Antiadrenergic Effects of Adenosine in Pressure Overload Hypertrophy


Abstract—In the present study, we sought to evaluate whether the antiadrenergic action of adenosine in the heart is altered in pressure overload hypertrophy produced in rats by suprarenal aortic banding. Epicardial and coronary effluent adenosine and inosine concentrations and release were significantly elevated in compensated pressure overload hypertrophy but not in hearts with left ventricular failure. In pressure overload hearts, the contractile response to $\beta$-adrenergic stimulation was less inhibited by incremental concentrations of either adenosine or the selective A$_1$ receptor agonist chloro-N$^6$-cyclopentyl adenosine than in controls. Furthermore, the extent of desensitization to the antiadrenergic actions of adenosine in pressure overload hypertrophy appeared to be proportional to the extent of chamber dilation and dysfunction. A 60-minute infusion of adenosine produced a sustained antiadrenergic effect that lasted up to 45 minutes after the infusion was terminated in both controls and hearts with compensated hypertrophy. This effect was not observed in the decompensated left ventricular failure group. Subsequent infusion with adenosine of the A$_{2A}$ receptor antagonist 8-(3-chlorostyryl)-caffeine to counteract the proadrenergic effect of A$_{2A}$ receptor stimulation did not alter the decreased sensitivity to the antiadrenergic actions of adenosine in hypertrophied hearts. Finally, isolated myocytes from hypertrophied hearts demonstrated a decreased ability to suppress isoproterenol-elicited increases in $[\text{Ca}^{2+}]$, transients in the presence of adenosine and the A$_{2A}$ receptor antagonist compared with myocytes from control hearts. Myocardial adenosine concentrations increase during the compensated phase of pressure overload hypertrophy but then decrease when there is evidence of decompensation. The antiadrenergic actions of adenosine transduced via the myocardial A$_1$ receptor are diminished in pressure overload hypertrophied hearts. These factors may render these hearts more vulnerable to the detrimental effects of chronically increased sympathetic activity. (Hypertension. 2001;37:862-868.)

Key Words: receptors, adenosine \(\blacktriangleright\) hypertrophy \(\blacktriangleright\) heart failure

Increased sympathetic tone appears to play a pivotal role in the natural history of heart failure by contributing to the progression of myocardial dysfunction.\(^1\) Adenosine protects the heart from excessive catecholamine exposure by inhibiting presynaptic norepinephrine release\(^2\) and attenuating metabolic and contractile responses to $\beta$-adrenergic stimulation.\(^3,4\) Because pressure overload hypertrophy (POH) is characterized by the gradual transition to heart failure,\(^5\) we hypothesized that if the negative feedback modulation of $\beta$-adrenergic responses by adenosine was diminished, POH hearts would be vulnerable to the long-term sequelae of increased sympathetic activity. In the present study, we assessed whether in POH (1) myocardial adenosine concentrations are altered, (2) adenosine $A_1$ receptor signaling is attenuated, (3) adenosine receptor subtypes other than the $A_1$ receptor contribute to the antiadrenergic actions of adenosine, and (4) the antiadrenergic actions of adenosine are altered with respect to $\text{Ca}^{2+}$ transients $[\text{Ca}^{2+}]$, in isolated myocytes.

Methods

Experimental Animals

Animals were maintained and used in accordance with recommendations in the Guide for the Care and Use of Laboratory Animal (Institute of Laboratory Animal Resources, National Research Council, US Department of Health, Education, and Welfare, NIH publication No. 85-23, 1996) and the guidelines of the Institutional Animal Care and Use Committee of the University of Massachusetts Medical School (Worcester). Male Sprague-Dawley rats (200 g) were anesthetized with an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (15 mg/kg) and underwent abdominal aortic banding as previously described.\(^6\)

Echocardiographic Studies

Ten weeks after surgery, animals were anesthetized as described, and 2-dimensional Doppler echocardiographic studies were performed and analyzed as previously described.\(^6\) Briefly, the animal was placed in the prone position, and a 7.5-MHz transducer and Hewlett-Packard Sonos 1500 sector scanner (Hewlett-Packard) were used.

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Then 2-dimensionally directed M-mode echocardiographic dimensions of the left ventricle (LV) were obtained in the parasternal short-axis view at the level of the papillary muscles and recorded on strip-chart paper. Doppler-derived mitral inflow velocities were obtained in the apical 4-chamber view. Fractional shortening at the endocardium and LV mass were calculated as described previously.5–7

Isolated Perfused Heart Preparation

The isolated perfused heart preparation used in this study was previously described by Perlini et al.8 Briefly, rats were anesthetized, and blood pressure was measured via a 24-gauge angiocath inserted into the carotid artery. The heart was then excised, weighed, and perfused with physiological saline (PSS). The flow rates of the PSS were adjusted to achieve perfusion pressures of ~100 and 70 mm Hg for hearts from banded and control rats, respectively. Flow rates were held constant throughout the experiment. These differing flow rates and perfusion pressures were chosen in recognition of the difference between the in vivo coronary perfusion pressure to which the control and banded groups were chronically exposed.9 Hearts were perfused either in the standard noninverted position or in the inverted position as previously described.10 The latter technique was used so that epicardial transudates uncontaminated by coronary effluent could be sampled.

Hearts were paced at 300 bpm. An appropriately sized balloon (Hugo Sachs) was placed in the LV cavity. The balloon volume was kept constant at a diastolic pressure of 10 to 15 mm Hg. The maximum rate of LV pressure development (±dP/dt max) was obtained by differentiating the pressure signal (model 13-4615-71; Gould Instrument Systems Inc). Epicardial and coronary effluent concentrations of adenosine were sampled and processed as previously described.8

Measurement of Lactate, Adenosine, and Inosine Concentrations

The lactate content of coronary effluents was analyzed enzymatically with lactate oxidase to catalyze the oxidation of lactate (Lactate Analyzer; YSI). Coronary effluent and epicardial transudate adenosine concentrations were determined according to previously described methods.8,10 Briefly, coronary effluent samples were analyzed isocratically with HPLC (Waters Chromatography Division). Epicardial fluid samples (5 μL) were derivatized with chloroacetaldehyde. The fluorescent adenosine derivative ethenoadenosine was obtained in the apical 4-chamber view. Fractional shortening at the endocardium and LV mass were calculated as described previously.5–7

In protocol 1, isoproterenol challenges were obtained at the end of each 5-minute infusion of increasing concentrations of adenosine ranging from 10 nmol/L to 100 μmol/L in the presence (1 set of rats) and absence (1 set of rats) of a continuous infusion of 1 μmol/L 8-(3-chlorostyril)-caffeine (CSC), a selective A2A receptor antagonist.13 The antagonist was used to eliminate the counteracting effects of adenosine-induced A1 receptor activation to ensure predominant A1 receptor–mediated effects. The effect of DMSO, the vehicle of CSC, on isoproterenol-mediated responses in the absence or presence of 1 μmol/L adenosine was also assessed. In protocol 2, in a different subset of rats, hearts were perfused with increasing concentrations of 2-chloro-N'-(cyclopentyl) adenosine (CCPA), a selective A1 receptor agonist,16 ranging from 1 nmol/L to 10 μmol/L. Each concentration was infused for 5 minutes, and the contractile response to isoproterenol was recorded at the end of each infusion.

Sustained Antiadrenergic Actions of Adenosine

Contractile responses to isoproterenol were determined for 3 successive challenges (control) separated by 10-minute recovery periods. Then, adenosine (33 μmol/L) or vehicle was infused for 60 minutes, and isoproterenol challenges were repeated at the end of the adenosine or vehicle infusion and at 15, 30, and 45 minutes of washout.

A1 Receptor Activation

We assessed the effect of increasing concentrations (0.1 nmol/L to 1 μmol/L at 5-minute intervals) of 4-aminobenzyl-5-N-methylcarboxyamido-adenosine (AB-MECA), a selective A1 receptor agonist,17 on contractile response to isoproterenol. We further assessed the effect of a 45-minute infusion of 1 μmol/L AB-MECA on the contractile response to isoproterenol in both control and POH groups.

Lactate Release

Coronary effluent was collected anaerobically for O2 tension and lactate determinations in 4 control hearts and 5 hearts with POH perfused at coronary flow rates as defined earlier.

[Ca2+]i in Isolated Ventricular Myocytes

After a period of stabilization, values for myocyte [Ca2+]i were obtained with exposure to a continuous infusion of 0.2 μmol/L isoproterenol. During this infusion, adenosine was administered at 1, 10, and 100 μmol/L in the presence of 1 μmol/L CSC or the DMSO vehicle of CSC. For each adenosine concentration, a separate myocyte was used. To determine whether CSC altered adrenergic responsiveness independent of adenosine, isoproterenol responses were obtained in a separate group of cells in the presence or absence of 1 μmol/L CSC.

Experimental Design

Prior observations regarding the actions of adenosine were taken into account with the design of the present study. The antiadrenergic action of adenosine mediated via the A1 receptor involves not only short-term effects3,4 but also sustained cellular responses.3 The short-term effect of adenosine is counteracted by the simultaneous activation of the stimulatory A2A receptor.7,14 The sustained antiadrenergic effect is observed after a >5-minute exposure to adenosine and is evident even after the adenosine concentration has returned to baseline levels.1 It is not known whether activation of the inhibitory adenosine receptor, the A1 receptor, inhibits the effects of β-adrenergic stimulation.

β-Adrenergic responses in the isolated perfused heart preparation were obtained after 20-second infusions of 10 nmol/L (final PSS concentration) isoproterenol. The same dose was used for all subsequent isoproterenol challenges.

Isolated Perfused Heart Studies

Animals from both POH and control groups were used for these experiments.

A1 Receptor–Mediated Effects

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Chemicals

Isoproterenol and DMSO were purchased from Sigma Chemical Co. Boehringer Mannheim supplied adenosine, and dispase was obtained from GBBCO. Fura-2 acetomethyl ester was purchased from Calbiochem. CSC, AB-MECA, and CCPA were supplied by Research Biochemicals. Isoproterenol and adenosine were dissolved in 0.1% sodium metabisulfite or H2O, respectively. CSC, AB-MECA, and CCPA stock solutions were all dissolved in DMSO.

Data and Statistical Analyses

POH animals with pulmonary congestion (lung weights >2 SD of controls) were deemed decompensated (D-POH), and those without...
congestion (lung weights ≤2 SD of controls) were regarded as compensated (C-POH). For the construction of the concentration-inhibition dose-response curves, the effect of each concentration of adenosine or CCPA was expressed as the percent change in +dP/dt relative to preinfusion control. The concentration of adenosine or CCPA that produced 50% of the maximal inhibitory response (IC50) was determined from nonlinear regression analysis using sigmoid curve fitting. The concentration of a ligand required to produce a maximal [Ca2+]i transient was calculated as the difference between maximum [Ca2+]i transient, during systole and minimum [Ca2+]i recorded during diastole. Myocardial oxygen consumption and the O2 supply/demand ratio were calculated as previously described.18 ANOVA for repeated measurements followed by either the Dunnett or Student-Newman-Keuls test was used to determine statistical significance (P<0.05) within each group, and factorial ANOVA was used for comparisons between the different interventions. Student’s t test was applied to paired comparisons. Comparisons between Ca2+ transient responses in control myocytes and POH myocytes were made using the Mann-Whitney nonparametric test. Values are expressed as mean±SEM.

### Results

#### General Characteristics

The control, C-POH, and D-POH groups had similar body weights, but mean blood pressure was increased in both POH groups (Table). The heart weights were significantly increased in both banded groups, but the hearts in the D-POH group weighed 25% more than those in the C-POH group. The lungs weighed ~200% more in the D-POH group than in the C-POH and control groups. Coronary flow was ~19% and ~54% higher in the C-POH and D-POH groups, respectively, compared with controls. However, coronary flow normalized to heart weight was ~16% lower in both banded groups (data not shown). The myocardial O2 consumption was 28% higher in the POH group, but coronary effluent PO2, O2 supply/demand ratio, and lactate release were similar in both groups (data not shown). The baseline contractile state, as measured by +dP/dtmax, was similar in all 3 groups. However, isoproterenol-elicited contractile responses were attenuated in the D-POH group compared with the control group. The baseline and isoproterenol-elicited contractile data for experimental groups not presented in the Table were similar in magnitude to the data shown.

The LV end-diastolic dimension was increased and endocardial fractional shortening was decreased in the D-POH group compared with the C-POH group and controls (Table). LV mass was significantly increased in both POH groups compared with the control group. In the D-POH group, LV mass increased by ~40% compared with the C-POH group. The peak early mitral inflow velocity, early-to-atrial velocity ratio, and deceleration velocity of the peak early velocity were significantly higher in the D-POH group compared with the control and C-POH groups, which is consistent with elevated left atrial pressures. The LV mass and Doppler-derived filling parameters in POH were of similar magnitude in all of the other experimental groups (data not shown).
Nucleoside Concentrations
Coronary effluent adenosine and inosine concentrations and release were elevated in the C-POH group compared with controls (Figure 1). However, in the D-POH group, these increases were not apparent; concentration and release values were significantly lower than those in the C-POH group and not different from those in the control group. Epicardial adenosine concentrations increased from 191 \( \pm 26 \) pmol/mL in the control group to 452 \( \pm 46 \) pmol/mL in the C-POH group \( (P, 0.001) \), but in the D-POH group, the levels were similar (267 \( \pm 43 \) pmol/mL, \( P, 0.37 \)) to those of the control group.

Adenosine A\(_1\) Receptor Signaling
In contrast to the controls, increasing concentrations of adenosine resulted in a relatively flattened concentration-inhibition curve in the POH group (Figure 2A). Infusion of the A\(_3\) receptor antagonist CSC produced a leftward shift in the concentration-inhibition curve of adenosine in both control and POH groups (Figure 2B). CSC unmasked the antiadrenergic effect of adenosine at a concentration (0.1 \( \mu \text{mol/L} \)) that under control conditions did not result in significant attenuation of isoproterenol-elicited responses in both the control and POH groups. Thus, A\(_3\) receptor antagonism in the presence of adenosine resulted in unopposed A\(_1\) receptor-mediated antiadrenergic effects. The IC\(_{50}\) value \( (\sim \log M) \) for adenosine-induced antiadrenergic effects in the presence of CSC was \( \sim 4\)-fold higher in the POH group than in the control group (5.83 \( \pm 0.16 \) versus 6.39 \( \pm 0.10 \), \( P, 0.04 \); Figure 2B, inset).

Increasing concentrations of CCPA were accompanied by a decreasing contractile responsiveness to isoproterenol in both the control and POH groups (Figure 2C). Adenosine A\(_1\) receptor sensitivity, as characterized by the IC\(_{50}\) value of CCPA, was \( \sim 3\)-fold higher in the POH group than in the control group (Figure 2C, inset). Several Doppler echocardiographic variables were correlated to the IC\(_{50}\) value of CCPA, with fractional shortening exhibiting the best correlation. Fractional shortening was inversely correlated to the IC\(_{50}\) value for CCPA \( (r = -0.67, P, 0.001) \); suggesting that hearts with marked LV dysfunction were the least sensitive to the A\(_1\) receptor agonist.

Sustained Antiadrenergic Actions of Adenosine
A 60-minute infusion of 33 \( \mu \text{mol/L} \) adenosine significantly suppressed the contractile response to isoproterenol by 48\%, followed by a slow and incomplete recovery of the contractile response to isoproterenol in control hearts (Figure 3). At 45 minutes of washout, there still was a significant depression (24\%) of adrenergic responsiveness (Figure 3A). The C-POH group demonstrated a similar response to prolonged infusion of adenosine as the control group (Figure 3B). However, in the D-POH group, prolonged adenosine exposure produced only a 29\% reduction in the contractile response to isoproterenol, followed by a complete recovery within 15 minutes of washout (Figure 3B).

A\(_3\) Receptor–Mediated Actions
Neither the infusion of progressively increasing concentrations of the selective A\(_3\) receptor agonist AB-MECA nor the
prolonged infusion (45 minutes) of this agonist inhibited the isoproterenol-elicited contractile responses in the control group (n = 6) and the POH group (n = 6) (data not shown).

Figure 2. Concentration-inhibition curves showing the effects of increasing concentrations of adenosine (A and B) and CCPA (C) on the contractile responses to isoproterenol (10 nmol/L) expressed as the percent change from baseline response. For adenosine (control ○, n = 12; POH ●, n = 9), the dose-response relationships were obtained in the absence (A) and presence (B) of 1 μmol/L CSC. C. Control group (○, n = 21) and POH group (●, n = 21). Inset, IC50 values (filled column, POH; open column, control). +dP/dt indicates maximal rate of pressure development. *P<0.05 vs corresponding control. †P<0.05 vs baseline value.

Figure 3. The effect of a 60-minute infusion of 33 μmol/L adenosine or vehicle (A v) on the contractile responses to isoproterenol (10 nmol/L) are expressed as in the legend to Figure 2. *P<0.01 vs respective vehicle. †P<0.01 vs end of infusion. #P<0.05 vs C-POH and control.

Myocardial Adenosine Concentrations
The concentration of adenosine in the myocardial interstitium determines in part the magnitude of negative-feedback modulation of β-adrenergic responses. The C-POH group had higher concentrations of adenosine in the epicardial transudates, increased adenosine and inosine levels in the coronary effluents, and a greater release of nucleosides (adenosine plus inosine) into their effluents than control hearts. However, these increases were not evident in the D-POH group. The
increased oxygen supply/demand ratio. This ratio was such but rather appear to be critically dependent on the trigger of myocardial oxygen consumption as previously that the formation and release of adenosine are not increased myocardial adenosine release. It has been shown that the differences in coronary flow between control and POH groups. Second, it is further doubtful that the differences in nucleoside transport vary among different animals and that the findings in this study of POH may be unique to the rodent species.

**Antiadrenergic Signaling**

The antiadrenergic actions of adenosine are primarily transduced via the myocardial A<sub>1</sub> receptor and involve both short-term and sustained effects. Data from intact heart and isolated myocyte experiments suggest that both short-term and sustained A<sub>1</sub> receptor–mediated antiadrenergic signaling are diminished in hearts with POH. In contrast to normal hearts, increasing concentrations of adenosine resulted in a relatively flat concentration-inhibition curve in the POH group. Furthermore, when the proadrenergic influences of A<sub>3</sub> receptor activation were blocked by CSC, allowing unopposed A<sub>1</sub> receptor–mediated effects, the IC<sub>50</sub> value for adenosine was ≈4 times higher in the POH group than in the control group. Similarly, increasing concentrations CCPA resulted in a flatter concentration-inhibition curve and increased IC<sub>50</sub> values in the POH group compared with controls. Finally, isolated myocytes from hearts with POH demonstrated a decreased ability to suppress isoproterenol-elicited increases in [Ca<sup>2+</sup>], transients in the presence of adenosine and the A<sub>3</sub> receptor antagonist CSC compared with myocytes from control hearts.

The extent of desensitization of the A<sub>1</sub> receptor may at first glance be modest. However, it should be recognized that the POH group consisted of a spectrum of hearts with varying degrees of compensation. The highest IC<sub>50</sub> values were obtained in the most compromised hearts. Also, at the end of a 60-minute infusion of 33 μmol/L adenosine, the contractile response to isoproterenol was similarly depressed in the control and C-POH groups (Figure 3). On the other hand, the isoproterenol-elicited responses were significantly less depressed after the infusion of adenosine in the D-POH group compared with the control and C-POH groups (Figure 3). The sustained or persistent antiadrenergic effect of adenosine also appeared to be altered in POH, especially when there was evidence of decompensation. This effect seems to be preserved in the C-POH group but not in the D-POH group.

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**Figure 4.** Effect of CSC on the antiadrenergic action of adenosine in ventricular myocytes from control (A: 1 μmol/L, n=4; 10 μmol/L, n=7; and 100 μmol/L, n=4) and POH (B: 1 μmol/L, n=4; 10 μmol/L, n=9; and 100 μmol/L, n=12) groups as determined from [Ca<sup>2+</sup>]<sup>i</sup> measurements. Data are shown as the percentage change in [Ca<sup>2+</sup>]<sup>i</sup> amplitude in response to 0.2 μmol/L isoproterenol. *P<0.01 vs vehicle.

findings in C-POH are in accord with earlier studies that showed increased myocardial adenosine release in aged rat and guinea pig hearts and in dogs with volume-overload heart failure. However, the reason for the enhanced myocardial adenosine release in C-POH and not in D-POH is not readily apparent. Several factors can be eliminated. First, it is unlikely that the expected increase in myocardial oxygen consumption associated with pressure overload contributed to increased myocardial adenosine release. It has been shown previously that the formation and release of adenosine are not triggered by changes in myocardial oxygen consumption as such but rather appear to be critically dependent on the increased oxygen supply/demand ratio. This ratio was unchanged in the POH groups. Second, it is further doubtful that the differences in coronary flow between control and POH hearts contributed to the differences in nucleoside release. The flow rate of the perfusate was adjusted to achieve a perfusion pressure of ≈100 mm Hg in hearts with POH and ≈70 mm Hg in control hearts. This approach has been shown previously to achieve adequate myocardial perfusion in hypertrophied hearts. In this study, the coronary flow rate per gram LV weight was slightly lower in the POH group compared with the control group. This difference did not translate into differences in the coronary venous Po<sub>2</sub>, O<sub>2</sub> supply/demand ratio, and lactate release. Moreover, the D-POH group with the larger hearts, where a decreased O<sub>2</sub> supply/demand ratio would be expected, had lower myocardial adenosine concentrations than the C-POH group.

Other possibilities were considered to explain the difference in adenosine release between hearts with POH and controls. Progressively increasing adrenergic stimulation may be involved, because myocardial norepinephrine levels increase with the progression to heart failure and endogenous norepinephrine increases the activity of ecto-5'-nucleotidase, which hydrolyzes AMP to adenosine. However, this possibility seems unlikely, because the D-POH group is more likely to have marked increases in myocardial norepinephrine concentrations, but this group had much lower myocardial adenosine concentrations than the C-POH group. There may be as yet undefined metabolic alterations or changes in membrane transport in the POH hearts that change over time and affect myocardial adenosine release or production. It should be further recognized that adenosine concentrations and transport vary among different animals and that the findings in this study of POH may be unique to the rodent species.
This study also assessed whether the inhibitory A3 receptor is upregulated in POH. Neither increasing concentrations of the selective A3 agonist AB-MECA nor the prolonged infusion of this agonist suppressed isoproterenol-elicited responses in normal hearts and in hearts with POH. Therefore, it appears unlikely that POH is associated with enhanced A3 receptor sensitivity.

Conclusions
Myocardial adenosine concentrations increase during the compensated phase of POH but then decrease with progression to the decompensated state. Short-term and sustained adenosine-mediated antiadrenergic signaling in the intact heart and in isolated myocytes is diminished in POH. These factors may render POH more vulnerable to the detrimental effects of chronically increased sympathetic activity.

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