Differential Desensitization of A₁ Adenosine Receptor–Mediated Inhibition of Cardiac Myocyte Contractility and Adenylate Cyclase Activity

Relation to the Regulation of Receptor Affinity and Density

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Effects of chronic exposure of cultured atrial myocytes to R-N⁶-(2-phenylisopropyl)-adenosine (R-PIA) on the A₁ adenosine receptor–mediated inhibition of adenylate cyclase activity and myocyte contractility were examined. Chronic exposure of atrial myocytes cultured from 14-day-old chick embryos to R-PIA desensitized the myocyte to the inhibitory effects of R-PIA on contractility and adenylate cyclase activity in a time- and dose-dependent manner. Desensitization of the negative inotropic response was only partial, whereas the adenosine receptor–mediated inhibition of adenylate cyclase activity was almost completely absent after 24 hours of R-PIA (1 μM) exposure. Furthermore, the contractile response to R-PIA desensitized more slowly than the desensitization of A₁ adenosine receptor–mediated inhibition of adenylate cyclase (t½=11.4±0.7 hours versus 7.5±1 hours, mean±SEM, n=12 and 6, respectively). Thus, the two A₁ adenosine receptor–linked functional responses desensitized differently in response to chronic exposure of the myocyte to R-PIA. Binding of the antagonist radioligand [³H]8-cyclopentyl-1,3-dipropylxanthine ([³H]CPX) in membranes from myocytes preexposed to R-PIA demonstrated a time-dependent decrease in receptor density without any change in the affinity for the antagonist radioligand. Computer analyses of agonist competition with [³H]CPX binding in membranes from control and R-PIA–treated myocytes revealed a conversion of the high-affinity A₁ adenosine receptor to a low-affinity form such that after 24 hours of 1 μM R-PIA exposure, all of the receptors were in a low-affinity form. On the other hand, downregulation of the receptor was partial, with nearly 60% of the receptor remaining after 24-hour treatment with 1 μM R-PIA. These data indicate that the mechanism of desensitization of the A₁ adenosine receptor–mediated negative inotropic response differs from that of the desensitization of the adenylate cyclase. Uncoupling of the A₁ adenosine receptor from a high-affinity state was closely associated with desensitization of the inhibition of adenylate cyclase; downregulation of the A₁ adenosine receptor appeared to parallel desensitization of the negative inotropic response. (Circulation Research 1990;67:406–414)

Cardiac actions of adenosine are mediated by specific adenosine receptors of the A₁ subtype located on the surface of cardiac myocytes.¹⁻⁹ Previous studies have demonstrated that A₁ adenosine receptors are present on spontaneously contracting cultured atrial myocytes and are negatively coupled to adenylate cyclase and to myocyte contractility.⁸ Pretreatment of intact chick embryo with R-N⁶-(2-phenylisopropyl)-adenosine (R-PIA) resulted in downregulation of the A₁ adenosine receptor and a concomitant desensitization of R-PIA–induced antagonism of forskolin-stimulated positive inotropic response in embryonic chick ventricular slices.¹⁰ Chronic subcutaneous administration of R-PIA also attenuated adenosine receptor–mediated inhibition of adenylate cyclase activity in rat adipose tissues.¹¹ However, the concentration of R-PIA to which the chick ventricle and rat adipose tissue in the intact animal were exposed is not known.
The kinetics and dose dependence of adenosine agonist–induced desensitization of these adenosine functional responses could not be readily obtained. Furthermore, little is known regarding whether desensitization of the cardiac contractile response to adenosine agonist develops coordinately with desensitization of $A_1$ adenosine receptor–linked adenylate cyclase. The role of downregulation of the $A_1$ adenosine receptor and that of the conversion of high-affinity receptor to a low-affinity form in mediating the desensitization of these two functional responses remain unknown.

In the present study, we used spontaneously contracting atrial myocytes cultured from 14-day-old chick embryos as a model system, which made possible a direct comparison between desensitization of adenosine physiological responses and changes in receptor density and affinity for the agonist under the condition in which adenosine agonist concentration in culture medium can be experimentally varied. We investigated the possibility of a differential desensitization of the $A_1$ adenosine receptor–linked contractile and adenylate cyclase responses and studied the regulation of affinity state as well as receptor number in the desensitization process.

Materials and Methods

Atrial myocytes from chick embryos 14 days in ovo were maintained in culture as previously described.$^8,12$ To keep the potential endogenous adenosine to a minimum in the culture medium, adenosine deaminase (ADA) was added (2 IU/ml) to the culture medium as previously described.$^13$ For assay of adenylate cyclase activity, cells were broken and Dounce homogenized in buffer containing 50 mM Tris-HCl, 5 mM MgCl$_2$, 1 mM EGTA, 1 mM EDTA, 32 $\mu$g/ml leupeptin, 32 $\mu$g/ml lima bean trypsin inhibitor, 32 $\mu$g/ml soybean trypsin inhibitor, and 0.01% CHAPS, pH 7.4. Cell homogenates were then pretreated with 20 $\mu$g/ml alamethicin and with 5 IU/ml ADA at 25°C for 10 minutes before the assay. Unless otherwise indicated, experiments were carried out in the presence of the protease inhibitors, alamethicin, ADA, and CHAPS.

Adenylate Cyclase and Lowry Assays

Assays of adenylate cyclase activity were carried out as previously described.$^9$ Proteins were determined according to the method of Lowry et al,$^{14}$ using bovine serum albumin as the standard.

Contractility

Measurement of cardiac contractility from single myocytes was performed with an optico-video motion detection system as previously described.$^{15}$

Radioligand Binding Studies

Cultured atrial cells were harvested and homogenized with a Dounce in 50 mM Tris-HCl, pH 7.4, containing 0.3 IU/ml ADA. Homogenates were centrifuged at 30,000g for 20 minutes and the membrane pellets resuspended in the same buffer and further incubated at 25°C for 10 minutes before the binding assay. Radioligand binding was carried out using [$^3$H]-8-cyclopentyl-1,3-dipropylxanthine ([H]CPX) (DuPont, Boston) as the adenosine receptor radioligand. The percent of nonspecific binding at the radioligand $K_d$ was about 60–65%, similar to that described previously.$^8$

Data Analysis

Analyses of the competition studies were carried out by the LIGAND program$^{16}$ as previously described.$^8$ Best-fit curves for binding data and the affinity constants for monophasic and biphasic curves were generated. Saturation isotherms were also analyzed by the computerized nonlinear, least-squares analysis, which yielded $K_d$ and $B_{\text{max}}$ values similar to those obtained by linear regression analyses of the transformed Scatchard plot, consistent with the results of the previous study.$^8$

In the curve-fitting analyses of competition experiments, $F$ test was used to determine whether a two-site model fit the data better than a one-site model ($F$ value indicating $p<0.01$); the Runs test was used to determine whether there was no systematic departure of the data points from the fitted curve ($p>0.05$). Data are expressed as mean±SEM.

Materials

ATP, GTP, lima bean trypsin inhibitor, soy bean trypsin inhibitor, leupeptin, L-isoproterenol, and alamethicin were all purchased from Sigma Chemical Co., St. Louis. ADA was obtained from Boehringer Mannheim Corp., Indianapolis. [H]ATP was from ICN Chemicals and Radioisotopes, Irvine, Calif. [$^{32}$C]Cyclic AMP was from Du Pont; embryonic chick eggs were purchased from Spafas Inc., Storrs, Conn.

Results

Desensitization of $A_1$ Adenosine Receptor–Linked Adenylate Cyclase in Cultured Atrial Myocytes

To determine the chronic effect of adenosine agonist exposure on the ability of the receptor to mediate inhibition of adenylate cyclase activity, the culture was treated with R-PIA for varying times, and the ability of R-PIA to inhibit adenylate cyclase activity was determined in membranes prepared from both control and agonist-treated cultures. Exposure of the culture to 1 $\mu$M R-PIA abolished the subsequent ability of R-PIA (1 $\mu$M) to cause inhibition of isoproterenol-stimulated adenylate cyclase activity (Table 1). Inhibition of adenylate cyclase activity caused by 1 $\mu$M R-PIA (maximal inhibition of adenylate cyclase activity) desensitized in both a dose-dependent (IC$_{50}$, 10±6 nM; $n=6$) and a time-dependent (t$_{1/2}$, 7.5±1 hours; $n=6$) manner (Figures 1 and 2, respectively). The $A_1$ adenosine receptor–selective antagonist CPX (10 nM) blocked the desensitization caused by treatment of the culture with R-PIA (data not shown), providing further evidence that desensitization of adenylate cyclase inhibition was mediated by the $A_1$ subtype. Adenylate cyclase
was also desensitized to the inhibitory effect of other $A_1$ adenosine receptor–selective agonists, such as N$^\ominus$-cyclopentyladenosine (CPA) (isoproterenol alone [10 $\mu$M], 944±22 pmol cyclic AMP/mg/10 min versus isoproterenol plus CPA [1 $\mu$M], 936±19; mean±SEM of triplicates, typical of two experiments), indicating that the desensitization is an $A_1$ adenosine receptor–specific phenomenon. We next determined whether desensitization of the maximal inhibition of adenylate cyclase activity is associated with a change in the $ED_{50}$ for R-PIA–induced inhibition of adenylate cyclase activity. In membranes prepared from cell exposed to 1 $\mu$M R-PIA for 6 hours, the $ED_{50}$ for R-PIA–induced inhibition of adenylate cyclase activity was 40±15 nM ($n=4$) and was similar to that obtained in membranes of control culture (30±11, $n=4$).

Desensitization of Contractile Response to $A_1$ Adenosine Receptor Agonist

To determine whether chronic exposure of the culture to R-PIA also causes desensitization of contractile response and whether such desensitization parallels desensitization of adenylate cyclase response, we studied the effect of chronic exposure of the culture to R-PIA on the subsequent contractile response to R-PIA. In culture not preexposed to R-PIA, the atrial myocyte responded to R-PIA with a decrease in contractile amplitude (Figure 3, upper tracing). However, in cells preexposed to 1 $\mu$M R-PIA for 24 hours, subsequent exposure to the maximal concentration of R-PIA (10 $\mu$M) caused an attenuated negative inotropic response (Figure 3, lower tracing). The contractile response to 10 $\mu$M R-PIA (the maximal contractile response) desensitized in a time-dependent (12±1.4 ±0.7 hours; $n=12$) and dose-dependent (IC$_{50}$: 20±8 nM; $n=10$) manner (Figures 2 and 4, respectively). The $A_2$ adenosine receptor–selective antagonist CPX blocked the ability of R-PIA to induce desensitization of negative inotropic response, providing further evidence that desensitization of contractile response is mediated by adenosine receptor of the $A_2$ subtype. Desensitization of negative inotropic response also occurred with other $A_1$ adenosine receptor–selective agonists such as CPA (control culture, percent decrease in contractile amplitude in response to 10 $\mu$M CPA was 34.8±4%, $n=17$, versus R-PIA–treated culture [1 $\mu$M for 24 hours], 17±3.5%, $n=34$; $p<0.01$, t test), indicating that desensitization of contractility is an $A_1$ adenosine receptor–specific event. We next determined whether attenuation in the maximal contractile response of the R-PIA–treated culture is associated with an alteration in $ED_{50}$ for the adenosine agonist–mediated negative inotropic response. Dose–response relations for R-PIA–induced negative inotropic effect in both control and desensitized atrial myocytes are summarized in Figure 5. $ED_{50}$ for R-PIA–induced negative inotropic response was 265±72 nM ($n=12$) in R-PIA–treated culture and was similar to that obtained in the control culture (310±80 nM, $n=11$; $p>0.05$, t test).

The kinetics of the $A_1$ adenosine receptor–mediated negative inotropic responses was compared with that of the adenylate cyclase inhibition. The percent maximal contractile response determined in myocyte preexposed to R-PIA was significantly higher than the percent maximal inhibition of ade-
nlate cyclase for each time point (Figure 2, p<0.01, two-tailed t test). In contrast to desensitization of A<sub>1</sub> adenosine receptor–mediated inhibition of adenylylate cyclase activity, more than 60% of the initial contractile response remained after 24 hours of 1 μM R-PIA treatment, whereas R-PIA–induced inhibition of adenylylate cyclase activity was completely lost. Thus, the activation of adenosine receptor appeared capable of eliciting a negative inotropic response even when the receptor was no longer coupled to the adenylylate cyclase. To further examine whether the negative inotropic response to R-PIA involves a cyclic AMP–independent mechanism, we tested the effect of R-PIA on the contractile amplitude in the presence of 0.5 mM dibutyl cyclic AMP, which caused a modest increase in contractile amplitude in both control (percent increase in amplitude, 23.3±5%, n=8) and the R-PIA–treated (28.7±6.7%, n=8) cultures. R-PIA (10 μM) was capable of inhibiting the increase in contractile amplitude stimulated by dibutyl cyclic AMP in the control culture, whereas the R-PIA–induced decrease in contractility from culture preexposed to R-PIA was attenuated (Table 2). The percent decrease in contractile amplitude caused by R-PIA in treated culture was about half that in control culture.

Receptor Binding Studies in Control and Agonist-Treated Myocyte Membranes

To study changes in receptor density and affinity in atrial myocytes chronically exposed to R-PIA, [3H]CPX binding studies were performed in membranes from control and agonist-treated cells (Figure 6). In membranes prepared from R-PIA–treated culture (1 μM for 24 hours), the density of A<sub>1</sub> adenosine receptor was significantly lower than the density in membranes of control culture (R-PIA–treated, 18±1.1 fmol/mg protein, n=10, versus control, 31±1.6, n=14). Affinity of the receptor for the antagonist radioligand [3H]CPX was not different between control and agonist-treated cultures (control, 3±0.23 nM, n=14, versus R-PIA–treated, 2.7±0.3, n=10). These results indicated that A<sub>1</sub> adenosine receptors are downregulated in response to chronic exposure of the culture to R-PIA.

To determine whether downregulation of A<sub>1</sub> adenosine receptor is associated with any change in the proportion of the high- and low-affinity receptor sites, competition of R-PIA with [3H]CPX in membranes of control and agonist-treated cultures was carried out. The competition curve for R-PIA revealed both high-affinity (R<sub>H</sub>) and low-affinity (R<sub>L</sub>) receptor sites. In membranes of control culture, R<sub>H</sub> was 51±3% (n=5). However, in membranes from cultures preexposed to R-PIA (1 μM for 24 hours), the competition curve for R-PIA was steep and best described by a one-site model (LIGAND) (Hill coefficient, 0.91±0.2, n=5), indicating that only the low-affinity sites were present (Figure 7). Competition carried out in the presence of GTP using membranes from R-PIA–treated cultures was best fit by a one-site model with a Hill coefficient of 1.1±0.1 (n=4). The affinity constant was 442±110 nM and was similar to the affinity constant for the low-affinity site (K<sub>L</sub>) obtained in Table 3 (p>0.05, t test).

To compare the kinetics of receptor downregulation and shift in affinity state, time courses for both
the downregulation of A1 adenosine receptor and the conversion of $R_h$ to $R_t$ in cultures chronically exposed to R-PIA were obtained. Both processes were time dependent and occurred over hours (Table 3, Figure 2). However, while all of the high-affinity sites were converted to the low-affinity form after 24 hours of exposure of the culture to 1 μM R-PIA, a significant number of A1 adenosine receptors remained after similar exposure to R-PIA (62±3%, n=10). The difference in the percentage of basal number of A1 adenosine receptor and basal high-affinity receptor was statistically different in cultures treated with 1 μM R-PIA for 8 hours and for 24 hours (p<0.01, t test for both comparisons).

**Discussion**

Previous studies demonstrated that chronic exposure of intact chick embryos to the A1 adenosine receptor–selective agonist R-PIA caused downregulation of the A1 adenosine receptor with concomitant desensitization of adenosine receptor–mediated antagonism of forskolin-stimulated positive inotropic response in ventricular slices.10 Another study, using chronic subcutaneous administration of R-PIA to rats, demonstrated desensitization of A1 adenosine receptor–mediated inhibition of adenylate cyclase activity in adipocyte membranes.11 However, it is not clear whether desensitization of contractile response and that of adenosine receptor–linked adenylate cyclase occur in the same tissue and whether the two processes develop coordinately. Such studies might provide information on whether the adenosine receptor–mediated inhibition of adenylate cyclase is involved in mediating the negative inotropic response of adenosine in the atrial myocyte. For example, a clear discrepancy between the development of desensitization of A1 adenosine receptor–mediated decrease in basal contractility and that of desensitization of the receptor-linked adenylate cyclase would suggest against a prominent role of the adenosine-induced inhibition of adenylate cyclase in modulating the basal contractile state of the atrial myocyte. Finally, the role of receptor downregulation and shift in affinity state in mediating the desensitization of

**Figure 3.** Contractile response to R-N6-(2-phenylisopropyl)-adenosine (R-PIA) in control and agonist-treated culture. Upper tracing: After a 20-minute equilibration period, cell was superfused with HEPES-buffered medium containing 10 μM R-PIA, and changes in amplitude of cell motion were monitored (arrow). Lower tracing: Cell was preexposed to 1 μM R-PIA for 24 hours and washed free of R-PIA during superfusion with HEPES-buffered medium. After achieving steady state, the amplitude of cell motion was recorded and then switched to medium containing 10 μM R-PIA (arrow).

**Figure 4.** Effects of prior exposure of the control to various concentrations of R-N6-(2-phenylisopropyl)-adenosine (R-PIA) on the subsequent ability of adenosine agonist to cause negative inotropic response. Measurement of contractility was carried out as described in Figure 3 legend. R-PIA (10 μM)–induced percent decrease in contractile amplitude in culture preexposed to various concentrations of R-PIA for 24 hours was normalized to the percent decrease in contractile amplitude caused by R-PIA in the control culture as percent maximum. Data are plotted as percent maximal contractile response to R-PIA vs. the concentration to which the culture was preexposed. Data are represented as mean±SEM, n=10.
these two functional responses remains unknown. In the present study, we used the spontaneous contracting atrial myocyte cultured from 14-day-old chick embryo as a model system to investigate these possibilities. Because R-PIA concentration can be varied experimentally in the culture medium, the kinetics and dose dependence of the desensitization of contractile and adenylate cyclase responses could be feasibly obtained and compared. A direct correlation between desensitization of the adenosine responses and changes in A<sub>1</sub> adenosine receptor number and affinity state could also be determined. Prior study demonstrated that A<sub>1</sub> adenosine receptors are present and are negatively coupled to adenylate cyclase activity and myocyte contractility<sup>4</sup> and that adenosine receptors in these membranes are coupled to a pertussis toxin-sensitive G protein to form a high-affinity state of the receptor. The present data indicate that desensitization of adenosine receptor–mediated inhibition of adenylate cyclase activity and of basal myocyte contractility occurs noncoordinately. Loss of inhibition of adenylate cyclase activity during desensitization was total and was associated with conversion of the high-affinity site to a low-affinity form, whereas desensitization of the negative inotropic response was partial, as was the downregulation of the total number of A<sub>1</sub> adenosine receptors.

To better quantify the loss of inhibition of adenylate cyclase activity, the present study used isoproterenol stimulation of the cyclase before determining the inhibition in the membrane preparation. The ability of R-PIA to cause inhibition of isoproterenol-stimulated adenylate cyclase activity was completely lost as a result of chronic exposure of the myocyte to R-PIA. In contrast, prior exposure of the myocyte to the same concentration of R-PIA for the same time resulted in only a partial desensitization of the contractile response to R-PIA. The ability of the adenosine receptor to mediate a decrease in basal contractility when the receptor is no longer capable of coupling to an inhibition of adenylate cyclase activity suggests that inhibition of the basal adenylate cyclase cannot explain the ability of receptors to mediate the decrease in basal contractile amplitude. Because of a selective uncoupling of A<sub>1</sub> adenosine receptor from inhibition of adenylate cyclase while the receptor remained coupled to a negative inotropic response, the present data provide direct evidence that the negative modulation of basal contractility by adenosine in atria is the result primarily of the activation of

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<th>TABLE 2. Contractile Response to R-N&lt;sup&gt;6&lt;/sup&gt;-(2-Phenylisopropyl)-Adenosine in the Presence of 0.5 mM Dibutyryl Cyclic AMP</th>
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<td>% Maximum</td>
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Values are mean±SEM of eight cells from four cultures. After achieving a steady state in the presence of dibutyryl cyclic AMP, the contractile amplitude obtained with 10 μM R-N<sup>6</sup>-(2-phenylisopropyl)-adenosine (R-PIA) was normalized to that obtained prior to the infusion of R-PIA as percent maximum.

*Atrial cultures treated with 1 μM R-PIA for 24 hours.
other A₁ adenosine receptor–coupled effectors such as the direct, cyclic AMP–dependent stimulation of K⁺ channel and possibly, inhibition of Ca²⁺ channel.¹² The finding that R-PIA caused a decrease in the contractile amplitude of these cultured atrial cells in the presence of dibutyryl cyclic AMP, consistent with previous studies, further supports the notion that the activation of adenosine receptor is capable of negatively modulating the contractility of atrial myocyte via a cyclic AMP–dependent pathway.

The desensitization of the A₁ adenosine receptor–mediated inhibition of myocyte contractility and adenylate cyclase activity involved an attenuation of the maximal response to R-PIA without any change in ED₅₀ in cells preexposed to the adenosine agonist. The atrial myocyte was desensitized to the effects of all R-PIA concentrations on both the contractility and the adenylate cyclase. Thus, the differential sensitivity of the two functional responses to R-PIA cannot be explained by a differential shift in ED₅₀ for the R-PIA effects on these two responses in the desensitized myocyte.

A₁ adenosine receptors exist in two affinity forms in membrane preparation of these cultured myocytes with a high-affinity site sensitive to guanine nucleotide and pertussis toxin treatment.⁸ Prior exposure of the culture to R-PIA shifted the high-affinity state of the adenosine receptor to a low-affinity form that was not sensitive to the effect of GTP. The conversion from high- to low-affinity state was total, with loss of all high-affinity sites on exposure of the myocyte to 1 μM R-PIA for 24 hours. On the other hand, down-regulation of A₁ adenosine receptors appeared to be partial, with about 60% of the basal level of receptor remaining after 24 hours of exposure to 1 μM R-PIA. The conversion of the high-affinity A₁ adenosine receptor to a low-affinity form correlated closely with the loss of A₁ adenosine receptor–mediated inhibition of adenylate cyclase activity, whereas such conversion differed from that of the contractile response to R-PIA. The parallelism between the kinetics of the disappearance of high-affinity receptor and that of the loss of adenylate cyclase inhibition is similar to the findings of Puttfarcken et al on desensitization of opioid inhibition of adenylate cyclase in 7315c cell membranes. Together, these data indicate that the ability of the receptor to form a high-affinity state is associated with the ability of the adenosine receptor to mediate inhibition of adenylate cyclase activity. These results do not necessarily contradict those of a previous study, which suggested that only the low-affinity form of A₁ adenosine receptor can be detected in the intact rat ventricular myocyte because of the high concentration of endogenous guanine nucleotides and that the low-affinity constant for adenosine agonist corresponded to the IC₅₀ values for inhibition of cyclic AMP accumulation. In the previous and present studies on cultured embryonic chick atrial cells, GTP was present and necessary for the adenosine receptor to mediate adenylate cyclase inhibition in membrane homogenates, whereas the high-affinity form of the receptor can be detected only in membranes in the absence of GTP or other guanine nucleotides. The high-affinity form of the receptor, although representing a unique binding property in membranes in the absence of GTP, does not predict the extent of A₁ receptor coupling to a high-affinity form in the intact, native plasma membranes of cultured myocytes. Nevertheless, the present data are consistent with the hypothesis that formation of the high-affinity adenosine receptor is involved in mediating the inhibition of adenylate cyclase activity.

On the other hand, in atrial myocytes partially desensitized to the contractile effect of R-PIA, the A₁ adenosine receptor appeared capable of mediating
the contractile response even in the absence of any high-affinity $A_1$ adenosine receptor. The simplest interpretation of our data is that although high-affinity $A_1$ adenosine receptors are closely coupled to adenylyl cyclase inhibition, they are not directly involved in mediating all the effectors and currents responsible for the negative inotropic response. However, the high-affinity formation is likely a transient part of a continuous cycle linking high- and low-affinity receptor states in the intact cell.\(^{20,21}\)

Also, the high-affinity $A_1$ adenosine receptor is detected only in membranes in the absence of GTP and may not reflect the transient formation of the high-affinity receptor in intact myocytes. Thus, the present data, while raising the possibility that the low-affinity receptor form is coupled to some of the effectors involved in mediating the negative inotropic effect, must be interpreted in the context of these considerations.

The present study differs from studies carried out by others\(^{22,23}\) who examined the effects of chronic muscarinic agonist exposure on the muscarinic receptor number and the muscarinic functional responses in cultured chick heart cells. The ability of muscarinic agonist to increase $^4$K efflux and to decrease the beating rate in heart cells preexposed to carbamylcholine was completely absent after the chronic, prior exposure of the culture. The difference in the desensitization of $A_1$ adenosine receptor-mediated contractile response and that of the muscarinic cholinergic receptor-mediated responses is not clear but may be related to a higher density of the muscarinic receptor in these cultured cells. Alternatively, the mechanism regulating the negative inotropic response studied presently may differ from the mechanism controlling the negative chronotropic response, and these two mechanisms may have differential sensitivity to the chronic effect of agonist exposure. Overall, the study indicates that adenylyl cyclase inhibition and the negative inotropic response mediated by atrial $A_1$ adenosine receptors have differential sensitivity to the chronic effects of adenosine agonist. The ability of adenosine receptors to mediate a decrease in the basal contractility when they are no longer capable of coupling to an inhibition of adenylyl cyclase supports the notion that $A_1$ adenosine receptor–coupled effectors other than adenylyl cyclase are critical in causing the direct, negative inotropic effect of adenosine in atria. Uncoupling of the $A_1$ adenosine receptor from a high-affinity state correlated with uncoupling of the receptor from an inhibition of adenylyl cyclase, whereas downregulation of the receptor paralleled desensitization of the contractile response.

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References

8. Liang BT: Characterization of the adenosine receptor in cultured embryonic chick atrial myocytes: Coupling to modulation of contractility and adenylyl cyclase activity and identification by direct radio ligand binding. J Pharmacol Exp Ther 1989;249:775–784
12. Liang BT, Galper JB: Differential sensitivity of $\alpha_1$ to $\alpha_2$ to ADP-ribosylation by pertussis toxin in the intact cultured embryonic chick ventricular myocyte: Relationship to the role of G-proteins in the coupling of muscarinic cholinergic receptors to inhibition of adenylyl cyclase activity. Biochem Pharmacol 1988;37:4549–4555


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