Cardiac effects of adenosine in A2A receptor knockout hearts: uncovering A2B receptors

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Morrison, R. Ray, M. A. Hassan Talukder, Catherine Ledent, and S. Jamal Mustafa. Cardiac effects of adenosine in A2A receptor knockout hearts: uncovering A2B receptors. Am J Physiol Heart Circ Physiol 282: H437–H444, 2002.—To clarify the relative roles of A2 adenosine receptor subtypes in the regulation of coronary flow and myocardial contractility, coronary vascular and functional responses to adenosine and its analogs were examined in isolated wild-type (WT) and A2A receptor knockout (A2AKO) mouse hearts. Nonselective agonists adenosine and 5′-N-ethyl-carboxamido-adenosine (NECA) increased coronary flow in A2AKO hearts, albeit with a rightward shift of concentration-response curves and decreased maximal vasodilation compared with WT hearts. 2-p-(2-carboxy-ethyl)phenethylamino-5′-N-ethyl-carboxamidoadenosine (CGS-21680, a selective A2A receptor agonist) increased coronary flow in WT hearts but did not affect A2AKO hearts. Adenosine and NECA each elicited equal maximal increases in developed pressure in WT and A2AKO hearts, whereas CGS-21680 did not affect developed pressure in A2AKO hearts. Alloxazine, a selective A2B receptor antagonist, attenuated NECA-induced coronary vasodilation (from 202 ± 14% to 128 ± 9% of baseline, P < 0.05) and NECA-induced increases in developed pressure (from 133 ± 8% to 112 ± 7% of baseline, P < 0.05) in A2AKO hearts. Together, these findings support the conclusion that A2B adenosine receptor activation increases coronary flow and developed pressure in isolated murine hearts.

Langendorff mouse hearts; adenosine analogs; coronary circulation; developed pressure

ADENOSINE EXERTS its well-described cardiovascular effects through interaction with at least four known adenosine receptor subtypes: A1, A2A, A2B, and A3 adenosine receptors (2, 8, 18, 26, 32, 35). Whereas A1 adenosine receptor activation results in negative chronotropic, dromotropic, and inotropic effects as well as protective antiadrenergic responses (2, 8, 32), activation of A2A receptors is largely responsible for coronary vasodilation (1, 3, 13, 16, 26, 27, 33, 35, 36). It has been speculated that A2B receptor activation contributes to adenosine-induced coronary vasodilation (13, 22), and the recent demonstration of A2B receptors in the coronary endothelium (31) supports this possibility. However, the lack of potent and selective A2B adenosine receptor agonists and antagonists leaves only indirect evidence of A2B-mediated coronary vasodilation (17, 22, 38), and the relative roles of the A2 receptor subtypes in regulating coronary flow and myocardial function remain incompletely understood (35).

Recent data indicate that coregulatory interactions between the different adenosine receptor subtypes are more complex than previously appreciated. For instance, the demonstration that A2A adenosine receptors are present on ventricular myocytes (7, 20, 23, 37) has led to strong evidence that A2A receptor activation attenuates A1 receptor-mediated antiadrenergic activity (28) and increases myocardial contractility in isolated hearts independent of effects on coronary flow (24). It is also becoming clear that A2A adenosine receptor activation protects against ischemia-reperfusion (particularly during reperfusion) in the heart (5, 21) and other tissues (12, 29, 30), and the mechanisms involved are being actively investigated. It is not known whether A2B receptor activation modulates these protective effects, but tissue distribution of this receptor subtype suggests a coregulatory role.

The demonstration that A2B adenosine receptor activation causes vasodilation in other tissues (14, 34) and augments contractility in isolated ventricular myocytes (20) suggests that A2B receptors may indeed modify these cardiac effects. Yet, without pharmacological ligands that are both selective and potent at A2B receptors, isolating the unique effects of A2B activation has remained difficult. The advent of targeted gene deletion allows more precise examination of these receptor-mediated responses than previously possible through agonist/antagonist studies alone. With the combination of receptor knockout technology and the traditional receptor-ligand approach, the physiological roles of A2 adenosine receptor subtypes can be elucidated despite the unavailability of selective and potent A2B receptor agonists and antagonists.

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TEMPERATURE was held constant at 23° C; humidity was 60 ± 10%. An inverted light-dark cycle of 12:12 h was used (lights off at 1700 h). Experiments were conducted in accordance with national legislation and with the Declaration of Helsinki regarding the use of experimental animals.

Langendorff isolated heart model. Isolated heart experiments were performed in accordance with the methods of Matherne and Headrick as previously described (11, 15, 25, 38). Animals were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg), a thoracotomy was performed, and the hearts were excised into heparinized (5 U/ml) ice-cold perfusate. The aorta was rapidly cannulated with a 20-gauge, blunt-ended needle, and retrograde coronary perfusion was initiated at constant pressure of 80 mmHg with modified Krebs bicarbonate buffer containing (in mM) 120 NaCl, 25 NaHCO_3, 4.7 KCl, 1.2 KH_2PO_4, 1.2 MgSO_4, 15 glucose, 2 pyruvate, and 0.05 EDTA. The perfusate was equilibrated with 95% O_2-5% CO_2 at 37°C, giving a pH of 7.4 and PO_2 of ~550 mmHg. The left atrium was removed, and the left ventricle was vented with a small polyethylene apical drain. A fluid-filled balloon constructed of plastic film was inserted into the left ventricle across the mitral valve and connected to a pressure transducer permitting continuous measurement of left ventricular pressure. Hearts were immersed in a water-jacketed bath maintained at 37°C, and the ventricular balloon was filled to yield a left ventricular diastolic pressure of 2–5 mmHg. Coronary flow was continuously monitored via a Doppler flow probe (Transonic Systems; Ithaca, NY) located in the aortic perfusion line. Aortic and left ventricular developed pressures were recorded on a MacLab multichannel data acquisition system (ADInstruments; Castle Hill, Australia) connected to a MacIntosh G4 computer. The ventricular pressure signal was digitally processed (using MacLab Chart software version 3.5.6, ADInstruments) to yield diastolic and systolic pressures as well as heart rate.

Experimental protocol. All hearts were equilibrated for 30 min before experimentation. Coronary flow, heart rate, and developed pressure (systolic pressure minus diastolic pressure) were examined in WT and A2AKO hearts, and baseline data for these parameters were sampled at the end of equilibration. Dose-response relationships were constructed for adenosine, 5'-N-ethyl-carboxamidoadenosine (NECA, a non-selective adenosine receptor agonist), and 2-p-(2-carboxyethyl)-phenethylamino-5'-N-ethyl-carboxamidoadenosine (CGS-21680, a selective A2A adenosine receptor agonist) by infusing these agonists into the coronary perfusate through an injection port directly proximal to the aortic cannula. Infusion rate was controlled to a maximum of 1% of coronary flow with a microinjection infusion pump (Harvard Apparatus; Holliston, MA). After baseline data were acquired, each heart was exposed to progressively increasing concentrations of a single agonist to develop a dose-response relationship. Each concentration of agonist was infused for 5 min (with plateau effect being achieved uniformly between 3 and 5 min), and data were sampled at the end of the 5-min infusion period. After the infusion of each concentration of agonist, a minimum of 5 min of perfusion was allowed for complete drug washout (with recovery to baseline parameters occurring uniformly between 3 and 5 min). Animals were sampled at the end of each washout period and used as a reference for normalizing response to subsequent agonist concentration. Notably, the washout period was sufficient to fully replace the volume of superfusate present in the water-jacketed bath, assuring complete clearance of each preceding dose before infusing a subsequent concentration of agonist.

Antagonist experiments. A subset of experiments was performed in A2AKO hearts to block the observed NECA-induced changes in coronary flow and developed pressure. Alloxazine, a selective A2A adenosine receptor antagonist, was used for these experiments. A2AKO hearts were dissected, cannulated, and equilibrated as described above. After baseline data for each heart were sampled at the end of the 30-min equilibration period, 100 nM NECA was infused at 1% of coronary flow for 5 min, and the plateau effect on coronary flow, heart rate, and left ventricular developed pressure was recorded. This initial infusion of NECA was allowed to wash out for 10 min and then 10 µM alloxazine was infused at 1% of coronary flow for 15 min. Ten minutes into the alloxazine infusion, data were sampled and normalized as a new “baseline,” and 100 nM NECA was added to the coronary perfusate at 1% of coronary flow for the remaining 5 min of the alloxazine infusion. Data were sampled at the end of this two-drug infusion for comparison with data resulting from infusion of NECA alone. After alloxazine and NECA were allowed to wash out for 10 min, a repeat infusion of 100 nM NECA was performed for comparison with the first NECA infusion.

Chemicals. A stock solution of adenosine (Sigma, St. Louis, MO) was prepared using distilled water. Stock solutions of NECA (Sigma), CGS-21680 (Sigma), and alloxazine (benzo[g]-pteridine-2,4-(1H,3H)-dione; RBI) were prepared in dimethyl sulfoxide (DMSO, Sigma), and serial dilutions to desired concentrations were made in distilled water. (A subset of experiments demonstrated that the effect of DMSO at 1% of coronary flow for 5 min elicited less than a 5% increase in baseline coronary flow; data not shown.) All stated chemical concentrations reflect actual drug concentration when delivered as 1% of coronary flow.

Statistical analysis. Baseline functional data for WT and A2AKO groups were analyzed by t-test. Differences in dose response between WT and A2AKO groups at individual agonist concentrations were analyzed by one-way ANOVA with Bonferroni’s correction for multiple comparisons applying Student-Neuman-Keuls post hoc test. Differences in dose response within each group were analyzed by one-way repeated-measures ANOVA with Bonferroni’s correction for
multiple comparisons. EC$_{50}$ values were derived as previously described (11, 15) by plotting responses for individual hearts in each group and fitting the following single-site, four-parameter logistic equation to the data

\[ \text{Response} = A + B \times \frac{D}{1 + \left(\frac{D}{EC_{50}}\right)^{n}} \]

where $A$ is the response at zero dose (baseline preinfusion value), $B$ is the response at infinite dose (maximal response), $D$ is the drug concentration, EC$_{50}$ is the effective concentration mediating 50% maximal response, and slope factor describes the steepness of the dose-response curve. The equation was fit to raw data using KaleidaGraph (Abelbeck Software: Reading, PA), and individual EC$_{50}$ values were derived from each curve fit. Resultant EC$_{50}$ values were compared by t-test. Coronary flow, developed pressure, and heart rate responses to antagonist infusion for WT and A$_2$A KO hearts were compared by t-test. For all tests, statistical significance was accepted at $P < 0.05$.

**RESULTS**

**Baseline function in isolated WT and A$_2$A KO hearts.** Table 1 summarizes baseline functional parameters (coronary flow, heart rate, and developed pressure) after 30 min of equilibration in isolated mouse hearts perfused at constant pressure. The average body weight and heart weight were significantly higher in age-matched A$_2$A KO mice compared with WT mice. Whereas this is consistent with previous findings in the A$_2$A KO knockout model (19), the heart weight-to-body weight ratios of WT and A$_2$A KO knockout animals were not different. There were no significant differences in baseline coronary flow, heart rate, or developed pressure between WT and A$_2$A KO hearts (Table 1).

**Effects of adenosine on coronary flow in WT and A$_2$A KO hearts.** Adenosine and its analogs NECA and CGS-21680 caused concentration-dependent increases in coronary flow (vasodilation) in isolated WT hearts perfused at constant pressure (Fig. 1, A–C). For both WT and A$_2$A KO hearts, maximal response to each agonist varied, as did the potency for producing coronary vasodilation. In A$_2$A KO hearts, concentration-response curves for adenosine- and NECA-induced coronary vasodilation were shifted to the right and demonstrated attenuated maximal responses compared with WT hearts (Fig. 1, A and B).

<table>
<thead>
<tr>
<th>Table 1. Baseline functional data in wild-type and A$_2$A adenosine receptor knockout mouse hearts</th>
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<tbody>
<tr>
<td><strong>Wild Type</strong> (n = 20)</td>
</tr>
<tr>
<td><strong>Age, wk</strong></td>
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<tr>
<td><strong>Body weight, g</strong></td>
</tr>
<tr>
<td><strong>Heart weight, mg</strong></td>
</tr>
<tr>
<td><strong>Heart-to-body weight ratio, %</strong></td>
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<tr>
<td><strong>Coronary flow, ml·min$^{-1}$·g$^{-1}$</strong></td>
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<tr>
<td><strong>Heart rate, beats/min</strong></td>
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<td><strong>Developed pressure, mmHg</strong></td>
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All values are means ± SE; n, number of hearts. Functional parameters were measured after 30 min of normothermic aerobic perfusion using a standard Langendorff preparation. *P < 0.05 wild-type vs. knockout hearts.

Table 2 summarizes EC$_{50}$ values for agonist-induced changes in coronary flow and left ventricular developed pressure for both WT and A$_2$A KO hearts. The relative order of potency for coronary vasodilation in WT hearts based on EC$_{50}$ values for each agonist was CGS-21680 (15.1 ± 2.37 nM) > NECA (18.7 ± 1.53 nM) > adenosine (565 ± 134 nM). Coronary flow EC$_{50}$ values for adenosine and NECA in A$_2$A KO hearts were significantly higher than those in WT hearts ($P < 0.05$, Table 2), correlating with the rightward shift of the concentration-response curves for these agonists (Fig. 1, A and B).
Table 2. Agonist EC$_{50}$ values for coronary flow and left ventricular developed pressure in wild-type and A$_{2A}$ adenosine receptor knockout hearts

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>Knockout</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>CF</td>
<td>565±134*</td>
<td>2,021±463</td>
</tr>
<tr>
<td>LVDP</td>
<td>342±36*</td>
<td>1,955±541</td>
</tr>
<tr>
<td>NECA</td>
<td>7</td>
<td>18.7±1.5*</td>
</tr>
<tr>
<td>CF</td>
<td>98.8±19.5</td>
<td>NR</td>
</tr>
<tr>
<td>LVDP</td>
<td>103±34</td>
<td>NR</td>
</tr>
<tr>
<td>CGS-21680</td>
<td>7</td>
<td>15.1±2.3*</td>
</tr>
<tr>
<td>CF</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>LVDP</td>
<td>11.9±1.4*</td>
<td>NR</td>
</tr>
</tbody>
</table>

All values are means ± SE; n, number of hearts in both groups. EC$_{50}$ values (expressed as nM) were derived from dose-response curves for each agonist during normothermic aerobic perfusion. CF, coronary flow; LVDP, left ventricular developed pressure; NR, no response to agonist. *P < 0.05 wild-type vs. knockout hearts.

The maximal increase in coronary flow caused by 10 μM adenosine was 446 ± 19% of baseline in WT hearts, and the maximal effect was reduced to 298 ± 30% of baseline in A$_{2A}$KO hearts (P < 0.05, Fig. 1A). Terminal bradycardia precluded evaluation of maximal coronary flow responses to NECA at 1 μM in WT hearts (data not illustrated). However, the coronary vasodilation induced by 500 nM NECA was reduced from 325 ± 21% in WT hearts to 257 ± 20% of baseline in A$_{2A}$KO hearts (P < 0.05, Fig. 1B). Likewise, 500 nM CGS-21680 resulted in maximal coronary flow of 506 ± 44% of baseline in WT hearts, whereas this selective A$_{2A}$ receptor agonist had no effect on coronary flow in A$_{2A}$KO hearts (P < 0.05, Fig. 1C).

Effects of adenosine on developed pressure in WT and A$_{2A}$KO hearts. In WT hearts, adenosine and its analogs NECA and CGS-21680 caused concentration-dependent increases in left ventricular developed pressure with a similar maximal response (Fig. 2, A–C). The maximum percent change in developed pressure in WT hearts for adenosine, NECA, and CGS-21680 was 167 ± 3% (n = 6), 177 ± 9% (n = 7), and 176 ± 7% (n = 7) of baseline, respectively (not significant; Fig. 2, A–C). In A$_{2A}$KO hearts, the selective A$_{2A}$ receptor agonist CGS-21680 had no effect on developed pressure (Fig. 2C). EC$_{50}$ values for agonist-induced increases in left ventricular developed pressure are summarized in Table 2. Adenosine (10 μM) elicited the same maximal increase in developed pressure for WT and A$_{2A}$KO hearts (167 ± 3% and 170 ± 15% of baseline, respectively; not significant; Fig. 2A), although the EC$_{50}$ for adenosine-induced increase in developed pressure was lower in WT versus A$_{2A}$KO hearts (P < 0.05, Table 2). Likewise, 500 nM NECA produced the same maximal response in WT and A$_{2A}$KO hearts (177 ± 9% and 181 ± 8% of baseline, respectively; not significant; Fig. 2B); however, the EC$_{50}$ for developed pressure could not be determined for NECA due to terminal bradyarrhythmias at 1 μM.

Effects of adenosine on heart rate in WT and A$_{2A}$KO hearts. Neither adenosine nor the selective A$_{2A}$ receptor agonist CGS-21680 had significant effect on the heart rate of WT and A$_{2A}$KO hearts (Fig. 3, A and C). NECA caused a concentration-dependent decrease in heart rate in both WT and A$_{2A}$KO hearts beginning at 50 nM (Fig. 3B).

Fig. 2. Concentration-response curves for developed pressure (expressed as percent change from baseline) for adenosine (A), NECA (B), and CGS-21680 (C) in isolated perfused mouse hearts. Values are means ± SE. *Significant differences between WT and KO groups, P < 0.05.
In the absence of alloxazine, 100 nM NECA increased coronary flow and developed pressure in A2AKO hearts to 202 ± 14% and 133 ± 8% of baseline, respectively (Fig. 4, A and B; n = 5). With 10 μM alloxazine, the NECA-induced increases in coronary flow and developed pressure were significantly attenuated to 128 ± 9% and 112 ± 7% of normalized baseline values, respectively (P < 0.05; Fig. 4, A and B; n = 5). To demonstrate that this decrease did not result from tachyphylaxis to NECA, a repeat infusion of 100 nM NECA (alone) was performed. This resulted in the same increase in coronary flow and developed pressure as the first infusion of NECA (to 211 ± 19% and 129 ±
6% of baseline, respectively; not significant; data not illustrated). The NECA-induced decrease in heart rate was unaffected by A2B receptor antagonism with alloxazine (Fig. 4C, n = 5).

DISCUSSION

The primary intent of this study was to examine coronary vascular and functional responses to adenosine and its analogs in isolated hearts from WT and A2A KO mice. The data demonstrate that nonselective agonists adenosine and NECA increase coronary flow in hearts lacking A2A adenosine receptors, albeit with a rightward shift of the concentration-response curves and attenuated maximal response. The maximal increase in developed pressure induced by adenosine and NECA was the same in WT and A2A KO hearts despite observed differences in coronary flow between the groups. NECA-induced increases in both coronary flow and developed pressure in A2A KO hearts were attenuated by alloxazine, an A2B adenosine receptor antagonist. Taken together, these findings support the conclusion that A2B adenosine receptor activation increases coronary flow and developed pressure in isolated murine hearts.

Coronary vascular responses in WT and A2A KO hearts. The diverse cardiovascular effects of adenosine are determined in part by tissue distribution, agonist affinity, and receptor signaling pathways associated with each of the adenosine receptor subtypes (2, 26, 32, 35). High-affinity A2A and low-affinity A2B adenosine receptors coexist in coronary endothelial cells (31) and ventricular myocytes (20). Early studies in most mammals indicate that coronary vasodilation is primarily mediated by A2A adenosine receptor activation (1, 3, 13, 23, 27, 38). Evidence for A2B receptor-mediated coronary vasodilation is rather indirect because of the lack of potent and selective A2B adenosine receptor agonists and antagonists (17, 22, 38). Because activation of high-affinity A2A adenosine receptors results in marked responses, it has been difficult to determine whether and to what extent activation of low-affinity A2B receptors alters vasoactive and/or functional responses in the heart. That is, the physiological effects of A2A receptor activation may overshadow any contributing responses resulting from activation of A2B adenosine receptors.

With the use of an A2A adenosine receptor knockout model in concert with a selective and potent A2A receptor agonist (CGS-21680), the cardiac effects of A2A activation can now be distinctly isolated. The data presented demonstrate that the CGS-21680-induced increase in coronary flow in WT hearts was absent in A2A KO hearts (Fig. 1C), whereas the nonselective agonists adenosine and NECA increased coronary flow in both WT and A2A KO hearts (Fig. 1, A and B). Together these findings necessarily infer the activation of an adenosine receptor subtype other than A2A adenosine receptors to account for the coronary vasodilation elicited by adenosine and NECA in A2A KO hearts (Fig. 1, A and B).

The adenosine- and NECA-induced increases in coronary flow in A2A KO hearts were observed at higher concentrations (Fig. 1, A and B) and therefore with higher EC50 values (Table 2) than in WT hearts. This rightward shift of concentration-response curves and attenuated maximal response to nonselective agonists suggests that the receptor subtype responsible for coronary vasodilation in A2A KO hearts (Fig. 1, A and B) has a low-affinity binding site. It is noteworthy that whereas NECA is the most potent A2B agonist available (9), it is a nonselective activator of A1, A2A, A2B, and A3 receptors with inhibitory constant (K_i) values of 3–30 nM, 1–20 nM, 0.5–5 μM, and 10–30 nM, respectively (9, 10). Thus, whereas NECA has K_i values for all other adenosine receptors in the nanomolar range (high affinity), its K_i for A2B receptors is in the micromolar range (low affinity). Likewise, adenosine has higher affinity for A2A receptors than A2B receptors with K_i values of 1–20 nM and 5–20 μM, respectively (9, 10). The observation that coronary vasodilation in A2A KO hearts occurs at high concentrations of adenosine and NECA is consistent with this effect being mediated through activation of low-affinity A2B adenosine receptors.

Functional responses in WT and A2A KO hearts. The selective A2A adenosine receptor agonist CGS-21680 increased developed pressure in WT hearts but had no effect on developed pressure in A2A KO hearts (Fig. 2C). Adenosine and NECA resulted in the same maximal increase in developed pressure in both WT and A2A KO hearts (Fig. 2, A and B). This equal increase in contractility occurs despite attenuated vasodilation by these agonists in A2A KO hearts (Fig. 1, A and B). Therefore, nonselective adenosine agonists increase developed pressure in A2A KO hearts independent of changes in coronary flow. Monahan et al. (24) recently reported that A2A receptor activation increases contractility independent of changes in coronary flow in isolated rat hearts perfused at constant pressure. The current data indicate the same is true in isolated mouse hearts lacking A2A receptors. That is, adenosine and NECA increase contractility in A2A KO hearts (to an extent not accounted for by changes in coronary flow) through activation of a receptor subtype other than A2A adenosine receptors.

Having eliminated A2A receptors by examining contractile performance in A2A KO hearts, only A1, A2B, and A3 receptors remain as candidate sites of action for adenosine and NECA to elicit the observed increase in developed pressure. It is well documented that A1 adenosine receptor activation results in negative inotropic and antiadrenergic effects through coupling to Gi proteins resulting in decreased intracellular cAMP through inhibition of adenylyl cyclase (2, 10, 35). Thus activation of A1 receptors is not a plausible explanation for the agonist-induced increase in developed pressure in A2A KO hearts. Neither is A3 receptor activation because it is also known to decrease cAMP through inhibition of adenylyl cyclase (37, 41, 42), although its effect on contractile performance is not known. The increased developed pressure elicited by nonselective
adrenoceptors in this model can only occur through activation of A2B adenosine receptors. This is consistent with the observation that activation of A2A as well as A2B receptors increases contractility in chick cardiac myocytes through Gα coupling and a resultant increase in cAMP through stimulation of adenylyl cyclase (20).

The selective and potent A2A adenosine receptor agonist CGS-21680 had no effect on heart rate in either WT or A2AKO hearts (Fig. 3C). Likewise, the nonselective agonist adenosine did not affect spontaneous heart rate in either WT or A2AKO hearts (Fig. 3A). It is unclear why adenosine did not produce bradycardia at concentrations known to activate A1 receptors (10). Nonetheless, the concordant heart rate response (an A1-mediated effect) between WT and A2AKO hearts was examined. Alloxazine did not inhibit A1-mediated heart rate responses, the effect of NECA in A2AKO hearts (Fig. 4, A and B). A repeat infusion of NECA in the absence of alloxazine elicited the same increase in both coronary and developed pressure in A2AKO hearts (Fig. 4C). That is, at a concentration greater than its Ki for A1 receptors, alloxazine did not inhibit A1-mediated heart rate responses, yet it indeed blocked the NECA-induced increases in coronary flow and developed pressure (Fig. 4, A and B). Thus, the increase in coronary flow and developed pressure elicited by adenosine and NECA in A2AKO hearts cannot be explained by A1 receptor activation, rather it supports an A2B-mediated effect that can be inhibited by alloxazine.

It is not known whether A3 adenosine receptor activation effects coronary flow. Recent work in our laboratory has demonstrated increased coronary vasodilation with adenosine and CGS-21680 in A3 receptor knockout hearts compared with WT hearts (39). Whereas a detailed analysis of this response is currently under investigation, this finding suggests that if A3 receptor activation coregulates coronary flow in mice, it does so by either inhibition or negative modulation of A2A receptor-mediated vasodilation. Thus it is unlikely that the current observation of adenosine- and NECA-induced coronary vasodilation in A2AKO hearts results from nonspecific activation of A3 receptors.

Study limitations. Studies examining the physiological consequences of A2B adenosine receptor activation continue to be limited by the lack of antagonists that are both selective and potent for this receptor. Whereas the selectivity of alloxazine has proven sufficient to examine A2B receptor-mediated effects in this and other models (20, 34), its lack of potency requires very high doses to elicit receptor blockade. The current work circumvents this issue by examining A2B receptor antagonism in A2AKO hearts, but it is recognized that neither the coronary vasodilation nor the increased developed pressure induced by NECA could be completely eliminated by alloxazine (Fig. 4, A and B). Thus, whereas the potency of the antagonist used may remain limiting, its selectivity is sufficient to conclusively establish A2B-mediated responses in this model. Additionally, contractile function was assessed in the present study using constant pressure perfusion; however, further characterization of adenosine-mediated contractile function in A2AKO hearts may require studies using constant flow perfusion and/or isolated myocyte preparations (24, 40). Finally, like all recent studies taking advantage of an ever-increasing array of gene-modified animal models, consideration must be given to the possibility that other receptor systems may have been affected by targeted expression or deletion of a single receptor subtype (11). That is, the functional genomics of A2AKO animals has not yet been examined and will be an important component of future work with this model.

Conclusions and future directions. The present study combines receptor knockout technology with the traditional pharmacological approach to examine A2 adenosine receptor subtypes in regulation of coronary flow and functional responses in isolated mouse hearts. The results provide the first direct evidence that activation of A2B adenosine receptors contributes to coronary vasodilation and cardiac contractility. This strategy can now be utilized to further examine the heretofore-elusive nature of A2B receptor effects and cell-signaling processes in the heart under pathophysiological conditions such as hypoxia or ischemia-reperfusion. Clarifying the relative roles of adenosine receptor subtypes in the cardiovascular system remains an essential step in the development of adenosinergic therapies for the clinical management of heart disease.

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