Cardioprotection by adenosine \(A_2A\) agonists in a canine model of myocardial stunning produced by multiple episodes of transient ischemia

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Glover DK, Ruiz M, Takehana K, Petruzella FD, Rieger JM, Macdonald TL, Watson DD, Linden J, Beller GA. Cardioprotection by adenosine \(A_2A\) agonists in a canine model of myocardial stunning produced by multiple episodes of transient ischemia. Am J Physiol Heart Circ Physiol 292: H3164–H3171, 2007. First published February 16, 2007; doi:10.1152/ajpheart.00743.2005.—We sought to determine whether administration of a very low, nonvasodilating dose of a highly selective adenosine \(A_2A\) receptor agonist (ATL-193 or ATL-146e) would be cardioprotective in a canine model of myocardial stunning produced by multiple episodes of transient ischemia. Twenty-four anesthetized open-chest dogs underwent either 4 (n = 12) or 10 cycles (n = 12) of 5-min left anterior descending coronary artery (LAD) occlusions interspersed by 5 or 10 min of reperfusion. Regional flow was measured with microspheres. In 12 of 24 dogs, \(A_2A\) receptor agonist was infused intravenously beginning 2 min prior to the first occlusion and continuing throughout reperfusion at a dose below that which produces vasodilatation (0.01 \(\mu g\cdot kg^{-1}\cdot min^{-1}\)). Myocardial flow was similar between control and \(A_2A\) receptor agonist-treated animals, confirming the absence of \(A_2A\) receptor agonist-induced vasodilatation. During occlusion, there was severe dyskinesis with marked LAD zone thinning in all animals. After 180 min of reperfusion following the last cycle, significantly greater recovery of LAD zone thickening was observed in \(A_2A\) receptor agonist-treated vs. control animals in both the 4-cycle (91 ± 7 vs. 56 ± 12%, respectively; \(P < 0.05\)) and the 10-cycle (65 ± 9 vs. 8 ± 16%, respectively; \(P < 0.05\)) occlusion groups. The striking amount of functional recovery observed with administration of low, nonvasodilating doses of adenosine \(A_2A\) agonist ATL-193 or ATL-146e supports their further evaluation for the attenuation of postischemic stunning in the clinical setting.

left ventricular dysfunction; demand ischemia; reperfusion injury

MYOCARDIAL STUNNING IS A FORM OF reversible reperfusion injury characterized by prolonged depression of left ventricular (LV) contractile function in viable myocardium despite restoration of normal or near-normal resting blood flow (7, 28). Clinical studies have demonstrated that many patients with coronary artery disease experience repetitive episodes of myocardial ischemia in the same vascular territory while going about their daily activities (14). Echocardiographic and radionuclide studies have demonstrated that severe postischemic contractile dysfunction, or myocardial stunning, occurs following periods of exercise or pharmacological stress-induced ischemia (15, 30, 34). Importantly, animal studies have shown that frequent episodes of myocardial ischemia that occur in close temporal proximity have a cumulative effect on the deterioration of myocardial contractility, and some (29, 33, 52) have proposed that repetitive myocardial stunning following demand ischemia is the mechanism for myocardial hibernation. In addition, this phenomenon has been proposed as one mechanism for regional systolic dysfunction in unstable coronary syndromes.

It is now thought that the major pathophysiological mechanism underlying myocardial stunning involves damage to myofibrillar and/or sarcoplasmic-reticular proteins by highly reactive oxygen species, particularly the superoxide anion (\(O^\cdot\)) and hydroxyl radical (\('OH\)), that are produced in the first few minutes of reperfusion with resulting disruption in calcium homeostasis (4–6, 51). Increased cytokine production with ischemia-reperfusion might also play a pathogenic role in myocardial stunning.

Recent experimental evidence indicates that adenosine markedly attenuates stunning after a period of myocardial ischemia (37, 50). Furthermore, adenosine receptor blockade has been shown to worsen myocardial stunning, presumably by inhibiting the protective effects of endogenous adenosine produced during the ischemic period (9, 40). The mechanism for the adenosine-mediated protection against myocardial stunning is unknown; however, it does not involve preconditioning, because it has been shown to be protective even when given just before reperfusion (37, 50).

We hypothesized that the anti-inflammatory property of adenosine is at least partly responsible for the cardioprotection against myocardial stunning. Specifically, adenosine, acting on \(A_2A\) receptors on the vascular endothelium and on all bone marrow-derived white cells and platelets, inhibits inflammatory processes, including neutrophil adhesion and extravasation and platelet aggregation. It also prevents the release of injurious cytokines, proteases, and reactive oxidative species such as \(O^\cdot\) and \('OH\). Accordingly, in the present study we tested this hypothesis by administering a very low, nonvasodilating dose of highly selective adenosine \(A_2A\) (\(AA_2A\)) receptor agonists in a canine model of myocardial stunning produced by repetitive episodes of brief coronary occlusion interspersed with reperfusion. Low doses of these \(AA_2A\) receptor agonists have previously been shown to markedly reduce inflammation and to protect tissue in a number of clinically relevant animal models of ischemia-reperfusion injury (13, 22, 41, 56).
MATERIALS AND METHODS

Surgical preparation. Twenty-four fasted, adult mongrel dogs (mean weight 25.6 ± 1.0 kg; range, 15.9–34.5 kg) were anesthetized with pentobarbital sodium (30 mg/kg), tracheally intubated, and mechanically ventilated with room air on a respirator (model 613; Harvard Apparatus) with positive end-expiratory pressure of 5 cm H2O. A left lateral thoracotomy was performed at the level of the fifth intercostal space, and the heart was suspended in a pericardial cradle. A proximal portion of the left anterior descending coronary artery (LAD) was dissected free of the epicardium and was loosely encircled with a suture. Animals were instrumented as previously described for measurement and recording of heart rate (HR), mean arterial pressure (MAP), left atrial pressure, LAD, and left circumflex coronary artery (LCx) flows (model T-206; Transonic Systems). To measure LV pressure and its first time derivative (dP/dt), a high-fidelity pressure-recording catheter (Millar Instruments) was inserted into a carotid artery sheath and was advanced while observing the pressure tracing until its tip rested within the LV cavity. Regional systolic thickening was measured by the epicardial crystal pulsed-Doppler technique (model DMM-10; Crystal Biotech) that has been previously validated in the canine model and has been used extensively by our group (11, 22, 60).

The femoral arteries were cannulated with 8-Fr polyethylene catheters for arterial blood gas monitoring (model 170; CIBA-Corning) and for arterial reference blood withdrawals for microsphere determination of regional blood flows (11, 22, 23, 27). The core temperature of the animal was held constant throughout each experiment by using a thermostatically controlled heat lamp with a set point equal to the initial core temperature of the animal prior to opening the chest cavity.

The investigation conformed with the “Guiding Principles in the Care and Use of Animals,” published by the American Physiological Society, and all experiments were performed with the approval of the Institutional Animal Care and Use Committee at the University of Virginia.

Experimental protocol. After instrumentation of the animals, steady-state baseline hemodynamic and wall-thickening measurements were made and regional myocardial blood flow was assessed with a left atrial injection of 2.5 × 106 radilabeled microspheres (either Cr51, Sr85, Nb95, or Sc46; 15 μm diameter). The microsphere technique is routinely used in our laboratory for measuring regional myocardial blood flow and has been previously described in detail (11, 22, 23, 27).

Stock solutions of the highly selective A2A agonist compounds ATL-193 (5 mg/ml; mol wt = 500.55) and ATL-146e (4.86 mg/ml; mol wt = 486.2) were previously prepared in 100% DMSO. On the day of each experiment, a dog weight-adjusted aliquot of the stock (range 9.6–21.3 μl) was diluted in 120 ml saline, giving a final concentration ranging from 0.40 to 0.86 μg/ml for the infusion solution. The cumulative dose of DMSO administered in these dogs was <0.5 μl/kg body wt.

In 12 dogs (group 1), mild-to-moderate myocardial stunning was produced by four cycles of transient LAD occlusion consisting of 5-min occlusion periods interspersed by 10-min reperfusion periods. Six of these twelve dogs received a treatment consisting of a low-dose intravenous infusion (0.01 μg·kg⁻¹·min⁻¹) of the A2A receptor agonist ATL-193 (23, 45) beginning 2 min prior to the first occlusion and continuing throughout the entire reperfusion period, whereas the remaining six control dogs received an intravenous infusion over the same period of time of an equivalent volume of saline (~97 ml).

In a second group of 12 dogs (group 2), a more severe model of myocardial stunning was used by performing 10 cycles of transient LAD occlusion (5 min occlusion, 5 min reperfusion). As in group 1, 6 of the 12 group 2 dogs were treated with a low-dose intravenous infusion (0.01 μg·kg⁻¹·min⁻¹) of ATL-193 (n = 2) or ATL-146e (n = 4) and 6 control dogs received an intravenous infusion of an equivalent volume of saline (~113 ml) over the same time period.

Both ATL-193 and ATL-146e have similar potency and very high selectivity for the A2A receptor subtype (45), and both compounds have been shown to produce similar hemodynamic effects at high, vasodilating doses in our laboratory (23). The use of one of the A2A agonists versus the other was based on the availability of the compounds at the time when these studies were being conducted.

At the end of the experimental protocol, the dogs were euthanized with an overdose of pentobarbital sodium and potassium chloride, and the heart was rapidly excised. The heart was then sliced into four rings from apex to base and subdivided into 72–1.0-g segments. Regional myocardial blood flow (ml·min⁻¹·g⁻¹) in each segment was quantified by gamma-well counting as previously described in detail (11, 22, 23, 27). Segments with endocardial flow of >0.3 ml·min⁻¹·g⁻¹ during LAD occlusion were classified as central LAD zone, whereas those segments with endocardial flow of >0.5 ml·min⁻¹·g⁻¹ were classified as LCx zone.

Statistical analysis. All statistical computations were made with SYSTAT software. Differences between groups over time were assessed by two-way repeated-measures ANOVA followed by Bonferroni’s post hoc testing when multiple comparisons were made. Single paired comparisons within a group were made by using a paired t-test. The results were expressed as means ± SE. Probability values <0.05 were considered significant.

RESULTS

Hemodynamic data. Mean hemodynamic data are summarized in Tables 1 and 2. In both group 1 (4 cycles) and group 2 (10 cycles), there were no significant variations over time in HR, MAP, or dP/dt, irrespective of the absence or presence of the A2A receptor agonist. Also, in both groups of dogs left atrial pressure increased slightly during occlusion and tended to return to baseline values afterward. In group 1 and group 2 dogs, ultrasonic flow decreased during each LAD occlusion.

In the group 2 dogs that underwent 10 occlusion-reperfusion cycles, reactive hyperemia was present in the reperfused LAD zone 5 min after the last cycle in both the control and A2A receptor agonist-treated subgroups but resolved quickly.

There was no evidence of vasodilatation caused by the A2A receptor agonist infusion in any of the treated dogs in group 1 or 2.

Regional myocardial blood flow. Regional myocardial blood flow data are presented in Tables 3 and 4. Overall, there were no significant differences in regional flows between control and A2A receptor agonist-treated animals in either group of dogs, with the exception of a small increase in endocardial flow in the normal zone of group 1 dogs at 180 min after reperfusion. In all dogs in both groups, regional flow in the endocardial, midwall, and epicardial layers of the LAD zone decreased significantly during occlusion, with endocardial flow being <0.15 ml·min⁻¹·g⁻¹. In all dogs in both groups, LAD zone flow returned to baseline following the last occlusion-reperfusion cycle. With the one exception noted above, normal LCx zone flow was not significantly different from baseline at any time point, confirming the absence of vasodilatation with the A2A agonist infusion at the low dose administered (0.01 μg·kg⁻¹·min⁻¹).

Regional myocardial systolic thickening. Normal LCx zone regional systolic thickening is shown in Table 5. Note that in both groups of dogs, regional systolic thickening in the normal zone was similar in the presence or absence of A2A agonist treatment at all time points, confirming that there was no...
positive inotropic effect of the AA₂ₐ agonist compounds at the low doses administered in this study.

Regional systolic thickening data from the occluded-reperfusion LAD zones of group 1 and group 2 dogs are presented in Figs. 1 and 2, respectively. In animals subjected to four cycles of coronary occlusion and reperfusion (group 1), the LAD zone was dyskinetic during each occlusion cycle, as shown by negative thickening values. In control animals (Fig. 1A), regional thickening was decreased by 65% immediately after the last occlusion-reperfusion cycle and remained significantly inhibited by ~45% for the next 3 h.

In the presence of AA₂ₐ agonist (Fig. 1B), systolic thickening was decreased by 43% immediately after the last coronary reperfusion and full recovery of function was observed as soon as 45 min after reperfusion (P = not significant vs. baseline). After 10 cycles of coronary occlusion and reperfusion (group 2), the LAD zone was akinetic in control dogs, as shown by a mean thickening value decreased by 88% when compared with baseline, and no improvement was observed over the course of the next 3 h (Fig. 2A), indicative of severe stunning. Intravenous infusion of a low dose of AA₂ₐ agonist (Fig. 2B) resulted in a significant improvement as early as 5 min after the last occlusion-reperfusion cycle in treated dogs, with a 55% decrease when compared with baseline (P < 0.01 vs. control). After 3 h of reperfusion, LAD systolic thickening returned to 65% of the baseline value (P < 0.05 vs. control). Similar results were obtained by using either ATL-193 or ATL-146e in this group.

Recovery of LV function in the LAD zone following either 4 or 10 cycles of transient ischemia is summarized in Fig. 3. As
shown, there was significantly greater recovery of thickening after reperfusion with AA2A agonist in both the 4- (56 vs. 91%) and 10-cycle (8 vs. 65%) groups. Note that this greater amount of recovery in the AA2A agonist-treated dogs was observed as early as 5 min after the 10th occlusion cycle.

**DISCUSSION**

In the present study we found that, in a canine model of moderate-to-severe myocardial stunning produced by multiple, brief episodes of transient ischemia, intravenous administration of a low dose of an AA2A receptor agonist (ATL-193 or ATL-146e) abolished or markedly attenuated the posts ischemic impairment in LV function. This is the first experimental study to demonstrate that this improvement in contractile function was unrelated to changes in coronary blood flow or other hemodynamic effects.

The exact mechanism by which the AA2A receptor agonist protected the myocardium against stunning in our study is unknown. One possibility is that the AA2A receptor agonist directly inhibited the production of oxygen free radicals that are known to play a major role in causing myocardial stunning (6). This concept is supported by experimental data from Jordan et al. (31) showing that another AA2A receptor agonist, CGS-21680, markedly reduced O2- production in reperfused canine myocardium and inhibited neutrophil adherence and accumulation. Although neutrophils are no longer believed to be the major source of oxygen free radicals involved in myocardial stunning (3), the vascular endothelium itself can produce free radicals via the enzymes xanthine oxidase and NADH/NADPH oxidase. Because AA2A receptors are also located on the endothelium, it is quite possible that stimulation of these receptors may act directly on these enzymes to inhibit the production of free radicals.

A second possibility is that the oxygen free radical concentration was reduced secondary to an increased production of nitric oxide by the constitutive form of nitric oxide synthase in the endothelium (eNOS). The AA2A receptor agonist CGS-21680 has been shown to increase the production of nitric oxide by both human and porcine cultured arterial endothelial cells (38, 44), as well as in porcine microvessels (25). Although nitric oxide is itself a free radical and is cytotoxic at high concentrations, at lower concentrations it is also a free radical scavenger of O2- and has been shown to be cytoprotective (1, 45). In a pig model of myocardial stunning, Engelman et al. (16) reported that intravenous administration of L-arginine, the nitric oxide precursor molecule, significantly reduced free radical-mediated lipid peroxidation, plasma levels of soluble adhesion molecules, myocardial stunning, and arrhythmias. Furthermore, studies in eNOS-knockout mice have clearly shown that deletion of eNOS exacerbates myocardial stunning (24), whereas overexpression of eNOS in cardiac myocytes was shown to be protective (8).

A third possibility is that AA2A receptor activation was protective by inhibiting the release of inflammatory cytokines and chemokines from tissue-resident mast cells, macrophages, or possibly even from the myocytes themselves. In addition to their role in initiating and amplifying posts ischemic inflammatory reactions, cytokines, particularly TNF-α, IL-6, and IL-1,

**Table 3. Regional myocardial blood flow, group 1**

<table>
<thead>
<tr>
<th>Occ/Rep LAD zone</th>
<th>Control Baseline</th>
<th>Treated</th>
<th>Control Occlusion</th>
<th>Treated</th>
<th>Control Reperfusion, 180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocardium</td>
<td>1.08 ± 0.15</td>
<td>1.35 ± 0.18</td>
<td>0.12 ± 0.03*</td>
<td>0.08 ± 0.03*</td>
<td>0.68 ± 0.09</td>
</tr>
<tr>
<td>Midwall</td>
<td>1.10 ± 0.14</td>
<td>1.34 ± 0.18</td>
<td>0.24 ± 0.07*</td>
<td>0.17 ± 0.05*</td>
<td>0.73 ± 0.08</td>
</tr>
<tr>
<td>Epicardium</td>
<td>1.09 ± 0.14</td>
<td>1.41 ± 0.22</td>
<td>0.37 ± 0.07*</td>
<td>0.19 ± 0.03*</td>
<td>0.79 ± 0.08</td>
</tr>
<tr>
<td>Transmural</td>
<td>1.09 ± 0.14</td>
<td>1.36 ± 0.19</td>
<td>0.25 ± 0.05*</td>
<td>0.15 ± 0.03*</td>
<td>0.73 ± 0.08</td>
</tr>
<tr>
<td>Normal LCx zone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocardium</td>
<td>1.17 ± 0.15</td>
<td>1.47 ± 0.18</td>
<td>1.00 ± 0.11</td>
<td>1.60 ± 0.31</td>
<td>0.84 ± 0.11</td>
</tr>
<tr>
<td>Midwall</td>
<td>1.07 ± 0.14</td>
<td>1.37 ± 0.18</td>
<td>0.90 ± 0.10</td>
<td>1.44 ± 0.29</td>
<td>0.82 ± 0.10</td>
</tr>
<tr>
<td>Epicardium</td>
<td>0.98 ± 0.13</td>
<td>1.29 ± 0.19</td>
<td>0.85 ± 0.09</td>
<td>1.33 ± 0.29</td>
<td>0.80 ± 0.11</td>
</tr>
<tr>
<td>Transmural</td>
<td>1.07 ± 0.14</td>
<td>1.37 ± 0.18</td>
<td>0.91 ± 0.10</td>
<td>1.45 ± 0.30</td>
<td>0.82 ± 0.11</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 experiments each given in ml·min⁻¹·g⁻¹. *P < 0.05 vs. baseline; †P < 0.05 vs. control.

**Table 4. Regional myocardial blood flow, group 2**

<table>
<thead>
<tr>
<th>Occ/Rep LAD zone</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocardium</td>
<td>0.96 ± 0.11</td>
<td>0.97 ± 0.05</td>
</tr>
<tr>
<td>Midwall</td>
<td>0.93 ± 0.08</td>
<td>0.96 ± 0.06</td>
</tr>
<tr>
<td>Epicardium</td>
<td>1.00 ± 0.10</td>
<td>1.01 ± 0.05</td>
</tr>
<tr>
<td>Transmural</td>
<td>0.96 ± 0.09</td>
<td>0.97 ± 0.05</td>
</tr>
<tr>
<td>Normal LCx zone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocardium</td>
<td>1.09 ± 0.12</td>
<td>1.09 ± 0.04</td>
</tr>
<tr>
<td>Midwall</td>
<td>1.00 ± 0.10</td>
<td>1.00 ± 0.04</td>
</tr>
<tr>
<td>Epicardium</td>
<td>0.96 ± 0.08</td>
<td>0.97 ± 0.04</td>
</tr>
<tr>
<td>Transmural</td>
<td>1.01 ± 0.09</td>
<td>1.02 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 experiments each given in ml·min⁻¹·g⁻¹. *P < 0.05 vs. baseline; †P < 0.05 vs. control.
can directly depress contractile function (55). Importantly, TNF-α can be measured in the coronary effluent from isolated hearts during ischemia-reperfusion and has been shown to contribute to postischemic contractile dysfunction (10, 26, 42). Furthermore, Cain et al. (10) demonstrated that adenosine reduced cardiac TNF-α production following reperfusion in isolated rat hearts and that TNF-α-binding protein confers protection similar to that of adenosine against stunning in human myocardium. One possible source of TNF-α in cardiac tissue is from tissue-resident mast cells and macrophages (18, 53). Frangogiannis et al. (18) demonstrated that tissue-resident cardiac mast cells degranulate following coronary reperfusion and release preformed TNF-α that eventually leads to the upregulation of IL-6. Importantly, both mast cells and macrophages are known to have functional AA2A receptors on their...
plasma membranes, and stimulation of these receptors inhibits these cells from degranulating (17).

In summary, although the exact mechanism for the reduction in myocardial stunning with the AA2A agonist treatment remains unknown, there are a number of ways in which these compounds can protect the heart by reducing the levels of injurious oxygen free radicals and by inhibiting the production and/or release of inflammatory cytokines with known negative inotropic properties. Further studies are required to address these mechanisms in detail.

Comparison with studies from other laboratories. Investigators from other laboratories (36, 39) have also shown a reduction in myocardial stunning by using the AA2A receptor agonist CGS-21680. In pigs subjected to myocardial ischemia (15 min LAD occlusion) and reperfusion, Lasley et al. (36) administered CGS-21680 directly into the coronary artery beginning 120 min after reperfusion and found that myocardial contractility was improved in the reperfused zone. In this study, the functional improvement cannot be attributed to an inhibition of oxygen free radicals or an anti-inflammatory effect because the treatment did not begin until 2 h after reperfusion. Although coronary flow also increased, the authors ruled out the flow increase as the mechanism for the increased inotropy. Nevertheless, at least part of the positive inotropic effect observed with CGS-21680 in the Lasley study (36) may have been a systemic effect because, despite the fact that CGS-21680 was given via the intracoronary route, heart rate increased significantly. One possibility is that the increased contractility may have resulted from stimulation of AA2A receptors in the peripheral nervous system, including the baroreceptors and chemoreceptors in the carotid body (21, 32). Stimulation of these autonomic receptors by AA2A receptor agonists is excitatory, has been shown to enhance sympathetic drive (49), and would be expected to increase contractility. This potential mechanism is supported by their finding that the intracoronary infusion of CGS-21680 produced tachycardia without an accompanying reduction in MAP. Although CGS-21680 had no effect on contractility in normal myocardium, this might be explained by the fact that stunned myocardium is known to be hypersensitive (57), i.e., factors that affect contractility in normal myocardium have a greater impact in stunned myocardium (2). It is interesting that in another study, CGS-21680 had no cardioprotective effect in a similar canine model of stunning produced by multiple, brief episodes of coronary artery occlusion and reperfusion (58). Although it is possible that this result could be dose-related, it might also represent differences in the biodistribution between CGS-21680 and the AA2A agonists ATL-146e or ATL-193 used in the present study. CGS-21680 is a carboxylic acid whose positive charge may hinder or prevent the molecule from leaving the intravascular space and reaching potential interstitial targets of action known to have AA2A receptors such as tissue-resident mast cells and/or macrophages.

Clinical implications. ATL-146e, one of the AA2A agonists used in this study, has already undergone safety testing and a Phase 2 clinical trial for high-dose bolus administration as a vasodilator for pharmacological stress myocardial perfusion imaging studies (Bristol-Myers Squibb Medical Imaging). The potential benefit of using a low dose of an AA2A agonist, such as ATL-146e, in the clinical setting to prevent or treat postischemic dysfunction secondary to stunning needs to be explored. The current experimental study in a canine model of repetitive stunning provides proof of principle that prolonged posts ischemic dysfunction can be significantly attenuated and recovery can be hastened by infusion of an AA2A agonist by using a dose that does not produce hypotension or other systemic hemodynamic changes. The striking amount of functional recovery observed with ATL-193 and ATL-146e supports their further evaluation for the attenuation of postischemic stunning after reperfusion therapy in patients. Also, if repetitive stunning is indeed a major pathophysiological mechanism in myocardial hibernation, a long-acting oral formulation of an AA2A agonist could prove to be beneficial for cardioprotection (12, 19, 35, 47, 48).

Study limitations. One limitation of our study was that it was conducted by using an open-chest, anesthetized canine model. Previous studies have shown that the magnitude of changes produced by the ischemic insult (i.e., free radical production, LV dysfunction) are greater in the anesthetized, open-chest model than observed under the same experimental conditions in conscious animals (57). In the present study, all dogs underwent the same experimental procedures and comparisons of LV function were made between the control and treated groups of dogs. If the oxygen free radical burst is indeed higher in anesthetized dogs, then this makes the amount of protection observed in this study all the more impressive.

A second limitation was that the AA2A receptor agonist was administered beginning prior to the first occlusion-reperfusion cycle. Thus it is difficult to ascertain whether the protection afforded by the treatment was due to a preconditioning effect, an anti-ischemic effect, or attenuation of reperfusion injury. As previously mentioned, we intentionally began the treatment prior to the first occlusion because prior studies have shown that a significant oxygen free radical burst occurs even after the first occlusion-reperfusion cycle, with additional and diminishing bursts occurring with subsequent cycles (6). It is unlikely that the antistunning effect observed in our study with ATL-146e was due to preconditioning or to an anti-ischemic effect. Although adenosine is known to be an important trigger of preconditioning, numerous investigators have shown that the preconditioning mechanism with adenosine involves stimulation of the adenosine A1 and possibly A3 receptor subtypes on cardiomyocytes (35). Furthermore, the improvement in postischemic dysfunction observed in our study was not likely due to an anti-ischemic effect of ATL-146e because coronary flow was not enhanced and systemic hemodynamics were unchanged.

A third limitation of this study was the fact that no postmortem tissue analysis was performed to document that the reperfused myocardium was not irreversibly injured by the repetitive occlusion and reperfusion cycles. However, this experimental model of stunning produced by multiple episodes of brief ischemia has been well documented in the literature in studies using the same or an even greater number of occlusion-reperfusion cycles of similar duration (6, 43, 54, 59). In these previous studies, there was a comparable degree of posts ischemic LV dysfunction with no evidence of myocardial necrosis by either postmortem triphenyltetrazolium chloride staining or electron microscopy in any experimental animal (6, 43, 54, 59).

Finally, it is impossible to determine from our study whether the mechanism for protection involves a reduction in the production of oxygen free radicals or an inhibition of the
release of inflammatory cytokines such as TNF-α because we did not measure any of these parameters. Future studies using electron spin-trap techniques to measure free radical production and/or immunohistochemical techniques to measure cytokines in this model may provide an answer to these important questions.

GRANTS

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DISCLOSURES

The A2A, agonists, ATL-193 and ATL-146e, used in this study are owned by Adenosine Therapeutics. T. Macdonald, J. Linden, G. A. Beller, and D. K. Glover have equity positions in, and J. M. Rioger is an employee of, this company.

REFERENCES