α₂C Adrenoceptors Inhibit Adenylyl Cyclase in Mouse Striatum: Potential Activation by Dopamine

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ABSTRACT

α₂C adrenoceptors occur in high density in the striatum, but the functional role of these receptors is uncertain. Mice with targeted inactivation of the α₂C adrenoceptor gene (Adra2c<sup>-/-</sup>) and genetically related control mice expressing the wild-type α₂C adrenoceptor (Adra2c<sup>+/+</sup>) were used to determine whether striatal α₂C adrenoceptors modulate adenylyl cyclase activation. In striatal slices from Adra2c<sup>-/-</sup> mice, the α₂C adrenoceptor antagonists RX821002 facilitated forskolin-stimulated cyclic AMP accumulation in a concentration-dependent manner. In contrast, RX821002 had no effect on forskolin-stimulated cAMP accumulation in striatal slices from Adra2c<sup>+/+</sup> mice or in striatal slices from Adra2c<sup>-/-</sup> mice treated with reserpine and α-methyl-p-tyrosine to deplete monoamine neurotransmitters. Given the sparse innervation of the striatum by noradrenergic neurons, the possibility that dopamine can activate the mouse α₂C adrenoceptor at physiologically relevant concentrations was investigated using normal rat kidney (NRK) cells transfected with the mouse α₂A or α₂C adrenoceptor cDNA (NRK-α₂A or NRK-α₂C cells). Inhibition of [<sup>3</sup>H]RX821002 binding by agonists in homogenates of transfected cells revealed an affinity of dopamine for α₂C adrenoceptors that was higher than the affinity of norepinephrine for its cognate receptor, the α₂A adrenoceptor. Both norepinephrine and dopamine inhibited forskolin-stimulated CAMP accumulation in intact NRK-α₂C cells. In NRK-α₂A cells, norepinephrine facilitated forskolin-stimulated CAMP accumulation, an effect not observed for dopamine. Together, these data demonstrate that the α₂C adrenoceptor is negatively coupled to adenylyl cyclase and is tonically activated in mouse striatal slices. The endogenous activator of the striatal α₂C adrenoceptor may be dopamine, as well as norepinephrine.

α₂ adrenoceptors mediate many physiological functions and consist of three distinct subtypes: α₂A, α₂B, and α₂C. The α₂C adrenoceptor subtypes are encoded by three different genes in mouse, rat, and humans (MacDonald et al., 1997) and are expressed (mRNA) or coexpressed in many brain regions (Scheinin et al., 1994; MacDonald and Scheinin, 1995; Wang et al., 1996). Although the α₂A adrenoceptor has a broad and dense distribution in the brain, the expression of the α₂C adrenoceptor gene is largely limited to the hippocampus, olfactory system, and basal ganglia (Nicholas et al., 1993; Scheinin et al., 1994; MacDonald and Scheinin, 1995; Talley et al., 1996; Tavares et al., 1996). In fact, radioligand binding to the α₂C adrenoceptor occurs in highest density in the striatum, relative to other brain regions (Ordway et al., 1993; Uhlen et al., 1997). α₂ adrenoceptors are located on noradrenergic neurons or are located in brain regions that are innervated by noradrenergic neurons because presynaptic markers of noradrenergic innervation (e.g., dopamine β-hydroxylase and norepinephrine transporters) are in these regions (Grzanna et al. 1977; Moore and Card, 1984; Ordway et al., 1993; Jursky et al., 1994; Ordway, 1995). However, the high concentration of α₂C adrenoceptor mRNA and protein in the striatum is peculiar given the paucity of noradrenergic innervation to this brain region (Moore and Card, 1984; Nicholas et al., 1993; Jursky et al., 1994; Scheinin et al., 1994; Rosin et al., 1996; Wang et al., 1996).

The lack of a selective ligand for the α₂C adrenoceptor has slowed the discovery of its functions in the brain. Recently, molecular biological techniques have advanced understanding of this receptor. For example, using mice lacking a functional α₂C adrenoceptor and mice overexpressing this receptor, Sallinen et al. (1997) demonstrated that this receptor mediates, at least in part, hypothermia in response to s.c. administration of the α₂ adrenoceptor agonist dexmedetomidine. Furthermore, the α₂C adrenoceptor modulates acoustic startle reflex and its prepulse inhibition, as well as isolation-induced aggression (Sallinen et al., 1998).

The functional role of the α₂C adrenoceptor in the striatum is uncertain. Using antisense oligonucleotide infusions directly into the rat striatum, we demonstrated that the α₂C adrenoceptor is negatively coupled to adenylyl cyclase and that this receptor appears to be tonically activated in striatal

ABBREVIATION: NRK, normal rat kidney.

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slices (Lu and Ordway, 1997b). Given the dense dopaminergic innervation and sparse noradrenergic innervation of the striatum, we postulated that the striatal \( \alpha_{2C} \) adrenoceptor may be activated promiscuously by dopamine. In the present study, we used mice with a targeted inactivation of the \( \alpha_{2C} \) adrenoceptor gene (\( \text{Adra2c}^{-/-} \)) and wild-type mice (\( \text{Adra2c}^{+/+} \)) were generated at Stanford University (Stanford, CA) and maintained at Roche Biosciences (Palo Alto, CA). The method of generation of the two mice has been detailed by Link et al. (1995). In brief, one copy of the murine \( \text{Adra2c} \) gene was inactivated in R1 129/Sv embryonic stem cells (Nagy et al., 1993), which lacks critical structural sequences required for G protein coupling and ligand binding. These cells were injected into C57BL/6J blastocyst, and (C57BL/6J x DBA/2J)F1 mice were used to breed resulting chimera mice. These mice were crossed back for several generations to C57BL/6J mice and intercrossed to form the colony. All experimental \( \text{Adra2c}^{-/-} \) mice were homozygous for the mutation. The genetic control of the wild-type strain was constituted primarily by C57BL/6J with a small contribution from 129/Sv and DBA/2J stains. The mice from both strains are viable and fertile and appear grossly normal. The mouse wild-type strain was constituted primarily by C57BL/6J with a small contribution from 129/Sv and DBA/2J stains. The mouse from both strains are viable and fertile and appear grossly normal.

Materials and Methods

Animals. Male mice with a targeted inactivation of the \( \alpha_{2A} \) adrenoceptor gene (\( \text{Adra2c}^{-/-} \)) and wild-type mice (\( \text{Adra2c}^{+/+} \)) were generated at Stanford University (Stanford, CA) and maintained at Roche Biosciences (Palo Alto, CA). The method of generation of the two mice has been detailed by Link et al. (1995). In brief, one copy of the murine \( \text{Adra2c} \) gene was inactivated in R1 129/Sv embryonic stem cells (Nagy et al., 1993), which lacks critical structural sequences required for G protein coupling and ligand binding. These cells were injected into C57BL/6J blastocyst, and (C57BL/6J x DBA/2J)F1 mice were used to breed resulting chimera mice. These mice were crossed back for several generations to C57BL/6J mice and intercrossed to form the colony. All experimental \( \text{Adra2c}^{-/-} \) mice were homozygous for the mutation. The genetic control of the wild-type strain was constituted primarily by C57BL/6J with a small contribution from 129/Sv and DBA/2J stains. The mice from both strains are viable and fertile and appear grossly normal. The mouse wild-type strain was constituted primarily by C57BL/6J with a small contribution from 129/Sv and DBA/2J stains. The mouse from both strains are viable and fertile and appear grossly normal.

Cell Culture. NRK cells stably transfected with mouse \( \alpha_{2A} \) and \( \alpha_{2C} \) adrenoceptors were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin, 100 \( \mu \)g/ml streptomycin, 2 mM L-glutamine, and gentamicin (G418; 250 \( \mu \)g/ml) at 37°C in 95% humidified air with 5% CO\(_2\). Transfected cell lines were obtained from Brian K. Kohlka (Stanford University, Stanford, CA). Culture medium and supplements were obtained from Gibco BRL (Grand Island, NY). Transfections of these NRK cells have been described by Daunt et al. (1997).

Homogenate Binding. Saturation and competition binding experiments using \( [\text{H}]\text{RX821002} \) (methoxy idazoxan) were performed as described by Bylund et al. (1988) and Ordway (1995), with minor modifications. Frozen cells were thawed quickly at 37°C, immediately put on ice, and then centrifuged at 1000g for 8 min. Pellets were washed twice with ice-cold PBS (10 ml) and centrifuged at 1000g for 8 min. Resulting pellets were homogenized in 10 ml of ice-cold Tris buffer (50 mM Tris, pH 8.0) using a Polytron (setting 6, for 20 s) and then centrifuged at 40,000g for 30 min. Pellets were then washed once with Tris buffer, centrifuged as above (40,000g for 30 min), homogenized in 25 mM glycylglycine buffer (pH 7.4), and centrifuged again. Final pellets were resuspended in ice-cold glycylglycine buffer.

Competition reactions were performed in glycylglycine buffer and were started by adding 50 \( \mu \)l of cell suspension (in duplicate) to borosilicate tubes containing 2.2 nM \( [\text{H}]\text{RX821002} \) and dopamine or norepinephrine (when noted) at 10 concentrations ranging from 20 nM to 2 nM. Binding reactions (total volume 0.25 ml) were incubated for 30 min at room temperature. Reactions were stopped by adding 3 ml of ice-cold Tris buffer and by vacuum filtration (Brandel, Gaithersburg, MD) through glass fiber filters (no. 25, Schleicher & Schuell, Keene, NH). Filters were washed twice with 3 ml of ice-cold Tris buffer.

Saturation curves of the binding of \( [\text{H}]\text{RX821002} \) were constructed using concentrations ranging from 0.05 to 10 nM. Non-specific binding was determined at each concentration of \( [\text{H}]\text{RX821002} \) with 50 \( \mu \)M phenolamine. Incubation, termination, and filtration were carried out as described above for competition experiments. Radioactivity was counted with a liquid scintillation counter (LS8801; Beckman, Irvine, CA). Protein was assayed according to the method of Lowry et al. (1951).

cAMP Assay. Levels of cAMP in slices of mouse striatum were measured by radioimmunoassay (Ordway et al., 1987). Mice were sacrificed by decapitation, and the brains were removed. To deplete monoamines, mice were treated with 2.5 mg/kg reserpine 24 h before and 50 mg/kg \( \alpha \)-methyl-\( \beta \)-tyrosine 1 h before decapsulation. A combination of reserpine and \( \alpha \)-methyl-\( \beta \)-tyrosine pretreatment can cause up to 99% and 80% depletion of striatal dopamine and norepinephrine, respectively (Koss and Christensen, 1979; White et al., 1988). Striata were dissected and chopped into 300-\( \mu \)m prisms using a McIlwain tissue chopper (Brinkmann Instruments, Westbury, NY). Strial slices prepared from two mice were dispersed in 25 ml of oxygenated (95% \( \text{O}_2 \)/5% \( \text{CO}_2 \)) Krebs-Ringer buffer (2.4 mM \( \text{MgSO}_4 \), 0.1 mM \( \text{KH}_2\text{PO}_4 \), 118 mM \( \text{NaCl} \), 4.8 mM KCl, 1.3 mM \( \text{CaCl}_2 \), and 0.01 mM disodium EDTA) at 37°C. Slices were preincubated for 1 h and washed with fresh oxygenated buffer every 15 min. After preincubation, slices were dispersed in 850-\( \mu \)l aliquots (about 300 \( \mu \)g protein/tube, in triplicate) into individual reaction tubes for 10 min. The volumes of drug and tissue aliquots were adjusted so that the final volume of the slice preparation was 1 ml. The phosphodiesterase inhibitor 3-isobutyl-methyxanthine (0.5 mM) and RX821002 (in concentrations noted) were added to each tube 15 min before the addition of forskolin. Most rapidly higher concentrations of \( \text{RX821002} \) and forskolin were used in the depletion experiments (compared with the experiment with \( \text{Adra2c}^{-/-} \) and \( \text{Adra2c}^{+/+} \) mice) in an effort to increase the net change in cAMP between the forskolin-alone and forskolin-plus-RX821002 conditions. After the addition of forskolin (in concentrations noted), reactions proceeded for 10 min and were stopped by the addition of ice-cold 2.5% perchloric acid (500 \( \mu \)l). Samples were sonicated for 15 s and centrifuged at 30,000g for 15 min. Pellets were dissolved in 0.1 N NaOH for protein measurement. The supernatants were neutralized with Ca\( \text{CO}_3 \) (40 mg/tube) and centrifuged twice at 30,000g for 15 min to remove excess Ca\( \text{CO}_3 \). Final supernatants were assayed for cAMP by radioimmunoassay. Aliquots of samples (100 \( \mu \)l) were incubated, in duplicate, with an [\( ^{125}\text{I} \)]dextran derivative of cAMP and antisum (New England Nuclear, Boston, MA) in an ice bath for 18 h. Antibodies were precipitated with ice-cold ethanol (95%). After centrifugation at 10,000g for 30 min, supernatants were aspirated and tubes were inverted and dried. Radioactivity was estimated using a gamma counter (Beckman Gamma 4000; Packard Instrument Company, Meriden, CT) at an efficiency of 80%. cAMP concentrations (pmol/mg protein) in each sample were determined by comparing the inhibition of \( ^{125}\text{I} \)cAMP binding to antibody caused by known concentrations of cAMP. Protein was assayed according to the method of Lowry et al. (1951).

The measurement of cAMP in NRK cells was similar to that described for slices of mouse striatum. Briefly, NRK cells were grown to confluency, and cells from two tissue culture flasks (250 ml) were dispersed in 25 ml of oxygenated (95% \( \text{O}_2 \)/5% \( \text{CO}_2 \)) Krebs-Ringer buffer at 37°C. Cells were preincubated for 30 min and washed with fresh oxygenated buffer every 15 min. The phosphodiesterase inhibitor 3-isobutyl-methyxanthine (0.5 mM), propranolol (1 \( \mu \)M, to block \( \beta \)-adrenoceptor-mediated responses), and RX821002 (when noted)
were added to each tube for 15 min. Dopamine or norepinephrine (at concentrations indicated) was added 5 min before the addition of forskolin (10 μM). All other procedures were performed as described above for slices of mouse striata.

Statistics. All saturation and competition binding data were analyzed using nonlinear regression analysis (Prism, version 1.0; GraphPad Software, San Diego, CA). Data were fit first to a model assuming binding to one site and then to a model assuming two sites of interaction. The best fit was determined statistically by a comparison of the sum of squares of residuals using the equation $F = \frac{(SS_1 - SS_2)/df_1}{df_2}$, in which $SS_1$ and $df_1$ are the sum of squares and degrees of freedom from the one binding-site model and $SS_2$ and $df_2$ are those from the two-binding site model. A two-site fit was considered a significantly better fit if the $F$ value was larger than that reported in the $F$ statistic table at $p < .05$ for the numerator of $df_1 - df_2$ and the denominator of $df_2$. Data from cAMP experiments were analyzed by one-way repeated measures ANOVA followed by a Student-Newman-Keuls test. All data are shown as mean ± S.E.M.

Results

Effects of RX821002 on Forskolin-Stimulated cAMP levels in Striatum. RX821002 is an $\alpha_2$ adrenoceptor antagonist that is not selective with respect to the $\alpha_2$ adrenoceptor subtypes. RX821002 alone has been shown to enhance forskolin-stimulated cAMP accumulation in a dose-dependent manner in rat striatal slices (Lu and Ordway, 1997b). Initial experiments were performed to verify that this effect occurs in the mouse striatum. RX821002 enhanced forskolin-stimulated cAMP accumulation in a concentration-dependent manner in slices of striata obtained from Adra2c$^{-/-}$ mice (Fig. 1). An enhancement of 60% was observed at the highest concentration of RX821002 (100 μM). To determine whether adenyl cyclase activation in the striatum is mediated by blockade of the $\alpha_2C$ adrenoceptor, experiments were performed to examine the ability of RX821002 (10 nM) to enhance forskolin (1 μM)-stimulated cAMP accumulation in Adra2c$^{-/-}$ and Adra2c$^{-/-}$ mice. As in Fig. 1, RX821002 significantly enhanced forskolin-stimulated cAMP accumulation in striatal slices of Adra2c$^{-/-}$ mice ($p < .05$; Fig. 2). In contrast to Adra2c$^{-/-}$ mice, no enhancement of cAMP accumulation by RX821002 was observed in striatal slices of Adra2c$^{-/-}$ mice. Rather, RX821002 tended to reduce forskolin-stimulated cAMP accumulation in Adra2c$^{-/-}$ mice, although this effect did not reach statistical significance. Both basal and forskolin-stimulated cAMP concentrations were moderately higher in Adra2c$^{-/-}$ striata than in Adra2c$^{+/+}$ striata.

The ability of an $\alpha_2$ adrenoceptor antagonist to enhance forskolin-stimulated cAMP accumulation in the absence of an exogenously added agonist implies that $\alpha_2$ adrenoceptors are activated tonically in the striatal slice preparation. To examine this possibility, the ability of RX821002 (100 nM) to enhance cAMP accumulation was evaluated in Adra2c$^{+/+}$ mice after the administration of the monoamine-depleting drugs reserpine and α-methyl-p-tyrosine (see Materials and Methods) or after vehicle administration. RX821002 failed to enhance forskolin (2 μM)-stimulated cAMP accumulation in striatal slices from monoamine-depleted mice (Fig. 3).

Affinities of Dopamine and Norepinephrine at Mouse $\alpha_2A$ and $\alpha_2C$ Adrenoceptors. Initial experiments examined the saturation binding of $[^3H]$RX821002 in homogenates of NRK-$\alpha_2A$ and NRK-$\alpha_2C$ cells. The $K_D$ and $B_{max}$ values of the binding of $[^3H]$RX821002 were 0.95 ± 0.27 nM and 8.02 ± 3.18 pmol/mg protein, respectively, in NRK-$\alpha_2A$ cells and 0.71 ± 0.08 nM and 9.21 ± 1.62 pmol/mg protein, respectively, in NRK-$\alpha_2C$ cells. Saturation isotherms of $[^3H]$RX821002 binding could not be fit significantly better by a model assuming two sites of interaction. Curves of the inhibition of $[^3H]$RX821002 binding by norepinephrine and...
Effects of Dopamine and Norepinephrine on Forskolin-Stimulated cAMP Accumulation in NRK-α2A and NRK-α2C Cells. NRK-α2A cells were incubated with forskolin alone or in the presence of increasing concentrations of dopamine or norepinephrine. At concentrations of 50 nM to 5 mM, dopamine reduced and norepinephrine increased forskolin-stimulated cAMP levels (data not shown). The inhibition produced by dopamine (40 μM) and the enhancement produced by norepinephrine (5 μM) were reversed by RX821002 (1 μM), demonstrating that these responses were mediated by α2A adrenoceptors (Fig. 5). In NRK-α2C cells, dopamine inhibited forskolin-stimulated cAMP accumulation in a concentration-dependent manner, with maximal inhibition occurring near 40 μM (Fig. 6A). Concentrations of dopamine greater than 40 μM, up to 500 μM, had no further effect. Norepinephrine also attenuated forskolin-stimulated cAMP accumulation concentration-dependently, between concentrations of 0.1 nM and 1 μM, in NRK-α2C cells (Fig. 6B). At concentrations of 10 μM and greater, the direction of change in forskolin-stimulated cAMP levels reversed, although cAMP levels were still significantly lower than the forskolin-alone condition at 10 μM. The inhibitions produced by 40 μM dopamine and 5 μM norepinephrine were reversed by RX821002 (1 μM; Fig. 7), confirming that these effects were mediated by α2C adrenoceptors.

Discussion

We reported previously that rat striatal α2C adrenoceptors are negatively coupled to adenyl cyclase (Lu and Ordway, 1997b). In that study, brimonidine (UK14,304) induced a
TABLE 1

<table>
<thead>
<tr>
<th>Receptor System</th>
<th>Dopamine</th>
<th>Norepinephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRK-(\alpha)A</td>
<td>(K_i) high affinity</td>
<td>3.3 ± 0.93</td>
</tr>
<tr>
<td></td>
<td>(K_i) low affinity</td>
<td>63 ± 14</td>
</tr>
<tr>
<td>(%) High affinity</td>
<td>27 ± 6.1</td>
<td>24 ± 5.3</td>
</tr>
<tr>
<td>NRK-(\alpha)C</td>
<td>(K_i) high affinity</td>
<td>0.18 ± 0.11</td>
</tr>
<tr>
<td>(%) High affinity</td>
<td>19.5 ± 0.27</td>
<td>6.1 ± 0.85</td>
</tr>
<tr>
<td>(%) High affinity</td>
<td>19 ± 2.7</td>
<td>38 ± 5.7</td>
</tr>
</tbody>
</table>

* \(p < .05\) compared with norepinephrine (Student’s \(t\) test). Each value is the mean ± S.E.M. of four separate determinations. \(K_i\) values given in \(\mu M\) concentrations.

![Image](https://via.placeholder.com/150)

**Fig. 5.** Inhibition and facilitation of forskolin-stimulated cAMP accumulation by dopamine (40 \(\mu M\)) and norepinephrine (5 \(\mu M\)), respectively, in intact NRK-\(\alpha\)A cells. Where noted, the \(\alpha\) adrenoceptor antagonist RX821002 (1 \(\mu M\)) was added 10 min before agonists. RX821002 alone (not shown) or with forskolin had no effects on cAMP levels. Each bar represents the mean value of six separate experiments. * \(p < .05\), ** \(p < .01\), significant differences from forskolin alone and from forskolin plus agonist in the presence of RX821002.

The obvious candidate transmitter for the activation of the \(\alpha_{2c}\) adrenoceptor in brain is its cognate neurotransmitter, norepinephrine. In fact, many brain regions that express the \(\alpha_{2c}\) adrenoceptor are innervated by noradrenergic neurons. Oddly, the striatum receives little or no noradrenergic innervation. Little or no dopamine \(\beta\)-hydroxylase immunostaining or radioligand binding to norepinephrine transporters has been found in striatum of rat brain (Grzanna et al., 1977; Jursky et al., 1994; Ordway, 1995). This occurs despite the fact that the striatum has the most dense \(\alpha_{2c}\) adrenoceptor gene expression (mRNA) and radioligand binding to \(\alpha_{2c}\) adrenoceptors relative to other brain regions (Ordway et al., 1993; Wang et al., 1996; Uhlen et al., 1997). Another possible candidate transmitter in the striatum is the related catecholamine, dopamine. There is a dense dopaminergic innervation of the striatum originating from the substantia nigra (Lindvall and Bjorklund, 1974). Concentrations of norepinephrine in striatum have been reported to be one-fifteenth to one-fortieth of the concentration of dopamine in the striatum (Laurent et al., 1975; Jacobowitz and Richardson, 1978). Versteeg et al. (1976) reported no detectable level of norepinephrine but dopamine concentrations of approximately 70 pg/\(\mu g\) protein in the rat striatum. Hence, there is an apparent paucity of noradrenergic innervation of striatal \(\alpha_{2c}\) adrenoceptors. Segawa et al. (1998) found that exogenously applied dopamine contracted femoral veins through the activation of \(\alpha_2\) adrenoceptors, demonstrating that dopamine can act as an agonist at peripheral \(\alpha_2\) adrenoceptors. Together, these observations led to the goal of the present study to examine the affinity and potency of dopamine at \(\alpha_{2c}\) adrenoceptors.

Here, we found that the affinities of dopamine at \(\alpha_2A\) and \(\alpha_{2c}\) adrenoceptors are similar to the affinities of norepinephrine at these receptors in NRK cells transfected with mouse \(\alpha_2A\) or \(\alpha_{2c}\) adrenoceptors. In fact, the affinity of dopamine at the mouse \(\alpha_{2c}\) adrenoceptor is approximately equivalent to the affinity of norepinephrine for the \(\alpha_{2c}\) adrenoceptor and 3- to 8-fold higher than the affinity of norepinephrine for the \(\alpha_2A\) adrenoceptor. Furthermore, dopamine inhibited forskolin-stimulated accumulation of cAMP in both NRK-\(\alpha_2A\) and NRK-\(\alpha_{2c}\) cells, effects that were reversed by the \(\alpha_2\) adrenoceptor antagonist RX821002. These results suggest that dopamine could indeed play a physiological role in the activation of \(\alpha_2\) adrenoceptors in the striatum.

Little is known with regard to the function of \(\alpha_{2c}\) adrenoceptors in brain because highly selective agonists and antagonists for subtypes of \(\alpha_2\) adrenoceptors have not yet been identified. However, considerable progress has been made recently in understanding functional roles of the individual \(\alpha_2\) adrenoceptor subtypes through the use of molecular biological techniques. For example, a decreased hypothermic response to dexmedetomidine (\(\alpha_2\) adrenoceptor agonist) and reduced brain concentrations of HVA have been reported in Adra2c\(^{-/-}\) mice (Sallinen et al., 1997). Furthermore, an increased sensitivity of the hypothermic response to dexmedetomidine and higher brain concentrations of HVA and DA concentration-dependent inhibition of forskolin-stimulated cAMP accumulation in rat striatal slices, an effect that was antagonized by RX821002, an \(\alpha_2\) adrenoceptor antagonist that lacks selectivity for any of the subtypes of \(\alpha_2\) adrenoceptors. Furthermore, RX821002 alone significantly enhanced forskolin-stimulated cAMP accumulation in a concentration-dependent manner in rat striatal slices (Lu and Ordway, 1997b). Enhancement of forskolin-stimulated adenylyl cyclase by an \(\alpha_2\) adrenoceptor antagonist implies that striatal \(\alpha_2\) adrenoceptors are tonically activated by endogenous agonist. Striatal infusions of an antisense oligonucleotide targeting mRNA encoding the \(\alpha_{2c}\) adrenoceptor results in a selective, but incomplete, reduction in the expression of striatal \(\alpha_{2c}\) adrenoceptors (Lu and Ordway, 1997a) and significantly enhances forskolin-stimulated cAMP accumulation (Lu and Ordway, 1997b). The enhancement of forskolin-stimulated adenylyl cyclase produced by \(\alpha_{2c}\) adrenoceptor antisense infusion resembles the effect of RX821002. The present study corroborated these previous findings by demonstrating that RX821002 enhances forskolin-stimulated CAMP accumulation in striatal slices from Adra2c\(^{+/+}\) mice but has no significant effect on this event in Adra2c\(^{-/-}\) mice or in monoamine-depleted Adra2c\(^{+/+}\) mice (see Figs. 2 and 3). Furthermore, RX82102 has no apparent intrinsic activity at \(\alpha_2A\) or \(\alpha_{2c}\) adrenoceptors, as indicated by a lack of effect of RX821002 on
Adrenoceptors also exhibit the ability to stimulate adenylyl cyclase through coupling to Gs protein in several cell types and from forskolin alone had no effects on cAMP levels. Each bar represents the mean value of six separate experiments. *p < .05, significant differences from forskolin alone and from forskolin plus agonist in the presence of RX821002.

have been observed in transgenic mice overexpressing the α2C adrenoceptor compared with wild-type mice (Sallinen et al., 1997). Sallinen et al. (1998) also showed that Adra2c−/− mice had enhanced startle responses, shortened aggressive response latencies, and decreased prepulse inhibition in an isolation-aggression test; mice overexpressing the Adra2c+/+ gene had opposite effects. The present demonstration of the functional coupling of the α2C adrenoceptor to adenylyl cyclase in the striatum suggests that the α2C adrenoceptor may modulate some striatal behaviors.

The classic biochemical response to α2 adrenoceptor activation is an inhibition of adenylyl cyclase via the coupling of the receptor to the Gs protein (Jansson et al., 1994a,b). α2 Adrenoceptors also exhibit the ability to stimulate adenylyl cyclase through coupling to Gs protein in several cell types that have been transfected with these receptors (Eason et al., 1992, 1994; Eason and Liggett, 1993; Jansson et al., 1994a, 1995). Hence, the stimulation or inhibition of cAMP production by α2 adrenoceptor activation is both subtype and target cell specific in transfected cells. In the present study, we found that norepinephrine and the selective α2 adrenoceptor agonists clonidine and brimonidine (data not shown for clonidine and brimonidine) increased cAMP production stimulated by forskolin in NRK-α2A cells. In contrast, dopamine only inhibited cAMP accumulation stimulated by forskolin, even at relatively high concentrations. These data indicate that the molecular mechanisms of α2A adrenoceptor activation by norepinephrine and dopamine are different. It is unlikely that this difference is related to differences in the general ability of these agonists to induce or stabilize coupling of the α2A adrenoceptor to G proteins. Curves of the inhibition of [3H]RX821002 binding revealed that there were approximately equal percentages of high-affinity binding for dopamine and norepinephrine in NRK α2A cells. Hence, differences in the effects of dopamine and norepinephrine on cAMP accumulation mediated by α2A adrenoceptors may result from induction of conformational changes in these receptors that are different for the two agonists, leading to differences in the affinity of binding of the receptor to Gs protein.

Interestingly, the percentage of high-affinity binding sites for dopamine was significantly lower than that for norepinephrine in NRK-α2C cells. The lower percentage of high-affinity binding sites for dopamine might indicate that dopamine is less capable of stabilizing or inducing the coupling of the α2 adrenoceptor to G protein. This may account for an apparent lower potency (as could be determined by estimating the concentration to produce a 50% inhibition, or EC50i in Fig. 6) of dopamine, relative to norepinephrine, for the inhibition of forskolin-stimulated cAMP accumulation, despite the near-equal affinities of these two monoamines for the α2C adrenoceptors. It should be noted that determination of potency of norepinephrine for α2C adrenoceptor-induced inhibition of forskolin-stimulated cAMP accumulation is complicated by the fact that a maximal inhibition could not be reliably obtained. This is because opposing enhancement of cAMP accumulation was initiated as the concentration of norepinephrine was increased, presumably by recruitment of Gs protein by the norepinephrine-α2 adrenoceptor complex. Hence, although maximal inhibition of cAMP accumulation was observed at 40 μM dopamine and 1 μM norepinephrine, the true maximum inhibition induced by norepinephrine was clouded by a concomitant activation.
In conclusion, RX821002 facilitated forskolin-stimulated cAMP accumulation in striatal slices in Adra2C<sup>++</sup> mice and failed to enhance forskolin-stimulated cAMP accumulation in Adra2C<sup>−−</sup> mice. These data, along with our previous findings (Lu and Ordway, 1997b), indicate that striatal α<sub>2C</sub> adrenoceptors are negatively coupled to adenyl cyclase and are under tonic activation by an endogenous agonist. The inhibition of forskolin-stimulated cAMP production in NRK-α<sub>2C</sub> cells by catecholamines and its antagonism by RX821002 further demonstrate that the α<sub>2C</sub> adrenoceptor is coupled to inhibition of adenyl cyclase. The high affinity of dopamine for the α<sub>2C</sub> adrenoceptor and the ability of dopamine to activate this receptor at physiologically relevant concentrations bolster the conjecture that dopamine may act as a promiscuous activator of α<sub>2C</sub> adrenoceptors in the brain, particularly in areas such as the striatum, where this receptor occurs in conjunction with dense dopaminergic innervation and sparse or absent noradrenergic innervation.

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