Phosphatase inhibitor cantharidin blocks adenosine A₁ receptor anti-adrenergic effect in rat cardiac myocytes

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Narayan, PRAKASH, Robert M. Mentzer, Jr., and Robert D. Lasley. Phosphatase inhibitor cantharidin blocks adenosine A₁ receptor anti-adrenergic effect in rat cardiac myocytes. Am. J. Physiol. Heart Circ. Physiol. 278: H1–H7, 2000.—Experiments were performed to examine whether the protein phosphatase inhibitor cantharidin blocks the anti-adrenergic effect of adenosine A₁ receptor stimulation. In electrically stimulated adult rat ventricular myocytes loaded with the intracellular calcium concentration ([Ca^2+]i) indicator fluo-3, isoproterenol (10 nM) increased systolic [Ca^2+]i, by 46%, increased twitch amplitude by 56%, and increased total cellular cAMP content by 140%. The adenosine A₁ receptor agonist 2-chloro-N⁶-cyclopentyladenosine (CCPA) reduced isoproterenol-stimulated [Ca^2+]i and contractility by 87 and 80%, respectively, but reduced cAMP content by only 18%. Cantharidin had no effects on myocyte [Ca^2+]i, contractility, or cAMP in the absence or presence of isoproterenol but blocked the effects of CCPA on [Ca^2+]i and contractility by ~44%. Cantharidin had no effect on CCPA attenuation of isoproterenol-induced increases in cAMP. Pretreatment with CCPA also reduced the increase in contractile parameters produced by the direct cAMP-dependent protein kinase A (PKA) activator 8-bromo-cAMP. These results suggest that activation of protein phosphatases mediate, in part, the anti-adrenergic effect of adenosine A₁ receptor activation in ventricular myocardium.

ADENOSINE has no direct inotropic effects in mammalian ventricular myocardium but reduces β-adrenergic receptor-stimulated increases in L-type calcium channel activity, action potential duration, intracellular calcium concentration ([Ca^2+]i) transient, and twitch amplitude (3, 4, 8). It is well recognized that this anti-adrenergic effect of adenosine is mediated via adenosine A₁ receptor coupling to a pertussis toxin-sensitive guanine nucleotide binding (G) protein, presumably G₁ or G₃ (4, 22), but there are conflicting reports of the mechanism(s) downstream to the activation of this inhibitory G protein. From the observations that adenosine A₁ receptor activation reduces catecholamine-stimulated adenylyl cyclase activity and cellular cAMP levels, it was hypothesized that decreased cAMP was the primary mediator of the anti-adrenergic effect of adenosine A₁ receptor activation (4, 5, 12).

There are reports however indicating that the anti-adrenergic effect of adenosine A₁ receptor activation cannot entirely be accounted for by this pathway (9, 10, 22, 24). Using several adenosine A₁ receptor analogs, Gupta et al. (9) and Neumann and colleagues (24) reported a dissociation between the reductions in isoproterenol-stimulated phosphorylation of phospholamban and troponin I and contractility versus the reduction in cAMP content in isolated guinea pig ventricular myocytes. These authors hypothesized that adenosine A₁ receptor activation exerted its anti-adrenergic effect via the stimulation of protein phosphatases. Additional support for this hypothesis is provided by studies with the muscarinic-cholinergic agonist acetylcholine, which exerts an anti-adrenergic effect similar to adenosine A₁ receptor (18). It has been reported that acetylcholine reduces the phosphorylation of phospholamban in isolated guinea pig ventricular myocytes stimulated with either the phosphodiesterase inhibitor ibutylmethylxanthine or the cAMP analog 8-bromo-cAMP (8-BrcAMP) (11) and increases protein phosphatase activity by decreasing protein phosphatase inhibitor-1 activity in isoproterenol-stimulated, isolated perfused guinea pig hearts (10). The selective phosphatase inhibitors okadaic acid and cantharidin block the acetycholine attenuation of isoproterenol-stimulated sarcotcemal calcium channel activity in guinea pig ventricular myocytes (13). Although there are no reports of protein phosphatase inhibitor effects on adenosine A₁ receptor signal transduction in cardiac myocytes, it has been reported that protein phosphatase inhibitors block adenosine receptor-mediated effects in polymorphonuclear leukocytes and bovine adrenal chromaffin cells (19, 26).

The purpose of the present study was to determine whether the protein phosphatase inhibitor cantharidin blocks the anti-adrenergic effects of adenosine A₁ receptor activation on [Ca^2+]i, contractility, and cAMP content in isoproterenol-stimulated rat ventricular myocytes.

METHODS

All animals in this study received humane care according to the guidelines set forth in the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National Research Council, National Institutes of Health Publication (No. 85-23, 1996) and according to the guidelines of the Department of Laboratory Animal Resources, University of Kentucky.

Isolation of myocytes. Ventricular myocytes were enzymatically dissociated from male Sprague-Dawley rats (350–400 g) by a previously described method (21) with minor modific-
tions. Rats were heparinized (500 U ip) and anesthetized with pentobarbital sodium (65 mg/kg ip). The heart was rapidly excised and retrogradely perfused with an HEPES buffer containing (in mM) 118 NaCl, 4.7 KCl, 1.2 MgSO4, 1.25 KH2PO4, 1.0 CaCl2, 25 HEPES, 11 glucose, and 0.01 EDTA. The buffer was gassed with 100% O2, pH was adjusted to 7.2 using NaOH, and temperature was maintained at 37°C. Perfusion was at a constant pressure of 70 mmHg, and after 5 min, perfusate was replaced with a nominally calcium-free buffer. After an additional 5 min, the buffer was supplemented with type IV collagenase (1 mg/ml, Worthington Biochemical, Freehold, NJ) and hyaluronidase (0.5 mg/ml, Sigma Chemical, St. Louis, MO), and the hearts were perfused in a recirculating mode for 15–20 min. During the final 5 min of collagenase infusion, calcium was gradually increased to 0.50 mM. The partially digested heart was then removed from the cannula, minced, and placed in a shaking water bath (37°C) with 15 ml of HEPES/collagenase buffer for ~30 min. Undigested tissue was filtered through a 250-µm wire mesh. The cell suspension was washed twice with enzyme-free buffer and resuspended in normal HEPES buffer (1.0 mM CaCl2, pH 7.4) at a final concentration of ~1 mg/ml. This protocol typically yielded over 70% rod-shaped myocytes. All cells were used within 6 h of isolation.

\[ [Ca^{2+}]_i \] and contractility measurements. Myocytes were loaded at room temperature with the acetoxy-methyl ester derivative of the Ca2+ probe fluo-3 (6 µM, Molecular Probes, Eugene, OR) for 15 min. After washout of the dye and a 30-min postincubation period to allow for destereification of the dye, aliquots of the fluo-3-labeled cell suspension were placed in a 300-µl temperature-controlled recording chamber (RC-24 chamber, TC-324B temperature controller, Warner Instrument, Hamden, CT) on the stage of an IX-70 Olympus inverted microscope (Olympus America, Melville, NY). The floor of the recording chamber was a 22 × 40 mm glass coverslip coated with laminin to enhance cell adherence. Cells were suffused with normal HEPES buffer (gassed with 100% O2, pH = 7.4 at 37°C) at a flow rate of 1 ml/min.

Intracellular fluo-3 was excited at 490 ± 10 nm using a 75-W xenon arc lamp through an epifluorescence attachment (505-nm dichroic mirror) and a ×20 UPlanFl objective lens (Olympus America). The emitted fluorescence, collected at 512 ± 10 nm by a charge-coupled device (CCD) camera (Coyote Bay, Manchester, New Hampshire), is proportional to \[ [Ca^{2+}]_i \] and contractility measurements. Myocytes were treated with the adenosine A1 receptor agonist 2-chloro-adenosine (CCPA, 200 nm, 5 min). Myocytes were reexposed to isoproterenol in the presence of CCPA. Additional cells (n = 4) in this group were treated with the combination of CCPA and the selective adenosine A1 receptor antagonist 8-cyclopyrrol-1,3-dipropylxanthine (DPCPX, 200 nM, 5 min). They were then exposed to 8-BrCAMP in the presence of CCPA + DPCPX.

In group 3, the effects of the serine-threonine phosphatase inhibitor cantharidin (15) were examined on isoproterenol-stimulated \([Ca^{2+}]_i\) and contractility. After the first exposure to isoproterenol, myocytes were treated for 5 min with cantharidin (500 nM) before the second isoproterenol treatment. In group 4, after recovery from the first exposure to isoproterenol, myocytes were treated with CCPA + cantharidin before the second isoproterenol exposure. Groups 5 and 6 utilized similar protocols to test whether cantharidin altered the effects of the β-blocker propranolol (300 nM, 5 min).

Groups 7 and 8 tested whether CCPA altered the effects of the cAMP analog 8-BrCAMP. In group 7, myocytes were exposed to two treatments with 8-BrCAMP (1 nM, 5 min) separated by a 20-min washout period. In group 8, recovery from the first exposure to 8-BrCAMP was followed by treatment with CCPA. Myocytes were then reexposed to 8-BrCAMP in the presence of CCPA.

For simultaneous measurement of contractility, the myocyte was continuously illuminated with low intensity light from the illumination optics of the microscope and its image collected by the CCD camera. Changes in cell length were quantified via edge-motion detection with a video dimension analyzer (Coyote Bay). Contractile amplitude, expressed as a percentage of baseline twitch amplitude, velocity of shortening (±vL/t), and velocity of relaxation (−vL/t) were computed as described by Spinale et al. (30).

Protocols. Myocytes were field stimulated throughout the experimental protocols at 0.5 Hz via platinum electrodes using a Grass SD9 stimulator (Grass Instruments, Quincy, MA). Each myocyte served as its own control. An equilibration period of 10 min was allowed for each myocyte following which baseline measurements were obtained. This ensured the presence of steady-state \([Ca^{2+}]_i\), and contractions. The following groups were studied (n = no. of myocytes): group 1: isoproterenol (n = 6); group 2: CCPA + isoproterenol (n = 6); group 3: cantharidin + isoproterenol (n = 4); group 4: cantharidin + CCPA + isoproterenol (n = 6); group 5: propranolol + isoproterenol (n = 4); group 6: cantharidin + propranolol + isoproterenol (n = 4); group 7: 8-BrCAMP (n = 4); and group 8: CCPA + 8-BrCAMP (n = 4).
ADENOSINE $A_1$ RECEPTOR AND PROTEIN PHOSPHATASES

Treatment times and concentrations were identical to the [Ca$^{2+}$] and contractility protocols. A subset of myocytes was used to study the effects of CCPA (n = 2), cantharidin (n = 2), and propranolol (n = 2) on cAMP levels. After treatments, the cell suspension was centrifuged at 1,000 g for 1 min and the supernatant discarded. Myocytes were then lysed with 0.1 N HCl, and the suspension was centrifuged at 3,000 g for 1 min. The resulting supernatant was used to measure cellular cAMP (immunoassay kit, R&D Systems, Minneapolis, MN), and the pellet was used to measure total cell protein using a modification of the Lowry assay (Bio-Rad, La Jolla, CA). Total cAMP was expressed as picomoles per milligram protein.

Materials. Isoproterenol, 8-BrCAMP, cantharidin, and propranolol were obtained from Sigma Chemical. CCPA and DPCPX were obtained from RBI (Natick, MA). Isoproterenol was used from fresh stock aliquots of 100 µM (dissolved in double distilled H$_2$O), which were discarded at the end of the experiment. 8-BrCAMP, CCPA, and propranolol were dissolved in double distilled H$_2$O; cantharidin and DPCPX were dissolved in dimethylsulfoxide. The vehicle concentrations did not exceed 0.05% in the buffer preparation.

Data analysis. Data are expressed as means ± SE (n, number of myocytes). Because none of the pretreatments (CCPA, DPCPX, cantharidin, and propranolol) influenced myocyte [Ca$^{2+}$]$i$ or contractility, comparison of data in these groups was done by single-factor analysis of variance (ANOVA) followed by Newman-Keuls post hoc analysis. Analysis of cAMP levels among the groups was done by ANOVA followed by Newman-Keuls post hoc analysis. A P value < 0.05 was considered statistically significant.

RESULTS

None of the treatments influenced diastolic [Ca$^{2+}$]$i$ (which averaged ~120 nM), and hence only systolic [Ca$^{2+}$]$i$ values, which averaged ~450 nM under baseline conditions, are reported. Myocytes were shortened by ~10% of their resting cell length, +6L/6t averaged 90.2 ± 5 µm/s, and −8L/6t averaged 75 ± 3 µm/s under these conditions. A 2-min infusion of isoproterenol increased systolic [Ca$^{2+}$]$i$ by 46 ± 2% (Fig. 1A) and −δCa$_{200}$/6t from 0.969 ± 0.03 to 1.672 ± 0.06 µM/s ($P < 0.05$ vs. baseline). Isoproterenol increased twitch amplitude by 56 ± 4% (Fig. 1B), +6L/6t by 140 ± 8.8%, and −8L/6t by 95 ± 4%. The effects of isoproterenol were completely reversible because both the magnitude and kinetics of [Ca$^{2+}$]$i$, and contractility recovered to baseline on washout of the drug. A subsequent exposure to isoproterenol produced effects identical to the first (data not shown).

In the second group of myocytes, a 5-min treatment with CCPA had no effect on systolic [Ca$^{2+}$]$i$ (433 ± 17 nM), −δCa$_{200}$/6t, twitch amplitude, +6L/6t, and −8L/6t. As seen in Fig. 1, A and B, in the presence of CCPA, isoproterenol produced only an ~10% increase in both systolic [Ca$^{2+}$]$i$, and contractility. The anti-adrenergic effect of adenosine A$_1$ receptor activation was also manifest in the kinetics of [Ca$^{2+}$]$i$, decline and contractility. In the presence of CCPA, isoproterenol increased −δCa$_{200}$/6t from 1.041 ± 0.08 to 1.20 ± 0.12 µM/s, +6L/6t by 61 ± 8%, and −8L/6t by 44 ± 3% ($P < 0.05$ vs. isoproterenol values). The anti-adrenergic effect of CCPA was negated by the adenosine A$_1$ receptor antagonist DPCPX (Fig. 1, A and B).

It was then examined whether the phosphatase inhibitor cantharidin inhibited the anti-adrenergic effect of CCPA. Treatment of myocytes with cantharidin (500 nM, 5 min) did not have any significant effects on systolic [Ca$^{2+}$]$i$, twitch amplitude or the kinetic parameters. Exposure to isoproterenol in the presence of cantharidin produced effects not significantly different from exposure to isoproterenol alone (Fig. 2, A and B). This dose of cantharidin also did not affect the response to a higher dose of isoproterenol (100 nM) because systolic [Ca$^{2+}$]$i$ increased by 820 ± 17 nM and twitch amplitude increased by 74 ± 2% (n = 2) in the absence or presence of cantharidin. Importantly, in myocytes pretreated with CCPA + cantharidin, exposure to isoproterenol (10 nM) increased both systolic [Ca$^{2+}$]$i$, and the extent of shortening by ~40% ($P < 0.05$ vs. CCPA + isoproterenol, Fig. 2, A and B). Isoproterenol increased −δCa$_{200}$/6t from 0.891 ± 0.06 to 1.449 ± 0.1 µM/s, +6L/6t by 114 ± 10%, and −8L/6t by 92 ± 6% in myocytes pretreated with cantharidin + CCPA ($P <
These results clearly demonstrate a blockade of the anti-adrenergic effect of CCPA with cantharidin.

The role of cellular cAMP in the anti-adrenergic effect of CCPA was examined in parallel experiments. Under baseline conditions, total cellular cAMP averaged 4.84 ± 0.58 pmol/mg protein. Exposure to isoproterenol increased cAMP 2.4-fold to 11.6 ± 0.4 pmol/mg protein. Treatment with CCPA alone had no effect on baseline cAMP (4.04 ± 0.39 pmol/mg protein), but in cells treated with CCPA + isoproterenol, cAMP increased to 9.5 ± 0.83 pmol/mg protein. Whereas this was only an 18% decrease from the isoproterenol group, it was significantly different from both baseline and isoproterenol groups (Fig. 3). In the cantharidin and cantharidin + isoproterenol groups, cellular cAMP contents did not differ from baseline and isoproterenol groups, respectively. In myocytes pretreated with CCPA + cantharidin and exposed to isoproterenol, cAMP content averaged 9.8 ± 0.5 pmol/mg protein, a value similar to the CCPA + isoproterenol group (Fig. 3).

A series of experiments was performed to determine whether the protein phosphatase inhibitor cantharidin altered the effects of the β-blocker propranolol. A 5-min treatment with propranolol (300 nM) had no effect on systolic [Ca²⁺]i and contractility but completely inhibited the effects of isoproterenol (Fig. 4, A and B). Propranolol alone had no effect on baseline cAMP (3.9 ± 0.14 pmol/mg protein) but blocked the isoproterenol-induced increase in cAMP (9.5 ± 0.83 pmol/mg protein). In myocytes pretreated with propranolol and exposed to isoproterenol, cAMP content averaged 9.8 ± 0.5 pmol/mg protein, a value similar to the CCPA + isoproterenol group (Fig. 3).
adenol-induced increases in cAMP (4.2 ± 0.03 pmol/mg protein). In contrast to its effects on CCPA, cantharidin had no effect on the propranolol blockade of isoproterenol-stimulated [Ca\(^{2+}\)] and contractility.

To further verify that a component of the adenosine A\(_1\) anti-adrenergic effect acts distal to adenylyl cyclase, the effects of CCPA were examined in the presence of the cAMP analog 8-BrcAMP. A 5-min exposure to 8-BrcAMP (1 mM) increased twitch amplitude, +8L/\(\Delta t\), and −8L/\(\Delta t\) by 26 ± 3, 62 ± 7, and 59.9 ± 6%, respectively. A second exposure to 8-BrcAMP produced effects identical to the first. In the presence of CCPA, however, 8-BrcAMP increased twitch amplitude, +8L/\(\Delta t\), and −8L/\(\Delta t\) by only 11 ± 2, 27 ± 2, and 21 ± 6%, respectively (P < 0.05 vs. 8-BrcAMP).

**DISCUSSION**

The present study examined the mechanism of the anti-adrenergic effect of adenosine A\(_1\) receptor activation in isolated rat ventricular myocytes. The A\(_1\) receptor agonist CCPA inhibited isoproterenol-induced increases in the magnitude and kinetics of both [Ca\(^{2+}\)], and contractility to a greater extent than the isoproterenol-induced increase in total cellular cAMP levels. The serine-threonine protein phosphatase inhibitor cantharidin, which exerted no effects alone, blocked the effects of CCPA on systolic [Ca\(^{2+}\)] and contractility to the same extent as the adenosine A\(_1\) receptor antagonist DPCPX. Activation of the A\(_1\) receptor also attenuated the increase in contractile parameters produced by the cAMP analog 8-BrcAMP. These results suggest that the anti-adrenergic effect of adenosine A\(_1\) receptor activation, at least in part, may involve the activation of a serine-threonine protein phosphatase.

Adenosine A\(_1\) receptor antagonism of the contractile and metabolic effects of β-adrenergic stimulation has been well documented (5). Although adenosine A\(_1\) receptor activation in the presence of catecholamines decreases adenylyl cyclase activity and cellular cAMP content (6, 12), there are several studies demonstrating a dissociation between the reductions in contractile parameters and cAMP levels (10, 22, 24, 27). For example, in the isolated perfused rat heart, adenosine A\(_1\) receptor stimulation with CCPA blunted the isoproterenol-stimulated increase in contractile parameters by 40% but reduced isoproterenol-stimulated cAMP content by only 14% (27). Gupta et al. (9) reported that the adenosine A\(_1\) receptor agonist N\(^6\)-phenylisopropyladenosine (PIA) significantly decreased isoproterenol-stimulated phosphorylation of phospholamban and troponin I in guinea pig ventricular myocytes with no change in cAMP content.

In the present study CCPA reduced the isoproterenol-induced increase in systolic [Ca\(^{2+}\)] and contractility by 87 and 80%, respectively, but attenuated the isoproterenol-induced increase in cAMP content by only 18%. It is known that myocardial cellular cAMP resides in two different compartments, viz., cytosolic and particulate, and the particulate cAMP pool is the more important arbiter of the β-adrenergic increases in [Ca\(^{2+}\)] and contractility (1, 14, 31). It is thus possible that adenosine A\(_1\) receptor activation may completely block the isoproterenol-induced increase in particulate cAMP, which may not be observed when measuring total cellular cAMP. However, it has been reported that the muscarinic receptor agonist carbachol, which exerts a similar anti-adrenergic effect as CCPA, did not reduce total or particulate cAMP levels in isoproterenol-stimulated isolated perfused rat hearts (32). Given the similarities between the muscarinic M\(_2\) and adenosine A\(_1\) anti-adrenergic effects, it is likely that A\(_1\) receptor antagonism of the contractile effects of β-adrenergic receptor stimulation cannot entirely be accounted for by a reduction in cAMP content.

The two effectors distal to cAMP, which A\(_1\) receptor activation could modulate, are cAMP-dependent protein kinase A (PKA) and a protein phosphatase. Because adenosine does reduce cAMP levels, it is expected that this would be associated with reduced PKA activity. This is indeed the case, although, as is the case with cAMP levels, there is a significant dissociation between A\(_1\) receptor modulation of PKA activity and contractile effects. In perfused rat hearts, application of adenosine decreased the isoproterenol-stimulated increase in PKA activity by only 30%, whereas contractility was reduced by 75% (5). In guinea pig myocardium, adenosine A\(_1\) receptor activation reduced isoproterenol-stimulated force development with little or no change in PKA activity (10). Studies on the anti-adrenergic effect of muscarinic M\(_2\) receptor stimulation indicate a similar disparity between catecholamine-induced increases in both the phosphorylation of regulatory proteins and contractility and alterations in PKA activity (10, 11).

In mammalian myocardium PKA and Ca\(^{2+}\)-calmodulin-dependent protein kinase phosphorylate serine-threonine residues on regulatory proteins such as phospholamban, a process that plays a physiological role in both the inotropic and kinetic parameters of contractility (20). At least two distinct enzymes, protein phosphatases 1 (PP1) and 2A (PP2A), by virtue of dephosphorylation of these regulatory proteins, reverse the effects of protein kinases (2, 6, 23, 28). Gupta et al. (10) reported that both acetylcholine and the adenosine A\(_1\) agonist PIA attenuated isoproterenol-induced increases in the activity of protein phosphatase inhibitor-1, an inhibitor of PP1 (28), in guinea pig ventricular myocardium. The authors suggested that adenosine and acetylcholine increase PP1 activity by inhibiting catecholamine-stimulated protein phosphatase inhibitor-1 activity (10). These data resulted in the hypothesis that the anti-adrenergic effects of muscarinic M\(_2\) and adenosine A\(_1\) receptor activation are due, in part, to the stimulation of a serine-threonine phosphatase. A direct stimulatory effect of adenosine on protein phosphatases has been reported in bovine adrenal chromaffin cells and polymorphonuclear leukocytes (19, 26).

The results of the present study provide additional support for this hypothesis, because the protein phosphatase inhibitor cantharidin blocked the anti-adrenergic effect of CCPA. Cantharidin alone had no effect on the baseline magnitudes and kinetics of the calcium transient or contractility nor did it influence the actions
of isoproterenol (at two different concentrations) on these parameters. Cantharidin alone did not alter cellular cAMP content nor did it affect cAMP levels in myocytes treated with isoproterenol or CCPA + isoproterenol. Cantharidin also did not modify the anti-adrenergic action of the β-adrenergic receptor blocker propranolol. However, cantharidin blocked the anti-adrenergic effects of CCPA to the same extent as the A₁ antagonist.

Cantharidin, an extract from blister beetles, is an inhibitor of serine-threonine protein phosphatases. In vitro studies indicate that cantharidin has a higher affinity for PP2A [inhibitory constant (Kᵢ) ~0.13 µM] than for PP1 (Kᵢ ~1.7 µM). Only at higher concentrations (>10 µM) does cantharidin appear to inhibit protein phosphatases 2B and 2C (15). Cantharidin has been used in numerous studies to examine the role of protein kinases and phosphatases in myocardial excitation-contraction coupling (7, 15–17, 23). At concentrations >3 µM, cantharidin exerts a positive inotropic effect in cardiac muscle by increasing transsarcomeral calcium flux (23). In the present study however cantharidin exerted no direct effects on contractility or [Ca²⁺]. Although it is possible that cantharidin could have altered PKA activity or increased the sensitivity of PKA to cAMP, cantharidin did not potentiate any of the effects of a submaximal dose (10 nM) of isoproterenol.

The results obtained in the 8-Br cAMP protocols provide additional evidence supporting our hypothesis that adenosine exerts significant anti-adrenergic effects distal to adenylyl cyclase. Incubation of ventricular myocytes with 8-Br cAMP, which acts by stimulating PKA, resulted in increases in twitch amplitude, +dL/dt, and −dL/dt, effects that were attenuated by pretreatment with CCPA. Isoproterenol-induced increases in +dL/dt and −dL/dt, although much greater in magnitude, were also attenuated by CCPA. The observations in the present study indicate that although the adenosine A₁ anti-adrenergic effect is mediated, in part, via reductions in cAMP and thus reductions in PKA activity, adenosine A₁ receptor activation also appears to involve the stimulation of one or more serine-threonine protein phosphatases.

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