Beneficial effects of adenosine A$_{2a}$ agonist CGS-21680 in infarced and stunned porcine myocardium

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Lasley, Robert D., M. Salik Jahania, and Robert M. Mentzer, Jr. Beneficial effects of adenosine A$_{2a}$ agonist CGS-21680 in infarced and stunned porcine myocardium. Am J Physiol Heart Circ Physiol 280: H1660–H1666, 2001.—Although there are conflicting results on whether adenosine infusion during reperfusion alters infarct size, there are several reports that indicate adenosine A$_{2a}$ agonists reduce infarct size. There are also reports that the A$_{2a}$ agonist CGS-21680 increases cAMP and contractility in ventricular myocytes. The purpose of this study was to determine whether low-dose intracoronary infusions of CGS-21680 during reperfusion exert any beneficial effects in irreversibly and reversibly injured myocardium. Open-chest pigs were submitted to 60 min of coronary artery occlusion and 3 h of reperfusion. Treated pigs were administered intracoronary CGS-21680 (0.2 μg·kg$^{-1}$·min$^{-1}$) for the first 60 min of reperfusion. Pigs submitted to regional stunning (15 min ischemia) were treated with intracoronary CGS-21680 (0.15 μg·kg$^{-1}$·min$^{-1}$) after 2 h of reperfusion. In the infarct protocol, CGS-21680 reduced infarct size from 62 ± 2% of the region at risk to 36 ± 2%. In stunned myocardium, CGS increased load-independent regional preload recruitable stroke work and area by ≥70%, but the same infusion in normal myocardium was associated with no inotropic effect. Both beneficial effects were associated with little systemic hemodynamic effects. These findings suggest that reperfusion infusions of low doses of the A$_{2a}$ agonist CGS-21680 exert beneficial effects in reversibly and irreversibly injured myocardium.

adenosine A$_{2a}$ receptor; myocardial stunning; preload recruitable stroke work; myocardial infarction

THE BENEFICIAL EFFECTS of adenosine in reversibly and irreversibly injured myocardium are now well recognized. Adenosine infusion before ischemia attenuates myocardial stunning and reduces infarct size, effects, which based on results with adenosine receptor agonists and antagonists, appear to be mediated primarily via activation of adenosine A$_1$ receptors (15). Furthermore, it is thought that these beneficial effects occur on the cardiac myocytes, because radioligand binding studies indicate that A$_1$ receptors are located primarily on the cardiomyocytes (19) and because A$_1$ agonist protection can be elicited in myocyte models of simulated ischemia (33). Although the results of some recent studies indicate the possible involvement of myocyte A$_3$ receptors (2, 32), there is, to date, no physical evidence of myocyte A$_3$ receptors.

Though the primary focus of the beneficial effects of adenosine has been on the modulation of myocyte signaling during ischemia, many of the initial studies on adenosine cardioprotection were designed to test whether reperfusion infusions of adenosine reduced infarct size. Studies with adenosine generally yielded conflicting results, despite the use of high doses of adenosine (9, 12, 22–24, 34). There are additional reports, however, of the beneficial effects of reperfusion treatments with adenosine A$_{2a}$ agonists (14, 21, 26). It is generally thought that these beneficial effects of adenosine A$_{2a}$ receptor activation are due to reduction of vascular injury and inhibition of neutrophil O$_2$ free radical production (14, 38).

Despite this renewed interest in the reperfusion effects of adenosine A$_{2a}$ agonists in the irreversibly injured heart, there have been few, if any, studies of the effects of reperfusion administration of A$_{2a}$ agonists in stunned myocardium. In fact the few studies on the effects of adenosine administration on posts ischemic function in stunned myocardium, including a previous study from our laboratory (25), have demonstrated little, if any, beneficial effects of reperfusion adenosine treatments in stunned myocardium (25, 28). However, there is now evidence that cardiac myocytes express adenosine A$_{2a}$ receptors (17), and there are reports that adenosine A$_{2a}$ receptor agonists exert a positive inotropic effect in ventricular myocytes (5, 37). Therefore, the purpose of this study was to determine whether low-dose reperfusion treatment with the selective adenosine A$_{2a}$ receptor agonist 2-[(2-carboxyethyl)-phenethylamino]-5’-N-ethylcarboxamidoadenosine (CGS-21680) reduces infarct size and enhances posts ischemic function in an in vivo porcine regional ischemia preparation.

METHODS

All animals in this study received humane care according to the guidelines set forth in The Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals.

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Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, Revised 1996). In addition, animals were used in accordance with the guidelines of the University of Kentucky Institutional Animal Care and Use Protocol.

Animal preparation. Farm pigs weighing 22–27 kg were used. Pigs were premedicated with ketamine (30 mg/kg im) and pentobarbital sodium (15–20 mg/kg iv) and maintained with additional pentobarbital sodium (1.5–2 mg/kg iv) every 15 min. A tracheotomy was performed, and the animals were mechanically ventilated with a mixture of room air and 100% O2. Tidal volume, respiratory rate, and fraction of O2 in inspired air were adjusted to maintain normal arterial blood gas and pH values. Core body temperature was monitored with an esophageal temperature probe and maintained with a heating pad between 37.5 and 38.0°C. Lactated Ringer solution was administered via an ear vein or femoral vein at 5–7 ml·kg⁻¹·h⁻¹, after an initial bolus administration of 300–400 ml. A femoral artery catheter was used to monitor arterial blood pressure and to obtain samples for blood-gas analysis.

The heart was exposed through a median sternotomy and suspended in a pericardial cradle. Left ventricular pressure (LVP) was measured with a 5-Fr high-fidelity, pressure-sensitive tip transducer (Millar Instruments; Houston, TX) placed in the left ventricular cavity via the apex and secured with a purse-string suture. A segment of the left anterior descending coronary artery (LAD), distal to the origin of the first diagonal branch, was dissected free of surrounding tissue. The area at risk was delineated by a brief (<20 s) occlusion of the LAD with a small vascular occluding clamp. A transit-time perivascular flow probe (Transonic Systems; Ithaca, NY) was placed around this segment to measure coronary blood flow (CBF).

Experimental protocols. The preparation was allowed to stabilize for 30 min after all instrumentation was complete before the experimental protocols were initiated. In the infarct studies, pigs were submitted to 60 min of LAD occlusion and 3 h of reperfusion. Before ischemia, phosphate-buffered saline (PBS) was infused through the intracoronary cannula in all pigs. At the onset of reperfusion, control pigs (n = 6) received intracoronary saline for the full 3-h reperfusion period. Treated animals (n = 6) received intracoronary CGS-21680 (0.2 μg·kg⁻¹·min⁻¹) for the first hour of reperfusion, after which intracoronary PBS was infused for the last 2 h of reperfusion. In the stunning protocol, pigs in both groups received intracoronary PBS before ischemia and for the first hour of reperfusion, pigs in the treatment group received CGS-21680 (0.150 μg·kg⁻¹·min⁻¹ intracoronary) for the final hour of reperfusion, whereas the PBS infusion was continued in the control pigs.

All animals received heparin (100 U/kg body wt iv) before the coronary occlusion. Lidocaine (2% solution; 2 mg/kg iv bolus) was administered immediately before occlusion in the infarct studies and just before reperfusion in the stunning studies. After 3 h of reperfusion, the ischemic area at risk was determined by reoccluding the LAD and infusing a 0.5% Evans blue solution into the left ventricle while occluding the aorta. The area at risk was devoid of the Evans blue stain. The animals were euthanized with an overdose of pentobarbital sodium while under deep anesthesia, and the hearts were excised. In the stunning experiments, crystal placement in the ischemic and nonischemic beds was verified after excision of the heart.

Regional ventricular function and load-insensitive contractility. In the stunning experiments, pigs were instrumented with piezoelectric crystals (Crystal Biotech; Houston, TX) to measure regional segment shortening via sonomicrometry. Pairs of crystals were placed in the LAD and left circumflex coronary artery-perfused beds. Crystals were placed in the midmyocardium (~4–6 mm deep) 5–15 mm apart and aligned in a manner such that the intercrystal axis was parallel to the direction of myocardial fiber shortening. End diastole was defined as the onset of pressure increase over time (+dP/dt), and end systole was defined as 20 ms before peak pressure decrease over time (−dP/dt). Segment shortening was defined as end-diastolic length (EDL) minus end-systolic length (ESL), and percent segment shortening (%SS) was calculated as (EDL − ESL/EDL) × 100%. All hemodynamic and sonomicrometry signals were fed through a 32-bit analog-digital converter into an online data acquisition computer with customized software (Augury, Coyote Bay Instruments; Manchester, NH). All hemodynamic data were continuously displayed on a computer monitor. Stroke work (Sw) was calculated by quantifying the area within the pressure-segment length loops generated during each cardiac cycle.

Load-insensitive measurements of preload recruitable SW (PRSW) and PRSW area (PRSWA) were generated from the segment length and LVP data collected during brief (7 s) veno cava occlusions. The inferior vena cava was occluded around its supradiaphragmatic portion by gradual tightening of an umbilical tape snare. During data acquisition, ventilation was held at end expiration to avoid effects of varying venous return on preload. PRSW and PRSWA were calculated according to the methods of Gower et al. (8). PRSW was based on linear regression of the relationship between SW and EDL calculated by the equation SW = Msw (EDL − Lw), where Msw is the slope of PRSW and Lw is the x-axis intercept. PRSWA was determined by the formula PRSWA = Msw/2 (1.2Lw,max − Lw), where Lw,max is the maximum x-axis intercept during the entire experiment. Baseline and caval occlusion data were saved at specific time points in the protocol for offline analysis. On average 9–11 beats were used in each calculation.

Area at risk and infarct size measurement. The isolated left ventricles were cut into four slices of equal thickness in a plane parallel to the atroventricular groove. Each slice was weighed and compressed between two transparent Plexiglas plates separated by a distance of 8 mm to achieve uniform thickness. The cross-sectional surface and ischemic areas of each slice were traced onto a transparency sheet. The slices were then incubated in a 1% triphenyltetrazolium chloride solution in PBS, at 37°C for 15 min. The presence of a brick red stain indicated viable tissue, whereas nonviable tissue (infarcted) remained pale. If any infarct was present, the tissue slices were again compressed between the Plexiglas plates and retraced. The areas on the tracings were quantified with a digitizer (Mustek 1200 IIL, parallel port scanner at 200 dpi) and graphic analysis software (Sigma Scan Pro Automated Image Analysis Software, Jandel Scientific, SPSS; San Rafael, CA). The percent area at risk was calculated for each slice by dividing the area at risk by the total slice area. The sum of the areas at risk of all slices was divided by the sum of the areas of all slices to obtain the percentage of the LV that was ischemic.

Statistical analysis. Results are expressed as means ± SE. Differences between groups were determined by two-way analysis of variance for treatment and time (ANOVA) followed by Duncan’s post hoc test. Differences within each group were determined by repeated-measures ANOVA fol-
lowed by Duncan’s post hoc test. A P value < 0.05 was considered statistically significant.

RESULTS

Infarction protocol. The systemic and cardiac hemodynamic data in the infarction protocol are shown in Table 1. There were no differences in mean arterial blood pressure (MAP), heart rate, or LAD CBF in either group before or during ischemia. During the first hour of reperfusion, the CGS-21680-treated group exhibited a higher heart rate than the control group, which persisted after discontinuation of the CGS-21680 treatment. The intracoronary infusion of CGS-21680 did not significantly alter CBF with the exception that at 60 min of reperfusion CBF was greater than preischemic CBF. There were no significant differences in reperfusion MAP or CBF values between the groups.

Figure 1 illustrates the region at risk and infarct size after 3 h of reperfusion. Control pigs had a region at risk of 22 ± 2% of the left ventricle, and in pigs treated with CGS-21680, 29 ± 3% of the left ventricle was ischemic. After 3 h of reperfusion infarct size in the control group was 62 ± 2% of the region at risk, whereas infarct size in the CGS-21680-treated pigs was reduced to 36 ± 2% (P < 0.01 vs. controls).

Stunning protocol. As shown in Table 2, there were no differences in any hemodynamic parameters between the groups before ischemia. Regional ischemia was associated with segment lengthening, but there were no differences in any hemodynamic parameter between the groups. After 2 h of reperfusion, MAP tended to be lower than preischemic values within each group, but again there were no differences between the groups. The intracoronary infusion of CGS-21680 was associated with reflex tachycardia as heart rate increased by ~20 beats/min (P < 0.05 vs. pre-CGS), and, as expected, CBF during CGS-21680 infusion was significantly increased.

Figure 2 summarizes the effects of the reperfusion intracoronary CGS-21680 treatment on LAD PRSW. Preischemic and 2-h reperfusion PRSW values in the two groups were similar, but after 1 h of CGS-21680 treatment, PRSW increased from 62 ± 4 to 107 ± 7 mmHg·mm·mm⁻¹ (P < 0.05). In contrast in the control group, PRSW values at 3 h of reperfusion (73 ± 6 mmHg·mm·mm⁻¹) were not significantly different from 2-h values (60 ± 9 mmHg·mm·mm⁻¹). The x-axis intercept of the PRSW relationship tended to shift rightward from preischemic values in both groups, but there were no differences between the groups. %SS in control pigs decreased from preischemic values of 20.3 ± 2.8 to 8.0 ± 1.7 and 8.5 ± 1.5% after 2 and 3 h reperfusion, respectively. In the CGS-21680-treated pigs, %SS decreased from 19.3 ± 2.4% before LAD occlusion to 6.0 ± 1.0% after 2 h of reperfusion. At the end of the 60-min CGS-21680 infusion, %SS increased to 9.0 ± 1.6% (P < 0.05 vs. pre-CGS). There was no evidence of necrosis (based on TTC-positive staining) in any of the pigs.

The effects of the A₂a agonist infusion on regional PRSWA are illustrated in Fig. 3, A and B. Both groups exhibited similar preischemic and 2-h reperfusion values of LAD PRSWA (Fig. 3A), with the reperfusion values in both groups depressed by ~40%, indicative of myocardial stunning. Within 30 min after the CGS-21680 infusion was initiated, PRSWA in the LAD bed increased from 205 ± 15 to 435 ± 92 mmHg·mm⁻³·mm⁻¹ (P < 0.05). This increase was maintained throughout the remainder of the intracoronary CGS-21680 infusion (PRSWA at 60 min treatment was 466 ± 73 mmHg·mm⁻³·mm⁻¹). In contrast, PRSWA in
the control stunned pigs increased only slightly over the final hour of reperfusion (from 242 ± 31 to 275 ± 42 mmHg•mm³•mm⁻¹). The PRSWA values in the non-stunned left circumflex artery bed in both control and A₂a agonist-treated pigs remained stable throughout the experiment, and the LAD infusion had no effect on PRSWA in this bed (Fig. 3B).

Two additional protocols were performed to determine whether the effects of CGS-21680 on regional contractility were due to increased CBF. In three normal pigs, CGS-21680 was infused into the LAD for 60 min at a dose similar to that in the stunned animals, which resulted in a 2.5-fold increase in CBF, but PRSWA remained essentially unchanged (Fig. 4). After termination of the CGS infusion, CBF returned to pre-CGS values and PRSWA remained unaltered. Two additional pigs were submitted to 15 min of LAD occlusion and 2 h of reperfusion, at which time an intracoronary infusion of the smooth muscle dilator papaverine (20 μg•kg⁻¹•min⁻¹) was started and maintained for 60 min. As shown in Fig. 5 papaverine increased LAD CBF approximately threefold in both pigs, but PRSWA was only marginally increased.

Table 2. Systemic hemodynamics and LAD coronary blood flow in stunning protocol

<table>
<thead>
<tr>
<th>HR, beats/min</th>
<th>MAP, mmHg</th>
<th>CBF, ml/min</th>
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<tbody>
<tr>
<td>Control CGS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>100 ± 3</td>
<td>82 ± 2</td>
</tr>
<tr>
<td>Ischemia</td>
<td>100 ± 4</td>
<td>76 ± 3</td>
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<tr>
<td>Reperfusion</td>
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<td></td>
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<tr>
<td>1 h</td>
<td>96 ± 5</td>
<td>72 ± 2</td>
</tr>
<tr>
<td>2 h</td>
<td>100 ± 2</td>
<td>72 ± 2</td>
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<tr>
<td>3 h</td>
<td>105 ± 7</td>
<td>71 ± 1</td>
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</table>

Values are means ± SE; n = 6 pigs per group. Baseline values were taken 5 min before LAD occlusion; ischemia values were at 5 min occlusion. One-hour reperfusion values in the CGS were taken just before initiating the intracoronary CGS infusion. *P < 0.05 vs. the control group; †P < 0.05 vs. baseline.

**DISCUSSION**

The results of the present study indicate that low-dose reperfusion treatment with the adenosine A₂a receptor agonist CGS-21680 not only reduces infarct size following prolonged ischemia but also exerts a positive inotropic effect in regionally stunned myocardium. The inotropic effect of CGS appeared to be independent of CBF, because it was not mimicked by the smooth muscle dilator papaverine. In addition the inotropic effect of CGS-21680 was evident only in stunned myocardium. The beneficial effects of CGS-21680 in both reversibly and irreversibly injured myocardium were associated with little hemodynamic effects. These
infiltration (14, 22), consistent with the ability of CGS-21680 to reduce neutrophil adherence to endothelium in vitro and inhibit neutrophil production of superoxide radical (14, 38). These observations are consistent with the presence of adenosine A2a receptors on endothelial cells and neutrophils, and all of these effects would be expected to be beneficial in reperfused myocardium. Because there appears to be a large receptor reserve for coronary adenosine A2a receptors (29), activation of only a small percentage of these receptors should exert significant cardiac effects without the deleterious effects caused by high doses of adenosine or adenosine agonists.

The novel finding in the present study was the ability of a reperfusion infusion of the A2a agonist CGS-21680 to increase contractility in vivo in stunned myocardium. Within 30 min of the commencement of an intracoronary infusion of this agent, PRSW and PRSWA exhibited significant increases from the 2-h reperfusion values, and contractility in the stunned bed remained elevated during the infusion. The A2a agonist infusion was associated with a small decrease in arterial blood pressure and reflex tachycardia; however, PRSW and PRSWA are relatively load-insensitive parameters of regional contractility. Glower et al. (8) reported that PRSWA was one of the most sensitive load-independent parameters of contractility in stunned myocardium. We (13) recently used this measurement to verify that ischemic preconditioning does not improve contractility in stunned porcine myocardium. Treatment with CGS-21680 also improved the recovery of regional segment shortening; however, this measurement of regional ventricular function is load dependent. Given the changes in arterial blood pressure and heart rate during the agonist infusion, this means of assessing regional contractility is not as reliable as PRSW and PRSWA.

Previous studies on the effects of adenosine during reperfusion in stunned myocardium have indicated little, if any, beneficial effects. Sekili et al. (28) reported that intracoronary adenosine infusion for the first hour of reperfusion did not attenuate regional myocardial stunning in dogs. These same authors also observed that a transient adenosine infusion during reperfusion, which increased regional CBF fourfold, did not in-
crease systolic wall thickening. In contrast, our laboratory previously reported that a brief (10 min) intracoronary infusion of adenosine in stunned canine myocardium (after 60 min reperfusion) did increase systolic wall thickening (25). This effect was associated with a five- to sixfold increase in CBF, and when the adenosine infusion was terminated, regional wall thickening decreased in parallel with blood flow. Our laboratory’s previous findings with adenosine are thus consistent with a previous report that increasing blood flow in stunned myocardium can increase regional ventricular function (30).

In the present study, the A<sub>2a</sub> agonist increased regional CBF twofold, but several observations suggest that the increased inotropy was not merely due to increased flow. First, in a subset of animals in which the infusion was terminated after 1 h, CBF returned to basal levels within 15 min, but regional PRSWA remained elevated. Furthermore, an intracoronary infusion of the smooth muscle dilator papaverine, titrated to increase CBF approximately threefold, did not exert any beneficial effect on PRSWA in stunned myocardium. These observations provide evidence that the effect of CGS-21680 in stunned porcine myocardium was independent of changes in CBF. Rather these data are consistent with the hypothesis that CGS-21680 exerted a positive inotropic effect in stunned myocardium.

Although the beneficial action of CGS-21680 on load-independent contractility in stunned myocardium was unexpected, there are two possible explanations for this effect. The first is that coronary A<sub>2a</sub> receptor activation may have stimulated the release of nitric oxide (NO) via endothelial NO synthase. There are several reports that adenosine, via A<sub>2a</sub> receptor activation, stimulates coronary NO release (1, 7, 10). Gao et al. (10) recently reported that infusion of CGS-21680 in postischemic isolated rat hearts increased recovery of LV function, an effect that was associated with increased NO release. Although there is a report that NO donors exert no effects on contractility in isolated papillary muscles (36), there are additional reports that NO donors exert biphase effects on contractility in ventricular myocardium, with low concentrations producing a positive inotropic effect (21, 35). However, all of these studies have been performed in normal myocardium, and NO may exert different effects in stunned myocardium.

A second possibility for the positive inotropic effect of CGS-21680 in stunned myocardium is the stimulation of cardiac myocyte A<sub>2a</sub> receptors. There is pharmacological (3, 5, 31, 37) and immunological (18) evidence that ventricular myocytes express adenosine A<sub>2a</sub> receptors. Although in two of the above studies the A<sub>2a</sub>-induced increases in cAMP were associated with increased myocyte contractility (5, 37), A<sub>2a</sub> agonists and adenosine do not increase contractility in normal intact ventricular myocardium (16, 25). In the present study CGS-21680 did not alter load-independent PRSWA in normal myocardium. Compartmentation of signal transduction may explain the different effects of CGS-21680 on contractility in normal and stunned myocardium. Boknik et al. (3), who reported that CGS-21680 increased guinea pig myocyte cAMP levels, but did not increase contractility, first suggested that A<sub>2a</sub> receptor-mediated increases in cAMP may be compartmentalized. These same authors (3) also provided evidence that CGS-21680 inhibited cAMP phosphodiesterase activity in guinea pig isolated ventricular myocytes. It is now well recognized that increases in cardiomyocyte contractility correlate to a much greater degree with increases in particulate, as opposed to total intracellular, cAMP levels (4, 11). There are additional reports that adenylyl cyclase subcellular localization is altered during myocardial ischemia (27), and myocardial cAMP levels are reported to be decreased in vivo stunned myocardium (17). Finally, it is reported (6) that the phosphodiesterase inhibitor milrinone exerted no effects on regional function in normal myocardium, but significantly improved contractility in stunned myocardium, a finding similar to what was observed with CGS-21680 in the present study.

It is unlikely that A<sub>2a</sub> receptor activation altered some aspect of reperfusion injury, because this treatment was not initiated until 2 h of reperfusion, a time point at which both groups exhibited similar degrees of stunning, and long after the release of potential mediators of reperfusion injury would have subsided. It is also not very likely that the positive inotropic effect of CGS-21680 was due primarily to a reflex mechanism, because this intracoronary infusion was associated with little hemodynamic effects, and similar hemodynamic effects in the nonstunned animals were associated with no inotropic effect.

In conclusion the results of the present study indicate that low doses of the adenosine A<sub>2a</sub> agonist CGS-21680 administered during reperfusion can reduce infarct size and increase contractility in stunned myocardium. These effects can be achieved with low-dose intracoronary infusions that exert only very minor hemodynamic effects. Although the current hypothesis is that this beneficial effect in irreversibly injured myocardium is mediated via effects on the vasculature and blood cells, it is possible that the effects of A<sub>2a</sub> agonists in stunned myocardium may be mediated via ventricular myocyte A<sub>2a</sub> receptor activation.

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