Novel $N^6$-substituted adenosine 5′-N-methyluronamides with high selectivity for human adenosine $A_3$ receptors reduce ischemic myocardial injury


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Tracey, W. Ross, William P. Magee, Joseph J. Oleynek, Roger J. Hill, Andrew H. Smith, David M. Flynn, and Delvin R. Knight. Novel $N^6$-substituted adenosine 5′-N-methyluronamides with high selectivity for human adenosine $A_3$ receptors reduce ischemic myocardial injury. Am J Physiol Heart Circ Physiol 285: H2780–H2787, 2003.—We recently reported the identification of a novel human adenosine $A_3$ receptor-selective agonist, (2S,3S,4R,5R)-3-amino-5-[(5-chloro-2-(3-methylisoxazol-5-ylmethoxy)benzylamino)purin-9-yl]-4-hydroxytetrahydrofuran-2-carboxylic acid methylamide (CP-608,039), with 1,260-fold selectivity for the human $A_3$ versus human $A_1$ receptor (DeNinno et al., J Med Chem 46: 353–355, 2003). However, because the modest (20-fold) rabbit $A_3$ receptor selectivity of CP-608,039 precludes demonstration of $A_3$-mediated cardioprotection in rabbit models, we identified another member of this class, (2S,3S,4R,5R)-3-amino-5-[(2,5-dichlorobenzylamino)purin-9-yl]-4-hydroxytetrahydrofuran-2-carboxylic acid methylamide (CP-532,903), which both retained human $A_3$ receptor selectivity (210-fold; human $A_3$/human $A_1$ $K_i$: 23/4,800 nM) and had improved rabbit $A_3$ receptor selectivity (90-fold; rabbit $A_3$/rabbit $A_1$ $K_i$: 23/2,000 nM). Infarct size was measured in Langendorff hearts or in vivo after 30 min of regional ischemia and 120 min of reperfusion. Five-minute perfusion with CP-532,903 before ischemia-reperfusion elicited a concentration-dependent reduction in infarct size in isolated hearts (EC$_{50}$: 97 nM; maximum reduction in infarct size: 77%, $P < 0.05$ vs. control). Furthermore, administration of CP-532,903 (150 nM) at reperfusion also significantly reduced infarct size by 64% ($P < 0.05$ vs. control), which was not different ($P > 0.05$) from the cardioprotection provided by the same concentration of drug given before ischemia. The selective rabbit $A_3$ receptor antagonist BWA1433 did not affect CP-532,903-dependent cardioprotection. In vivo, CP-532,903 (1 mg/kg) reduced infarct size by 50% in the absence of significant hemodynamic effects (mean arterial pressure, heart rate, rate-pressure product). CP-532,903 and CP-608,039 represent a novel class of human $A_3$ receptor-selective agonists that may prove suitable for investigation of the clinical cardioprotective efficacy of $A_3$ receptor activation. 

THE ABILITY TO PROVIDE CARDIOPROTECTION, i.e., the reduction or prevention of myocardial injury resulting from ischemia-reperfusion, is an area of significant unmet clinical need. The potential utility of adenosine receptor agonists in this arena became evident after Ely et al. (8) demonstrated that adenosine could provide protection from ischemic injury, and subsequent studies by Downey et al. (22, 23, 33, 36) and others (1, 10, 18) indicated that the cardioprotective effect of adenosine was mediated by activation of adenosine $A_1$ and/or $A_3$ receptors. Studies from both our group (14, 34, 35) and Auchampach et al. (2) have confirmed and extended these observations, both in vitro and in vivo, through the use of selective adenosine $A_3$ receptor agonists; importantly, these benefits were obtained in the absence of any changes in hemodynamics or cardiac function.

Clinically, adenosine has been reported to provide a cardioprotective benefit in the settings of coronary angioplasty, cardiac surgery, and myocardial infarction (12, 20, 25–27, 38), although adenosine carries with it the risk of clinically unacceptable changes in heart rate (HR) and blood pressure. In addition, $A_3$ receptor agonists reduce ischemic injury in isolated human cardiac tissue (3). Studies such as these highlight the significant therapeutic potential of a selective human $A_3$ receptor agonist. However, whereas modestly selective $A_3$ receptor agonists exist for some animal species, potent and selective human $A_3$ receptor agonists have yet to be identified.

Efforts in our laboratories to identify a compound(s) possessing highly potent human $A_3$ receptor agonist activity, while retaining at least 1,000-fold selectivity against other adenosine receptor subtypes, particularly $A_1$, $A_2a$, and $A_2b$ receptors, have led to the discovery of several compounds of interest. One of these compounds, (2S,3S,4R,5R)-3-amino-5-[(5-chloro-2-(3-methylisoxazol-5-ylmethoxy)benzylamino)purin-9-yl]-4-hydroxytetrahydrofuran-2-carboxylic acid methylamide (CP-608,039) (Fig. 1), possesses 1,260-fold selectivity for the human $A_3$ versus $A_1$ receptor and represents a novel class of human $A_3$ receptor-selective agonists (7). However, CP-608,039 has minimal rabbit $A_3$ versus $A_1$ receptor activity.

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binding was determined in the presence of 5 μM \(^{125}\text{I}\)ABA. Affinity constants \((K_i)\) were calculated for each individual experiment by fitting binding data via nonlinear least-squares regression analysis to the following equation: 

\[
\% \text{Inhibition} = 100/(1 + (C/IC_{50})^D)
\]

where \(X\) is the log [drug concentration], \(C\) is the log [drug concentration at 50% inhibition] \((IC_{50})\), and \(D\) is the Hill coefficient. At the 0.1 mM concentration of \(^{125}\text{I}\)ABA used in competition experiments, 70-fold < \(^{125}\text{I}\)ABA \(K_i\) for rabbit A1, 90-fold < \(^{125}\text{I}\)ABA \(K_i\) for rabbit A2, and 120-fold < \(^{125}\text{I}\)ABA \(K_i\) for rabbit A3 (13), \(IC_{50} = K_i\).

\(cAMP\) accumulation. CHO-K1 cells stably transfected with either rabbit A1 or A2 receptors were washed with PBS and then detached with 1.0 mM EDTA-PBS. Cells were collected by centrifugation at 300 g for 5 min, and the supernatant was discarded. The cell pellet was dispersed and resuspended in cell buffer (DMEM-F-12 containing 10 mM HEPES, 20 μM RO-20-1724, and 1 U/ml ADA). After preincubation of cells (100,000 cells/well) for 10 min at 37°C, 5 μM forskolin, with or without the appropriate concentration of test compound, was added, and the incubation was continued for 10 min.

Reactions were terminated by the addition of 0.1 N HCl, followed by centrifugation at 2,000 g for 10 min. Supernatants were removed, and cAMP levels were determined by radioimmunoassay (New England Nuclear; Boston, MA). The control basal and forskolin-stimulated cAMP accumulation were routinely 1 and 30 pmol·ml\(^{-1}\)·100,000 cells\(^{-1}\) for cells containing rabbit A1 receptors and 1 and 15 pmol·ml\(^{-1}\)·100,000 cells\(^{-1}\) for cells containing rabbit A3 receptors.

\textit{In vitro (Langendorff) preparation.} Male New Zealand White rabbits (3–4 kg, Covance; Denver, PA) were anesthetized by intravenous administration of pentobarbital sodium (30 mg/kg), followed by intubation and ventilation with 100% O\(_2\) using a positive pressure ventilator. A left thoracomy was performed, the heart was exposed, and a snare (2-0 silk) was placed loosely around a prominent branch of the left coronary artery. The heart was rapidly removed from the chest, mounted on a Langendorff apparatus, and maintained by retrograde perfusion (nonrecirculating) with a modified Krebs solution containing (in mM) 118.5 NaCl, 4.7 KCl, 1.2 MgSO\(_4\), 1.2 KH\(_2\)PO\(_4\), 24.8 NaHCO\(_3\), 2.5 CaCl\(_2\), and 10 glucose at a constant pressure of 80 mmHg and a temperature of 38.5°C. Perfuse pH was maintained at 7.4–7.5 by bubbling with 95% O\(_2\)-5% CO\(_2\).

The temperature of the heart was maintained by suspending it in a heated, water-jacketed organ bath. A fluid-filled latex balloon was inflated in the left ventricle (LV) and connected by stainless steel tubing to a fluid-pressure transducer; the balloon was inflated to provide a systolic pressure of 80–120 mmHg and a diastolic pressure of ≤10 mmHg. HR, LV systolic and diastolic pressures, and LV developed pressure (LVDP) were recorded using a PO-NE-MAH Data Acquisition and Archive System (Gould Instrument Systems; Valley View, OH). Total coronary flow rate (CF) was determined using an in-line flow probe (Transonic Systems; Ithaca, NY); CF was normalized for heart weight. Each heart was allowed to equilibrate for 30 min; if stable LV pressures within the parameters outlined above were not observed, the heart was discarded. Pacing was not used unless the HR fell below 180 beats/min before the 30-min period of regional ischemia; in this case, the heart was paced at 200 beats/min, which was the average spontaneous rate observed.

\textit{Langendorff experimental protocols.} CP-532,903 was perfused through the heart for 5 min, followed by a 10-min washout (Fig. 2); control hearts received vehicle alone. Thirty minutes of regional ischemia were then produced by tightening the snare around the branch of the coronary artery. At
the end of this period, the snare was released, and the heart was reperfused for an additional 120 min. In experiments in which the rabbit A₁ receptor-selective antagonist 8-(4-carboxyethenylphenyl)-1,3-dipropylxanthine (BWA1433) was used, the antagonist was perfused through the heart for a total of 15 min, beginning 5 min before the A₃ receptor agonist perfusion and continuing until 5 min after the A₃ receptor agonist perfusion was stopped (Fig. 2). A 5-min washout period followed before the ischemia-reperfusion. BWA1433 perfusion alone had no effect on infarct size in this model (34). Two additional groups of rabbits were studied to examine whether CP-532,903 was cardioprotective when administered at reperfusion. In hearts from these rabbits, either drug or vehicle perfusion was initiated 1 min before the coronary artery snare was released and continued for the duration of the reperfusion period (Fig. 2).

In vivo preparation. Forty-six fed New Zealand White male rabbits (3–4 kg) were anesthetized with pentobarbital sodium (30 mg/kg iv), and a surgical plane of anesthesia was maintained by a continuous infusion of pentobarbital sodium (16 mg·kg⁻¹·h⁻¹) via an ear vein catheter. A tracheotomy was performed through a ventral midline cervical incision, and the rabbits were ventilated with 100% oxygen using a positive pressure ventilator. Body temperature was maintained at 38.5°C using a heating pad connected to a temperature controller (model 72, Yellow Springs Instruments; Yellow Springs, MD). Fluid-filled catheters were placed in the left jugular vein for drug administration and in the left carotid artery for blood pressure measurements and for blood gas analysis using a blood gas analyzer (model 248, Bayer Diagnostics; Norwood, MA). The ventilator was adjusted as needed to maintain blood pH and Pco₂ within normal physiological ranges for rabbits. The heart was exposed through a left thoracotomy at the fourth intercostal space, and a 2-0 silk suture was placed around a prominent branch of the left coronary artery. Lead II ECG was measured using an ECG amplifier (Gould; Cleveland, OH) connected to surface ECG electrodes. Arterial pressure was measured using a calibrated strain-gauge transducer (Spectromed; Oxnard, CA) connected to the arterial catheter. HR and mean arterial pressure (MAP) were derived using the PO-NE-MAH Data Acquisition and Archive System. The rate-pressure product (RPP) was calculated as the product of HR and MAP. RPP has been previously used as an index of myocardial O₂ consumption in this model (16).
performed in duplicate.

Data are means ± SE; n = 4–6, performed in duplicate.

Determination of infarct size. After the completion of each experiment (in vitro or in vivo) and with the heart suspended and perfused on the Langendorff apparatus, the coronary artery snare was retightened, and a 0.5% suspension of fluorescent zinc cadmium sulfide particles (1–10 μm) was perfused through the heart to delineate the area at risk (AAR; nonlabeled) in the LV for infarct development. The heart was removed from the Langendorff apparatus, blotted dry, weighed, wrapped in aluminum foil, and stored overnight at −20°C. Frozen hearts were sliced into 2-mm transverse sections and incubated with 1% triphenyltetrazolium chloride in PBS for 20 min at 37°C to delineate the noninfarcted (stained) from infarcted (nonstained) LV tissue. The infarct area (IA) and AAR were calculated for each slice of the LV using video-captured images and ETC3000 image-analysis software (Engineering Technology Center; Mystic, CT), followed by adding the values for each tissue slice to obtain the total IA and total AAR for each heart. To normalize the infarct area for differences in the AAR between hearts, the infarct size was expressed as the ratio of IA to AAR (%IA/AAR).

Data expression and analysis. Data are expressed as means ± SE. Between-group comparisons of in vitro and in vivo AARs expressed as a percentage of LV areas (%AAR/LV) were compared using ANOVA. Comparisons of in vivo hemodynamic parameters between CP-532,903 and vehicle control over the course of the study were performed using ANOVA with repeated measures. In vitro hemodynamic comparisons were performed by t-test, whereas in vitro and in vivo %IA/AAR values were compared using a Mann-Whitney test; a Bonferroni correction was applied to multiple comparisons. A P value of < 0.05 was considered statistically significant.

Drugs and drug preparation. CP-532,903, CP-608,039, and BWA1433 were synthesized at Pfizer Global Research and Development (Groton, CT). [125I]ABA was prepared by New England Nuclear, and ADA was obtained from Boehringer Mannheim (Indianapolis, IN). DMEM-F-12 culture media and fetal calf serum were obtained from GIBCO-BRL (Grand Island, NY). When administered to the isolated hearts, CP-

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<tr>
<td>CP-532,903</td>
<td>4,800</td>
<td>23</td>
<td>210</td>
<td>2,000</td>
<td>23</td>
<td>90</td>
</tr>
<tr>
<td>CP-608,039</td>
<td>7,300</td>
<td>5.8</td>
<td>1,260</td>
<td>1,750</td>
<td>82</td>
<td>20</td>
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<tr>
<td>BWA1433</td>
<td>3</td>
<td>746</td>
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Values are affinity constants (K_i; in nM). K_i was determined by measuring displacement of N^6-(4-amino-3-[125I]iodobenzyl)adenosine binding as described in MATERIALS AND METHODS. A3-to-A1 ratios are rounded off. Human data for CP-608,039 and rabbit data for BWA1433 are from DeNinno et al. (7) and Hill et al. (13), respectively.

Fig. 3. Inhibition of N^6-(4-amino-3-[125I]iodobenzyl)adenosine ([125I]ABA) binding to human A1 (solid circles) and A3 receptors (solid squares) by CP-532,903 (A) and to rabbit A1 (circles) and A3 receptors (squares) by CP-532,903 (solid symbol) and CP-608,039 (open symbol) (B). [125I]ABA binding to CHO-K1 cells stably transfected with adenosine receptors was measured as described in MATERIALS AND METHODS, and smooth curves were fitted to the data via nonlinear least-squares regression analysis. Data are means ± SE; n = 4–6, performed in duplicate.

Fig. 4. CP-532,903 inhibition of forskolin-stimulated cAMP accumulation in CHO-K1 cells expressing rabbit A1 (solid circles) or A3 receptors (solid squares). cAMP measurement was as described in MATERIALS AND METHODS, and smooth curves were fitted to the data via nonlinear least-squares regression analysis according to the following equation: %Forskolin-stimulated cAMP = 100/[1 + (X/10^D)^-H]), where X is the log [drug concentration], C (IC50) is the log [drug concentration at 50% inhibition], and D is the Hill slope. Data are means of duplicate determinations of a representative experiment.
Radioligand displacement studies demonstrated that CP-532,903 was both more selective (90-fold) and potent for the rabbit A3 receptor than CP-608,039 (Fig. 3 and Table 1) while retaining good selectivity and potency for the human A3 receptor (Fig. 3 and Table 1) as previously demonstrated that BWA1433 alone has no effect on infarct size (34). For the in vivo studies, CP-532,903 was dissolved in 0.01 N HCl in normal saline.

RESULTS

For the Langendorff studies, baseline HR, CF, and LVDP values for each of the treatment groups were similar before the regional ischemia and are shown in Table 2. LVDP and CF were significantly (*P < 0.05) reduced in all groups by occlusion of the coronary artery, confirming that ischemia was achieved in all groups (Table 2). %AAR/LV was 55 ± 3% (n = 11) for the control group; other groups did not differ significantly (P ≥ 0.05) from the controls.

CP-532,903 elicited a concentration-dependent reduction in infarct size in the isolated rabbit hearts (Fig. 5), with an EC50 of 0.97 nM. The maximum reduction in infarct size was 77% (control: 53 ± 2% IA/AAR; 150 nM CP-532,903: 12 ± 2% IA/AAR). The rabbit A1 receptor-selective antagonist BWA1433 (13, 34) had no effect on the cardioprotection elicited by 150 nM CP-532,903 (Fig. 5; 13 and 12 ± 2% IA/AAR with and without BWA1433, respectively). We (34) have previously demonstrated that BWA1433 alone has no effect on infarct size in this model.

The cardioprotective efficacy of CP-532,903 when administered at reperfusion was also examined. CP-

<table>
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<tr>
<th>Group</th>
<th>n</th>
<th>HR</th>
<th>CF</th>
<th>LVDP</th>
<th>HR</th>
<th>CF</th>
<th>LVDP</th>
</tr>
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<tr>
<td>Control</td>
<td>11</td>
<td>217 ± 7</td>
<td>7.1 ± 0.4</td>
<td>102 ± 3</td>
<td>206 ± 6</td>
<td>5.2 ± 0.5*</td>
<td>70 ± 4*</td>
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<tr>
<td>CP-532,903 (0.15 nM)</td>
<td>6</td>
<td>204 ± 6</td>
<td>7.2 ± 0.4</td>
<td>102 ± 1</td>
<td>200 ± 7</td>
<td>5.5 ± 0.4*</td>
<td>75 ± 4*</td>
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<td>CP-532,903 (1.5 nM)</td>
<td>6</td>
<td>200 ± 3</td>
<td>7.7 ± 0.1</td>
<td>108 ± 2</td>
<td>202 ± 4</td>
<td>6.1 ± 0.2*</td>
<td>81 ± 4*</td>
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<tr>
<td>CP-532,903 (15 nM)</td>
<td>8</td>
<td>206 ± 4</td>
<td>7.5 ± 0.3</td>
<td>102 ± 3</td>
<td>204 ± 4</td>
<td>6.3 ± 0.2*</td>
<td>76 ± 5*</td>
</tr>
<tr>
<td>CP-532,903 (150 nM)</td>
<td>6</td>
<td>215 ± 10</td>
<td>7.0 ± 0.3</td>
<td>102 ± 3</td>
<td>212 ± 10</td>
<td>5.1 ± 0.2*</td>
<td>75 ± 4*</td>
</tr>
<tr>
<td>CP-532,903 (150 nM) +</td>
<td>5</td>
<td>196 ± 4</td>
<td>5.9 ± 0.2</td>
<td>91 ± 4</td>
<td>191 ± 8</td>
<td>3.8 ± 0.2*</td>
<td>50 ± 8*</td>
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Values are means ± SE; n, number of rabbits. HR, heart rate (in beats/min); CF, total coronary flow (in ml·min⁻¹·g⁻¹); LVDP, left ventricular developed pressure (in mmHg). *P < 0.05 vs. preischemia values.

532,903 was dissolved in DMSO and diluted in buffer; the final DMSO concentration was <0.1%, which had no effect on infarct size (34). For the in vivo studies, CP-532,903 was dissolved in 0.01 N HCl in normal saline.

Fig. 5. Effect of CP-532,903 on the ratio of the infarct area to the area at risk (%IA/AAR) in isolated rabbit hearts. CP-532,903 was perfused through the hearts for 5 min in the presence or absence of BWA1433, followed by 30 min of regional ischemia and 120 min of reperfusion, as described in MATERIALS AND METHODS. IAs and AARs were determined by image analysis, and the IA was normalized to the AAR (%IA/AAR). CP-532,903 elicited a concentration-dependent reduction in infarct size. Data are means ± SE for each group; n = 6–11. *Significantly different (P < 0.05) from control. Inset: cardioprotection elicited by CP-532,903 (150 nM) was unaffected by a selective A1 receptor antagonist, BWA1433 (50 nM). Data from each heart are presented along with means ± SE for each group; n = 5–6.

Fig. 6. Effect of CP-532,903 on the %IA/AAR in isolated rabbit hearts when administered at reperfusion. CP-532,903 (150 nM) was perfused through the hearts starting 1 min before the release of the coronary artery snare and was continued for the duration of the reperfusion period, as described in MATERIALS AND METHODS. IAs and AARs were determined by image analysis, and the IA was normalized to the AAR (%IA/AAR). Data from each heart are presented along with means ± SE for each group; n = 4–6. *Significantly different (P < 0.05) from control.
532,903 (150 nM) significantly reduced infarct size by 64% compared with control hearts (control: 51 ± 2% IA/AAR; CP-532,903: 18 ± 4% IA/AAR, P < 0.05; Fig. 6), which was not significantly different (P ≥ 0.05) from the cardioprotection provided by the same concentration of drug administered before ischemia (77% reduction in infarct size; Fig. 5). The AARs were not different (P ≥ 0.05) between the drug-treated and control groups; ischemia was achieved in both groups, as shown by similar and significant (P < 0.05) drops in CF and LVDP (control values: %AAR/LV, 48 ± 6%; change in CF, 2.3 ml·min⁻¹·g⁻¹; change in LVDP, 72 mmHg, n = 4).

In the in vivo model, the %AAR/LV value for the untreated control group was 37 ± 2% (n = 21); the %AAR/LV values for the remaining in vivo groups were not significantly different (P ≥ 0.05) from the controls. The effects of CP-532,903 on MAP, HR, and RPP in the anesthetized rabbit are illustrated in Fig. 7; no significant effects of CP-532,903 on any of these hemodynamic variables were observed compared with the vehicle control. CP-532,903 produced a significant (P < 0.05) dose-dependent reduction in infarct size in the anesthetized rabbit (Fig. 8). The %IA/AAR was decreased by 50% at a dose of 1 mg/kg, from 60 ± 3% (vehicle control) to 30 ± 6%.

DISCUSSION

We (7) recently reported that CP-608,039 represents the first highly potent and selective human adenosine A₃ receptor agonist. Further evaluation of CP-608,039 revealed that its selectivity for the rabbit A₃ receptor was minimal (20-fold), such that a clear demonstration of A₃ receptor-mediated cardioprotection in standard rabbit models of ischemia-reperfusion injury (17, 35)
was viewed as potentially problematic (given the lack of a rabbit A3-selective antagonist). Therefore, we identified another closely related member of this novel class (N\textsuperscript{6}-substituted adenosine 5′-N-methyluronamides), CP-532,903, which possessed the improved rabbit A\textsubscript{3}/A\textsubscript{1} selectivity (90-fold) necessary to demonstrate A\textsubscript{3}-mediated cardioprotection while retaining good human A\textsubscript{3} receptor selectivity (210-fold). Before the cardioprotection studies were performed, CP-532,903 was confirmed functionally to be a rabbit A\textsubscript{3} receptor agonist.

These studies show that CP-532,903 is a potent cardioprotective agent (EC\textsubscript{50}: 0.97 nM), reducing infarct size in a concentration-dependent manner in the rabbit heart both in vitro and in vivo. The maximum reduction in infarct size we observed in the isolated hearts (77% at 150 nM) was similar to that achieved with other A\textsubscript{3} receptor-selective agonists (34, 35). Given that the K\textsubscript{i} of CP-532,903 for the rabbit A\textsubscript{1} receptor was more than 10-fold higher than the maximum cardioprotective concentration, it is unlikely the reduction in infarct size we observed was A\textsubscript{1} receptor mediated. Nevertheless, we confirmed that this was not the case by attempting to block CP-532,903-dependent cardioprotection with the rabbit A\textsubscript{1} receptor-selective antagonist BWA1433; we (34) have previously demonstrated that 50 nM BWA1433 selectively blocks the rabbit A\textsubscript{1} receptor in our model. CP-532,903-dependent cardioprotection was unaffected by BWA1433; furthermore, there were also no hemodynamic or cardiac function changes observed with CP-532,903 either in vitro or in vivo, which, if present, would have indicated possible A\textsubscript{1} or A\textsubscript{2A} receptor-mediated effects. Clearly, it would have been advantageous to demonstrate inhibition of CP-532,903 (or CP-608,039)-dependent cardioprotection with an A\textsubscript{2A} receptor-selective antagonist, but such a compound does not presently exist for the rabbit receptor.

CP-532,903 was also cardioprotective when administered at reperfusion; moreover, the degree of cardioprotection was not different from that provided by the same drug concentration (150 nM) given before ischemia. These data suggest the time of administration of an A\textsubscript{3} receptor agonist is not critical for reducing infarct size and support previous studies (15, 24) in isolated rabbit and rat hearts in which the A\textsubscript{3} receptor agonist 2-chloro-N\textsuperscript{6}-(3-iodobenzyl)adenosine-5′-N-methyluronamid (Cl-IB-MECA) attenuated reperfusion injury. In contrast, Flood et al. (9) reported that 100 nM Cl-IB-MECA did not diminish myocardial injury when given at reperfusion in isolated mouse hearts. However, this lack of efficacy may be explained by the observations of Maddock and colleagues (24), i.e., 1) that Cl-IB-MECA had a biphasic concentration-response curve in the rat heart, and 2) that concentrations >30 nM administered at reperfusion were not cardioprotective.

A complete understanding of the mechanism(s) underlying A\textsubscript{3} receptor-mediated acute cardioprotection remains elusive (for reviews, see Refs. 21 and 28). Nevertheless, the available evidence implicates coupling of cardiac A\textsubscript{3} receptors via RhoA to phospholipase D (19, 30), generation of diacylglycerol, and activation of protein kinase C (6), followed by opening of mitochondrial ATP-sensitive K\textsuperscript{+} channels (5, 32, 35, 37). Additional evidence suggests the involvement of tyrosine kinase signaling pathways (e.g., p44/p42 MAPK, p78 MAPK, Lck) (11, 31) at least one of which (p38 MAPK via MAPKAPK2) (29) may ultimately lead to phosphorylation of heat shock proteins such as heat shock protein 27. Whether these pathways operate in parallel or in series remains to be clarified. Regardless of the signaling pathway(s) uncertainties, what is clearly evident is that several clinical studies (12, 20, 25–27, 38) strongly suggest an adenosine receptor agonist could have clinical utility for reducing myocardial ischemic injury. Furthermore, A\textsubscript{3} receptor agonists reduce ischemia-reperfusion injury in human myocardial tissue (3). A selective human A\textsubscript{3} receptor agonist theoretically would have significant clinical advantages over adenosine or an A\textsubscript{1} agonist, as the A\textsubscript{3} agonist should be devoid of the cardiodepressant and vasodilatory effects associated with A\textsubscript{1} and A\textsubscript{2A} receptor stimulation; we and others (2, 34, 35) have confirmed this hypothesis in preclinical models.

In summary, CP-532,903 and CP-608,039 (7) were identified from our efforts to design highly selective and potent agonists for the human A\textsubscript{3} receptor. The results from the present study indicate that the novel class of human A\textsubscript{3} receptor agonists represented by CP-532,903 is cardioprotective and free of hemodynamic liabilities, lending further impetus for the development of these compounds for use in providing clinical cardioprotection.

REFERENCES


H2786

NOVEL A3 AGONISTS REDUCE MYOCARDIAL ISCHEMIC INJURY


