A New Cyclic AMP-independent, Gs-mediated Stimulatory Mechanism via the Adenosine A2a Receptor in the Intact Cardiac Cell*

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The objectives of this study were to investigate the mechanism underlying the adenosine A2a receptor (A2aR)-mediated positive inotropic response and to define its contractile function using chick embryo ventricular cells as a model. Activation of the A2aR caused a marked stimulation of calcium entry and cell contractility, which were blocked by verapamil or nifedipine. The effects elicited by maximal concentrations of the A2aR agonist 2-(4-carboxyethyl)phenylethylamine-5'-N-ethylcarboxamidoadenosine and the β-adrenergic agonist isoproterenol were additive, indicating that the two receptors do not share a common stimulatory mechanism. The cAMP antagonist (R)-adenosine cyclic 3′:5′-monophosphorothioate was ineffective in inhibiting the A2aR-mediated stimulation of contractility or the L-type calcium channel, while it completely abolished the isoproterenol effects. Activation of the A2aR had no effect on Na+/Ca2+ exchange or inositol 1,4,5-trisphosphate accumulation. Blocking of the A2aR resulted in unopposed A1 receptor-mediated inhibitory effects and led to an inhibition of basal contractility and an enhanced anti-adrenergic effect by A1 agonist. The adenosine A2a receptor mediates a new cyclic AMP-independent mechanism and a new contractile function in the cardiac cell.

Multiple pathways mediate stimulation of cardiac cell contractility. The classical and best characterized pathway is that mediated by the β-adrenergic receptor, giving rise to an increase in cAMP with subsequent calcium entry and increase in myocyte contractility (1–4). The α-adrenergic receptor-mediated augmentation of cardiac contractility involves stimulation by inositol phosphates and protein kinase C that is cyclic AMP-independent (5–8). Adenosine exerts pronounced contractile effects in the heart. In the cardiac ventricular cell, adenosine attenuates the increase in the force of contraction elicited by β-adrenergic agonist, known as the anti-adrenergic effect, while adenosine by itself has no direct effect on the basal level of contractility. The anti-adrenergic effect is mediated by the inhibitory A1 subtype of the adenosine receptor (for review, see Refs. 9–13). Using fetal chick embryo ventricular cells as a model, prior studies demonstrated the presence of a high affinity adenosine A2a receptor capable of mediating positive inotropic response when the A1 receptor is blocked (14, 15). The marked positive inotropic response mediated by the A2aR receptor suggests a significant physiological or pathophysiological role of this receptor in the cardiac cell. Since the inhibitory A1 receptor coexists with the stimulatory A2a receptor and since the A1 receptor mediates the anti-adrenergic effect of adenosine, the question arises regarding specifically the function of the A2aR in modulating the basal as well as the β-adrenergic stimulated contractile states. Furthermore, the mechanism underlying the A2aR receptor-mediated positive inotropic response is not known. Although activation of the A2aR receptor can be coupled to stimulation of cAMP accumulation, it is not known whether cAMP mediates the increase in myocyte contractility.

The objectives of this study, using cultured fetal ventricular cells as a model system, were to investigate the mechanism underlying the adenosine A2a receptor-mediated positive inotropic response and to study the contractile function observed by the A2aR receptor. Cardiac ventricular cells cultured from chick embryos retain many of the biological properties of the intact heart and represent a useful model for study of cardiac function and contractility (16–23). Activation of adenosine receptors in these cultured heart cells produced physiological effects similar to those elicited by adenosine in the mammalian heart (14, 15, 23). The present data demonstrate that the A2aR receptor and the β-adrenergic receptor do not share a common pathway leading to stimulation of myocyte contractility or calcium entry; that a cyclic AMP-independent, Gs-mediated mechanism is largely responsible for the A2aR receptor-mediated stimulatory response; and that a physiological action of the activated A2aR receptor is to oppose the inhibitory effect of A1 receptor agonist on basal and β-adrenergic stimulated contractile states.

EXPERIMENTAL PROCEDURES

Methods

Preparation of Cultured Cardiac Cells—Atrial and ventricular cells were cultured from chick embryos 14 days in ovo according to previously described procedures (15, 23). Cells grew to confluence on day 3 of culture and exhibited rhythmic spontaneous contraction. Unless otherwise indicated, all experiments were carried out on day 3 of culture. Cultures were treated with adenosine deaminase (2 units/ml) for 24 h to keep the endogenous adenosine at a minimal level; they were also treated with pertussis toxin (5 ng/ml for 24 h) to uncouple the inhibitory A1 receptor from its effector(s) as described previously (14, 15). Blocking of the A1 receptor facilitated quantitation of the A2a receptor-mediated functional responses. In experiments in which cholera toxin was used to activate the stimulatory G protein (Gαs), the cultures were treated with 2 μg/ml cholera toxin for 3 h. This dose and duration gave consistent stimulation of cyclic AMP and 45Ca2+ influx.

Determination of Contractile Amplitude—Measurement of contractile amplitude in cultured cardiac cells was carried out according to previously described methods (15, 18). The contractile amplitude of the cultured cell was determined by an optro-video motion detection sys-
tem with a video motion analyzer (Colorado Video Inc., Boulder, CO) as described previously. The perfusion medium contained the various adenine analogs as indicated as well as the following components: 4 mM HEPES (pH 7.4), 137 mM NaCl, 3.6 mM KCl, 0.5 mM MgCl2, 0.6 mM CaCl2, 5.5 mM glucose, and 8% horse serum. Measurement of contractile amplitude was carried out on only one cell/cover slip, and each culture dish was assayed in triplicate. After achieving a steady state of beating in medium without adenine analogs, the medium was switched to that containing the indicated adenine drugs. Both the basal contractile amplitude and the amplitude measured during adenine analog exposure were determined. The stimulatory effect of the various adenine analogs on the contractile state was predominantly on the amplitude measured during adenosine analog exposure. There was no significant consistent effect of any of the analogs on the spontaneous rate of contraction.

Measurement of Cyclic AMP Level—Cultured ventricular cells were treated with pertussis toxin and adenine deaminase as described above. On day 3 of culture, the media were replaced with culture medium lacking fetal bovine serum, and cells were incubated with the indicated agonist(s) and antagonist(s). Cyclic AMP was extracted and assayed according to the previously described radioimmun assay method (Ref. 15; Amersham Corp.). The effect of agonist on cyclic AMP accumulation was linear for 10 min, at which time, cyclic AMP was extracted for assay.

Measurement of 45Ca Uptake into Myocardial Cells—Determination of 45Ca uptake was made according to a modification of a previously described method (18). Cultures were incubated with 1-(3,4,5-trihydroxymethyl)imidazole (52.2 Ci/mmol) for 24 h prior to the 45Ca uptake measurement. [3H]Leucine incorporated into the cellular protein allowed normalization of 45Ca content to milligrams of cellular protein. For measurement of 45Ca uptake, growth media in which ventricular cells were grown and maintained on 12-well culture plates were replaced with HEPES-buffered solution (pH 7.35) containing 1.0 mM CaCl2, 4 mM KCl, and 0.5 mM MgCl2 (buffer A) at 37°C for 10 min and then incubated in the same medium except that calcium was omitted and 1 mM EGTA was added (buffer B) and subsequently incubated in buffer A containing 45Ca (5-10 μCi/ml) and adenine analogs for the times indicated. The step of exposure of myocytes to calcium-free medium helped minimize mixing of 45Ca with unlabelled Ca2+ near or at the cell surface, optimized 45Ca at the cell surface for subsequent uptake, and resulted in reproducible quantitation of 45Ca uptake. Cells were then washed free of the 45Ca media by four rinses with ice-cold buffer A containing 1 mM lanthanum. This washing procedure removed >99% of the extracellular marker 45Ca-labeled EDTA and thus ensured complete removal of the extracellular 45Ca. The presence of lanthanum in the wash buffer allowed displacement of any 45Ca that may have attached to the extracellular surface of the cells. Influx of 45Ca was quantitated for 90 s to allow determination of its uptake into the rapidly exchanging pool, which has been shown to be due either to calcium entry through the L-type calcium channel or to Na+/Ca2+ exchange (18). Determination of 45Ca influx during this initial phase of its uptake in the presence of various agents permitted assessment of their effects on the activity of the calcium channel or Na+/Ca2+ exchange (24, 25). For all data comparing the effect of different agents on 45Ca influx, one-way ANOVA followed by group comparison with the Student’s t test was carried out at each 15-, 30-, and 60-s time points of 45Ca influx (Fig. 2 C) and 45Ca uptake (inhibition = 24.8 ± 2.4% inhibition of the CGS21680-stimulated calcium response at the 90-s time point [n = 5]). (R)-cAMP-S alone had no effect on the basal contractility or the basal uptake of 45Ca (data not shown). Similar percent inhibition by (R)-cAMP-S was also evident at the 15-, 30-, and 60-s time points of 45Ca influx (Fig. 2 C). Increasing (R)-cAMP-S to 100 μM did not result in further inhibition of the CGS21680-stimulated increase in 45Ca influx (inhibition = 26.4 ± 1% at the 90-s time point [n = 4]). The ability of CGS21680 to stimulate myocyte contractility appeared to be

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minimally affected by (Rp)-cAMP-S (Fig. 2C). However, a small inhibition of contractile amplitude by (Rp)-cAMP-S may be difficult to quantitate and cannot be ruled out completely. Although the data are consistent with a role of cyclic AMP in mediating the calcium influx response to the A2a agonist, they demonstrate the existence of a cAMP-independent mechanism in causing a stimulation of calcium influx and provide further evidence that the A2a and β-adrenergic receptors use different pathways to cause stimulatory responses.

Cellular Mechanisms Underlying A2a Receptor-mediated Stimulation of Calcium Influx—Activation of the A2a receptor could cause an increase in the trans-sarcoplaemmal calcium influx by stimulating the voltage-sensitive L-type calcium channel or by augmenting Na⁺/Ca²⁺ exchange. Verapamil (1 μM) blocked the spontaneous contraction of cultured ventricular cells and completely attenuated the CGS21680-elicted increase in ⁴⁵Ca influx in the absence (Fig. 3A) or presence (data not shown) of (Rp)-cAMP-S, indicating that the effect on calcium influx is due to stimulation of the L-type calcium channel. Similar results were obtained using nifedipine (1 μM) as the calcium channel antagonist (data not shown). To examine whether Na⁺/Ca²⁺ exchange is involved in this calcium response, the effect of CGS21680 was determined in media in which choline chloride substituted for NaCl in the presence of verapamil. Decreasing the extracellular Na⁺ induced a marked stimulation of ⁴⁵Ca influx in the presence of verapamil (Fig. 3B), indicating a Na⁺/Ca²⁺ exchange-mediated increase in calcium influx. The addition of CGS21680 did not cause any further stimulation of ⁴⁵Ca influx. In the absence of verapamil, CGS21680 increased the extent of ⁴⁵Ca influx further, above that caused by lowering the extracellular sodium concentration (Fig. 3C).

Stimulation of cAMP accumulation by adenosine A2a agonist is likely mediated by the Gs-induced activation of adenyl cyclase (9–13). Although the present data do not indicate a major role for the A2a receptor-Gs-cAMP pathway in the calcium influx response, activation of Gs mediated by the A2a receptor may be involved in causing a cAMP-independent stimulation of calcium influx. Direct activation of Gs by cholera toxin, which stimulated cAMP accumulation (67 ± 4%, mean ± S.E., n = 5), induced a large increase in ⁴⁵Ca influx that was only partially blocked by 100 μM (Rp)-cAMP-S (Fig. 3D). The percent inhibition of cholera toxin-stimulated ⁴⁵Ca influx by (Rp)-cAMP-S was 40 ± 8, 54 ± 15, 43 ± 8, and 48 ± 6% at the
15-, 30-, 60-, and 90-s time points, respectively (p < 0.05, paired t test). The (R)p-cAMP-S-insensitive portion of the increase in 45Ca influx was abolished by 1 μM verapamil (Fig. 3D), which was also able to block all of the 45Ca influx caused by isoproterenol or by cholera toxin (data not shown). Thus, direct activation of Gs by cholera toxin can stimulate, through the L-type calcium channel, both cAMP-dependent and -independent increases in 45Ca influx.

CGS21680 (at 1 or 10 μM) had no effect on the level of inositol 1,4,5-trisphosphate (41 ± 5 pmol/mg (control) versus 45 ± 4 pmol/mg (1 μM CGS21680) and 38 ± 6.5 pmol/g (10 μM CGS21680)) (data were means ± S.E. of quadruplicates and were representative of three other experiments). As a positive control, both the muscarinic cholinergic agonist carbamylcholine (300 μM) and the purinergic agonist ATP (300 μM) caused a large increase in the level of inositol trisphosphate (carbamylcholine, 70 ± 5 pmol/mg; and ATP, 92.5 ± 6 pmol/mg) (data were typical of three other experiments). Thus, at the concentrations of CGS21680 that caused a marked increase in calcium influx or myocyte contractility, there was no stimulation of inositol 1,4,5-trisphosphate accumulation. These data indicate that the CGS21680-mediated stimulatory contractile or 45Ca influx response is not mediated by inositol 1,4,5-trisphosphate.

Adenosine A2a Receptor Activation Attenuates A1 Receptor-mediated Anti-adrenergic Contractile and Calcium Influx Effects—The present data predict that concomitant activation of both the A2a and A1 receptors by adenosine agonist should alter the basal as well as the β-adrenergic stimulated levels of con-
tractility. (R)-PIA is an A1 receptor-selective agonist that can also activate the A2a receptor at the higher concentrations (14, 26). Prior studies indicated that (R)-PIA was an A1 receptor agonist capable of inhibiting the isoproterenol-stimulated increase in chick embryo cardiac cells (14, 15, 23). The present data demonstrate that (R)-PIA (10 μM) can stimulate both 45Ca influx and contractility in cells in which the A1 receptor pathway is blocked (Fig. 4A). The A2a receptor-selective antagonist CSC blocked the (R)-PIA-induced stimulation of both 45Ca influx and contractility in cells in which the A1 receptor pathway is blocked (Fig. 4A). The A2a receptor-selective antagonist CSC blocked the (R)-PIA-induced stimulation of 45Ca influx and contractility, indicating that the stimulatory effects of (R)-PIA are mediated by the A1 receptor. (R)-PIA further increased the level of 45Ca influx and myocyte contractility stimulated by isoproterenol (Fig. 4B and C), an additive effect that was consistent with agonist activity of (R)-PIA at the A2a receptor. Thus, (R)-PIA can activate both the A1 and A2a receptors in these ventricular cells. Whether activation of the A2a receptor can modulate the basal and anti-adrenergic contractile responses elicited by A1 receptor agonist was examined next.

Blocking of the A2a receptor caused not only a depression of the basal contractility (Fig. 5A), but also a further inhibition of the isoproterenol-stimulated positive inotropic response by (R)-PIA (Fig. 5B). A previous study demonstrated that atrial myocytes cultured from the same chick embryos exhibited no positive inotropic response to CGS21680 or other adenosine receptor agonists (14), indicating the absence of a functional A2a receptor in the atrial myocyte. If atrial myocytes do not express A2a receptors, the presence of CSC should not influence the ability of (R)-PIA to inhibit the basal or isoproterenol-stimulated increase in contractility. In fact, Fig. 5C demonstrates that CSC had no effect on the ability of (R)-PIA to inhibit basal contractile amplitude (basal contractile amplitude 577 ± 62%, mean ± S.E., n = 10 (R)-PIA) versus 78 ± 1.6% n = 10 (R)-PIA plus CSC; p > 0.1, paired t test). Furthermore, CSC did not affect the (R)-PIA-mediated inhibition of the isoproterenol-stimulated increase in contractility (maximal isoproterenol stimulation by (R)-PIA alone of 76 ± 3% versus percent maximum of 75 ± 4% by (R)-PIA plus CSC; p > 0.1, paired t test). These data serve as a control for the experiments carried out in the ventricular cells and indicate that CSC has no intrinsic nonspecific contractile effect.

**Fig. 3.** Mechanism underlying the adenosine A2a receptor-mediated stimulation of 45Ca influx. The effect of verapamil on the CGS21680-stimulated increase in 45Ca influx was determined in cells preincubated with verapamil (1 μM) for 5 min prior to exposure to both verapamil (1 μM) and CGS21680 (1 μM) during 45Ca uptake (A). Verapamil abolished the increase in 45Ca influx stimulated by CGS21680 (p < 0.01). The effect of substituting extracellular NaCl with choline chloride (137 mM) on 45Ca influx was investigated in the presence or absence of CGS21680 (1 μM) plus verapamil (1 μM) (B). The level of 45Ca influx determined in the presence of CGS21680 plus choline chloride and verapamil was similar to that obtained with choline chloride plus verapamil (p > 0.1). In the absence of verapamil, the extent of 45Ca uptake stimulated by CGS21680 plus choline chloride was significantly greater than that determined in the presence of choline chloride alone (p < 0.01), which was, in turn, greater than the extent of 45Ca influx in the control (CON; p < 0.01) (C). The effects of cholera toxin (CTX) on 45Ca influx were determined in the presence or absence of (R)-cAMP-S (100 μM) and/or verapamil (1 μM) (D). Cholera toxin (2 μg/ml) stimulated a marked increase in 45Ca influx (p < 0.01). (R)-cAMP-S abolished part of the cholera toxin-stimulated 45Ca influx (p < 0.05); verapamil reduced the remaining portion of the increase in 45Ca influx to the control level (p < 0.05).
Adenosine plays an important role in modulating the contractile state of the cardiac cell (for review, see Refs. 9–13). Previous studies carried out in this laboratory have demonstrated the existence of a high affinity adenosine A2a receptor capable of mediating a marked positive inotropic response in cultured fetal chick embryonic ventricular cells (14, 15). However, the mechanism underlying the A2a receptor-mediated positive inotropic effect is not known. Although the A2a receptor is coupled to stimulation of cyclic AMP accumulation, it is not clear whether cyclic AMP mediates all of the positive inotropic effect of A2a receptor agonist. It is not known whether the A2a receptor and the β-adrenergic receptor share a similar mechanism of positive inotropic effect. Furthermore, the role of the A2a receptor in regulating the contractile state of the cardiac cell is not known. Since the inhibitory A1 receptor coexists with the stimulatory A2a receptor in these cultured ventricular cells and since the A1 receptor mediates the anti-adrenergic effect of adenosine, the question arises regarding the specific role of the A2a receptor in modulating the basal as well as the β-adrenergic stimulated contractile states. The main findings of this study are as follows. The mechanism underlying the A2a receptor-mediated positive inotropic effect involves a cyclic AMP-independent, A2a receptor/Gs-mediated stimulation of the L-type calcium channel and differs from the mechanism of the β-adrenergic stimulated inotropic response. A normal function of the activated A2a receptor is to attenuate the A1 receptor-mediated anti-adrenergic response.

Four lines of evidence support the existence of a new mechanism responsible for the A2a receptor-mediated positive inotropic response. First, the positive inotropic effects elicited by maximally effective concentrations of isoproterenol and CGS21680 were additive, indicating that the β-adrenergic and adenosine A2a receptors do not share a common positive inotropic mechanism. Similarly, the stimulation of calcium influx caused by maximally effective concentrations of isoproterenol and CGS21680 was also additive, providing further evidence for the distinct pathways used by the two receptors. Second, while the β-adrenergic receptor is coupled to pronounced stimulation of cyclic AMP accumulation, A2a receptor activation caused only a modest increase in cyclic AMP, with nearly 10-fold less stimulation of the cyclic AMP level. Third, at a concentration of isoproterenol that caused an increase in the cyclic AMP level similar to the increase elicited by the maximally effective concentration of CGS21680, there was no increase in either myocyte contractile amplitude or calcium influx. Fourth, while (R)-CAMP-S was able to block all of the positive inotropic and calcium influx responses elicited by isoproterenol, (R)-CAMP-S could inhibit only a small portion of the calcium influx response stimulated by CGS21680. These data indicate that a distinct mechanism, different from that used by the β-adrenergic receptor, mediates the stimulatory contractile and calcium influx responses to adenosine A2a receptor agonist.

Since verapamil or nifedipine (1 μM) was able to block all of the CGS21680-stimulated increase in calcium influx, an A2a receptor-mediated stimulation of the L-type calcium channel appeared to be responsible for the increase in calcium influx. Lowering the extracellular sodium in the presence of verapamil caused a marked stimulation of calcium influx, secondary to a Na⁺/Ca²⁺ exchange-mediated increase in calcium entry. The inability of CGS21680 to cause a further increase in calcium influx in this type of medium suggests that CGS21680 does not stimulate Na⁺/Ca²⁺ exchange. In the absence of verapamil, CGS21680 was able to cause a further increase in the level of calcium influx that was stimulated by lowering the extracellu-
stimulatory Adenosine A2a Receptor in the Cardiac Cell

A

R-PIA  R-PIA + CSC

0.5 μm

2 sec

B

ISO  ISO + R-PIA  ISO + R-PIA + CSC

0.5 μm

2 sec

C

R-PIA  R-PIA + CSC

0.5 μm

2 sec

Fig. 5. Adenosine A2a receptor effects on A1 receptor-mediated anti-adrenergic contractile and calcium responses. Atrial and ventricular cells were prepared and pretreated with adenosine deaminase only. The effects of CSC (1 μM) on the basal and anti-adrenergic responses to (R)-PIA (10 μM) were examined. After achieving steady state, ventricular cells were exposed to (R)-PIA and then to (R)-PIA plus CSC (A). The level of contractile amplitude determined in the presence of (R)-PIA plus CSC was less than that in the control or the amplitude obtained with (R)-PIA alone (one-way ANOVA followed by t test; p < 0.01). In another experiment, ventricular cells were exposed to isoproterenol (ISO; 0.3 μM), then to isoproterenol plus (R)-PIA, and finally to isoproterenol plus (R)-PIA and CSC (B). The level of contractile amplitude determined in the presence of isoproterenol plus (R)-PIA and CSC was less than that obtained with isoproterenol plus (R)-PIA (p < 0.01), which was, in turn, less than that obtained in the presence of isoproterenol alone (p < 0.01). Similar experiments were carried out to examine the effect of CSC (1 μM) on the (R)-PIA (10 μM)-mediated direct negative inotropic or anti-adrenergic response in atrial cells (C). In atrial cells, the levels of contractile amplitude obtained in the presence of CSC were not different from those determined in its absence (p > 0.1).

lar sodium. These data provide further evidence for the notion that the CGS21680-induced calcium effect is mediated by the L-type calcium channel and does not involve Na+/Ca2+ exchange. Since CGS21680 could not stimulate inositol 1,4,5-trisphosphate accumulation, it is unlikely that the inositol trisphosphate is involved in mediating the CGS21680-induced stimulatory responses.

Although (R)-cAMP-S inhibited partially the A2a agonist-mediated calcium influx response, the percent inhibition was modest (25%). These data indicate that a cAMP-independent pathway is capable of, and likely plays a primary role in, mediating the A2a agonist-induced increase in calcium influx. Activation of Gs by A2a receptor agonist likely mediates the cAMP-independent stimulation of the L-type calcium channel, based on the following evidence. First, direct activation of Gs by cholera toxin resulted in stimulation of both cAMP and calcium influx; cholera toxin-stimulated calcium influx exhibited both (R)-cAMP-S-sensitive and -insensitive components. Both components can be blocked by the L-type channel antagonists verapamil and nifedipine. Since activation of Gs can stimulate the L-type calcium channel independent of cAMP (32, 33), these data are consistent with the notion that cholera toxin-activated Gs can cause a cAMP-independent stimulation of the L-type calcium channel. Second, A2a agonist-stimulated calcium influx also exhibited (R)-cAMP-S-sensitive and -insensitive components, which were blocked by verapamil or nifedipine. These data indicate that the A2a receptor is also capable of mediating a cAMP-independent activation of the L-type channel. Similarities in the action and effect of CGS21680 compared with those of cholera toxin are consistent with the notion that the A2a agonist-induced augmentation of nifedipine-sensitive calcium influx involves a cAMP-independent, A2a receptor/Gs-mediated stimulation of the L-type calcium channel. Preliminary data demonstrate that transfection of the myocytes with rat Gαs causes an increased A2a agonist-mediated, nifedipine-sensitive 45Ca influx in the presence of (R)-cAMP-S, providing evidence that exogenous Gαs can couple the A2a receptor to a cAMP-independent stimulation of the calcium channel. Although β-adrenergic receptor activation, via Gs, can stimulate the L-type calcium channel directly independent of cAMP in the cardiac cell membrane (32, 33), the present data suggest that a cyclic AMP-independent, Gs-mediated stimulation of the L-type calcium channel, which is separate from those coupled to the β-adrenergic receptor, remains unknown.

The existence of an A2a receptor-mediated signaling mechanism distinct from that of the β-adrenergic receptor suggests that possibility that activation of the A2a receptor could mediate the possibility of a different mechanism of stimulation of the calcium channel independent of cAMP is not clear. Whether this differential ability of the two Gs-linked receptors to couple to activation of the calcium channel can be explained by a predetermined coupling of the A2a receptor to a pool of Gs capable of mediating a cAMP-independent stimulation of the calcium channel, which are separate from those coupled to the β-adrenergic receptor, remains unknown.

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Receptor with the selective antagonist CSC caused not only a decrease in basal contractile amplitude, but also a further inhibition of isoproterenol-stimulated contractility in response to (R)-PIA. These data indicate that activation of the A2 receptor, if unopposed by the A2a subtype, could cause a direct negative inotropic effect as well as an enhanced anti-adrenergic response in the ventricular cell. The lack of effect of CSC on the A2 receptor-mediated anti-adrenergic or direct negative inotropic effect in atrial cells, which do not express an A2 receptor, suggests that the effect of CSC in ventricular cells is not due to a nonspecific contractile response to CSC. These data are consistent with a prior study indicating that CSC acts as a selective antagonist at the adenosine A2a receptor in the cultured chick embryo ventricular cell (15). The data also indicate that potential agonist effect on the A2a receptor should be considered when studying the cardiac action of adenosine agonist.

Overall, this study demonstrates a new stimulatory signaling mechanism mediated by the adenosine A2a receptor and elucidates a novel contractile function of the A2a receptor in the intact cardiac ventricular cell. Whether other cell-surface cardiac receptor(s) are also coupled to such Gs-mediated stimulatory pathway remains to be determined. Demonstration of this new cAMP-independent, Gs-mediated stimulatory mechanism in the cardiac cell, however, should have significant general implications for the regulation of basic cardiac function.

Acknowledgments—We thank Dr. David Manning for useful discussion and Danielle Groce for capable technical assistance.

REFERENCES
1. Walsh, D. A., Clippinger, M. S., Sivaramakrishnan, S., and McCullough, T. E. (1979) Biochemistry 18, 871–877
2. Hartzell, H. C., Mery, P., Fischmeister, R., and Szabo, G. (1991) Nature 351, 573–576
3. Walsh, D. A., and Van Patten, S. M. (1994) FASEB J. 8, 1227–1235
4. Kameyama, M., Hofmann, F., and Trautwein, W. (1986) Pflugers Arch. Eur. J. Physiol. 407, 123–128
5. Buckner, R., and Scholz, H. (1984) Br. J. Pharmacol. 82, 223–232
6. Steinberg, S. F., Chaow, Y. K., Robinson, R. B., and Bilezikian, J. P. (1987) Endocrinology 120, 1889–1894
7. Otani, H., Otani, H., and Das, D. K. (1988) Circ. Res. 62, 8–17
8. Brown, J. H., Buxton, I. L., and Brunton, L. L. (1985) Circ. Res. 57, 532–537
9. Belardelli, L. B., Linden, J., and Berne, R. M. (1989) Prog. Cardiovasc. Dis. 32, 93–97
10. Olsson, R. A., and Pearson, J. D. (1990) Physiol. Rev. 70, 761–809
11. Liang, B. T. (1992) Trends Cardiovasc. Med. 2, 100–108
12. Stiles, G. (1992) J. Biol. Chem. 267, 6451–6454
13. Fredholm, B. B., Abbracchio, M. P., Burnstock, G., Daly, J. W., Harden, T. K., Jacobson, K. A., Leff, P., and Williams, M. (1994) Pharmacol. Rev. 46, 143–156
14. Xu, D., Kong, H., and Liang, B. T. (1992) Circ. Res. 70, 56–65
15. Liang, B. T., and Haltiwanger, B. (1995) Circ. Res. 76, 242–251
16. DeHaan, R. L. (1967) Dev. Biol. 16, 216–249
17. Galper, J. B., and Smith, T. W. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 5831–5835
18. Barry, W. H., and Smith, T. W. (1982) J. Physiol. (Lond.) 325, 243–260
19. Marsh, J. D., Lachance, D., and Kim, D. (1985) Circ. Res. 57, 171–181
20. Liang, B. T., Helmich, M. R., Neer, E. J., and Galper, J. B. (1986) J. Biol. Chem. 261, 9011–9021
21. Stimers, J. R., Liu, S., and Lieberman, M. (1991) J. Gen. Physiol. 98, 815–833
22. Xu, H., Miller, J., and Liang, B. T. (1992) Nucleic Acids Res. 20, 6425–6426
23. Liang, B. T. (1989) J. Pharmacol. Exp. Ther. 249, 775–784
24. Laurent, S., Kim, D., Smith, T. W., and Marshall, T. (1985) Circ. Res. 56, 676–682
25. Laurent, S., Marshall, T. D., and Smith, T. W. (1986) J. Clin. Invest. 77, 1436–1440
26. Brun, R. F., Lu, G. H., and Pugsley, T. A. (1986) Mol. Pharmacol. 29, 331–346
27. Connelly, P. A., Botelho, L. H. P., Sisk, R. B., and Garrison, J. C. (1987) J. Biol. Chem. 262, 4324–4332
28. Pereira, M. E., Segaloff, D. L., Ascoli, M., and Eckstein, F. (1987) J. Biol. Chem. 262, 6093–6100
29. Parker Botelho, L. H., Rothermel, J. D., Coombs, R. V., and Jastorff, B. (1988) Methods Enzymol. 159, 159–172
30. Wang, L.-Y., Salter, M. W., and MacDonald, J. F. (1991) Science 253, 1132–1135
31. Gu, Z. F., Jensen, R. T., and Maton, P. N. (1994) Biochemistry 33, 1436–1440
32. Yatani, Y., and Brown, A. M. (1989) Science 245, 71–74
33. Pelzer, S., Suba, Y. M., and Pelzer, D. J. (1993) J. Physiol. (Lond.) 459, 228P
34. Yatani, Y., and Brown, A. M. (1989) Science 245, 71–74