Myocardial Blood Flow and Myocardial Uptake of $^{201}$Tl and $^{99m}$Tc-Sestamibi During Coronary Vasodilation Induced by CGS-21680, a Selective Adenosine A$_{2A}$ Receptor Agonist

Zuo-Xiang He, MD; Eduardo Cwajg, MD; Wayne Hwang, MD; Craig J. Hartley, PhD; Etai Funk, BS; Lloyd H. Michael, PhD; Mario S. Verani, MD

**Background**—We investigated the hemodynamic and coronary vasodilatory effects of CGS-21680, a potent selective adenosine A$_{2A}$ agonist, as well as its potential use as a new stress modality in combination with perfusion scintigraphy.

**Methods and Results**—A stenosis of the left anterior descending coronary artery (LAD) was produced in dogs to reduce the reactive hyperemic response to <20%. Adenosine and CGS-21680 were then separately infused to maximize left circumflex coronary artery (LCx) flow velocity. $^{201}$Tl (0.5 mCi) and $^{99m}$Tc-sestamibi (5 mCi) were injected at the maximal dose of CGS-21680. Heart rate decreased with adenosine but increased during CGS-21680 infusion ($P<0.005$). The decrease in systolic blood pressure was more prominent with adenosine than with CGS-21680 ($P<0.005$). In the control LCx zone, maximal myocardial blood flow (MBF) (measured by radioactive microspheres) increased 3.1-fold during adenosine infusion ($P<0.005$) and 3.8-fold during CGS-21680 infusion ($P<0.005$). In the stenotic LAD zone, MBF did not change significantly. During adenosine and CGS-21680 infusion, stenosis/control zone MBF ratios were comparable ($0.32\pm0.11$ versus $0.27\pm0.10$, $P=NS$), and transmural $^{201}$Tl and $^{99m}$Tc-sestamibi count-activity ratios ($0.48\pm0.11$ and $0.51\pm0.09$, respectively) were also comparable ($P=NS$). Myocardial scintigraphy uncovered perfusion defects in all dogs.

**Conclusions**—CGS-21680 elicits coronary vasodilation comparable to that of adenosine and produces profound heterogeneity of MBF and of $^{201}$Tl and $^{99m}$Tc-sestamibi myocardial uptake, rendering it a promising agent for pharmacological myocardial perfusion imaging. (*Circulation*. 2000;102:438-444.)

**Key Words:** adenosine receptors ▪ CGS-21680 ▪ radioisotopes ▪ imaging ▪ coronary disease

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tress radionuclide myocardial perfusion imaging is the most commonly used noninvasive technique for the detection of coronary artery disease. Pharmacological stress with a coronary vasodilator$^{1,2}$ or an inotropic$^{3}$ drug is often used in patients unable to undergo an exercise stress test.$^{4-8}$

In animal models, $^{201}$Tl- as well as $^{99m}$Tc-labeled agents underestimate the regional flow disparity between the stenotic and the normal perfusion beds during pharmacological stresses, although the underestimation is greater with the $^{99m}$Tc-labeled agents than with $^{201}$Tl.$^{9-12}$ Nevertheless, clinical studies have shown that pharmacological stress myocardial perfusion imaging with either $^{99m}$Tc-labeled sestamibi$^{13,14}$ or tetrofosmin$^{15,16}$ has a good sensitivity for the diagnosis of coronary artery disease.

Coronary vasodilation induced by dipyridamole or adenosine is mediated by the stimulation of adenosine A$_{1}$ receptors.$^{17}$ The concomitant nonspecific stimulation of the adenosine A$_{1}$, A$_{2B}$, or A$_{3}$ receptors is thought to be responsible for most side effects produced by these drugs. Thus, selective adenosine A$_{3A}$ receptor agonists may be better tolerated than dipyridamole or adenosine. Several highly selective adenosine A$_{2A}$ receptor agonists have been synthesized.$^{18-22}$ Glover et al$^{23}$ recently demonstrated in dogs the potential utility of WRC-0470, another adenosine A$_{2A}$ agonist, for pharmacological stress myocardial perfusion imaging.

The goals of the present study were to compare (1) the myocardial blood flow changes during maximal coronary vasodilation produced by adenosine and by 2-$p$-(2-carboxyethyl) phenethylamino-5’-$N$-ethylcarboxamido adenosine (CGS-21680) (the latter is a highly selective adenosine A$_{2A}$ receptor agonist with a 170-fold selectivity for the A$_{2}$ versus the A$_{1}$ receptor$^{18,19}$) and (2) the myocardial uptake of $^{99m}$Tc-sestamibi and $^{201}$Tl during maximal coronary vasodilation with CGS-21680. The hypotheses tested in this study were that (1) CGS-21680 is a coronary vasodilator at least as potent as adenosine and (2) differences in myocardial uptake...
of both $^{201}$Tl and $^{99m}$Tc-sestamibi between the stenotic and normal perfusion beds, sufficient to allow scintigraphic detection of a coronary stenosis, can be achieved during coronary vasodilation with CGS-21680.

**Methods**

Baylor College of Medicine is an American Association for Accreditation of Laboratory Animal Care–approved facility, and the research protocol was approved by our Institutional Animal Care and Use Committee in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

**Animal Preparation**

Twelve adult mongrel dogs were anesthetized with 25 to 35 mg/kg IV sodium pentobarbital, intubated, and ventilated on a respirator with positive end-expiratory pressure of 4 cm H$_2$O. Additional small amounts of sodium pentobarbital were used as needed to maintain anesthesia. An ECG lead was continuously monitored. The right femoral vein was cannulated for the administration of fluids, medications, $^{99m}$Tc-sestamibi, and $^{201}$Tl. Both femoral arteries were also cannulated for blood withdrawal and for continuous monitoring of arterial blood pressure.

A thoracotomy was performed at the level of the fifth left intercostal space, and the heart was suspended in a pericardial cradle. A polyethylene catheter was inserted into the left atrial appendage for continuous pressure monitoring and for the injection of radiolabeled microspheres. The left anterior descending (LAD) and the left circumflex (LCx) coronary arteries were dissected free of the epicardium, and ultrasonic Doppler flow probes were placed around these vessels. A screw occluder for producing a coronary stenosis and a snare occluder for performing temporary total occlusions were placed around the LAD just proximal to the Doppler probe (Figure 1).

**Study Protocol**

The study protocol is shown schematically in Figure 2. After animal instrumentation and a 60-minute rest period had been completed, a first set of radioactive microspheres (50 μCi in 1.0- to 1.5-mL total volume) was administered into the left atrium to measure baseline coronary blood flow. Simultaneously, a 2-minute reference blood sample was withdrawn from the femoral artery. Microspheres (15 μm in diameter) were labeled with either $^{85}$Sr, $^{95}$Nb, $^{51}$Cr, or $^{46}$Sc and randomly selected to minimize bias. To measure the normal reactive hyperemic response, the LAD was briefly occluded for 10 seconds with the snare occluder while the Doppler flow velocity signal showing hyperemia was recorded on a strip-chart recorder. The peak-to-resting ratio (P/R) was calculated as the ratio of the maximum velocity during reactive hyperemia after the release of the snare to the resting velocity before the 10-second occlusion. The LAD occluder was then adjusted to produce a critical stenosis, defined as the point at which the baseline flow velocity was unchanged or reduced by <20% and the reactive hyperemic response to a 10-second ligation was reduced to <20% (P/R <1.20). After that, no further adjustments in the occluder were necessary. An intravenous infusion of adenosine was begun at a rate of 150 μg · kg$^{-1}$ · min$^{-1}$ and titrated upward (to a maximum of 1200 μg · kg$^{-1}$ · min$^{-1}$) to produce maximal LCx blood flow velocity. When the maximal flow was achieved, a second set of microspheres was injected to measure myocardial blood flow. The intravenous infusion of adenosine was stopped 2 minutes after the injection of microspheres, followed by a 60-minute resting interval.

After the LCx and LAD flows had returned to the baseline level, a third set of radioactive microspheres was used for flow measurement. Infusion of CGS-21680 hydrochloride (Research Biochemicals International) (Figure 3) was begun at a dose of 0.5 μg · kg$^{-1}$ · min$^{-1}$ and increased until maximal LCx flow velocity was achieved. The maximal required dose of CGS-21680 was 4 μg · kg$^{-1}$. Once the maximal Doppler coronary blood flow was reached, 0.5 mCi of $^{99m}$Tc, 5 mCi of $^{99m}$Tc-sestamibi, and a fourth set of radioactive microspheres were injected simultaneously. Ten minutes later, planar scintigraphy was performed. The dogs were then euthanized, and the hearts were removed and carefully sliced into 5 rings of approximately uniform 1-cm thickness from apex to base. The slices were trimmed of excess fat and adventitia, placed on a thin piece of cardboard, covered with cellophane wrap, and then imaged on the gamma camera.

**In Vivo and Ex Vivo Myocardial Imaging**

In vivo planar scintigraphy was performed using the peak photon energy of $^{99m}$Tc (140 keV) and a 20% window in the anterior, 45° left anterior oblique, and left lateral views. Acquisitions were for 5 minutes per view with a high-resolution, low-energy collimator. The slices were imaged directly on the inverted detector head with the same collimator and acquisition of 500,000 counts on the $^{99m}$Tc photopk with a 20% window. The planar images were interpreted qualitatively by 2 expert nuclear cardiologists (Z.X.H., M.S.V.). Only a definitive reduction in tracer uptake involving ≥10% of the image perimeter was considered abnormal.

**Quantification of Myocardial Blood Flow and $^{201}$Tl and $^{99m}$Tc-Sestamibi Activities**

Each of the 5 myocardial slices were equally divided into 8 transmural sections, which were then further subdivided into epicar-
dial, midwall, and endocardial thirds (except for the apical slice), resulting in a total of 104 samples for each dog. These samples were then counted in a gamma-well scintillation counter within 24 hours for $^{99m}$Tc and 48 to 72 hours later for $^{201}$Tl. The samples were re-counted for microsphere flows 3 weeks later when the $^{201}$Tl and $^{99m}$Tc had already totally decayed. The tissue radioactivity counts (cpm/g) were corrected for background, decay, and isotope spillover. The microsphere content in the 2-minute reference blood collection was used to calculate absolute flow (mL · min$^{-1}$ · g$^{-1}$) with a dedicated computer system.24

Transmural myocardial blood flow and $^{201}$Tl and $^{99m}$Tc-sestamibi activities on each myocardial section were calculated as the weighted average of the 3 myocardial segments. The 5 transmural sections with the highest flows at the time of $^{201}$Tl and $^{99m}$Tc-sestamibi injection were defined as the normal region and the 5 transmural sections with the lowest flows as the stenotic region. Stenotic-to-normal region ratios were calculated by dividing the average flow and $^{201}$Tl or $^{99m}$Tc-sestamibi activities in the stenotic region by the average values in the normal regions. Coronary flow reserve was calculated by dividing the myocardial flow during pharmacological vasodilation by the resting myocardial blood flow.10–12

Statistical Analysis
Data are presented as mean±SD. Changes from baseline to adenosine or CGS-21680 infusion were tested by Wilcoxon signed-rank test, and differences between 2 groups were compared by the Mann-Whitney test. Comparison between categorical variables used a $\chi^2$ test. Regression analysis was used to compare the microsphere proximal flow ratio (stenosis:normal region) to the $^{201}$Tl and $^{99m}$Tc-sestamibi uptake ratios (determined by well counting). ANOVA was used to assess differences of flow among myocardial layers, and significant differences were further evaluated with a Bonferroni-adjusted $t$ test.

### TABLE 2. Comparison of Regional Myocardial Blood Flow During Administration of Adenosine and CGS-21680

<table>
<thead>
<tr>
<th>Section</th>
<th>Baseline-1</th>
<th>Adenosine</th>
<th>Baseline-2</th>
<th>CGS-21680</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAD zone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epicardial</td>
<td>0.61±0.17</td>
<td>2.24±1.01*</td>
<td>0.58±0.21</td>
<td>2.80±0.94*</td>
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<tr>
<td>Mid-wall</td>
<td>0.70±0.20</td>
<td>2.32±1.22*</td>
<td>0.66±0.23</td>
<td>2.53±0.99*</td>
</tr>
<tr>
<td>Endocardial</td>
<td>0.79±0.26</td>
<td>1.98±1.36*</td>
<td>0.74±0.24</td>
<td>1.89±0.91*</td>
</tr>
<tr>
<td>Endo/epi</td>
<td>1.30±0.22</td>
<td>0.89±0.38†</td>
<td>1.32±0.22</td>
<td>0.68±0.19*</td>
</tr>
<tr>
<td>Transmural</td>
<td>0.70±0.20</td>
<td>2.20±1.16*</td>
<td>0.66±0.22</td>
<td>2.42±0.93*</td>
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<tr>
<td>LCx zone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epicardial</td>
<td>0.57±0.19</td>
<td>0.90±0.48†</td>
<td>0.61±0.24</td>
<td>0.99±0.55†</td>
</tr>
<tr>
<td>Midwall</td>
<td>0.55±0.15</td>
<td>0.61±0.37†</td>
<td>0.59±0.26</td>
<td>0.57±0.29</td>
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<tr>
<td>Endocardial</td>
<td>0.58±0.23†</td>
<td>0.53±0.45</td>
<td>0.61±0.23</td>
<td>0.45±0.35</td>
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<tr>
<td>Endo/epi</td>
<td>1.03±0.28</td>
<td>0.61±0.33†</td>
<td>1.02±0.32</td>
<td>0.51±0.43†</td>
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<tr>
<td>Transmural</td>
<td>0.57±0.18</td>
<td>0.68±0.40</td>
<td>0.61±0.22</td>
<td>0.65±0.33</td>
</tr>
</tbody>
</table>

Values are mL · min$^{-1}$ · g$^{-1}$. *$P<0.005$; †$P<0.05$ vs baseline; ‡$P<0.05$ vs LCx.

### Results

#### Hemodynamic Data
The hemodynamic data are summarized in Table 1. Heart rate decreased insignificantly during adenosine infusion; in contrast, it increased significantly during CGS-21680 infusion. Both systolic and diastolic arterial blood pressure decreased significantly during adenosine and CGS-21680 infusion. However, the decrease produced by CGS-21680 was significantly less than that caused by adenosine.

#### Doppler Flow Data
During control, before coronary stenosis was produced, Doppler flows were 16.2±5.2 and 12.6±3.7 mL/min in the LAD and LCx, respectively. The peak/resting flow ratio after a 10-second total occlusion before stenosis was 3.32±0.76 for the LAD and 3.87±0.71 for the LCx. After critical stenosis, LAD flow was reduced by an average of 15% to 13.7±4.8 mL/min, and the peak/resting flow ratio after a 10-second occlusion was reduced to 1.18±0.15. Thus, the peak hyperemic flow in the LAD with a critical stenosis was approximately the same as the resting flow in the LAD before stenosis (13.7 mL/min×1.18=16.2 mL/min).

The peak/resting flow ratio was 1.26±0.82 in the LAD and 2.76±0.90 in the LCx during adenosine administration and 1.21±0.53 in the LAD and 3.12±0.88 in the LCx during CGS-21680 infusion. After CGS, it took 30 minutes for the flow in the control LCx to return halfway to the predrug baseline level.

#### Regional Myocardial Blood Flow
Regional myocardial blood flow at baseline and during adenosine and CGS-21680 infusion is summarized in Table 2.
Baseline transmural myocardial blood flows in the LAD and the LCx vascular territories were similar during control before adenosine or CGS-21680 infusion (P=NS).

In the LCx zone, epicardial, midwall, and endocardial coronary blood flow increased significantly during adenosine infusion (P<0.005). Transmural myocardial blood flow also increased during adenosine infusion (P<0.005). In contrast, in the stenotic LAD zone, only epicardial myocardial blood flow increased slightly during adenosine infusion (P<0.05), whereas transmural flow did not increase significantly (Table 2). The maximal epicardial blood flow reserve elicited by adenosine was significantly greater than that of the endocardial flow of both the LCx and the LAD vascular territories (Table 3).

Likewise, in the LCx zone, epicardial, midwall, and endocardial coronary blood flow increased significantly during CGS-21680 infusion (P<0.005). Transmural LCx artery myocardial blood flow increased during CGS-21680 infusion (P<0.005). In the stenotic LAD zone, only the epicardial blood flow increased slightly during adenosine infusion (P<0.05), whereas transmural myocardial blood flow was unchanged. The increase in epicardial blood flow elicited by CGS-21680 was significantly greater than that in the endocardium of both the LCx and the LAD zones (Table 3).

The epicardial blood flow reserve elicited by CGS-21680 in the LCx zone was significantly greater than that elicited by adenosine (P<0.05). CGS-21680 also showed a tendency to elicit a greater transmural blood flow reserve than adenosine in the LCx zone (Table 3). Coronary blood flow reserve elicited in the LAD zone was similar with adenosine and CGS-21680 (Table 3).

Endocardial-to-Epicardial Myocardial Blood Flow Ratio
Both adenosine and CGS-21680 produced a fall in endocardial-to-epicardial myocardial blood flow ratios (Table 2). There was a significant, albeit modest, difference in endocardial-to-epicardial flow ratios between the LCx and LAD zones at baseline; however, endocardial-to-epicardial flow ratios produced by adenosine or CGS-21680 infusion were similar in the LCx and the LAD zones (Table 2).

Stenotic-to-Normal Myocardial Blood Flow Ratios
The LAD/LCx myocardial blood flow ratios at baseline and during adenosine and CGS-21680 infusion are summarized in Table 4. The stenotic-to-normal blood flow ratios in the epicardial, midwall, and endocardial segments fell significantly during both adenosine and CGS-21680 administration. Transmural flow ratios were 0.32±0.11 during adenosine and 0.27±0.10 during CGS-21680 infusion. The stenotic-to-control flow ratios across the left ventricular wall during CGS-21680 are illustrated in Figure 4.

Regional Myocardial 201Tl and 99mTc-Sestamibi Uptakes
The stenotic-to-normal 201Tl and 99mTc count activity ratios during CGS-21680 were similar and are summarized in Figure 5. Transmural stenotic/normal 201Tl and 99mTc-sestamibi ratios during CGS-21680 administration were also similar. Both 201Tl and 99mTc-sestamibi activities underestimated the true reduction in flow ratios (Figure 6). There was,
however, a strong correlation between the microsphere flow
and the $^{201}$Tl uptake (linear) and $^{99m}$Tc-sestamibi uptake
(exponential) during CGS-21680 administration. The rela-
tionship between flow and sestamibi uptake plateaued at high
flows (Figure 7). There was also a significant correlation
between the stenosis/control microsphere flow ratio and the
stenosis/control uptake ratio (measured by well counting) for
$^{201}$Tl ($r=0.81$, $P=0.001$) and for $^{99m}$Tc-sestamibi ($r=0.74$, $P=0.006$).

In all 12 dogs, CGS-21680 produced myocardial perfusion
defects, which were readily detected in the in vivo and ex
vivo scintigrams (Figure 8).

**Discussion**

Our study demonstrates conclusively that (1) CGS-21680
markedly increases the blood flow by nearly 4-fold in the
myocardium supplied by a normal coronary artery, but not
in the regions supplied by a significantly stenotic artery;
(2) CGS-21680 and adenosine produce a similar increase in transmural myocardial blood flow, but the former causes significantly less systemic hypotension than the latter; (3) the increase in myocardial blood flow elicited by both adenosine and CGS-21680 is greater in the epicardium than in the endocardium, particularly with CGS-21680, but the LAD/LCx myocardial blood flow ratios are similar in the epicardial, midwall, or endocardial segments; and (4) in the presence of a significant coronary stenosis, CGS-21680 produces heterogeneous myocardial uptake of both $^{201}$Tl and $^{99m}$Tc-sestamibi, which can be imaged with standard gamma cameras.

The robustness of our microsphere data was confirmed by similar directional changes in coronary blood flow assessed by the Doppler flow probe. Both techniques showed a very substantial increase in coronary flow in the control artery, with little increase in the stenotic artery during coronary vasodilation. The increase in flow was more prominent in the epicardial than in the endocardium with both adenosine and CGS-21680, although only the latter achieved statistical significance. This preferential epicardial vasodilation has been previously documented with “maximal” adenosine doses (up to 1000 $\mu g \cdot kg^{-1} \cdot min^{-1}$), similar to the dosages used in our experiments. The greater increase in the epicardial layer flow with CGS-21680 than with adenosine is probably a reflection of the slightly greater potency of the former compound in the doses used in our experiments. Alternatively, this might be attributed to the smaller reduction in blood pressure with CGS-21680, leading to higher coronary perfusion pressures.

**Adenosine Receptors**

The coronary vasodilatory effects of dipyridamole and adenosine are mediated by stimulation of the $A_2A$ receptors, whereas their side effects are secondary to the undesirable stimulation of adenosine $A_1$, $A_2B$, and $A_1$ receptors.

Recent studies have demonstrated that in rats, CGS-21680 is a selective coronary vasodilator. Makujina et al. in ex vivo experiments, found the coronary arteries to be more responsive than the internal mammary artery or the saphenous vein, both of which displayed only marginal relaxation with CGS-21680. The clinical significance of the latter observation is uncertain, because no studies have been performed in vivo. CGS-21680 also has significant effects on heart rate, probably as a result of reflex activation of the sympathetic nervous system rather than of any direct effect on the sinus node. During adenosine administration at maximal doses, the expected reflex tachycardia is blunted because of the direct inhibitory effect of the $A_1$ receptors on sinus node automaticity.

**Clinical Implications**

Side effects occur in $\approx 50\%$ of patients receiving dipyridamole and in $\approx 70\%$ to $80\%$ of patients receiving adenosine. There are several contraindications to the use of dipyridamole or adenosine, including bronchospasm, hypotension, acute myocardial infarction within the first 24 hours, and second-degree or higher atrioventricular block. Although both dipyridamole and adenosine have a good safety record, there have been instances of death and other serious side effects with these agents.

Glover et al. recently demonstrated that WRC-0470, another selective adenosine $A_{2A}$ agonist, increased myocardial blood flow without lowering mean arterial blood pressure. Together, their and our results demonstrate that selective stimulation of adenosine $A_{2A}$ receptors is a potentially effective means to perform pharmacological stress myocardial perfusion imaging.

**Study Limitations**

There are several limitations in our study. First, anesthesia may have influenced the hemodynamic and coronary vasodilatory effects of adenosine and CGS-21680. Second, because of the shorter half-life of adenosine, we deliberately always infused adenosine before CGS-21680. Randomizing the order of drug administration would have been preferable but would have extended and possibly compromised the protocol. Third, in vivo and ex vivo images were not quantified, and therefore, the principal findings of this study are derived from tissue counting. Finally, the distribution and richness of adenosine receptors in dogs differ from those in other species, including mammalian species. Whether CGS-21680 had significant effects on other adenosine receptors in the doses we used cannot be inferred from the present study. This was not, however, the purpose of this study.

In conclusion, in the presence of a significant coronary artery stenosis, CGS-21680 produces consistent myocardial blood flow heterogeneity and heterogeneous myocardial uptake of $^{201}$Tl and $^{99m}$Tc-sestamibi, which can be imaged with standard gamma cameras. The present results lend support to...
a trial of this agent in human beings after appropriate toxicity studies are done.

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References

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