Effects of Adenosine A1- and A2A-Receptor Agonists on Enhancement of Dopamine Release From the Striatum in Methamphetamine-Sensitized Rats

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ABSTRACT—We report here both adenosine A1- and A2A-receptor agonists inhibit the expression of methamphetamine (MAP)-induced behavioral sensitization in rats. Animals were treated with MAP (1.0 mg/kg, i.p.) every 3 days with a total of 5 administrations. The augmentation of dopamine release from the striatum was demonstrated by MAP re-administration (0.5 mg/kg, i.p.) after 7-day withdrawal by microdialysis. The augmentation of dopamine release was inhibited by pre-treatment not with N6-cyclohexyladenosine (0.01 mg/kg, i.p.) but by with 2-p-(2-carboxyethyl)phenethylamino-5'-N-ethylcarboxy-amide adenosine (0.1 mg/kg, i.p.). These results suggested that adenosine A1 and A2A receptors play an inhibitory role in sensitization via different mechanisms.

Keywords: Sensitization, Dopamine, Adenosine

Studies of the mechanism of behavioral sensitization to amphetamine or methamphetamine (MAP) have focused on possible alterations in the dopamine system (1). Adenosine is known to play an important role in synaptic transmission in the central nervous system (2). Adenosine A1 receptors are distributed in various regions of the rat brain (3), and their stimulation induces a decrease in cAMP level (4). On the other hand, adenosine A2A receptors are distributed in the striatum, the accumbens, etc., and their stimulation induces increases in the cAMP level (5). Previous reports indicated that both receptors suppress dopamine transmission (6). Antagonistic adenosine A1 - dopamine D2 and adenosine A2 - dopamine D2 receptor interactions have been reported (7, 8).

We reported previously that MAP-induced sensitization causes the enhancement of striatal dopamine release and that metabotropic glutamate receptors might be involved in this dopamine release (9). We also demonstrated that both adenosine A1 and A2A receptors inhibit the expression of MAP-induced behavioral sensitization (10). Therefore, the striatal dopamine release may also be influenced by adenosine-related drugs in MAP-sensitized rats. In the present study, we investigated the effects of an adenosine A1-receptor agonist, N6-cyclohexyladenosine (CHA), and an adenosine A2A-receptor agonist, 2-p-(2-carboxyethyl)phenethylamino-5'-N-ethylcarboxy-amide adenosine (CGS21680), on the striatal dopamine release in the expression of MAP-induced sensitization.

The animals used in this experiment were male rats of the Wistar strain (Kyudo Experimental Animal Co., Saga), weighing between 230 – 250 g at the start of the experiment. The rats were housed in groups of 4 or 5 under constant temperature (23 ± 2°C) and a 12-h light/dark cycle (light period: 07:00 – 19:00). The rats were allowed free access to food and water throughout the experiments.

The drugs used in the present study were MAP (Dainippon Pharmaceuticals Ltd., Osaka); CHA and CGS21680 (Research Biochemicals International, Natick, MA, USA).

The rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and were fixed on a stereotactic instrument. A guide cannula (0.5-mm outer diameter, AG-8; Eicom Co., Kyoto) was placed just above the striatum (0.7-mm anterior to the bregma, 2.6-mm lateral to the midline, 3.2-mm ventral to the surface of the skull measured at the bregma).

After at least a 2-day recovery period after the operation, we examined the acute effects of MAP (0.5 and 1.0 mg/kg, i.p.). Then, we determined the effect of pretreatment with CHA (0.01 mg/kg, i.p.) or CGS21680 (0.1 mg/kg, i.p.) on...
the increase of dopamine release by acute MAP administration (1.0 mg/kg, i.p.). Animals were treated with CHA or CGS21680 30 min before MAP administration. Thereafter, the effects of CHA or CGS21680 on MAP-induced sensitization were studied. Rats were injected with MAP (1.0 mg /kg) or saline i.p. every 3 days with a total of 5 injections in their home cages. After a 7-day withdrawal period, rats were challenged with MAP (0.5 mg/kg, i.p.). To study the effects of CHA (0.01 mg/kg, i.p.) or CGS21680 (0.1 mg /kg, i.p.) on sensitization, each drug was injected i.p. 30 min before MAP challenge. The expression of MAP-induced behavioral sensitization was significantly inhibited by 0.01 mg/kg of CHA or 0.1 mg/kg of CGS21680 (10). Therefore, these doses were used in this study.

Brain microdialysis was carried out in unanesthetized, freely moving rats. A concentric dialysis probe (3.0-mm active membrane length and 0.2-mm outer diameter, A-I-8-03; Eicom Co.) was inserted into the striatum through the guide cannula so that the tip of the probe was located 6.2-mm ventral to the skull surface. During the dialysis experiment, the probe was connected to an infusion pump (EP-60, Eicom Co.) and was perfused with Ringer’s solution (147 mM NaCl, 4 mM KCl, 2.3 mM CaCl2) at a rate of 2.0 µl/min. Perfusate samples collected at 20-min intervals (40 µl) were directly injected into the HPLC-ECD system for quantification of dopamine. The HPLC-ECD consisted of a pump (EP-10, Eicom Co.) coupled to a reversed-phase column (5 mm, 4.6 × 150 mm, Develosil ODS-HG-5; Nomura Chemical Co., Ltd., Aichi) and an ECD (ECD-100, Eicom Co.). A graphite working electrode (WE-3G, Eicom Co.) was set at +0.60 V against the Ag/AgCl reference electrode. The mobile phase was composed of 36.8 mM citric acid, 52.6 mM sodium acetate, 0.6 mM sodium 1-octanesulfonate, 14 mM EDTA and 11% methanol. Flow rate was 1.0 µl/min.

After the basal dopamine level was stabilized (at least 2 h after probe insertion), drugs were injected i.p., and then dopamine release was measured for up to 3 h. Data are expressed as percentages of baseline levels, and significance was analyzed by repeated measures analysis of variance (ANOVA) followed by Student’s t-test. The baseline value of the extracellular concentration of dopamine in dialysates from the striatum was 17.7 ± 1.0 pg/20 min (mean ± S.E.M., n=24) in saline-treated rats. The value was not different from that in MAP-sensitized rats (17.8 ± 0.7 pg/20 min, n=27). Therefore, the basal dopamine release in each group was set as 100%.

Dopamine release from the striatum was significantly increased by acute treatment with 1.0 mg/kg of MAP, but not 0.5 mg/kg. Neither CHA (0.01 mg/kg, i.p.) nor CGS21680 (0.1 mg/kg, i.p.) had any effect on dopamine release from the striatum. Neither drug had any effect on the increase of dopamine release by MAP administration (data not shown). Figure 1 shows the time course of changes in dopamine level after MAP challenge (0.5 mg/kg, i.p.). Dopamine release in the striatum was significantly increased by MAP challenge in MAP-sensitized rats. Figure 2 shows the time course of changes in dopamine level after MAP challenge with or without CHA pretreatment (0.01 mg/kg, i.p.). The increase of dopamine release by MAP challenge was not influenced by pretreatment with CHA. Figure 3 shows the time course of changes in dopamine level after MAP challenge with or without CGS21680 pre-treatment (0.1 mg/kg, i.p.). The increase of dopamine release by MAP challenge was significantly inhibited by pretreatment with CGS21680.

We reported previously that MAP-induced behavioral sensitization is inhibited by pretreatment with CHA or CGS21680 (10). However, the present study indicated that these drugs may block this sensitization via different mechanisms. CHA did not block the increase in dopamine release induced by acute MAP treatment or MAP challenge. It is reported that supersensitivity of dopamine D1 receptors is induced in the accumbens on the expression of MAP-induced sensitization (11). Dopamine D1 receptors are known to increase cAMP production. cAMP induces an increase in adenosine as a result of hyperactivation of...
adenylate cyclase through nucleoside transporter (12). So, the increase in adenosine is suggested by the expression of sensitization. Moreover, pretreatment with CHA induced the inhibition of locomotor activity before MAP challenge, although a single administration at the dose used in this study did not have any effect (data not shown). Taken together, the increase in adenosine function may be induced on the expression of sensitization. Further studies are needed to clarify this point.

However, adenosine A2A receptors were reported to distribute in post-synaptic sites (13). CGS21680 blocked the increase of dopamine release only by MAP challenge. The substantia nigra receives GABAergic input from the striatum through cholinergic neurons. Therefore, adenosine A2A receptors are distributed in the cholinergic terminals. Adenosine A2A receptors of cholinergic terminals are activated by CGS21680, and thus GABAergic neurons might also be activated. Therefore, dopaminergic neurons might be inhibited by activated GABAergic neurons, and dopamine release in the striatum might be blocked.

The striatum receives two main afferent pathways, i.e., dopaminergic and glutamatergic input from the substantia nigra and the cortex, respectively. Many studies have indicated that corticostriatal glutamatergic projections are involved in regulation of dopamine release from terminals of nigrostriatal dopaminergic neurons. Both in vivo and in vitro studies have reported that glutamate stimulates the release of dopamine from the striatum (14, 15). These studies suggested that the facilitatory regulation of glutamatergic input on dopamine release is in part, mediated by the activation of presynaptic receptors. The existence of adenosine A2A receptors on the nerve terminals of glutamatergic neurons has yet not be confirmed. However, our results were highly suggestive of this possibility.

In conclusion, adenosine A1 and A2A receptors may inhibit MAP-induced sensitization via different mechanisms.

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REFERENCES
1 Robinson TE and Camp DM: Long-lasting effects of escalating doses of d-amphetamine on brain monoamines, amphetamine-induced stereotyped behavior and spontaneous nocturnal loco-
motion. Pharmacol Biochem Behav 26, 821 – 827 (1987)
2 Brundege JM and Dunwiddie TV: Modulation of excitatory synaptic transmission by adenosine released from single hippocampal pyramidal neurons. J Neurosci 16, 5603 – 5612 (1996)
3 Williams M and Braunwalder A: Effects of purine nucleotides on the binding of \(^{[3]H}\)cyclopentyladenosine to adenosine A-1 receptors in rat brain membranes. J Neurochem 47, 88 – 97 (1986)
4 Fredholm BB and Dunwiddie TV: How does adenosine inhibit transmitter release? Trends Pharmacol Sci 9, 130 – 134 (1988)
5 Jarvis MF and Williams M: Direct autoradiographic localization of adenosine A2 receptors in the rat brain using the A2-selective agonist, \(^{[3]H}\)CGS 21680. Eur J Pharmacol 168, 243 – 246 (1989)
6 Inoue H, Arai I, Shibata S and Watanabe S: \(N^\circ\)-nitro-L-arginine methyl ester attenuates the maintenance and expression of methamphetamine-induced behavioral sensitization and enhancement of striatal dopamine release. J Pharmacol Exp Ther 277, 1424 – 1430 (1996)
7 Ferre S, von Euler G, Johansson B, Fredholm BB and Fuxe K: Stimulation of high-affinity adenosine A2 receptors decreases the affinity of dopamine D2 receptors in rat striatal membranes. Proc Natl Acad Sci USA 88, 7238 – 7241 (1991)
8 Ferre S, O’Connor WT, Snaprud P, Ungerstedt U and Fuxe K: Antagonistic interaction between adenosine A2A receptors and dopamine D2 receptors in the ventral striopallidal system. Implications for the treatment of schizophrenia. Neuroscience 63, 765 – 773 (1994)
9 Arai I, Shimazoe T, Shibata S, Inoue H, Yoshimatsu A and Watanabe S: Enhancement of dopamine release from the striatum through metabotropic glutamate receptor activation in methamphetamine sensitized rats. Brain Res 729, 277 – 280 (1996)
10 Shimazoe T, Yoshimatsu A, Kawashimo A and Watanabe S: Roles of adenosine A1 and A3A receptors in the expression and development of methamphetamine-induced sensitization. Eur J Pharmacol 388, 249 – 254 (2000)
11 Higashi H, Inanaga K, Nishi S and Uchimura N: Enhancement of dopamine actions on rat nucleus accumbens neurones in vitro after methamphetamine pre-treatment. J Physiol (Lond) 408, 587 – 603 (1989)
12 Rosenberg PA, Knowles R, Knowles KP and Li Y: Beta-adrenergic receptor-mediated regulation of extracellular adenosine in cerebral cortex in culture. J Neurosci 14, 2953 – 2965 (1994)
13 Ferre S, Fredholm BB, Morelli M, Popoli P and Fuxe K: Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. Trends Neurosci 20, 482 – 487 (1997)
14 Keefe KA, Zigmond MJ and Abercrombie ED: Extracellular dopamine in striatum: influence of nerve impulse activity in medial forebrain bundle and local glutamatergic input. Neuroscience 47, 325 – 332 (1992)
15 Ochi M, Inoue H, Koizumi S, Shibata S and Watanabe S: Long-term enhancement of dopamine release by high frequency tetanic stimulation via a \(N\)-methyl-D-aspartate-receptor-mediated pathway in rat striatum. Neuroscience 66, 29 – 36 (1995)