stimulation of Adenosine A2b Receptors Begun After Myocardial Infarction Prevents Cardiac Remodeling in Rats

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