Sensitization of Neuronal A2A Adenosine Receptors after Persistent D2 Dopamine Receptor Activation

Timothy A. Vortherms and Val J. Watts
Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, Indiana

Received July 16, 2003; accepted September 17, 2003

ABSTRACT
Acute activation of Gα1o-coupled D2 dopamine receptors inhibits A2A adenosine receptor stimulation of adenylate cyclase. This antagonistic interaction between D2 dopamine and A2A adenosine receptors has been well documented; however, the effects of persistent activation of D2 dopamine receptors on subsequent A2A adenosine receptor signaling have not been explored. The present study investigated the effects of short-term (3-h) and long-term (18-h) activation of D2L dopamine receptors on subsequent A2A adenosine receptor stimulation of adenylate cyclase in CAD-D2L and NS20Y-D2L neuroblastoma cells. Short- and long-term activation of D2L dopamine receptors markedly increased 5′-N-methylcarboxamidoadenosine (MECA)-stimulated cyclic AMP accumulation 1.4-fold and 1.7-fold, respectively. D2L receptor-induced sensitization of A2A adenosine receptor stimulation of adenylate cyclase was blocked by the D2 antagonist spiperone and pertussis toxin pretreatment. In addition, persistent activation of A2A adenosine receptors resulted in 50% desensitization of subsequent MECA-stimulated cyclic AMP accumulation; however, MECA-induced desensitization of A2A adenosine receptors did not prevent completely quinpirole-induced sensitization of adenylate cyclase. These studies revealed a novel mode of regulation between D2L dopamine and A2A adenosine receptors and suggest a cooperative interaction in the regulation of cyclic AMP signaling.

The D2 dopamine and A2A adenosine receptors belong to the superfamily of G protein-coupled receptors that transduce extracellular stimuli to activate intracellular signaling pathways. Agonist activation of the Gα1o-coupled A2A adenosine receptor stimulates cyclic AMP accumulation, whereas acute activation of the Gα1o-coupled D2 dopamine receptor inhibits cyclic AMP accumulation to provide antagonistic control on receptor-mediated adenylate cyclase activity at the cellular level (Ferre et al., 1997). Consistent with this regulatory role on adenylate cyclase activity, D2 dopamine and A2A adenosine receptors are colocalized on striatopallidal GABAergic neurons in the striatum (Schiffmann et al., 1991; Fink et al., 1992). This colocalization also implicates D2 and A2A receptor interactions as a molecular target for intervention of basal ganglia disorders associated with the pharmacotherapy of Parkinson’s disease and schizophrenia (Ferre et al., 1997; Aoyama et al., 2000; Fredruzzii et al., 2002). The ability of D2 dopamine receptors to control locomotor activity seems to involve regulation of AC5 in the striatum (Lee et al., 2002). In addition, A2A adenosine receptor-stimulated cyclic AMP accumulation in the striatum was markedly reduced in mice deficient of AC5 (Lee et al., 2002). These studies suggest that individual adenylate cyclase isoforms may act as coincident detectors of Gα2- and Gα1o-coupled receptors. Furthermore, disruption of the balance between D2 dopamine and A2A adenosine receptor signaling may lead to changes in adenylate cyclase activity, resulting in impaired locomotor function.

D2 dopamine and A2A adenosine receptor interactions may also involve additional molecular mechanisms. Recent studies have demonstrated that G protein-coupled receptors can form homo- and heterodimers and that protein–protein interactions between receptors can influence agonist affinities as well as activation of signaling pathways (Hillion et al., 2002; Franco et al., 2003). For example, agonist activation of A2A adenosine receptors decreased the potency of dopamine for the D2L dopamine receptor in Ltk− fibroblast cells independent of regulation of adenylate cyclase (Dasgupta et al., 1996). Immunofluorescence experiments have also shown that D2 dopamine and A2A adenosine receptors colocalize in SH-SY5Y neuroblastoma cells and that agonist activation of A2A adenosine or D2 dopamine receptors results in desensitization of A2A adenosine receptors (Hillion et al., 2002). Although the mechanistic details remain unclear, heteroreceptor-induced desensitization and internalization may result from direct protein–protein interactions between D2 dopamine and A2A adenosine receptors at the cellular

ABBREVIATIONS: AC(1–9), adenylate cyclase type (1–9); CAD, Cath.a. differentiated cells; DARPP-32, dopamine- and cyclic AMP-regulated phosphoprotein 32 kDa; EBSS, Earle’s balanced salt solution; ANOVA, analysis of variance; PKA, cyclic AMP dependent protein kinase A.
membrane (Hillion et al., 2002). These physical interactions may provide another molecular mechanisin for the antagonistic receptor interactions in addition to regulation of adenylate cyclase.

Cooperative modes of interaction between D2 dopamine and A2A adenosine receptors have also been suggested. For example, A2A adenosine receptor activation of Goβγ subunits released from Gαq,coupled D2 dopamine receptors can conditionally activate AC2 (Watts and Neve, 1997; Yao et al., 2002). Such synergistic activation of adenylate cyclase via D2 dopamine and A2A adenosine receptors has been implicated in ethanol consumption (Yao et al., 2002). In addition, persistent activation of D2 dopamine receptors has been shown to increase subsequent Gαq-coupled receptor agonist-stimulated cyclic AMP accumulation (Watts and Neve, 1996).

This D2 receptor-induced heterologous sensitization of adenylate cyclase may represent an additional cooperative mode of interaction between D2 dopamine and A2A adenosine receptors (Watts, 2002). However, the ability of D2 dopamine receptors to sensitize specifically A2A adenosine receptor-stimulated cyclic AMP accumulation has not been explored.

The functional interactions between D2 dopamine and A2A adenosine receptors in the regulation of adenylate cyclase activity in neuronal cells form the basis of the present study. We examined the ability of D2 dopamine receptor activation to alter A2A adenosine receptor stimulation of adenylate cyclase in NS20Y neuroblastoma cells and the neuronal Cath.a. differentiated (CAD) cells stably expressing the D2L dopamine receptor (NS20Y-D2L and CAD-D2L cells) (Qi et al., 1997; Watts et al., 1998). These cells provide neuronal model systems derived from the central nervous system that endogenously express functional A2A adenosine receptors. In the present study, we confirmed that acute activation of Gαq-coupled D2L dopamine receptors inhibits A2A adenosine receptor-stimulated cyclic AMP accumulation. In contrast, persistent activation of D2L dopamine receptors results in a compensatory increase in A2A adenosine receptor activation of adenylate cyclase. These results suggest another mode of regulation between D2 dopamine and A2A adenosine receptors, supporting the hypothesis that pathogenic and therapeutic-induced increases in dopaminergic tone in the basal ganglia may potentiate A2A adenosine receptor signaling.

Materials and Methods

Materials. [3H](Cyclic AMP (25 Ci/mmol) was purchased from PerkinElmer Life Sciences (Boston, MA). Quinpirole, spiperone, 5'-N-methylcarboxamidoadenosine (MECA), 9-chloro-2-(2-furyl)(1,2,4) triazol[1,5-c]quinazolin-5-amine (CGS 15943), 2-carboxethyl) phenethylamino-5'-N-methylcarboxamidoadenosine (CGS 21680), 4-(3-butoxy-4-methoxybenzyl)imidazolidin-2-one (Ro 20-1724), pertussis toxin, MECA, or a combination of these drugs as indicated in the figure legends. Statistical analysis was completed using repeated measures ANOVA and post-hoc comparisons were made using the program Prism (GraphPad Software Inc., San Diego, CA). All values for cyclic AMP accumulation are expressed as picomoles cyclic AMP per well.

Data Analysis. Statistical comparisons were made using repeated measures ANOVA followed by the indicated (Bonferroni’s) post hoc analysis for comparison of multiple stimulations or stimulation and pretreatment conditions. F values for repeated measures ANOVA and p values for post hoc comparisons are indicated in the figure legends. Statistical analysis was completed using the program Prism (GraphPad Software Inc.).

Results

Acute Regulation of Adenylate Cyclase in CAD-D2L Cells. We explored the antagonistic interactions of D2L dopamine and A2A adenosine receptors in CAD-D2L cells. CAD cells were stably transfected with the rat D2L dopamine receptor (~200 fmol/mg protein) as described previously (Johnston et al., 2002). Acute activation of endogenous A2A adenosine receptors by the adenosine analog MECA and the A2A-selective agonist CGS 21680 resulted in robust cyclic AMP accumulation above basal levels, and this A2A-stimulated cyclic AMP accumulation was blocked by the nonselective adenosine antagonist CGS 15943 (Fig. 1). In addition, CGS 15943 markedly attenuated the residual agonist (Watts and Neve, 1996) and Ro 20-1724 (100 M). Incubations were carried out for 15 min at 37°C, and then the assay buffer was decanted. The culture plates were placed on ice, and cells were lysed with 100 to 200 µl of 3% trichloroacetic acid. The 48-well plates were stored at 4°C overnight before quantification. For sensitization experiments, cells were preincubated for 3 or 18 h in the presence of quinpirole, spiperone, pertussis toxin, MECA, or a combination of these drugs as indicated, at 37°C in a humidified incubator in the presence of 5% CO2. After drug pretreatment, the cells were washed three times for 3 to 4 min with 200 µl of EBSS assay buffer, placed on ice, and then A2A agonists were added. Sensitization experiments were done in the presence of spiperone (1 µM) to block activation of D2L receptors by residual agonist (Watts and Neve, 1996) and Ro 20-1724 (100 µM) was added to inhibit phosphodiesterase activity. The cells were then incubated for 15 min at 37°C. The medium was removed, and the cells were lysed with 3% trichloroacetic acid. The 48-well plates were stored at 4°C until quantification of cyclic AMP was carried out.

Quantification of Cyclic AMP. Cyclic AMP was quantified using a competitive binding assay adapted from Watts and Neve, 1996. Briefly, duplicate samples of the cell lysate (10–15 µl) were added to reaction tubes. [3H](Cyclic AMP (~1 nM final concentration) and cyclic AMP binding protein (~100–150 µg in 500 µl of buffer) were diluted in cyclic AMP binding buffer (100 mM Tris-HCl, pH 7.4, 100 mM NaCl, and 5 mM EDTA) and then added to each reaction tube. The reaction was incubated on ice at 4°C for 2 h and then harvested by filtration ( GF/C filters; Whatman, Maidstone, UK) by using a 96-well Packard Filtermate cell harvester. Filter plates were dried overnight at room temperature, and 40 µl of Packard Microscint O scintillation fluid was added to each well. Radioactivity was determined using a Packard TopCount scintillation counter. Cyclic AMP concentrations in each sample were determined in duplicate from a standard curve ranging from 0.01 to 300 pmol of cyclic AMP. Dose-response curves for cyclic AMP accumulation were analyzed by nonlinear regression by using the program Prism (GraphPad Software Inc., San Diego, CA).

Cyclic AMP Accumulation Assay. Cells were seeded at concentrations between 50,000 and 100,000 cells/well in 48-well cluster plates. For acute experiments, cells were washed in 200 µl of Earle’s balanced salt solution (EBSS) assay buffer (EBSS containing 2% calf bovine serum, 0.025% ascorbic acid, and 15 mM Na+·HEPES) for 10 min at room temperature. The medium was then decanted, and the cells were placed on ice. Quinpirole (10 µM) in the absence or presence of spiperone (1 µM) was added to the cells before the addition of MECA or CGS 21680 (1 µM). Where indicated, the CGS 15943 (1 µM) was also added to the cells before acute agonist activation of A2A adenosine receptors. In addition, acute experiments were completed in the presence of Ro 20-1724 (100 µM). Incubations were carried out for 15 min at 37°C, and then the assay buffer was decanted. The culture plates were placed on ice, and cells were lysed with 100 to 200 µl of 3% trichloroacetic acid. The 48-well plates were stored at 4°C overnight before quantification. For sensitization experiments, cells were preincubated for 3 or 18 h in the presence of quinpirole, spiperone, pertussis toxin, MECA, or a combination of these drugs as indicated, at 37°C in a humidified incubator in the presence of 5% CO2. After drug pretreatment, the cells were washed three times for 3 to 4 min with 200 µl of EBSS assay buffer, placed on ice, and then A2A agonists were added. Sensitization experiments were done in the presence of spiperone (1 µM) to block activation of D2L receptors by residual agonist (Watts and Neve, 1996) and Ro 20-1724 (100 µM) was added to inhibit phosphodiesterase activity. The cells were then incubated for 15 min at 37°C. The medium was removed, and the cells were lysed with 3% trichloroacetic acid. The 48-well plates were stored at 4°C until quantification of cyclic AMP was carried out.

Materials. [3H](Cyclic AMP (25 Ci/mmol) was purchased from PerkinElmer Life Sciences (Boston, MA). Quinpirole, spiperone, 5'-N-methylcarboxamidoadenosine (MECA), 9-chloro-2-(2-furyl)(1,2,4) triazol[1,5-c]quinazolin-5-amine (CGS 15943), 2-carboxethyl) phenethylamino-5'-N-methylcarboxamidoadenosine (CGS 21680), 4-(3-butoxy-4-methoxybenzyl)imidazolidin-2-one (Ro 20-1724), pertussis toxin, MECA, or a combination of these drugs as indicated in the figure legends. Statistical analysis was completed using the program Prism (GraphPad Software Inc., San Diego, CA). All values for cyclic AMP accumulation are expressed as picomoles cyclic AMP per well.

Production and Maintenance of Cell Lines. CAD cells were stably transfected with the D2L dopamine receptor (CAD-D2L) by using pcDNA3-D2L and clones were isolated using G418 selection (600 µg/ml) as described previously (Johnston et al., 2002). NS20Y-D2L neuroblastoma cells were constructed as described previously (Watts et al., 1998). Cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 5% fetal bovine serum, 5% bovine calf serum, 50 U/ml penicillin, 50 µg/ml streptomycin, and 300 µg/ml G418 or 2 µg/ml puromycin. Cells were grown in a humidified incubator in the presence of 5% CO2 at 37°C.
Fig. 1. Acute regulation of A2A adenosine receptors in CAD-D2L cells. Cyclic AMP accumulation was measured under basal conditions or in the presence of 1 μM MEC or 1 μM CGS 21680 for 15 min. Where indicated, experiments were completed in the presence of 1 μM CGS 15943 (+CGS 15945). Data shown are the mean ± S.E.M. of three independent experiments assayed in duplicate. *, p < 0.05 compared with basal stimulation under the control condition; †, p < 0.05 compared with control for the indicated stimulation condition [Bonferroni’s post hoc repeated measures ANOVA; basal, F(3,12) = 131.3, p < 0.0001].

Acutely, the D2 dopamine receptor agonist, quinpirole, significantly attenuated basal and A2A agonist-induced cyclic AMP accumulation in CAD-D2L cells, and this inhibition was completely blocked by the D2 antagonist spiperone (Fig. 2). These results suggest that constitutively active A2A adenosine receptors can be antagonized by agonist activation of D2L dopamine receptors. These antagonistic interactions on adenylyl cyclase regulation are consistent with previous studies in cultured cells and demonstrated the ability of adenylyl cyclase to function as a coincident detector of Gs- and Gαi-coupled receptor signaling (Kull et al., 1999; Hillion et al., 2002).

**D2 Dopamine Receptor-Induced Sensitization of A2A Adenosine Receptor-Stimulated Cyclic AMP Accumulation.** Based on the antagonistic interactions between D2 dopamine and A2A adenosine receptors in CAD-D2L cells, we examined the ability of short-term (3 h) and long-term (18 h) activation of D2L dopamine receptors to alter subsequent A2A adenosine receptor stimulation of cyclic AMP accumulation. Treatment of CAD-D2L cells with quinpirole for 3 h resulted in a significant increase of subsequent basal and A2A agonist-stimulated cyclic AMP accumulation compared with vehicle-treated cells (Table 1). Consistent with short-term sensitization studies, treatment with the D2 agonist quinpirole for 18 h also resulted in a marked enhancement of subsequent basal and A2A adenosine receptor-stimulated cyclic AMP accumulation in CAD-D2L cells compared with vehicle-treated cells (Fig. 3). Specifically, quinpirole-induced sensitization increased both MEC- and CGS 21680-stimulated cyclic AMP accumulation compared with vehicle-treated cells. The magnitude of sensitization of MEC- and CGS 21680-stimulated cyclic AMP accumulation (1.7 ± 0.1-fold and 1.5 ± 0.1-fold, respectively) was similar to long-term quinpirole-induced sensitization of forskolin-stimulated cyclic AMP accumulation in CAD-D2L cells (Johnston et al., 2002).

Although acute stimulation of A2A adenosine receptors (after 18-h quinpirole treatment) was completed in the presence of spiperone to prevent residual D2 dopamine receptor activation (Watts and Neve, 1996), additional experiments examined sensitization of acute A2A adenosine receptor signaling in the absence of spiperone. These studies revealed that quinpirole pretreatment for 18 h enhanced subsequent MEC (170 ± 5 to 257 ± 19 pmol/well, n = 3) and CGS 21680 (200 ± 5 to 300 ± 13 pmol/well, n = 3) stimulated cyclic AMP accumulation, demonstrating that sensitization of A2A adenosine receptor signaling was similar in the presence or absence of spiperone. This compensatory increase in Gαs-stimulated cyclic AMP accumulation after long-term activation of D2L dopamine receptors is consistent with previous studies in C6-D2L glioma cells and HEK-D2L cells expressing recombinant isoforms of adenylyl cyclase (Watts and Neve, 1996; Cumbay and Watts, 2001).

Heterologous sensitization of basal and A2A-stimulated cyclic AMP accumulation after long-term (18 h) activation of D2L dopamine receptors was completely blocked by pretreatment with the D2 antagonist spiperone; however, treatment with spiperone alone did not significantly alter cyclic AMP accumulation in CAD-D2L cells (Fig. 3). In addition, pretreatment with pertussis toxin prevented D2L agonist-induced heterologous sensitization of basal and A2A-stimulated cyclic AMP accumulation in CAD-D2L cells. Pretreatment with pertussis toxin did not alter basal or MEC-stimulated cyclic AMP accumulation (Fig. 4). These studies implicate a role for

**TABLE 1**

Effects of short-term (3 h) activation of D2L dopamine receptors on cyclic AMP accumulation in CAD-D2L cells

<table>
<thead>
<tr>
<th>Acute Stimulation</th>
<th>Pretreatment (3 h)</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>Quinpirole</td>
<td></td>
</tr>
<tr>
<td>49 ± 3</td>
<td>65 ± 4*</td>
<td>1.3 ± 0.08</td>
</tr>
<tr>
<td>MECA</td>
<td>204 ± 12</td>
<td>291 ± 30*</td>
</tr>
<tr>
<td>CGS 21680</td>
<td>261 ± 23</td>
<td>422 ± 20*</td>
</tr>
</tbody>
</table>

*p < 0.05 compared with vehicle-treated cells under the indicated acute stimulation condition (Student’s paired t test).
In the development of enhanced \( \text{A2A} \) adenosine receptor signaling after persistent \( \text{D2L} \) dopamine receptor activation. We also extended our studies to the NS20Y neuroblastoma cell line stably expressing the \( \text{D2L} \) dopamine receptor. NS20Y neuroblastoma cells endogenously express \( \text{A2A} \) adenosine receptors and provide a model cell line mimicking the properties of striatal cholinergic cells (Amano et al., 1972). \( \text{D2} \) agonist-induced heterologous sensitization of forskolin-stimulated cyclic AMP accumulation has been described previously in NS20Y-\( \text{D2L} \) cells (Watts et al., 1998).

Acute experiments revealed that quinpirole inhibited MECA-stimulated cyclic AMP accumulation from 51.1 ± 8.2 to 6.5 ± 1.4 pmol/well (\( n > 3 \)). In contrast, persistent activation (18 h) of \( \text{D2L} \) dopamine receptors in NS20Y-\( \text{D2L} \) neuroblastoma cells resulted in a marked increase (1.6 ± 0.1-fold) of \( \text{A2A} \)-stimulated cyclic AMP accumulation. Quinpirole pretreatment (18 h) did not alter basal cyclic AMP accumulation (Fig. 5). Additional studies revealed that \( \text{D2} \) receptor-induced heterologous sensitization of MECA-stimulated cyclic AMP accumulation was blocked when quinpirole pretreatment was completed in the presence of spiperone (Fig. 5).

**Effects of \( \text{A2A} \) Adenosine Receptor Desensitization on \( \text{D2} \) Dopamine Receptor-Induced Heterologous Sensitization in \( \text{CAD-D2L} \) Cells.** Based on the functional interactions between \( \text{D2} \) dopamine and \( \text{A2A} \) adenosine receptors, we examined the ability of \( \text{A2A} \) agonist-induced desensitization of \( \text{A2A} \) adenosine receptors to alter \( \text{D2L} \) receptor-induced heterologous sensitization of adenylate cyclase. We examined the effects of long-term (18 h) pretreatment of quinpirole or MECA on basal cyclic AMP accumulation in \( \text{CAD-D2L} \) cells. Persistent quinpirole activation of \( \text{D2L} \) dopamine receptors increased basal cyclic AMP accumulation (1.8 ± 0.1-fold) above vehicle-treated cells (Fig. 6A). In contrast, persistent MECA activation of \( \text{A2A} \) adenosine receptors resulted in a significant decrease of basal cyclic AMP accumu-
mulation from $41.2 \pm 5.0$ pmol/well in vehicle-treated cells to $23.8 \pm 4.9$ pmol/well in MECA-treated cells (Fig. 6A). Co-treatment with both quinpirole and MECA in CAD-D2L cells did not affect basal cyclic AMP accumulation compared with vehicle-treated cells (Fig. 6A).

Subsequent studies examined the effects of A2A agonist pretreatment on quinpirole-induced heterologous sensitization of MECA-stimulated cyclic AMP accumulation. Quinpirole-induced heterologous sensitization of adenylate cyclase increased MECA-stimulated cyclic AMP accumulation 1.5 ± 0.1-fold above vehicle-treated cells (Fig. 6B). In contrast, persistent MECA activation of A2A receptors resulted in ca. 50% desensitization of subsequent A2A-stimulated cyclic AMP accumulation (Fig. 6B). We then examined the ability of MECA-induced desensitization of A2A adenosine receptor signaling to alter quinpirole-induced heterologous sensitization of adenylate cyclase in CAD-D2L cells. Pretreatment with both quinpirole and MECA resulted in decreased MECA-stimulated cyclic AMP accumulation compared with vehicle-treated cells; however, quinpirole-induced sensitization of MECA-stimulated cyclic AMP accumulation was not prevented by MECA pretreatment suggesting that A2A adenosine receptor desensitization did not completely ablate this signaling pathway (Fig. 6B).

Discussion

In the present study, we demonstrated a functional interaction between stably transfected D2L dopamine receptors and endogenously expressed A2A adenosine receptors in CAD-D2L and NS20Y-D2L cells, two neuronal cell lines derived from the central nervous system (Qi et al., 1997; Watts et al., 1998). Long-term (18 h) activation of D2L dopamine receptors markedly enhanced subsequent A2A adenosine receptor-stimulated cyclic AMP accumulation in the neuronal CAD-D2L and NS20Y-D2L cell lines. These receptor interactions may represent a novel mode of cooperative regulation between D2 dopamine and A2A adenosine receptors.

The acute antagonistic regulation of adenylate cyclase observed in CAD-D2L cells is consistent with previous reports in other neuronal model cell lines, including SH-SY5Y neuroblastoma cells, PC 12 neuroendocrine cells, and Chinese hamster ovary cells (Kull et al., 1999; Hillion et al., 2002; Kudlacek et al., 2003). Furthermore, agonist activation of A2A adenosine receptors decreases the potency of dopamine for the D2 dopamine receptor in rat and human striatal slices and in cultured cell lines expressing D2L dopamine and A2A adenosine receptors (Kull et al., 1999; Salim et al., 2000; Diaz-Cabiale et al., 2001). In the present study, A2A adenosine receptors in CAD-D2L cells exhibited constitutive activity that was blocked by the adenosine receptor antagonist CGS 15943 and inhibited by the D2 dopamine receptor ago-
nyst quinpirole. Constitutive activity of the A2A adenosine receptor has been described in stably transfected human embryonic kidney-293 cells and truncation of the carboxyl terminus of the A2A receptor ablates constitutive activity (Klinger et al., 2002). This basal tone of A2A adenosine receptor signaling is thought to be critical to increase phosphorylation of the dopamine- and cyclic AMP-regulated phosphoprotein, DARPP-32, which is thought to be important in regulation of dopaminergic signaling (Svenningsson et al., 2000). These observations suggest that adenylate cyclase and subsequently, DARPP-32, may be an important targets for differential regulation of locomotor function by D2 dopamine and A2A adenosine receptors.

Short-term (3-h) and long-term (18-h) activation of the D2 dopamine receptor resulted in a compensatory increase of basal and A2A receptor-stimulated cyclic AMP accumulation in CAD-D2L cells. It is tempting to hypothesize that pathogenesis or therapeutic intervention may enhance A2A receptor signaling via D2 dopamine receptor-induced heterologous sensitization. In fact, persistent l-DOPA treatment has been shown to increase cyclic AMP response element-binding protein phosphorylation in striatal neurons (Cole et al., 1994; Oh et al., 2003). A compensatory increase in A2A-stimulated cyclic AMP accumulation after quinpirole pretreatment was also observed in the striatal-like NS20Y-D2L cells (present study). In contrast, a previous study showed short-term (3-h) quinpirole pretreatment in SH-SY5Y cells stably expressing the D2 dopamine receptor did not induce significant heterologous sensitization (Hillion et al., 2002). The reason for this disparity is unclear, however, this may be due to differences in experimental methodology or more likely due to differences in cultured cellular models. Specifically, CAD-D2L cells express AC6 and AC9, whereas SH-SY5Y neuroblastoma cells endogenously express AC1, AC8, and AC9 and evidence suggests an adenylate cyclase isoform dependence for heterologous sensitization (Cumbay and Watts, 2001; Jiang and Juhnn, 2001; Johnston et al., 2002; Watts, 2002). Nevertheless, based on the model cell lines used in the present study, D2 receptor-induced sensitization of A2A adenosine receptor signaling could contribute significantly to the regulation of neuronal signaling.

D2 dopamine and A2A adenosine receptor regulation of cyclic AMP signaling pathways and cyclic AMP-dependent protein kinase (PKA) has also been implicated in modulating ethanol consumption and sensitivity (Moore et al., 1998; Thiele et al., 2000). The mechanism for PKA regulation of voluntary alcohol consumption is thought to occur through translocation of the catalytic subunit of PKA to the nucleus, resulting in prolonged cyclic AMP response element-binding protein phosphorylation (Constantinescu et al., 1999; Yao et al., 2002). This effect seems to involve modulation of AC2 that is conditionally activated by βγ subunits released from D2 dopamine receptors in the presence of activated Gα or protein kinase C (Tsuj et al., 1995; Watts and Neve, 1997; Yao et al., 2002). Recent studies have also demonstrated that D2 dopamine receptor activation enhances A2A adenosine receptor stimulation of adenylate cyclase in NG108-15 cells, which endogenously express AC2 and AC4 (Yao et al., 2002). Moreover, persistent activation of D2 dopamine receptors leads to heterologous sensitization of protein kinase C-stimulated AC2 activity (Watts and Neve, 1996; Cumbay and Watts, 2001). These observations are consistent with studies implicating a D2 dopamine receptor-mediated enhancement of cyclic AMP accumulation in the nucleus accumbens as a mechanism for modulating sensitivity to ethanol (Yao et al., 2002). Together, these studies suggest that persistent activation of D2 dopamine receptors releases βγ subunits that may play a role in synergistic activation of adenylate cyclase after increases (e.g., ethanol-induced) in A2A-receptor activated Gα.

D2 dopamine receptor-induced heterologous sensitization of adenylate cyclase may represent a more general mechanism for enhancing Gα-coupled receptor signaling (i.e., A2A adenosine receptor) in an adenylate cyclase isoform-dependent manner (Thomas and Hoffman, 1996; Cumbay and Watts, 2001; Watts, 2002). The current study used cells endogenously expressing AC6 (CAD-D2L) and striatal-like NS20Y-D2L cells to demonstrate that persistent agonist activation of D2 dopamine receptors results in a compensatory increase in A2A adenosine receptor-stimulated cyclic AMP accumulation. AC6 and the closely related AC5 are members of the Ca2+-inhibited adenylate cyclases and AC5 is highly localized to the striatum, whereas AC6 is more widespread throughout the brain (Hanoune and Defer, 2001). In addition, persistent activation of Gαi0-coupled receptors results in heterologous sensitization of both AC5 and AC6 that is attenuated by sequestering βγ subunits (Avidor-Reiss et al., 1996; Thomas and Hoffman, 1996). This suggests that the current neuronal cell lines used to examine D2 receptor-induced heterologous sensitization of A2A receptor signaling may represent useful models for studying neuroadaptive responses in the striatum. Accordingly, it has been suggested that D2 dopamine and A2A adenosine receptor signaling in the striatum is selectively integrated by AC5. After genetic ablation of AC5, the ability of A2A adenosine and D1 and D2 dopamine receptors to regulate adenylate cyclase was markedly reduced (Lee et al., 2002). Furthermore, the neuroleptic effects of haloperidol and sulpiride, D2 receptor antagonists, were reversed, and each agent actually increased locomotion in an open field test. In contrast, the behavioral effects of A2A adenosine and D1 dopamine receptor activation were intact, suggesting that the effects of AC5 deletion on behavior were specific to D2 dopamine receptors (Lee et al., 2002). These studies highlight the importance of AC5 in D2 dopamine receptor signaling pathways for the maintenance of proper locomotor function. That D2 dopamine and A2A adenosine receptor signaling can be integrated selectively by a single adenylate cyclase support an important role of heterologous sensitization in regulating Gαi signaling.

D2 dopamine and A2A adenosine receptor interactions have been implicated as a molecular target for intervention of basal ganglia disorders, including Parkinson’s disease and schizophrenia (Perre et al., 1997). These neuropsychiatric disorders and the associated pharmacotherapy are thought to involve increased dopamine levels or increased dopamine receptor activity. Therefore, understanding the molecular mechanisms associated with persistent activation of D2 dopamine receptors may provide insight for the advancement of therapeutic intervention. It has been suggested that A2A adenosine receptor antagonists may increase the therapeutic benefit of l-DOPA replacement therapy in Parkinson’s disease (Kanda et al., 2000). In fact, several studies have demonstrated that decreased A2A adenosine receptor signaling may be effective in the regulation of locomotor activity. For example, genetic ablation of A2A adenosine receptors has been shown to decrease l-DOPA-induced dyskinesia behaviors in 6-hydroxydopamine-lesioned mice (Freduzzi et al.,
In addition, A2A adenosine receptor antagonists partially rescued impaired locomotor activity in D2 dopamine receptor-deficient mice (Aoyama et al., 2000). These studies suggest that decreased A2A receptor signaling may have beneficial effects on locomotor function. An alternative mode to decrease A2A receptor function may be desensitization as was observed after persistent A2A dopamine receptor activation. This agonist-induced desensitization may represent an additional mechanism for down-regulating A2A adenosine receptor signaling. Furthermore, MECA pretreatment markedly reduced drug-stimulated cyclic AMP accumulation in quinpirole-treated cells (Fig. 6), suggesting that D2 dopamine receptor-induced increases in cyclic AMP accumulation can be attenuated in the presence of A2A agonist treatment. Together, these studies suggest an important role of A2A adenosine receptors in the regulation and maintenance of proper dopaminergic signaling in the striatum.

In summary, the present study demonstrated a functional interaction between stably transfected D2L dopamine receptors and endogenously expressed A2A adenosine receptors in CAD-D2L and NS20Y-D2L cells. Persistent activation of D2 dopamine receptors results in a compensatory increase of A2A-stimulated cyclic AMP accumulation. The D2 antagonist siperone and pertussis toxin pretreatment blocked this heterologous sensitization of adenylylate cyclase in CAD-D2L cells, implicating a role for Gi/o in this neuroadaptative response. This compensatory increase in A2A adenosine receptor signaling after persistent activation of D2 dopamine receptors may provide an additional mode of interaction for D2 dopamine and A2A adenosine receptor regulation of adenylylate cyclase. Furthermore, the regulatory properties of D2 dopamine and A2A adenosine receptors on adenylylate cyclase described in the current study support the hypothesis that A2A adenosine receptor signaling is a critical component in the regulation of proper dopaminergic tone.

Acknowledgments

We thank Joshua Lisinichia for technical assistance.

References

Aoyama S, Kase H, and Borrelli E (2000) Rescue of locomotor impairment in dopamine D2 receptor signaling. Furthermore, MECA pretreatment markedly reduced drug-stimulated cyclic AMP accumulation in quinpirole-treated cells (Fig. 6), suggesting that D2 dopamine receptor-induced increases in cyclic AMP accumulation can be attenuated in the presence of A2A agonist treatment. Together, these studies suggest an important role of A2A adenosine receptors in the regulation and maintenance of proper dopaminergic signaling in the striatum.

In summary, the present study demonstrated a functional interaction between stably transfected D2L dopamine receptors and endogenously expressed A2A adenosine receptors in CAD-D2L and NS20Y-D2L cells. Persistent activation of D2 dopamine receptors results in a compensatory increase of A2A-stimulated cyclic AMP accumulation. The D2 antagonist siperone and pertussis toxin pretreatment blocked this heterologous sensitization of adenylylate cyclase in CAD-D2L cells, implicating a role for Gi/o in this neuroadaptative response. This compensatory increase in A2A adenosine receptor signaling after persistent activation of D2 dopamine receptors may provide an additional mode of interaction for D2 dopamine and A2A adenosine receptor regulation of adenylylate cyclase. Furthermore, the regulatory properties of D2 dopamine and A2A adenosine receptors on adenylylate cyclase described in the current study support the hypothesis that A2A adenosine receptor signaling is a critical component in the regulation of proper dopaminergic tone.

Acknowledgments

We thank Joshua Lisinichia for technical assistance.

References

Aoyama S, Kase H, and Borrelli E (2000) Rescue of locomotor impairment in dopamine D2 receptor signaling. Furthermore, MECA pretreatment markedly reduced drug-stimulated cyclic AMP accumulation in quinpirole-treated cells (Fig. 6), suggesting that D2 dopamine receptor-induced increases in cyclic AMP accumulation can be attenuated in the presence of A2A agonist treatment. Together, these studies suggest an important role of A2A adenosine receptors in the regulation and maintenance of proper dopaminergic signaling in the striatum.

In summary, the present study demonstrated a functional interaction between stably transfected D2L dopamine receptors and endogenously expressed A2A adenosine receptors in CAD-D2L and NS20Y-D2L cells. Persistent activation of D2 dopamine receptors results in a compensatory increase of A2A-stimulated cyclic AMP accumulation. The D2 antagonist siperone and pertussis toxin pretreatment blocked this heterologous sensitization of adenylylate cyclase in CAD-D2L cells, implicating a role for Gi/o in this neuroadaptative response. This compensatory increase in A2A adenosine receptor signaling after persistent activation of D2 dopamine receptors may provide an additional mode of interaction for D2 dopamine and A2A adenosine receptor regulation of adenylylate cyclase. Furthermore, the regulatory properties of D2 dopamine and A2A adenosine receptors on adenylylate cyclase described in the current study support the hypothesis that A2A adenosine receptor signaling is a critical component in the regulation of proper dopaminergic tone.

Acknowledgments

We thank Joshua Lisinichia for technical assistance.

References

Aoyama S, Kase H, and Borrelli E (2000) Rescue of locomotor impairment in dopamine D2 receptor signaling. Furthermore, MECA pretreatment markedly reduced drug-stimulated cyclic AMP accumulation in quinpirole-treated cells (Fig. 6), suggesting that D2 dopamine receptor-induced increases in cyclic AMP accumulation can be attenuated in the presence of A2A agonist treatment. Together, these studies suggest an important role of A2A adenosine receptors in the regulation and maintenance of proper dopaminergic signaling in the striatum.

In summary, the present study demonstrated a functional interaction between stably transfected D2L dopamine receptors and endogenously expressed A2A adenosine receptors in CAD-D2L and NS20Y-D2L cells. Persistent activation of D2 dopamine receptors results in a compensatory increase of A2A-stimulated cyclic AMP accumulation. The D2 antagonist siperone and pertussis toxin pretreatment blocked this heterologous sensitization of adenylylate cyclase in CAD-D2L cells, implicating a role for Gi/o in this neuroadaptative response. This compensatory increase in A2A adenosine receptor signaling after persistent activation of D2 dopamine receptors may provide an additional mode of interaction for D2 dopamine and A2A adenosine receptor regulation of adenylylate cyclase. Furthermore, the regulatory properties of D2 dopamine and A2A adenosine receptors on adenylylate cyclase described in the current study support the hypothesis that A2A adenosine receptor signaling is a critical component in the regulation of proper dopaminergic tone.

Acknowledgments

We thank Joshua Lisinichia for technical assistance.

References

Aoyama S, Kase H, and Borrelli E (2000) Rescue of locomotor impairment in dopamine D2 receptor signaling. Furthermore, MECA pretreatment markedly reduced drug-stimulated cyclic AMP accumulation in quinpirole-treated cells (Fig. 6), suggesting that D2 dopamine receptor-induced increases in cyclic AMP accumulation can be attenuated in the presence of A2A agonist treatment. Together, these studies suggest an important role of A2A adenosine receptors in the regulation and maintenance of proper dopaminergic signaling in the striatum.

In summary, the present study demonstrated a functional interaction between stably transfected D2L dopamine receptors and endogenously expressed A2A adenosine receptors in CAD-D2L and NS20Y-D2L cells. Persistent activation of D2 dopamine receptors results in a compensatory increase of A2A-stimulated cyclic AMP accumulation. The D2 antagonist siperone and pertussis toxin pretreatment blocked this heterologous sensitization of adenylylate cyclase in CAD-D2L cells, implicating a role for Gi/o in this neuroadaptative response. This compensatory increase in A2A adenosine receptor signaling after persistent activation of D2 dopamine receptors may provide an additional mode of interaction for D2 dopamine and A2A adenosine receptor regulation of adenylylate cyclase. Furthermore, the regulatory properties of D2 dopamine and A2A adenosine receptors on adenylylate cyclase described in the current study support the hypothesis that A2A adenosine receptor signaling is a critical component in the regulation of proper dopaminergic tone.

Acknowledgments

We thank Joshua Lisinichia for technical assistance.

References

Aoyama S, Kase H, and Borrelli E (2000) Rescue of locomotor impairment in dopamine D2 receptor signaling. Furthermore, MECA pretreatment markedly reduced drug-stimulated cyclic AMP accumulation in quinpirole-treated cells (Fig. 6), suggesting that D2 dopamine receptor-induced increases in cyclic AMP accumulation can be attenuated in the presence of A2A agonist treatment. Together, these studies suggest an important role of A2A adenosine receptors in the regulation and maintenance of proper dopaminergic signaling in the striatum.

In summary, the present study demonstrated a functional interaction between stably transfected D2L dopamine receptors and endogenously expressed A2A adenosine receptors in CAD-D2L and NS20Y-D2L cells. Persistent activation of D2 dopamine receptors results in a compensatory increase of A2A-stimulated cyclic AMP accumulation. The D2 antagonist siperone and pertussis toxin pretreatment blocked this heterologous sensitization of adenylylate cyclase in CAD-D2L cells, implicating a role for Gi/o in this neuroadaptative response. This compensatory increase in A2A adenosine receptor signaling after persistent activation of D2 dopamine receptors may provide an additional mode of interaction for D2 dopamine and A2A adenosine receptor regulation of adenylylate cyclase. Furthermore, the regulatory properties of D2 dopamine and A2A adenosine receptors on adenylylate cyclase described in the current study support the hypothesis that A2A adenosine receptor signaling is a critical component in the regulation of proper dopaminergic tone.

Acknowledgments

We thank Joshua Lisinichia for technical assistance.