A2A-Adenosine Receptor Reserve for Coronary Vasodilation

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Background—Adenosine is a potent coronary vasodilator and causes an increase of coronary blood flow by activation of A2A-adenosine receptors (A2A-AdoRs). The purpose of this study was to test the hypothesis that the high potency of adenosine and adenosine analogues to cause coronary vasodilation is explained by the presence of a large A2A-AdoR reserve ("spare receptors").

Methods and Results—A novel, irreversible antagonist of A2A-AdoRs was used to inactivate receptors and reduce the response to agonist. Agonist-induced increases of coronary conductance before and after exposure of hearts to the irreversible antagonist were compared. Three agonists were studied: 2-p-(2-carboxyethyl)-phenethylamino-5’-N-ethyl-carboxamidoadenosine (CGS21680), adenosine, and 2-chloro-N6-cyclopentyladenosine (CCPA). Data were analyzed to determine agonist Kₐ (equilibrium dissociation constant) and EC₅₀ values. Values of Kₐ for activation of A2A-AdoRs by CGS21680, adenosine, and CCPA were 105, 1800, and 2630 nmol/L, respectively. In contrast, values of EC₅₀ for CGS21680, adenosine, and CCPA to increase coronary conductance were 1.5, 85, and 243 nmol/L, respectively. By use of the law of mass action, it was calculated that half-maximal responses to CGS21680, adenosine, and CCPA occurred when only 1.3%, 5%, and 9%, respectively, of A2A-AdoRs were occupied by agonist.

Conclusions—Receptor reserves for 3 A2A-AdoR agonists were large. The receptor reserve for A2A-AdoRs to cause an increase of coronary conductance can explain both the high potency of adenosine to cause coronary vasodilation and the observation that an A2A-AdoR agonist can cause coronary vasodilation without systemic effects. (Circulation. 1998;98:711-718.)

Key Words: adenosine ■ pharmacology ■ receptors ■ circulation ■ vasodilation

Adenosine is a potent coronary vasodilator.1-3 The EC₅₀ value of ~0.1 μmol/L for exogenous adenosine to cause coronary vasodilation is ~10-fold lower than the EC₅₀ values for adenosine to elicit negative inotropic, dromotropic, or chronotropic responses in the guinea pig heart.4 The coronary circulation also appears to be more sensitive than the peripheral circulation to adenosine.2,5,6 And the concentration-response relationship for interstitial adenosine-induced coronary vasodilation is reported to be steep.7 These observations suggest that transduction of adenosine receptor activation to coronary vasodilation is very efficient.

The action of adenosine to cause coronary vasodilation is mediated by the A₂A-adenosine receptor (A₂A-AdoR).5-10 Evidence of a role for the A₂A-AdoR is stronger than evidence of a role for the A₂B-AdoR as mediator of this response.10 Adenosine and adenosine analogues have a much higher affinity for A₂A-AdoRs than for A₂B-AdoRs,11,12 and the potency of these compounds to cause coronary vasodilation implies that A₂A-AdoRs are involved in the response. Nanomolar concentrations of the selective A₂A-AdoR agonist CGS21680 (2-p-(2-carboxyethyl)phenethylamino-5’-N-ethylcarboxamidoadenosine) cause coronary vasodilation,13,14 and this action is antagonized by the selective A₂A-AdoR antagonists 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a]][1,3,5]triazin-5-yl amino]ethyl) phenol (ZM241385)14 and 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo[4,3-e]-1,2,4-triazolo [1,5-c]pyrimidine (SCH58261).10 A high density of A₂A-AdoRs has been detected in coronary arteries of pig hearts by analysis of [³H]SCH58261 binding data.15

In theory, the high potency of adenosine to cause coronary vasodilation could be the result of either a high affinity (low Kₐ value) of adenosine for the A₂A-AdoR or a large receptor reserve ("spare receptors") for A₂A-AdoR-mediated coronary vasodilation. Receptor reserve is a pharmacological term for the phenomenon wherein the increment of functional response caused by an increase of agonist concentration is proportionately greater than the increment of receptor occupancy. When receptor reserve is present, a near-maximal response can be achieved by occupancy of a relatively small fraction of the total receptor population by agonist.18 Receptor reserve is both agonist dependent, reflecting the intrinsic efficacy of a given agonist to stabilize the active state of a receptor, and tissue dependent, reflecting the density of receptors and the efficiency of the biochemical signaling path.
from receptor activation to response. Increases of receptor density, receptor-to-response coupling efficiency, and agonist intrinsic efficacy may be associated with increases of receptor reserve and sensitivity of a tissue to agonist.

To test the hypothesis that receptor reserve for A_{2A}-AdoR agonist–mediated coronary vasodilation is large, we measured agonist equilibrium dissociation constants (K_{A} values) and receptor reserves for adenosine, CGS21680, and 2-chloro-N^6-cyclopentyladenosine (CCPA) to increase coronary conductance of the guinea pig isolated perfused heart. CGS21680 is a potent, highly selective A_{2A}-AdoR agonist, adenosine is the natural ligand, and CCPA is a relatively selective agonist of the A_{1}-adenosine receptor and has low affinity for A_{2A}-AdoRs. We were able to demonstrate that the high potency of these agonists is dependent on a large receptor reserve (spare receptors) for A_{2A}-AdoR–mediated coronary vasodilation and not on high affinity of the agonists for the A_{2A}-AdoR. By comparing receptor reserves and K_{A} values for 3 agonists that have very different potencies and structures, we were also able to show that receptor reserve is in part dependent on the identity of the agonist.

**Methods**

**Chemicals**

Adenosine and adenosine deaminase type VII were purchased from Sigma Chemical Co. Sodium nitroprusside, CGS21680, CCPA, 5'-N-ethylcarboxamidoadenosine (NECA), 8-cyclopentyl-1,3-dipropylxanthine (CPX), and 8-cyclopentyl-1,3-dimethylxanthine (CPT) were purchased from Research Biochemicals, SCH58261 and [H]SCH58261 were gifts from Schering-Plough Research Institute (Milan, Italy). ZM241385 was a gift from Dr Simon Poucher, Zeneca Pharmaceuticals (London, UK). To prepare stock solutions, adenosine was dissolved in water, whereas all other drugs were dissolved at a concentration of 10 mmol/L in DMSO. Dilutions of stock solutions were prepared in saline. DMSO content of coronary perfusates ranged from none (experiments with adenosine) to 0.1% (during perfusion of 10 mmol/L of CGS21680 or CCPA) and up to 0.3% (during perfusion of 30 mmol/L CCPA). DMSO alone at 0.1% (vol/vol) did not cause any change of coronary perfusion pressure of isolated hearts perfused at a constant flow of 10 mL/min, and 0.3% DMSO caused a very small change (±1 mm Hg) of pressure.

**Synthesis of FSPTP**

SCH58261 (30 mg, 0.086 mmol) was reacted with fluorosulfonic acid (0.1 mL) at 10°C for 2 hours. The mixture was then quenched with ice water (1 mL) and the precipitate, a white solid, was collected by filtration (yield: 89%, 31.48 mg). The melting point was 264°C (MeOH); 1H NMR (DMSO-d_6) δ 3.32 (t, 2H, J = 6); 4.55 (t, 2H, J = 6); 6.72 (dd, 1H, J = 2, J = 4); 7.22 (dd, 1H, J = 4); 7.53 (d, 2H, J = 8); 7.95 (d, 1H, J = 2); 7.99 (d, 2H, J = 8); 8.08 (bs, 2H); 8.15 (d, 3H). The chemical name of this compound is 5-amino-7-(2-[4-(fluorosulfonyl)phenethyl]-2-(2-furyl)-pyrazol-4,3-yl)-1,2,4-triazolo[1,5-c]pyrimidine, abbreviated herein as FSPTP.

**Isolated Perfused Heart Preparation**

Adult Hartley guinea pigs of either sex weighing between 250 and 300 g were anesthetized with methoxyflurane. The chest was quickly opened and the heart removed. The heart was perfused retrogradely (the method of Langendorff) at a flow rate of 10 mL/min with modified Krebs-Henseleit (K-H) solution of the following composition (mmol/L): NaCl 117.9, KCl 4.5, CaCl_2 2.5, MgSO_4 1.18, KH_2PO_4 1.18, pyruvate 2.0, glucose 5.5, Na_2 EDTA 0.57, ascorbic acid 0.007, and NaHCO_3 25.0. The K-H solution was gassed with a mixture of 95% O_2 and 5% CO_2, adjusted to pH 7.4, and warmed to 35.0±0.5°C for perfusion of the coronary vasculature.

The hearts were paced electrically at a fixed cycle length of 300 ms (200 beats/min) by a bipolar electrode placed on either the left atrium or the right ventricle. To facilitate both atrial pacing of the heart and (when desired) placement of a unipolar electrode for recording of a His bundle electrogram (HBE), parts of the right and left atrial tissues were excised. The HBE was recorded and analyzed to determine the stimulus-to-His bundle (S-H) conduction time.

To measure coronary perfusion pressure, a pressure transducer was connected to the aortic cannula via a T connector positioned ~2 cm above the aorta. Coronary perfusion pressure (in mm Hg) was monitored throughout an experiment and recorded on a strip chart. The range of coronary perfusion pressures for hearts used in this study was 50 to 74 mm Hg in the absence of drug. Agonists of A_{2A}-AdoRs caused a maximal reduction of perfusion pressure to 24 to 34 mm Hg, i.e., a range of perfusion pressures that is within the range of pressures in which autoregulation of coronary flow occurs in the guinea pig isolated heart. Coronary conductance (in mL·min^{-1}·mm Hg^{-1}) was calculated as the ratio between coronary perfusion rate (10 mL/min) and coronary perfusion pressure (in mm Hg). For determination of steady-state responses to drugs, drug stock solutions were diluted with K-H solution and infused at a constant rate to the coronary perfusate until a stable measurement was recorded. Concentration-response data were obtained by exposure of hearts to increasing concentrations of drug in a cumulative manner.

**Estimations of the Equilibrium Dissociation Constant (K_{A}), Receptor Inactivation by FSPTP, and Receptor Reserve**

The method of Furchgott and Bursztyn was used to estimate both the equilibrium dissociation constant (K_{A}) for agonist binding to the A_{2A}-AdoR that mediates an increase of coronary conductance and the fraction of functional receptors (q) remaining after exposure of hearts to the irreversible A_{2A}-AdoR antagonist FSPTP. FSPTP is presumed to bind to the A_{2A}-AdoR and then to react with a nearby free amino group to form a covalent nitrogen-to-sulfur bond (and free HF) that irreversibly links the receptor and the antagonist. Measurements of steady-state increases of coronary conductance caused by either CGS21680 (0.1 nmol/L to 10 μmol/L), adenosine (1 nmol/L to 30 μmol/L), or CCPA (10 nmol/L to 30 μmol/L) were obtained both before and after treatment of the isolated perfused guinea pig heart with FSPTP. Pairs of concentrations of agonist that caused equal increases of coronary conductance before and after inactivation of a fraction of A_{2A}-AdoRs with FSPTP were selected. These data were used to determine values of K_{A} and q as previously described.

A mathematical expression of the law of mass action was used to calculate fractional receptor occupancy as a function of agonist concentration with the use of the experimentally determined K_{A} value. Plots of the relationships between agonist concentration and both response and fractional receptor occupancy were made. By combining concentration-response data and concentration-occupancy data, we made a determination of response as a function of receptor occupancy. The extent of receptor reserve for an agonist to increase coronary conductance was estimated at near-maximal effect and at 50% of maximal effect as follows (1 and 2, respectively): (1) percent receptor reserve = 100−percent receptor occupancy required to produce 95% of the maximal response and (2) by comparison of values of agonist K_{A} (agonist concentration that caused 50% receptor occupancy) and EC_{50} (agonist concentration that caused 50% of maximal effect). Values of relative intrinsic efficacy (ε) were calculated as follows: ε_{1}/ε_{2}=[(EC_{50}/(EC_{50}+K_{A1}))/[(EC_{50}/(EC_{50}+K_{A2}))], where (1) and (2) indicate 2 different agonists.

**Data Analysis**

Data are presented as mean±SEM. Significance of differences among group means in experiments in which control and all treatment responses were obtained from the same heart was determined by repeated-measures ANOVA (1-way) followed by Student-Newman-Keuls test. Significance of differences among group means
in experiments in which control and treatment responses were obtained from each heart but different hearts were used for different treatments was determined by repeated-measures ANOVA (2-way) followed by Student-Newman-Keuls test. A value of \( P < 0.05 \) was considered to indicate a significant difference. The significance of the difference between percent receptor occupancy versus percent response plots for CCPA and for CGS21680 was determined by 2-factor repeated-measures ANOVA with 1-way replication (SPSS version 7.5, SPSS, Inc.).

Parameters describing results of saturation (ie, \( B_{max}, K_D \) and Hill coefficient) and competition radioligand binding assays (IC\(_{50}, K_I\), and Hill coefficient) were determined with the radioligand analysis program RADLIG version 4.0 (Elsevier-Biosoft).

The concentrations of agonist that caused half-maximal (EC\(_{50}\)) and maximal responses were estimated by analysis of concentration-response data with a nonlinear regression algorithm (Marquardt-Levenberg) to fit data to a multiparameter logistic equation (Table Curve, Jandel Scientific).

Mean values of EC\(_{50}\) and \( K_D \) were calculated as the antilogarithms of mean values of pEC\(_{50}\) and pK\(_D\). The latter values are normally distributed and are thus stated as mean with SEM.

**Results**

**A\(_{2A}\)–AdoR**-Mediated Coronary Vasodilation

Coronary conductance of the guinea pig isolated perfused heart was increased by adenosine, the selective A\(_{2A}\)-AdoR agonist CGS21680, the reputedly selective A\(_1\)-AdoR agonist CCPA, and NECA, an unselective A\(_1\)- and A\(_2\)-AdoR agonist. Equal increases of coronary conductance of 0.07 mL \( \cdot \) min\(^{-1}\) \( \cdot \) mm Hg\(^{-1}\) were elicited by 60 nmol/L adenosine, 1 nmol/L CGS21680, 120 nmol/L CCPA, and 5 nmol/L NECA (Figure 1). The selective A\(_{2A}\)-AdoR antagonists SCH58261 (100 nmol/L) and ZM241385 (300 nmol/L) nearly completely attenuated these increases of coronary conductance caused by CGS21680, CCPA, adenosine, and NECA (Figure 1), suggesting that responses to all 4 agonists were mediated by activation of A\(_{2A}\)-AdoRs. The responses of hearts perfused at a constant pressure of 50 mm Hg were either quantitatively (values of agonist EC\(_{50}\) and maximal conductance) or qualitatively (agonist responses attenuated by SCH58261 and by ZM241385) similar to responses of hearts perfused at constant flow.

**Irreversible Binding of FSPTP to the A\(_{2A}\)-AdoR**

The para-fluorosulfonyl derivative of SCH58261, a selective antagonist of the A\(_{2A}\)-AdoR, \textsuperscript{10,15,21,22} was prepared (see Methods) for use in the present study as an irreversible antagonist of the A\(_{2A}\)-AdoR. The binding of FSPTP to A\(_{2A}\)-AdoRs in porcine striatal membranes was studied. Preincubation of striatal membranes for 20 minutes with 400 nmol/L FSPTP, followed by extensive washing (10\( \times \)) of membranes to remove unbound FSPTP, reduced the density of \([\text{H}]\text{SCH58261}\)-specific binding sites (A\(_{2A}\)-AdoRs) from 1167 to 596 fmol/mg protein but did not alter the affinity of the remaining sites for \([\text{H}]\text{SCH58261}\) (Figure 2, top). To more clearly demonstrate the irreversibility of FSPTP binding, striatal membranes were preincubated with either SCH58261 or FSPTP, then washed to remove preincubation drug, and assayed for density of A\(_{2A}\)-AdoRs (Figure 2, bottom). Membranes were washed up to 10 times, and after every 2 washes, an aliquot of membranes was removed for measurement of \([\text{H}]\text{SCH58261}\) binding sites. The density of \([\text{H}]\text{SCH58261}\) binding sites in membrane preincubated with SCH58261 returned to 60% and 90% of control after 2 and 4 washes, respectively. In contrast, the density of \([\text{H}]\text{SCH58261}\) binding sites in membranes preincubated with FSPTP recovered from 7\( \pm \)5% of control at the end of 20 minutes’ incubation with FSPTP, to 33\( \pm \)2% of control after 4 washes to remove reversibly bound FSPTP (Figure 2, bottom). Additional washes (up to 10) did not further increase the density of binding sites. The results indicated that the majority of the binding of FSPTP was irreversible, and the washout of reversibly bound FSPTP was rapid.

**FSPTP Irreversibly and Selectively Antagonized an A\(_{2A}\)-AdoR–Mediated Increase of Coronary Conductance**

Antagonism by FSPTP of the action of adenosine to increase coronary conductance of the guinea pig isolated perfused heart was irreversible. Bolus administration of adenosine (10 \( \mu \)L, 0.2 nmol/L) caused a transient 0.07 mL \( \cdot \) min\(^{-1}\) \( \cdot \) mm Hg\(^{-1}\) (peak effect) increase of coronary conductance of the heart (not shown). This response was reproducible for \( \geq \)2 hours. FSPTP (100 nmol/L) antagonized the action of adenosine by 93\( \pm \)2%. After 1 and 2 hours of washout of FSPTP, the response of the isolated heart to adenosine had recovered to only 12\( \pm \)3% and 11\( \pm \)3% of control, respectively.

The A\(_{2A}\)-AdoR agonist CGS21680, the relatively selective A\(_1\)-AdoR agonist CCPA, and adenosine increased coronary conductance of the guinea pig isolated heart (Figures 1 and 3). Actions of these agonists to increase coronary conductance were irreversibly antagonized by 100 nmol/L FSPTP (Figure 3). The action of CCPA (20 nmol/L) to prolong the S-H interval (an action mediated by A\(_1\)-AdoRs) was fully antagonized by the A\(_1\)-AdoR antagonist CPX (100 nmol/L) but was not attenuated by 100 nmol/L FSPTP (not shown). Furthermore, although both sodium nitroprusside (1 \( \mu \)mol/L) and the A\(_{2}\)-AdoR agonist CGS21680 (1 nmol/L) increased coronary conductance of the guinea pig isolated heart, this response was irreversible and was antagonized by FSPTP.
coronary conductance by 0.07 to 0.08 mL·min⁻¹·mm Hg⁻¹, 100 nmol/L FSPTP completely and irreversibly antagonized the action of CGS21680 but not that of sodium nitroprusside. In addition, the increases of coronary conductance caused by the muscarinic cholinergic agonist carbamylcholine or the β₂-adrenergic receptor agonist procaterol were not attenuated by FSPTP. Collectively, the data indicate that FSPTP is a selective, irreversible A₂A-AdoR antagonist that is an appropriate tool for determination of receptor reserves for adenosine, CGS21680 and CCPA to cause coronary vasodilation.

Receptor Reserves for CGS21680, Adenosine, and CCPA to Increase Coronary Conductance

The magnitudes of receptor reserves were determined by comparison of concentration-response relationships for the action of each agonist to increase coronary conductance before and after irreversible inactivation by FSPTP of a portion of the A₂A-AdoR population. Results of a single representative experiment with each agonist are shown in Figure 4. As expected, inactivation of A₂A-AdoRs caused reductions of both potency (EC₅₀) and efficacy (maximal effect) of CGS21680, adenosine, and CCPA (Figure 4). Experimental results were analyzed by the method of Furchgott and Bursztyn¹⁹ to determine agonist Kₘ values. A summary of experiments to determine values of agonist EC₅₀ and Kₘ for agonist EC₅₀ (before treatment of heart with FSPTP) and Kₘ is given in the Table. Comparison of Kₘ values for the 3 agonists indicates that the affinities of adenosine and CCPA for the A₂A-AdoRs that mediate coronary vasodilation were ~17- and 25-fold lower, respectively, than the affinity of CGS21680 for the same receptors. Receptor reserve, as indicated by the value of Kₘ/EC₅₀, was significantly greater for CGS21680 than for adenosine and significantly greater for adenosine than for CCPA. Comparison of calculated values of intrinsic efficacies of CGS21680 and adenosine relative to that of CCPA indicated that occupancies of an A₂A-AdoR by CGS21680 and adenosine caused roughly 6-fold and 2-fold greater vasodilator responses, respectively, than occupancy of an A₂A-AdoR by CCPA (Table).

The reductions of agonist potency and efficacy observed after treatment of hearts with FSPTP were not due to either a time-related deterioration or an agonist-induced desensitization of the isolated heart preparation. To demonstrate this, a series of hearts were exposed twice, 50 minutes apart, to...
increasing concentrations of a single agonist (either CGS21680, CCPA, or adenosine). The values of EC$_{50}$ and maximal agonist-induced increases of coronary conductance for the first and second agonist concentration-response series were not significantly different. This finding indicates that agonist-induced desensitization of coronary responsiveness was not apparent in our experiments.

A$_{2A}$-AdoR occupancy as a function of agonist concentration is shown in Figure 5. Note that the agonist concentration-response curves for all 3 agonists lie to the left of the agonist concentration-receptor occupancy curves. This finding indicates the presence of receptor reserve (spare receptors) for each agonist to increase coronary conductance. This is clearly illustrated in Figure 6, wherein responses to CGS21680, adenosine, and CCPA are plotted as a function of A$_{2A}$-AdoR occupancy. Occupancies of 1.3%, 5%, and 9% of receptors by CGS21680, adenosine, and CCPA, respectively, caused 50% of the maximal vasodilator response. Occupancy of 8% of receptors by CGS21680 caused a near-maximal (95%) response, whereas occupancy of 30% of receptors by adenosine and 48% of receptors by CCPA was needed to cause a near-maximal (95%) response. Thus, nearly 90% and 50% of A$_{2A}$-AdoRs are “spare” for CGS21680 and CCPA, respectively, to cause a maximal increase of coronary conductance of the guinea pig isolated heart. Approximately 70% of A$_{2A}$-AdoRs appear to be spare for an adenosine-mediated maximal increase of coronary conductance.

**Discussion**

The results of our studies with the novel irreversible antagonist FSPTP indicate that there is a large reserve for A$_{2A}$-AdoR–mediated coronary vasodilation in the guinea pig heart. Activation by adenosine of 5% of A$_{2A}$-AdoRs was sufficient to cause a half-maximal vasodilator response. The existence of an A$_{2A}$-AdoR reserve for coronary vasodilation can explain both the high potency of A$_{2A}$-AdoR agonists (including adenosine) to cause coronary vasodilation and the unexpected vasodilator responses to relatively selective A$_{1}$-AdoR agonists that activate A$_{2A}$-AdoRs only at high concentrations, such as the adenosine analogues CCPA (Figures 1 and 4), CPA,23 and R-PIA.8,9

The potency of an agonist to elicit vasodilation depends on the affinity of the agonist for the receptor (ie, agonist binding), the density of receptors, the intrinsic efficacy of the bound agonist to activate the receptor, and the efficiency of coupling of receptor activation to response. The affinities of CGS21680, adenosine, and CCPA for the A$_{2A}$-AdoR (K$_{a}$ values in the Table) were low compared with the potencies of each agonist to cause coronary vasodilation (EC$_{50}$ values in the Table). Thus, the high potency of these compounds

| Pharmacological Parameters Describing A$_{2A}$-Ado–Mediated Vasodilator Actions of CGS21680, Adenosine, and CCPA in Guinea Pig Isolated Hearts |
|-------------|-----------|-------------|---------------|-------------|-------------|
| Agonist     | n | EC$_{50}$ (pEC$_{50}$±SEM) | K$_{a}$ (pK$_{a}$±SEM) | K$_{a}$/EC$_{50}$ | Relative $\varepsilon$ |
| CGS21680    | 4 | 1.48 (8.83±0.12) | 105 (6.98±0.002)$^\dagger$ | 79±21 | 6.1 |
| Adenosine   | 4 | 84.9 (7.07±0.06)$^*$ | 1800 (5.74±0.10) | 22±2$^*$ | 1.9 |
| CCPA        | 4 | 243 (6.61±0.06) | 2630 (5.57±0.10) | 11±2 | 1.0 |

$^\varepsilon$ indicates intrinsic efficacy. Relative $\varepsilon$ was determined as indicated in Methods. Values of EC$_{50}$ and K$_{a}$ in nmol/L are mean values from n experiments similar to those shown in Figure 4. K$_{a}$ values were determined by the method of Furchgott and Bursztyn$^9$ (see Methods).

$^*$P<0.05 vs CGS21680 and CCPA.

$^\dagger$P<0.05 vs adenosine and CCPA.
cannot be explained by their affinities for A2A-AdoRs. Rather, a high density of A2A-AdoRs and efficient receptor-effector signal transduction appear to explain the responsiveness of the coronary circulation to adenosine and adenosine analogues. This conclusion is strengthened by the finding of a large A2A-AdoR reserve for each of 3 agonists (adenosine, CCPA, and CGS21680) that have markedly different structures and affinities, indicating that the presence of a receptor reserve for coronary vasodilation is not unique to a single A2A-AdoR agonist but is a property of the coronary circulation.

We estimate that there is a 70% receptor reserve for adenosine to cause a maximal increase of coronary conductance. In comparison, the A1 AdoR reserve for adenosine to activate the inwardly rectifying potassium current of single, isolated atrial myocytes is small (2%), and the A1 AdoR reserve for adenosine to inhibit a β-adrenergic receptor-mediated increase of calcium inward current of guinea pig atrial myocytes was found to be 30%. The differences in receptor reserves for various actions of adenosine in the heart may help to explain the observed hierarchy of cardiac responses to both exogenous adenosine and hypoxia (endogenous adenosine). Thus, coronary vasodilation occurs at lower concentrations of exogenous adenosine than does either prolongation of A-V nodal conduction time or slowing of atrial rate, and an increase of coronary blood flow is the first response to cardiac hypoxia.

The magnitude of receptor reserve for CGS21680 to increase coronary conductance of the guinea pig isolated heart was much greater than that for CCPA to cause the same response. One interpretation of this finding is that the most active conformation of the A2A-AdoR may be better stabilized by some agonists than by others. CGS21680 may be one such agonist with a high intrinsic efficacy to cause receptor stimulation, whereas CCPA may have a low intrinsic efficacy. Our data indicate that the intrinsic efficacy of CGS21680 was 6-fold higher than the intrinsic efficacy of CCPA to cause coronary vasodilation (Table).

**Study Limitations**

Several limitations and assumptions of the methodological approach used in this study need to be noted. First, the location (presumably endothelial and/or vascular smooth muscle) of the A2A-AdoR that mediates an increase of coronary conductance is not clarified by our experiments. Second, although our results demonstrate that antagonism by FSPTP of the A2A-AdoR is long-lasting (ie, apparently exceeding the duration of our experiments), it is not clear whether the binding of FSPTP to the A2A-AdoR is irreversible or just slowly reversible. If binding were slowly reversible, then the magnitude of receptor reserve may have been slightly underestimated in this study. Third, we assume that occupancy by the agonist of a similar number
of receptors before and after exposure of hearts to FSPTP causes a similar magnitude of response (coronary vasodilation). Lastly, the law of mass action for agonist-receptor interactions is assumed to apply to coronary A$_{2A}$-AdoRs. Receptors are assumed to be independent of one another (ie, noninteracting), and agonist-receptor interactions are assumed to be reversible. Our findings that incubation of membrane A$_{2A}$-AdoRs with FSPTP caused a reduction of binding sites for [H]SCH58261 but did not alter the $K_d$ for binding of [3H]SCH58261 to remaining sites (Figure 2) and that the responses to adenosine, CGS21680, and CCPA did not show evidence of desensitization support the validity of these assumptions.

The estimate of receptor reserve for adenosine is likely to be less accurate than those for CGS21680 and CCPA. The reason for this is that formation and metabolism of adenosine by the heart cause the concentration of adenosine in the interstitium to be different from that in the coronary perfusate (arterial compartment). Analysis using a mathematical model of adenosine transport and metabolism in guinea pig heart indicated that the concentration of adenosine in the interstitium was 2- to 3-fold lower than the concentration of adenosine in the arteries during arterial administration of adenosine and was not a linear function of arterial adenosine concentration. The use of inhibitors of adenosine transport (eg, dipyridamole) and metabolism [eg, 5'-iodotubercidin and erythro-9-(2-hydroxy-3-nonyl)adenine] is unfortunately not a solution to this problem because these compounds cause coronary vasodilation in the absence of exogenous adenosine, by increasing the accumulation of endogenous adenosine. Because both coronary arterial endothelial and smooth muscle cell A$_{2A}$-AdoRs may potentially mediate the vasodilator action of adenosine but are likely exposed to unequal concentrations of adenosine, it is difficult to predict whether our estimate of receptor reserve for adenosine is too high, too low, or about right. Our estimate of receptor reserve for adenosine is most likely to be accurate if the A$_{2A}$-AdoRs that mediate the coronary vasodilator action of adenosine are located on the luminal surfaces of arterial endothelial cells.

**Consequences of the Large Receptor Reserve for A$_{2A}$-AdoR–Mediated Coronary Vasodilation**

Results of this study indicate that activation by adenosine of 5% of the total number of coronary A$_{2A}$-AdoRs can cause half-maximal coronary vasodilation. The concentration of adenosine needed to activate this 5% portion of the A$_{2A}$-AdoR population was 85 nmol/L. In contrast, the concentration of adenosine needed to occupy 50% of the receptors was 1.8 μmol/L (Table). In the absence of receptor reserve, therefore, 1.8 μmol/L adenosine would be required to cause a half-maximal coronary vasodilation. Thus, a major consequence of the large A$_{2A}$-AdoR reserve for adenosine-mediated coronary vasodilation is an increased sensitivity of the coronary vasculature to adenosine.

The existence of a large receptor reserve for A$_{2A}$-AdoR–mediated coronary vasodilation has consequences for the interpretation of experiments to investigate the role of endogenous adenosine as a mediator of hypoxia and/or ischemia-induced vasodilation. Attempts to block the actions of adenosine by use of antagonists in these experiments may be unsuccessful because >70% of A$_{2A}$-AdoRs must be bound by an antagonist before a maximal response to adenosine is attenuated, and 95% of receptors must be bound by an antagonist to attenuate the maximal response by half. Thus, it may be improper to conclude that adenosine has no role in mediation of an observed coronary vasodilation if this conclusion is based on the inability of AdoR antagonists to attenuate an increase of conductance.

In summary, our data show that there is a large receptor reserve for an A$_{2A}$-AdoR–mediated increase of coronary conductance. These data explain the apparent contradiction between the high potency of adenosine and adenosine analogues to cause coronary vasodilation and the much lower affinity of the same agonists to bind A$_{2A}$-AdoRs. Also, the data partially explain the observations that intravenous administrations of low doses of either adenosine, WRC-0470 (a selective A$_{2A}$-AdoR agonist), or CGS21680 can cause coronary vasodilation without causing systemic hypotension. Further investigation will be needed to determine A$_{2A}$-AdoR reserve in other regional vascular beds for comparison with our findings for the coronary circulation. These data may provide the rationale for use of certain A$_{2A}$-AdoR agonists for selective vasodilation of the coronary or other regional circulations.

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