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Short communication

The enhancement of dopamine D_1 receptor desensitization by adenosine A_1 receptor activation

Yan Cao *, Ke-Qiang Xie, Xing-Zu Zhu ✠

Department of Pharmacology, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai 201203, PR China

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Abstract

The present study was designed to examine the effects of adenosine A_1 receptor on dopamine D_1 receptor desensitization in a human embryonic kidney 293 cell line stably cotransfected with human adenosine A_1 receptor and dopamine D_1 receptor cDNAs (A_1D_1 cells) by means of cAMP accumulation assay. Long-term exposure of A_1D_1 cells to dopamine D_1 receptor agonist (±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride (SKF38393) caused a rapid desensitization of dopamine D_1 receptor. Coadministration of adenosine A_1 receptor agonist N^6-cyclopentyladenosine (CPA) potentiated the effect of SKF38393. This enhancement effect of CPA was blocked by adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) but not by pertussis toxin, indicating that this effect of CPA was mediated by adenosine A_1 receptor and was G_i protein independent. Furthermore, the blockade of endogenous adenosine by adenosine deaminase or DPCPX attenuated dopamine D_1 receptor desensitization. Collectively, these results suggest that adenosine A_1 receptor plays an important role in the regulation of dopamine D_1 receptor by potentiating ligand-induced desensitization.

Keywords: Desensitization; Dopamine D_1 receptor; Adenosine A_1 receptor; Receptor interaction

1. Introduction

Antagonistic adenosine–dopamine interactions have been widely reported in the central nervous system in behavioral and biochemical studies (Ferre et al., 1997). Adenosine agonists inhibit and adenosine antagonists, such as caffeine, potentiate the behavioral effects induced by dopamine agonists. Thus, adenosine agonists and antagonists are suggested to be potent atypical neuroleptics and antiparkinsonian drugs, respectively (Ferre et al., 1997). The evidence suggests that this adenosine–dopamine antagonism is at least in part caused by an intramembrane interaction between specific subtypes of dopamine and adenosine receptors, namely, between adenosine A_1 receptors and dopamine D_1 receptors and between adenosine A_{2a} receptors and dopamine D_2 receptors (Ferre et al., 1997). The antagonism between adenosine A_1 receptor and dopamine D_1 receptor is evident in crude membrane preparations from cell lines expressing the two receptors and from rat striatum (Ferre et al., 1994, 1998; Uustare et al., 2006). Recently, it has been shown that this receptor–receptor interaction could involve A_1/D_1 heteromeric receptor complexes since such complexes could be demonstrated in cotransfected A_1/D_1 fibroblast Ltk^- cells by means of coimmunoprecipitation (Gines et al., 2000). Therefore the antagonistic interaction of these two receptors could be the result of a physical interaction of adenosine A_1 receptor with dopamine D_1 receptor in this heteromeric complex. However, to what extent this antagonistic interaction may alter agonist-induced desensitization of these receptors has not been well documented. Here the effects of adenosine A_1 receptor agonist on the ligand-mediated regulation of dopamine D_1 receptor were studied in a human embryonic kidney 293 (HEK293) cell line stably cotransfected with human adenosine A_1 receptor and dopamine D_1 receptor cDNAs.

* Corresponding author. Tel.: +86 21 5080 6600 2308.
E-mail address: ycao@sibs.ac.cn (Y. Cao).
✠ Deceased.

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2. Materials and methods

2.1. Transfection and maintenance of HEK293 cells

Cells from the HEK293 cell line previously transfected with the human adenosine A_1 receptor cDNA (Sun et al., 2005a) were used and grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and geneticin (200 μg/ml). For the generation of HEK293/A_1D_1 cell line, dopamine D_1 receptor cDNA (cloned into pcDNA3.1/Hygro(+), Invitrogen, USA) (Sun et al., 2005b) was transfected into HEK293/A_1 cells by the Lipofectamine^{2000} reagent (Invitrogen, USA). These transfected HEK293/A_1 cells were selected with hygromycin (250 μg/ml) in DMEM medium to generate a single clone of HEK293/A_1D_1 cell line (A_1D_1 cells) which stably expressed both adenosine A_1 receptor and dopamine D_1 receptor. Cells were incubated in a humid atmosphere of 5% CO_2 and 95% air at 37 °C.

2.2. Membrane preparation

The A_1D_1 cells were lifted from Petri dishes with a cell scraper. Harvested cells were washed twice with ice-cold PBS and centrifuged at 420 ×*g* for 5 min at 4 °C. The cell pellet was resuspended with hyponic buffer (Tris–HCl 5 mM, EDTA 2 mM, pH 7.4, leupeptin 1 mg/l, pepstatin A 1 mg/l, aprotinin 1 mg/l, phenylmethanesulfonyl fluoride 1 mM) and sonicated (18 s) three times on ice. The homogenate was centrifuged at 960 ×*g* for 10 min at 4 °C. The precipitated nucleic fraction was discarded and the supernatant was centrifuged at 40,000 ×*g* for 30 min at 4 °C. The pellet was washed with 50 mM Tris–HCl buffer (pH 7.4) and centrifuged again under the same conditions (Xie et al., 2006). Finally, the pellet was resuspended in the same buffer, and protein concentration was determined by using the BCA (bicinchoninic acid) Protein Assay Kit (Pierce) as described previously (Sun et al., 2005a).

2.3. Radioligand binding experiments

Saturation experiments with adenosine A_1 receptor antagonist [propyl-^{3}H]8-cyclopentyl-1,3-dipropylxanthine ([^{3}H]DPCPX) were carried out with 8 concentrations (0.05–10.0 nM) of [^{3}H]DPCPX (128.0 Ci/mmol, Amersham, USA) by incubation in 50 mM Tris–HCl buffer (pH 7.4) for 1 h at 37 °C. Nonspecific binding (in the presence of 10 μM unlabeled DPCPX) was about 5%–15% of the total binding. Saturation experiments with dopamine D_1 receptor antagonist [*N*-methyl-^{3}H]R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine ([^{3}H]SCH23390) were carried out with 8 concentrations (0.1–10.0 nM) of [^{3}H]SCH23390 (79.0 Ci/mmol, Amersham, USA) by incubation in Tris–HCl buffer (Tris–HCl 50 mM, NaCl 120 mM, KCl 5 mM, $MgCl_2$ 1 mM, $CaCl_2$ 2 mM, pH 7.4) for 30 min at 37 °C. Nonspecific binding (in the presence of 10 μM butaclamol, a dopamine receptor antagonist) was about 10%–15% of the total binding. The incubation was stopped by fast filtration through glass-fiber filters (GF/B, Whatman) by washing three times with 5 ml of 50 mM ice-cold Tris–HCl buffer (pH 7.4) with an automatic cell harvester (Brandel). The radioactivity was counted with Beckman LS6500 liquid scintillation analyzer (Sun et al., 2005a).

2.4. cAMP determination

Treatments were performed for 0.5, 1, 2 and 6 h with 10 μM (±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride (SKF38393, Sigma) in the presence or absence of different concentrations of N^6-cyclopentyladenosine (CPA, Sigma). Experiments with pertussis toxin were performed with A_1D_1 cells exposed to pertussis toxin (100 ng/ml) overnight before cAMP determination. Treatments with adenosine deaminase (10 U/ml; Roche), or adenosine A_1 receptor antagonist DPCPX (10 μM) were performed by adding the compounds 30 min before SKF38393 in the presence or absence of CPA in the culture media, and they were present all along the desensitization protocol. After two washes at 4 °C with culture medium containing 30 μM phosphodiesterase inhibitor rolipram (Sigma), cAMP accumulation was induced by stimulating dopamine D_1 receptors for 15 min with 10 μM SKF38393 and 30 μM rolipram at 37 °C. The reaction was terminated with 1 M perchloric acid to a final concentration of 0.5 M. Samples were neutralized with 2 M KOH, and the cAMP content in the supernatants was determined with a protein binding assay (Sun et al., 2005b). The samples were then centrifuged at 14,000 ×*g* for 5 min, and 50 μl of the supernatant from each well was subsequently transferred to a 5-ml tube containing 50 μl of reaction buffer (50 mM acetate acid and 4 mM EDTA, pH 7.4) and 100 μl of [^{3}H]cAMP. 8 μl of acetic anhydride/triethylamine (33%) was added to each tube followed by adding 100 μl of cAMP-binding protein. After incubation at 4 °C overnight, radioactivity in the supernatant from each tube was counted with Beckman LS6500 liquid scintillation analyzer. cAMP concentrations were calculated using a standard curve according to the protocol of the assay kit cAMP (Sun et al., 2005b).

2.5. Statistics

Results were expressed as mean±S.E.M. and were analyzed using a two-way analysis of variance (ANOVA) followed by Bonferroni post hoc test. The level of significance was set at $P<0.05$.

3. Results

The clone chosen for subsequent studies (A_1D_1 cells) had a density of adenosine A_1 receptor labeled with [^{3}H]DPCPX (mean±S.E.M.: B_{max}, 809.7±10.1 pmol/g of protein; K_d value of 2.30±0.11 nM; $n=3$) and dopamine D_1 receptor labeled with [^{3}H]SCH23390 (mean±S.E.M.: B_{max}, 269.4±3.6 pmol/g of protein; K_d value of 0.51±0.05 nM; $n=3$). In A_1D_1 cells, pretreatment with dopamine D_1 receptor agonist SKF38393 (10 μM) resulted in desensitization of the subsequent

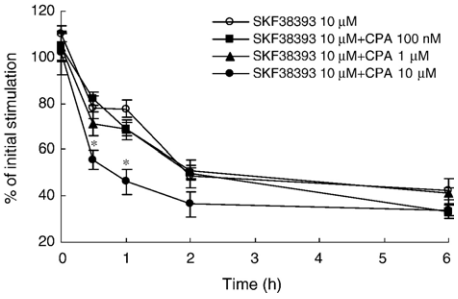

Fig. 1. Concentration-dependent influence of adenosine A_1 receptor agonist CPA on the desensitization time-course of dopamine D_1 receptor in stably cotransfected HEK293 cells. Cells were treated with 10 μM SKF38393 for 0.5 h, 1 h, 2 h, and 6 h in basal conditions (○), or in the presence of adenosine A_1 receptor agonist CPA (100 nM, ■; 1 μM, ▲; 10 μM, ●). Data were expressed as mean±S.E.M., $n=4$. Two-way ANOVA indicated that both treatment and time had a significant effect on dopamine D_1 receptor desensitization ($P<0.01$). *$P<0.05$ compared with the basal condition (Bonferroni post hoc test).

SKF38393 response as manifested by a reduction (of about 30%) in the maximum cAMP accumulation. Maximum desensitization of the dopamine D_1 receptor response was observed to occur by about 2 h of SKF38393 pretreatment. This effect was potentiated by co-pretreatment with CPA in a concentration-dependent manner (Fig. 1). This result suggested that adenosine A_1 receptor activation could enhance dopamine D_1 receptor desensitization. Consistent with this, the enhancement effect by CPA was blocked by pretreatment with adenosine A_1 receptor antagonist DPCPX (Fig. 2). However, a G_i protein inhibitor pertussis toxin pretreatment had no effect on the enhancement effect by CPA (Fig. 2). This indicated that the effect of CPA was not induced via an interaction of the adenosine A_1 receptor with G_i. To rule out the presence of endogenous adenosine in the culture medium, adenosine deaminase, an enzyme capable of converting adenosine to inosine, was added. In this condition, the dopamine D_1 receptor desensitization was delayed (Fig. 2). This effect of adenosine deaminase suggested that endogenous adenosine produced by the cells and released into the culture medium, was responsible for dopamine D_1 receptor desensitization. This agreed well with the demonstration that DPCPX pretreatment attenuated the desensitization of dopamine D_1 receptor (Fig. 2).

4. Discussion

The present studies firstly showed that tonic activation of adenosine A_1 receptor potentiated dopamine D_1 receptor desensitization in a HEK293 cell line stably cotransfected with human adenosine A_1 receptor and dopamine D_1 receptor cDNAs. Moreover, this effect was blocked by adenosine A_1 receptor antagonist DPCPX. These results indicate that the enhancement of dopamine D_1 receptor desensitization depends on adenosine A_1 receptor activation. Since adenosine A_1 receptor is coupled to G_i protein, the addition of adenosine A_1 receptor agonist CPA might lower the basal cAMP level. Pertussis toxin is a good tool to investigate G protein involvement because of its selectivity of action by inducing an ADP-ribosylation of the G_α subunit of the G_i (and G_o) protein family (Gilman, 1987). However, pertussis toxin pretreatment failed to block the effect of adenosine A_1 receptor agonist on dopamine D_1 receptor desensitization. Thus, the decrease of the cAMP level by adenosine A_1 receptor agonist pretreatment was not caused by adenosine A_1 receptor agonist-induced G_i protein activation. Moreover, adenosine deaminase or DPCPX pretreatment attenuated the desensitization of dopamine D_1 receptor, indicating that adenosine released by the cells exerts a tonic enhancement of the desensitization of dopamine D_1 receptor. However, DPCPX pretreatment has no effect at the 6 h time point (Fig. 2). We speculate that after 6 h of agonist exposure, most dopamine D_1 receptors would internalized from cell surface. Sustained exposure to agonist for more than 1 h resulted in significant dopamine D_1 receptor internalization occurring well after the desensitization of dopamine D_1 receptor (Ng et al., 1995). Thus, the effect of adenosine A_1 receptor on dopamine D_1 receptor signaling might be very weak at this time point.

Previous evidence has demonstrated that the well documented antagonistic A_1–D_1 receptor–receptor interaction could be the result of a physical interaction of adenosine A_1 receptor with dopamine D_1 receptor in the heteromeric complex, leading to an uncoupling of dopamine D_1 receptor from its G_s-like protein in this functionally interacting heteromeric complex (Ferre et al., 1998; Gines et al., 2000). A decrease of cAMP formation induced by preincubation with SKF38393 was found in the present study, but not by Gines et al. (2000). Such differences might be a result of the fact that cells other than HEK293 cells were used in the previous studies. Moreover, endogenous adenosine is a ubiquitous physiological regulator and neuromodulator capable of multiple physiological actions in various systems (Ribeiro et al., 2002). The addition of adenosine deaminase in the present studies

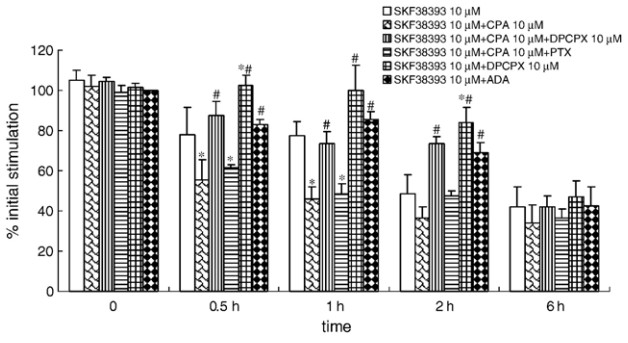

Fig. 2. The enhancement of dopamine D_1 receptor desensitization by CPA depends on adenosine A_1 receptor. A_1D_1 cells were treated with 10 μM SKF38393 for 0.5 h, 1 h, 2 h, and 6 h in basal conditions (open bars), or in the presence of CPA (10 μM), or in the presence of the specific adenosine A_1 receptor antagonist DPCPX (10 μM), or in the presence of adenosine deaminase, or in the presence of CPA (10 μM) and DPCPX (10 μM) in combination. Experiments with pertussis toxin were performed with A_1D_1 cells exposed to pertussis toxin (100 ng/ml) overnight followed by treatment with 10 μM SKF38393 and 10 μM CPA. PTX, pertussis toxin; ADA, adenosine deaminase. Data were expressed as mean±S.E.M., $n=3$. Two-way ANOVA indicated that both treatment and time had a significant effect on dopamine D_1 receptor desensitization ($P<0.01$). *$P<0.05$ compared with the basal condition (Bonferroni post hoc test). #$P<0.05$ compared with in the presence of CPA (10 μM) (Bonferroni post hoc test).

demonstrated the involvement of endogenous adenosine in the regulation of dopamine D_1 receptor desensitization. Uustare et al. (2006) have reported the involvement of G_i protein in the acute antagonistic A_1–D_1 receptor–receptor interaction recently. This agreed well with our previous studies (Cao et al., 2006) and other groups' results (Ferre et al., 1998). However, the G_i protein inhibitor pertussis toxin pretreatment failed to block the effect of adenosine A_1 receptor agonist on dopamine D_1 receptor desensitization. These results suggested antagonistic A_1–D_1 receptor–receptor interaction after long-term exposure to agonists might be different from the acute antagonism. One hour exposure with both adenosine A_1 receptor and dopamine D_1 receptor agonists maintained the heteromeric association of the two receptors but decreased the amount of adenosine A_1 receptor/dopamine D_1 receptor aggregations (clusters) (Gines et al., 2000). Thus, the enhancement of dopamine D_1 receptor desensitization may be caused mainly by a prolonged allosteric change in dopamine D_1 receptor brought about by the A_1–D_1 receptor–receptor interaction within the heteromeric complex. The allosteric change in dopamine D_1 receptor could be related to subsequent phosphorylation changes and/or association with β-arrestin-like molecules (Lefkowitz, 2000; McDonald and Lefkowitz, 2001; Tiberi et al., 1996), leading overall to a reduced dopamine D_1 receptor/G_s coupling. This is in contrast to the A_{2a}–D_2 receptor–receptor interaction, where long-term exposure to adenosine A_{2a} receptor and dopamine D_2 receptor agonists in dopamine D_2 receptor-cotransfected SH-SY5Y cells resulted in coaggregation, cointernalization and codesensitization of adenosine A_{2a} receptor and dopamine D_2 receptor (Hillion et al., 2002). Thus, it suggests that the intramembrane A_1–D_1 receptor–receptor interaction in this heteromeric complex is relevant not only for acute antagonism of dopamine D_1 receptor signaling (Cao et al., 2006; Ferre et al., 1997, 1994, 1998) but also for a persistent long-term antagonism of dopamine D_1 receptor signaling to the G_s protein. This antagonistic mechanism may contribute to the adenosine A_1 receptor/dopamine D_1 receptor functional antagonism found in the brain and offers a basis for the design of novel agents to treat Parkinson's disease and neuron psychiatric disorders in which dopamine D_1 receptor has been implicated (Ferre et al., 2001; Goldman-Rakic et al., 2004).

However, the enhancement of dopamine D_1 receptor desensitization by adenosine A_1 receptor agonist differs from the previous finding that autocrine activation of adenosine A_1 receptor blocks dopamine D_1 receptor desensitization in COS7 cells transiently transfected with dopamine D_1 receptor and adenosine A_1 receptor cDNAs (Le Crom et al., 2002). Difference in the cellular context could result in discordant observations. It has been shown that when expressed in different cell types, the internalization profiles for some, but not all G protein-coupled receptors, are different (Menard et al., 1997; Zhang et al., 1996). Previous studies showed that COS7 cells expressed relatively less G protein-coupled receptor kinase (GRK) and β-arrestin protein than HEK293 cells (Aramori et al., 1997; Barlic et al., 1999; Menard et al., 1997; Zhang et al., 1996). Tiberi et al. (1996) have shown that phosphorylation of dopamine D_1 receptor in HEK293 cells is predominantly mediated by GRKs. Thus, the enhancement of dopamine D_1 receptor desensitization by adenosine A_1 receptor agonist could not be observed in COS7 cells. Moreover, a HEK293 cell line stably cotransfected with dopamine D_1 receptor and adenosine A_1 receptor cDNAs was used in the present studies. The failure in demonstrating this interaction in transiently transfected COS7 cells could probably be due to a relative low number of cells containing both receptors.

Agonist exposure to dopamine D_1 receptor can cause rapid desensitization (Gardner et al., 2001; Kim et al., 2004). In contrast, it is generally believed that desensitization of the adenosine A_1 receptor differs from the other adenosine receptors because of its relatively slower rate. The slowly desensitizing adenosine A_1 receptor is not phosphorylated in response to agonist exposure, and is desensitized only after agonist exposure for several hours or even days (Ferguson et al., 2000; Gao et al., 1999; Ramkumar et al., 1991). This slower rate of desensitization might be important *in vivo* during ischemia by helping to maintain adequate tissue perfusion in the presence of elevated levels of adenosine. Since the enhancement effect of CPA occurred at agonist exposure for 30 min in the present studies and the slow desensitization rate of adenosine A_1 receptor, we speculate that adenosine A_1 receptor desensitization might not be responsible for the enhancement effect of CPA. However, it will be very interesting and crucial to evaluate the relationship between adenosine A_1 receptor desensitization and dopamine D_1 receptor desensitization in the further studies.

Previous studies showed the acute antagonistic interaction between dopamine D_1 receptor and adenosine A_1 receptor (Cao et al., 2006; Ferre et al., 1997, 1994, 1998). Our findings shed new light on the interaction between these two receptors, a persistent long-term antagonistic modulation of dopamine D_1 receptor signaling by adenosine A_1 receptor. This gives us a novel understanding of the desensitization at dopamine D_1 receptor, a key target for treatment of neuropsychiatric diseases (Ferre et al., 2001; Goldman-Rakic et al., 2004). We speculate that adenosine A_1 receptor antagonists may therefore be used in Parkinson's disease because they counteract the desensitization of dopamine D_1 receptor like receptors after prolonged L-DOPA and/or dopamine D_1 receptor agonist treatment (Ferre et al., 2001). Thus, this provides a new way of avoiding desensitization of key receptors in heteromeric complexes after prolonged agonist treatment, namely by developing drugs that act on the coreceptors. Further studies will be needed to elucidate how the prolonged allosteric changes in dopamine D_1 receptor brought about by adenosine A_1 receptor activation affect the phosphorylation changes and/or the association with the β-arrestin-like molecules.

Our data provide novel evidence that tonic activation of adenosine A_1 receptor potentiates dopamine D_1 receptor desensitization. Such concerned interactions between functionally distinct neurotransmitter receptors appear to be part of a general overall mechanism by which neurons are able to receive and integrate diverse extracellular signals.

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