Adenosine $A_{2a}$-receptor activation increases contractility in isolated perfused hearts

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Monahan, Thomas S., Darrell R. Sawmiller, Richard A. Fenton, and James G. Dobson, Jr. Adenosine $A_{2a}$-receptor activation increases contractility in isolated perfused hearts. Am J Physiol Heart Circ Physiol 279: H1472–H1481, 2000.—Adenosine $A_{2a}$-receptor activation enhances shortening of isolated cardiomyocytes. In the present study the effect of $A_{2a}$-receptor activation on the contractile performance of isolated rat hearts was investigated by recording left ventricular pressure (LVP) and the maximal rate of LVP development ($+dP/dt_{\text{max}}$). With constant-pressure perfusion, adenosine caused concentration-dependent increases in LVP and $+dP/dt_{\text{max}}$ with detectable increases of 4.1 and 4.8% at $10^{-6}$ M and maximal increases of 12.0 and 11.1% at $10^{-4}$ M, respectively. The contractile responses were prevented by the $A_{2a}$-receptor antagonists chlorostyryl-caffeine and aminourfyltriazolotriazinyl-aminoethyphenol (ZM-241385) but were not affected by the $\beta_1$-adrenergic antagonist atenolol. The adenosine $A_1$-receptor antagonist dipropylcyclopentylxanthine and pertussis toxin potentiated the positive inotropic effects of adenosine. The $A_{2a}$-receptor agonist ethylcarboxamidoadenosine and dimethoxyphenyl-methylphenylethyl-adenosine also enhanced contractility. With constant-flow perfusion, $10^{-6}$ M adenosine increased LVP and $+dP/dt_{\text{max}}$ by 5.5 and 6.0%, respectively. In the presence of the coronary vasodilator hydralazine, adenosine increased LVP and $+dP/dt_{\text{max}}$ by 7.5 and 7.4%, respectively. Dipropylcyclopentylxanthine potentiated the adenosine contractile responses with constant-flow perfusion in the absence and presence of hydralazine. These increases in contractile performance were also antagonized by chlorostyryl-caffeine and ZM-241385. The results indicate that adenosine increases contractile performance via activation of $A_{2a}$ receptors in the intact heart independent of $\beta_1$-adrenergic receptor activation or changes in coronary flow.

Adenosine $A_1$ receptor; $\beta_1$-adrenergic receptor; myocardial contractility; constant-pressure-perfused heart; constant-flow-perfused heart; $A_1$ antagonists; $A_{2a}$ antagonists; hydralazine

Adenosine is known to elicit multiple effects in the heart, including heart rate reduction (37), vasodilation (3), and cardioprotection (1, 24, 27, 36), mediated by activation of adenosine $A_1$ (24, 37), $A_{2a}$ (3, 36, 40), and $A_3$ (1, 27, 36) receptors. One well-characterized effect of adenosine on myocardial contractile performance is its ability to reduce the contractile and metabolic responses elicited by $\beta_1$-adrenergic stimulation (8, 31). This antiadrenergic effect of adenosine is mediated by $A_1$ receptors, which activate the inhibitory heterotrimeric G protein, $G_i$, reducing $\beta_1$-adrenergic-elicited adenyl cyclase activity, cAMP accumulation, protein kinase A activity, and myocardial protein phosphorylation (9, 13, 15, 30).

Adenosine also has a stimulatory influence on myocardial contractile performance elicited by activation of $A_{2a}$ receptors (11, 39, 40). This effect of adenosine is putatively mediated by activation of the stimulatory G protein, $G_s$, and involves presumably a cAMP-dependent pathway (11, 25, 40). However, a cAMP-independent pathway may also be involved (11, 26). Although the natural nucleoside adenosine has been shown to increase contractility of papillary muscles isolated from rats (23), guinea pigs (4), and dogs (6), it was not determined in these studies whether the increase in contractile performance was mediated by activation of $A_{2a}$ receptors. Some reports have suggested that $A_{2a}$-receptor agonists do not increase the contractility of isolated ventricular myocytes from rats (32), rabbits (32), or guinea pigs (2, 32, 35). However, other reports indicate that $A_{2a}$-receptor agonists increase contractile performance of rat (11, 40), chick embryonic (25, 26, 39), and human (34) ventricular myocytes. Thus, if the latter findings are correct, an increase in the contractility of the intact heart should also result via $A_{2a}$-receptor-mediated mechanisms. The purpose of the present study was to investigate the role of $A_{2a}$-receptor activation by adenosine in the enhancement of contractile performance of intact hearts isolated from rats. The results from this study indicate that adenosine increases ventricular contractile performance in the intact heart independently of adrenergic stimulation, perfusion pressure, and coronary flow and that this increase in contractility is prevented by $A_{2a}$-receptor antagonists. These results further suggest a physiological role of the $A_{2a}$ receptor as a mediator of positive inotropy.
METHODS AND MATERIALS

Preparation of Isolated Perfused Hearts

Male Sprague-Dawley rats (310 ± 7 g; Harlan Sprague Dawley, Indianapolis, IN) were initially anesthetized with pentobarbital sodium (40 mg/kg ip). The hearts were rapidly excised, immersed in ice-chilled physiological saline (PS), and immediately perfused via the aortic cannula with non-recirculated PS at 37°C. A perfusion pump maintained a column of PS at a height of 88 cm in the perfusion apparatus, providing a constant coronary perfusion pressure of 65 mmHg. In constant-flow preparations, the perfusion pump delivered PS directly to the perfusion apparatus at a desired constant flow. The PS was prepared daily and contained (in mM) 120 NaCl, 4.7 KCl, 2.5 CaCl₂, 25 NaHCO₃, 1.2 MgSO₄, 1.2 KH₂PO₄, and 10 glucose. The pH was maintained at 7.4 by gassing the PS with 95% O₂-5% CO₂. Coronary perfusion pressure was recorded by a pressure transducer connected via a side tube to the aortic perfusion cannula. Left ventricular pressure (LVP) was determined using a water-filled, latex balloon-tipped polyethylene cannula (1.5 mm ID) attached to a strain-gauge manometer (Micro-Med, Louis ville, KY). The balloon (Hugo Sachs, Hugstetten, Germany) was inserted into the lumen of the left ventricle. The diastolic pressure was set at 5–10 mmHg and held constant. The hearts were paced at 5 Hz with a stimulator (model SD9, Grass Instruments, Quincy, MA) via platinum wire electrodes inserted into the right and left atria. The voltage was 10% above threshold, and the pulse duration was 5 ms. LVP, maximum rates of LVP development (+dP/dtmax) and relaxation (−dP/dtmax), heart rate, and end-diastolic pressure were assessed continuously by an analog-to-digital converter (Heart Performance Analyzer, Micro-Med, Louisville, KY) at a frequency of 500 Hz. These data were averaged over 0.5-s intervals and stored on a personal computer (Gateway 2000, North Sioux City, SD). A system of dual outputs allowed the signal from the Heart Performance Analyzer to be simultaneously recorded on a multichannel polygraph (model 7758A, Hewlett-Packard, Waltham, MA). Coronary flow was determined volumetrically. The hearts were allowed to stabilize for ≥45 min before an experimental protocol was begun. After the stabilization period, hearts that did not have an LVP ≥75 mmHg were not utilized.

Experimental Protocols

In the first six protocols, hearts were constant-pressure perfused at 65 mmHg. This procedure resulted in coronary flows of 16 ± 1 ml/min (12 ± 1 ml•min⁻¹•g wet wt⁻¹). In protocols 7–9, hearts were constant-flow perfused, unless otherwise indicated, at 16 ± 1 ml/min, resulting in a perfusion pressure of 65 mmHg.

Protocol 1. The effect of adenosine on left ventricular contractility was determined. Adenosine was infused into the perfusion PS at 1% of the coronary flow rate for 3 min yielding final PS adenosine concentrations of 10⁻³–10⁻⁴ M. Concentrations were administered randomly. The maximum contractile response (LVP, +dP/dtmax, and −dP/dtmax) to each concentration of adenosine was achieved within 1 min after the beginning of the infusion. Coronary flow was determined, and coronary resistance was calculated by dividing the perfusion pressure (65 mmHg) by the coronary flow.

Protocol 2. The effects of the A₂a-receptor antagonists chlorostyryl-caffeine (CSC) and aminofuryltriazolotriazinyl-aminoethylphenol (ZM-241385) on the contractile response to adenosine were determined. The antagonists were infused to produce a final concentration of 10⁻⁷ M in the PS, and the contractile response to a 3-min administration of 10⁻⁵ M adenosine was determined periodically throughout this infusion. The contractile response to adenosine was also determined periodically throughout a 60-min period in the presence of 0.01% DMSO, the vehicle for CSC and ZM-241385. Because of the liability of CSC, it was prepared fresh immediately before use.

Protocol 3. The effect of the A₁-receptor antagonist dipropylcyclopentoxanthine (DPCPX) at 10⁻⁷ M on the adenosine-elicited contractile response was assessed. The antagonist was administered 5 min before and during a 3-min exposure to 10⁻⁵ M adenosine.

Protocol 4. The effects of the A₂a-receptor agonists ethylcarboxamido-adenosine (NECA) and dimethoxyphenyl-methylphenyl-adenosine (DPMA) at 10⁻⁶ M and carboxethylphenyl-aminoethylcarboxamido-adenosine (CGS-21689) at 10⁻⁷–10⁻⁸ M on left ventricular contractility were determined. The agonists were administered for 5 min.

Protocol 5. The effect of pertussis toxin on the adenosine-elicited contractile responses was assessed. Rats received activated pertussis toxin (25 μg/kg ip; Research Biochemicals, Natick, MA) 48 h before initiation of experiments. The toxin was activated by incubation in 50 mM KH₂PO₄ (pH 7.5), 1 mg/ml ovum albumin, and 250 mM dithiothreitol at 30°C for 18 h.

Protocol 6. The effect of the β-adrenergic antagonist atenolol (10⁻⁶ M) on the contractile responses elicited by 10⁻⁵ M adenosine and 10⁻⁹ M isoproterenol (a β₁-adrenergic agonist) was determined. The antagonist was infused for 5 min, and adenosine or isoproterenol was administered during the final 3 min.

Protocol 7. The effect of 10⁻⁵ M adenosine on left ventricular contractile performance was determined under constant-flow conditions. This was considered because, during constant-pressure perfusion, the vasodilation elicited by adenosine may have resulted in an increased flow that, on its own, could have augmented contractility. After achieving steady state under constant-pressure perfusion, the hearts were switched to constant-flow perfusion at their natural flow rate of 16 ± 1 ml/min. Subsequently, adenosine was administered at 10⁻⁸–10⁻⁴ M for 3 min, and LVP, +dP/dtmax, and coronary perfusion pressure were recorded.

Protocol 8. The effect of the A₁-receptor antagonist DPCPX (10⁻⁷ M) on the adenosine-elicited contractile response was assessed under constant-flow conditions. The antagonist was administered 5 min before and during a 3-min exposure to 10⁻⁵ M adenosine.

Protocol 9. Hydralazine, a known vasodilator (33), was employed in constant-flow experiments to vasodilate the hearts so that the addition of adenosine would contribute little to the total vasodilation. Pretreatment of hearts with 10⁻⁴ M hydralazine attenuated the reduction of coronary perfusion pressure (~1–5 mmHg) mediated by adenosine-induced vasodilation. Some hearts were also treated with 10⁻⁷ M DPCPX 5 min before and during the 3-min administration of 10⁻⁵ M adenosine to prevent the possible involvement of A₁ receptors. The contractile response to adenosine was determined before and after treatment of the hearts with 10⁻⁷ M CSC for 60 min or 10⁻⁤7 M ZM-241385 for 5 min and then after CSC or ZM-241385 was allowed to wash out from the heart for an additional 30 min.

During the course of this study, the adenosine-induced contractile response was reduced or entirely absent in hearts from hyperexcited rats compared with hearts from tranquil rats. If it was deemed necessary, the rats were moved to a quiet room for 2–3 days before experimentation. All animals were exposed to a 12:12-h light-dark cycle and given food and

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water ad libitum. In addition, because it was difficult to assess the contractile response in hearts displaying a high degree of ventricular arrhythmic activity, data from arrhythmic hearts are not included.

Animals

The animals were maintained and used in accordance with recommendations in the Guide for the Care and Use of Laboratory Animals [Institute of Laboratory Animals Resources, National Research Council (NIH Publication), vol. 25, no. 28, revised 1996] and the guidelines of the Institutional Animal Care and Use Committee of the University of Massachusetts Medical School.

Materials

Adenosine (Boehringer Mannheim, Indianapolis, IN) and NECA (Research Biochemicals, Natick, MA) were prepared as stock solutions of $10^{-2}$ M and $3 \times 10^{-3}$ M, respectively, in PS or deionized water (MilliQ water system, Millipore, Bedford, MA). CSC (Research Biochemicals) was prepared as a stock solution of $10^{-3}$ M in 50% DMSO, and isoproterenol (Sigma Chemical, St. Louis, MO) was prepared as a stock solution of $10^{-2}$ M in 0.1% (wt/vol) sodium metabisulfite. CGS-21680, DPMA, DPCPX (Research Biochemicals), and ZM-241385 (Tocris Cookson, Ballwin, MO) were prepared in stock solutions of $10^{-2}$ M in DMSO. All solutions were further diluted in PS or water before infusion. Pentobarbital sodium was obtained from Abbott Laboratories (North Chicago, IL), and all other salts, glucose, and solvents were of certified grade (Fisher Scientific, Boston, MA, or J. T. Baker, Phillipsburg, NJ).

Statistical Treatments

Contractile data (LVP and $\pm dP/dt_{\text{max}}$) were recorded via the Heart Performance Analyzer immediately before each treatment with adenosine, NECA, DPMA, CGS-21680, or vehicle and during the peak responses to the agents. In some studies the concentration-dependent effects of adenosine on all three indexes of left ventricular contractility (LVP, $+dP/dt_{\text{max}}$, and $-dP/dt_{\text{max}}$) were determined. These studies indicated that the effect of adenosine on contractile performance is adequately assessed through measurements of LVP and $+dP/dt_{\text{max}}$. Therefore, in most studies, LVP and $+dP/dt_{\text{max}}$ were utilized as the indexes of contractility. Values are means ± SE. Statistical analysis was performed by randomized block ANOVA, which allowed the contractile responses to each treatment to be compared with a control value generated from the same heart. $P < 0.05$ was accepted as indicating a statistically significant difference.

RESULTS

Adenosine Enhances Contractile Performance With Constant-Pressure Perfusion

Adenosine at $10^{-5}$ M increased LVP, $+dP/dt_{\text{max}}$, and $-dP/dt_{\text{max}}$ within 3 min (Fig. 1). On termination of the adenosine administration, the contractile variables returned to control levels within 5 min. Concentration-response curves revealed increases for LVP, $+dP/dt_{\text{max}}$, and $-dP/dt_{\text{max}}$ of 4.1, 4.8, and 4.6% at $10^{-6}$ M adenosine, increases of 8.0, 8.7, and 9.4% at $10^{-5}$ M adenosine, and maximum increases of 12.0, 11.1, and 14.3% at $10^{-4}$ M adenosine, respectively (Fig. 2). The hearts demonstrated contractile responses to 3-min infusions of adenosine every 10 min for 90 min. Repeated infusion of the vehicle for adenosine (PS or water) did not significantly change contractile performance.

Adenosine-Elicited Increase in Contractile Performance Is Prevented by $A_{2a}$-Receptor Antagonists

The $A_{2a}$-receptor antagonists CSC and ZM-241385, when administered alone at $10^{-7}$ M, did not significantly affect LVP and $+dP/dt_{\text{max}}$ (Fig. 4). However, both antagonists prevented the 8.8 and 8.1% increase in LVP and $+dP/dt_{\text{max}}$, respectively, caused by $10^{-5}$ M adenosine. A 60-min, continuous infusion of CSC was required to prevent the adenosine-elicited contractile response, whereas only a 5-min infusion of ZM-241385 was required for the same inhibitory effect. A 60-min infusion of 0.001–0.005% DMSO, the vehicle for CSC and ZM-241385, did not alter the contractile response to adenosine.

$A_1$-Receptor Antagonist, DPCPX, Potentiates the Adenosine-Elicited Increase in Contractile Performance

DPCPX was used in some studies to ascertain whether $A_1$-receptor stimulation could affect the adenosine-elicited response. In the absence of DPCPX, $10^{-5}$ M adenosine increased LVP by 9.5% and $+dP/dt_{\text{max}}$ by 9.8% (Fig. 5). However, in the presence of $10^{-7}$ M DPCPX, the adenosine-elicited increases in LVP and $+dP/dt_{\text{max}}$ were significantly potentiated to...
15.2 and 15.4%, respectively. DPCPX alone did not affect the basal level of LVP or \(+dP/dt_{max}\).

**A2a-Receptor Agonists, NECA and DPMA, Increase Left Ventricular Contractile Performance**

As with adenosine, NECA and DPMA at \(10^{-6}\) M increased LVP and \(+dP/dt_{max}\) (Fig. 5). NECA increased LVP by 4.5% and \(+dP/dt_{max}\) by 4.2%. DPMA increased LVP and \(+dP/dt_{max}\) by 4.1 and 3.5%, respectively. The A2a-receptor agonist CGS-21680 at \(10^{-7} - 10^{-5}\) M did not increase LVP and \(+dP/dt_{max}\) (data not shown). Furthermore, at these concentrations, CGS-21680 did not significantly increase coronary flow. Infusion of the appropriate vehicle (DMSO or water) did not affect LVP or \(+dP/dt_{max}\).

**Pertussis Toxin Potentiated the Adenosine-Elicited Increase in Contractile Performance**

Because the A1-receptor antagonist DPCPX potentiated the contractile response of adenosine, pertussis toxin was used as an alternative approach to eliminate the influence of A1-receptor stimulation, which is manifest via inhibitory G protein action. Treatment with pertussis toxin increased the \(10^{-6}, 10^{-5}\), and \(10^{-4}\) M adenosine-elicited increases in LVP from 6.0, 9.5, and 13.7% to 10.4, 15.9, and 21.0%, respectively (Fig. 6). The increases in \(+dP/dt_{max}\) caused by these adenosine concentrations were enhanced from 6.0, 9.8, and 14.0% to 10.4, 16.4, and 18.0%, respectively. Thus pertussis toxin, like DPCPX, potentiated the adenosine-elicited increases in the contractile variables by 31–74%.

**Adenosine-Elicited Contractile Response Is Unaffected by the \(\beta_1\)-Adrenergic Receptor Antagonist Atenolol**

Atenolol was used in some studies to assess whether catecholamines endogenously released could play a role in the adenosine-elicited increase in contractility. Atenolol alone had no effect on LVP and \(+dP/dt_{max}\) and did not affect the \(10^{-5}\) M adenosine-elicited 10.4–10.8% increases in these contractile variables.

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**Fig. 2.** Effect of \(10^{-8} - 10^{-4}\) M Ado on LVP (A), \(+dP/dt_{max}\) (B), and \(-dP/dt_{max}\) (C) of constant-pressure-perfused hearts. Values are means ± SE for 7 hearts. *Statistically significant increase from zero adenosine.

**Fig. 3.** Effect of \(10^{-9} - 10^{-4}\) M Ado on coronary flow (A) and coronary resistance (E; B) of constant-pressure-perfused hearts. Values are means ± SE for 7 hearts. *Statistically significant difference from zero adenosine.
however, the adrenergic antagonist did prevent the 17.8 and 18.3% increases in LVP and $+\frac{dP}{dt_{max}}$, respectively, caused by the $\beta$-adrenergic agonist isoproterenol at $10^{-5}$ M. Isoproterenol was employed at this concentration because it produced a contractile response similar in magnitude to that caused by $10^{-5}$ M adenosine. Isoproterenol at $10^{-4}$ M produced a twofold increase in contractility (data not shown), as previously reported for a perfused rat heart preparation (9).

Adenosine Increased Contractile Performance With Constant-Flow Perfusion

In constant-flow-perfused hearts having a coronary flow of 16 ± 1 ml/min, $10^{-5}$ M adenosine increased LVP, $+\frac{dP}{dt_{max}}$, and $-\frac{dP}{dt_{max}}$ by 5.5, 6.0, and 6.5%, respectively (Fig. 8). At $10^{-4}$ M adenosine the increases were 8.7, 8.9, and 9.4%, respectively, for these contractile variables. The coronary perfusion pressure was only significantly decreased by 5.2% at $10^{-4}$ M adenosine. This indicated that the increase in the contractile variables at $10^{-5}$ M adenosine was most likely independent of a change in perfusion pressure.

$A_2$-Receptor Antagonist DPCPX Potentiates the Adenosine-Elicited Contractile Performance Increase in Constant-Flow-Perfused Hearts

In constant-flow-perfused hearts, DPCPX potentiated the $10^{-5}$ M adenosine-induced increases in LVP and $+\frac{dP}{dt_{max}}$ (Fig. 9). DPCPX potentiated the adenosine-elicited increases in LVP and $+\frac{dP}{dt_{max}}$ from 5.6 and 5.9% to 8.8 and 8.6%, respectively. The potentiation was similar to that observed in the constant-pressure-perfused hearts (Fig. 5).

Adenosine Elicits an Increase in Contractile Performance in the Maximally Dilated Constant-Flow-Perfused Hearts

In constant-flow-perfused hearts, hydralazine, a vasodilator, was administered at $10^{-4}$ M to maximally dilate the coronary vasculature. The coronary flow increased and ranged from 18 to 20 ml/min to maintain a perfusion pressure of 65 mmHg. In the presence of hydralazine, $10^{-5}$ M adenosine increased LVP and $+\frac{dP}{dt_{max}}$ by 7.5 and 7.4%, respectively (Fig. 10). In the presence of DPCPX, the adenosine-induced increases in these contractile variables were...
and 11.2%, respectively. CSC at 10^{-7} M prevented these increases in LVP and \(\frac{dP}{dt_{\text{max}}}\). After a 30-min washout of CSC, 10^{-5} M adenosine in the absence of DPCPX increased LVP and \(\frac{dP}{dt_{\text{max}}}\) by 6.8 and 6.7%, respectively. ZM-241385, like CSC, at 10^{-7} M prevented the adenosine-induced increase in LVP and \(\frac{dP}{dt_{\text{max}}}\) (data not shown). The adenosine-elicited contractile response also returned on washout of ZM-241385. Overall the above results indicate that the adenosine-elicited increase in contractility of the perfused heart is independent of changes in coronary flow.

**DISCUSSION**

**Adenosine Enhancement of Contractile Performance**

The present study indicates that A_{2a}-receptor activation increases left ventricular contractile performance in intact hearts isolated from rats. Adenosine increased contractility in a concentration-dependent fashion, starting at 10^{-6} M (Fig. 2). Only the highest concentration of adenosine (10^{-4} M) significantly increased coronary flow (Fig. 3). The increases in contractility elicited by adenosine were prevented by the A_{2a}-receptor antagonists CSC (14, 19) and ZM-241385 (22, 29) at 10^{-7} M (Fig. 4). Because these compounds are primarily A_{2a}-receptor antagonists, the present findings would suggest that the contractile responses are probably the result of A_{2a}-receptor stimulation. In addition, the A_{2a}-receptor agonists NECA and DPMA increased left ventricular contractile performance, further indicating that the increase is mediated by A_{2a}-receptor activation (Fig. 5). The increase in left ventricular contractile performance mediated by adenosine was not antagonized by the \(\beta\)-adrenergic antagonist atenolol, indicating that the contractile response was not mediated by \(\beta\)-adrenergic stimulation (Fig. 7).

The antagonism of the adenosine-elicited contractile response by CSC required \(\approx 60\) min of continuous CSC treatment to completely develop. However, the antagonism of the adenosine-elicited contractile response was observed within 5 min after the administration of ZM-241385. The reason for this difference in the time required to achieve A_{2a}-receptor blockade is not known.
Adenosine Contractile Effects With Constant-Pressure and Constant-Flow Perfusion

The adenosine-elicited increase in contractile performance was presumably not caused by increased coronary flow elicited by adenosine-induced vasodilation, because it was also observed under constant-flow perfusion conditions (Fig. 8). An adenosine-elicited contractile response was observed after pretreatment of hearts with the coronary vasodilator hydralazine (Fig. 10). Moreover, hydralazine dilated the vascular bed, thereby reducing the vasodilatory effect of 10^{-4} M adenosine (Fig. 8). This prevented any reduction in perfusion pressure caused by adenosine. The increase in ventricular contractility elicited by adenosine under constant-flow perfusion conditions was antagonized by CSC and ZM-241385, further indicating that this increase in contractility is mediated by activation of the A_{2a} receptor.

The adenosine-elicited contractile responses were somewhat greater with constant-pressure than with constant-flow perfusion. This small difference in the contractile responses could be a flow-dependent component related to the Gregg phenomenon (7, 16) in constant-pressure-perfused hearts. The flow-dependent component is assumed to be small, because only the largest concentration of adenosine administered caused a significant increase in coronary flow. Thus the positive inotropic responses elicited by adenosine are independent of coronary flow changes.

Potentiation of Adenosine by Pertussis Toxin and A_{1}-Receptor Antagonism

Pertussis toxin, which is known to prevent A_{1}-receptor-mediated events in the heart (18, 20) by ADP-ribosylation of G_{i} protein, potentiated the adenosine-elicited increase in contractile performance. This

![Graphs showing effects of adenosine on LVP, dP/dt_{max}, coronary perfusion pressure](http://ajpheart.physiology.org/)
suggests that, in the absence of pertussis toxin, adenosine is also activating myocardial A1 receptors, which appear to oppose the A2a-receptor-elicited contractile effects of adenosine. Moreover, in the presence of the A1-receptor antagonist DPCPX (17), the contractile response to adenosine was greater than in the absence of the antagonist. This also indicates that with blockade of A1 receptors the A2a-receptor-mediated action is enhanced. The interaction of A1 receptors and A2a receptors has been observed previously where the use of A2a-receptor antagonists was reported to enhance the antiadrenergic action of A1-receptor agonists in perfused hearts (28).

**Myocardial Actions of Adenosine and Adenosine Analogs**

The results from this study suggest that A2a-receptor activation increases contractility in the intact heart, as previously reported for ventricular myocytes isolated from rats (11, 40), human myocardium (34), and chick embryos (25, 26, 39). In addition, these results confirm previous studies that indicate that stimulation of adenosine receptors increases contractility in isolated papillary muscles from rats (23), guinea pigs (4), and dogs (6). However, other investigators have shown that adenosine or A2-receptor agonists do not increase contractility in isolated ventricular myocytes from rats (32), rabbits (32), or guinea pigs (2, 32, 35). Utilizing papillary muscle isolated from guinea pigs, Stein et al. (35) reported that the A2a-receptor agonist CGS-21680 does not increase myocardial contractility. In addition, Shryock et al. (32) and Felsch et al. (12) reported that the A2a-receptor agonists CGS-21680, methylphenylethoxy-adenosine (WRC-0090), and NECA do not increase the contractile performance of hearts isolated from guinea pigs. It has also been shown that adenosine does not increase contractility of rat ventricular strips (5). The reason for these differences is not known, but several suggestions can be offered. First, the effect of A2a-receptor stimulation on myocardial contractile performance may be lost in studies where the animals were stressed before experimentation. During the course of this study, it was found that adenosine-induced contractile responses were reduced or absent in hearts isolated from hyperexcited rats compared with hearts from tranquil rats. Second, the effect of A2a-receptor stimulation on myocardial contractility might also vary depending on the underlying level of arrhythmia. In our study a contractile response to adenosine was not detectable in hearts that displayed a high level of ventricular arrhythmias during experimentation. In these hearts the contractile response to adenosine was comparable to, or smaller than, the oscillations in baseline contractility elicited by these arrhythmias. Third, the variable effects of A2a-receptor stimulation on myocardial contractility observed in previous studies might be related to the experimental system utilized for measuring contractility. It is possible that our experimental system, which utilized an analog-to-digital converter and computer software for assessing left ventricular contractility, facilitated the resolution of the small increases in contractility elicited by adenosine. Other studies that relied solely on polygraphic recordings of myocardial contractility might have missed this response. Fourth, the lack of a contractile response to A2a-receptor stimulation in some reports could also be due to differences in preparations such as those that are inherent in ventricular myocyte isolation. Finally, the effect of A2a-receptor activation on myocardial contractility might vary depending on the specific A2a-receptor agonist utilized. It was found here that the A2a-receptor agonists NECA and DPMA produced smaller increases in contractility than adenosine (Fig. 5). However, the A2a-receptor agonist CGS-21680 (11, 21) did not cause a significant increase in contractility or coronary flow. It is likely that different A2a-receptor agonists may have varying degrees of A2a-receptor availability or activation/transduction efficiencies in the intact heart.

**Mechanism of Adenosine Action**

The positive inotropic action of adenosine reported here appears to occur via A2a receptors. Agonists for this receptor have been reported in cardiac prepara-
tions to mediate responses via cAMP-dependent and -independent mechanisms (11, 26), possibly involving Ca\(^{2+}\) (26) but having only a minimal effect on intracellular Ca\(^{2+}\) transients (38). The mechanism(s) involved requires further study.

In conclusion, the present study shows that A\(_{2a}\) receptor activation increases contractility of the intact heart isolated from rats. This increase in contractility is independent of activation of \(\beta\)-adrenergic receptors or the increase in coronary flow mediated by adenosine-induced vasodilation. Although the adenosine A\(_{2a}\)-receptor-elicited positive inotropic response is rather small in magnitude, it may be important in providing contractile support of the mechanically depressed hypoperfused heart in which interstitial adenosine levels are increased (10). Furthermore, A\(_{2a}\)-receptor activation has been demonstrated to attenuate the antiadrenergic action of adenosine mediated by A\(_1\) receptors (28). Thus inhibition of the antiadrenergic effect of adenosine coupled with its intrinsic direct positive inotropic effect suggests that stimulation of A\(_{2a}\) receptors is likely to be capable of enhancing and maintaining myocardial contractility. The role of A\(_{2a}\) receptors in modulation of heart contractile function should be addressed further.

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REFERENCES

30. Romano FD, Macdonald SG, and Dobson JG Jr. Adenosine receptor coupling to adenylate cyclase of rat ventricular myocyte


