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Determination of the effects of caffeine and carbamazepine on striatal dopamine release by in vivo microdialysis

Motohiro Okada *, Kazuhiro Kiryu, Yuko Kawata, Kazuhisa Mizuno, Kazumaru Wada, Hiroichi Tasaki, Sunao Kaneko

Department of Neuropsychiatry, Hirosaki University, Hirosaki 036, Japan

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Abstract

The effects of carbamazepine and caffeine on adenosine receptor subtypes were determined using in vivo microdialysis in an attempt to elucidate their different psychotropic mechanisms of action. Adenosine and a selective adenosine A_1 receptor agonist decreased the striatal extracellular dopamine level, whereas caffeine, carbamazepine and a selective adenosine A_1 receptor antagonist increased it, but neither an adenosine A_2 receptor agonist nor an antagonist affected it. Under conditions of adenosine A_1 receptor blockade, adenosine, carbamazepine and a selective adenosine A_2 receptor agonist increased the striatal extracellular dopamine level, whereas caffeine and a selective adenosine A_2 receptor antagonist decreased it. These results suggest that adenosine A_1 receptor stimulation reduces the striatal extracellular dopamine level, and that adenosine A_2 receptor stimulation under conditions of adenosine A_1 receptor blockade increases it. Therefore, caffeine is an antagonist of both adenosine A_1 and A_2 receptor subtypes, and carbamazepine is an adenosine A_1 receptor antagonist as well as an adenosine A_2 receptor agonist. These properties support the hypothesis that the central actions of both carbamazepine and caffeine result from effects on both adenosine A_1 and A_2 receptors.

Keywords: Adenosine; Caffeine; Carbamazepine; Dopamine; Microdialysis

1. Introduction

Xanthines, including caffeine and theophylline, at high doses cause focal and generalized convulsions (Rall, 1990). Furthermore, caffeine, which is one of the most widely consumed psychotropic agents, may cause or exacerbate symptoms in patients with panic disorders (Apfeldorf and Shear, 1993) and anxiety or depression (Gilliland and Andress, 1981). The mechanisms responsible have yet to be elucidated, but it has been suggested that the adenosine receptor antagonistic effect of these xanthines may contribute to their psychotropic actions (Daval et al., 1989).

The frequently prescribed antiepileptic drug carbamazepine is effective in controlling affective disorders (Okuma et al., 1990), panic disorders (Tondo et al., 1989) and trigeminal neuralgia (Blom, 1962), but its mechanisms of action have not been elucidated fully. Carbamazepine lacks affinity for a wide variety of neurotransmitter receptors which are classically associated with psychiatric disorders (Marangos et al., 1983). Although there is increasing evidence from receptor binding assays that carbamazepine, at therapeutically relevant concentrations (from 20 to 50 μM), acts on adenosine receptors (Marangos et al., 1983; Fujiwara et al., 1986; Daval et al., 1989), it is controversial whether carbamazepine acts as an agonist or antagonist at adenosine receptors (Skerritt et al., 1983; Fujiwara et al., 1986; Daval et al., 1989).

Recently, it was proposed that adenosine is a homeostatic neuromodulator (Williams, 1989). The rat striatum is rich in both adenosine A_1 and A_2 receptors (Wojcik and Neff, 1983; Lee and Reddington, 1986), and the nigrostriatal dopaminergic pathway appears to be modulated by adenosine (Wood et al., 1989; Okada et al., 1996). Many of the modulatory effects of adenosine on the release of a range of 'classical' transmitters appear to be modulated by extracellular receptors, which are negatively (A_1) and positively (A_2) coupled to adenylate cyclase (Wood et al., 1989; Barraco and Stefano, 1990; Zetterström and Fillenz, 1990; Okada et al., 1996). Adenosine A_1 receptor activation leads to the inhibition of dopamine release (Wood et al., 1989; Barraco and Stefano, 1990; Zetterström and

* Corresponding author. Tel.: (81-172) 39-5066; Fax: (81-172) 39-5067; e-mail: okadamot@cc.hirosaki-u.ac.jp

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Fillenz, 1990; Okada et al., 1996). The adenosine A_2 receptor has been subdivided pharmacologically into two subtypes designated A_{2A} and A_{2B}, which bind to adenosine with relatively high and low affinity, respectively (Reddington and Lee, 1991), but the effects of each of these receptor subtypes on extracellular dopamine levels have yet to be determined.

In order to clarify the functions of adenosine receptors and to determine the effects of carbamazepine and caffeine on them, we used in vivo microdialysis to measure striatal dopamine release after local administration of various adenosine receptor ligands, including carbamazepine and caffeine, to freely moving rats.

2. Materials and methods

2.1. Materials

Male Wistar rats (Clea, Japan), weighing 250–300 g, were housed under conditions of constant temperature (25 ± 2°C) with a 12-h light-dark cycle. The experimental protocols used in this study were approved by the appropriate institutional review committee and met the guidelines of the responsible governmental agency.

2.2. Microdialysis system preparation

Each rat was anesthetized with diethylether and placed in a stereotaxic frame. A concentric I-type dialysis probe (0.22-mm diameter; 3-mm exposed membrane; Eicom, Japan) was implanted in the striatum (A = 0.2 mm, L = 3.0 mm, V = −3.4 mm relative to the bregma) and the perfusion experiments were started 24–36 h after the rats had recovered from anesthesia. The perfusion rate was always 2 μl/min, using modified Ringer's solution (MRS) composed of (in mM): 145 Na^+, 2.7 K^+, 1.2 Ca^{2+}, 1.0 Mg^{2+}, 154.4 Cl^-, 0.2 ascorbate, and buffered with 10 mM phosphate buffer and 1.1 mM Tris buffer to adjust the pH to 7.40. Dialysate was injected automatically every 20 min into a high-performance liquid chromatograph with an electrochemical detector (ECD-HPLC) system by an autoinjector (Okada et al., 1992, 1995, 1996).

2.3. ECD-HPLC conditions for measuring extracellular dopamine levels

The HPLC system, used for determination of the extracellular dopamine level, was equipped with an ECD (ECD-100, Eicom, Japan) and a graphite carbon electrode set at +750 mV (versus an Ag/AgCl reference electrode). The analytical column (Superspher RP-18, 75 mm × 4 mm internal diameter, particle size 4 μm) was purchased from Kanto Kagaku (Japan). The mobile phase comprised 0.1 M citrate/0.1 M sodium acetate buffer, containing 10% (v/v) methanol, 150 mg/l octansulfonic sodium and 0.1 mM EDTA-2 Na. The final pH was 2.5 and the column temperature was maintained at 25°C with the flow rate set at 1.0 ml/min (Okada et al., 1992, 1995, 1996).

2.4. Study design

Chemical agents used in this study are summarized in Table 1.

2.4.1. Experiment 1 (effects of adenosine receptor ligands on striatal extracellular dopamine level)

In order to study the effects of adenosine receptor function on striatal extracellular dopamine level, perfusion with MRS was commenced, and when the striatal extracellular dopamine level had stabilized the perfusion medium was switched to MRS containing the required agents: the non-selective endogenous agonist adenosine (0.05, 0.5, 5 and 50 μM), the selective adenosine A_1 receptor agonist 2-chloro-N^6-cyclopentyladenosine (CCPA: 0.1 and 1 μM), the selective adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dimethylxanthine (CPT: 0.5, 5 and 50 μM), the selective adenosine A_2 receptor agonist N^6-[2-(3,5-dimethoxyphenyl)-2-(methylphenyl)ethyl]adenosine (DPMA: 0.5 and 5 μM), the selective adenosine A_2 receptor antagonist 3,7-dimethyl-1-propargylxanthine (DMPX: 1 and 10 μM), the selective adenosine A_{2A} receptor agonist 2-[4-(2-carboxyethyl)phenethylamino]-5′-*N*-ethyl-carboxamide-adenosine (CGS21680: 1, 10 and 100 μM), the non-selective adenosine receptor antagonist caffeine (0.5, 5 and 50 μM), and carbamazepine (100 μM).

2.4.2. Experiment 2 (effects of adenosine receptor ligands on striatal extracellular dopamine level under conditions of adenosine A_1 receptor blockade)

In order to study the interactions between the selective adenosine A_1 receptor antagonist CPT and adenosine receptor ligands on striatal extracellular dopamine level, perfusion with MRS containing 50 μM CPT was commenced, and when the basal striatal extracellular dopamine level had stabilized the perfusion medium was switched to MRS containing 50 μM CPT plus the required agents: adenosine (5 and 50 μM), caffeine (5 and 50 μM), DPMA (0.5 and 5 μM), DMPX (1 and 10 μM), CGS21680 (1, 10 and 100 μM) or carbamazepine (100 μM).

2.5. Diffusion rate of carbamazepine

To estimate the rate of carbamazepine diffusion through the membrane, dialysis probes were perfused at a flow rate of 2 μl/min and placed in the perfusing solution in vitro. The temperature was maintained at 37°C with a perfusion warmer during dialysis. The amount of carbamazepine that diffused through the dialysis tube into the extramembrane solution in 120 min was determined by HPLC according to the method of Juergens (1987).

Table 1
Chemical agents used in this study

Agent name (abbreviation)	Character	Source
Adenosine	Non-selective adenosine receptor agonist	Sigma
Caffeine	Non-selective adenosine receptor antagonist	Nacalai Tesque
2-Chloro-N^6-cyclopentyladenosine (CCPA)	Adenosine A_1 receptor agonist	Research Biochemicals International
8-Cyclopentyl-1,3-dimethylxanthine (CPT)	Adenosine A_1 receptor antagonist	Research Biochemicals International
2-[4-(2-Carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamideadenosine (CGS21680)	Adenosine A_{2A} receptor agonist	Research Biochemicals International
N^6-[2-(3,5-Dimethoxyphenyl)-2-(methylphenyl)ethyl] adenosine (DPMA)	Adenosine A_2 receptor agonist	Research Biochemicals International
3,7-Dimethyl-1-propargylxanthine (DMPX)	Adenosine A_2 receptor antagonist	Research Biochemicals International
Carbamazepine		Ciba-Geigy

2.6. Statistics

The differences between the mean striatal extracellular dopamine level under control conditions and during adenosine receptor ligand treatment were analyzed using repeated measurements one-way analysis of variance with a randomized blocked design test, and Dunnett's multiple comparison test. Differences of $P < 0.05$ were considered significant.

3. Results

In vitro experiments had shown that the recovery rate of probes for dopamine (external to internal probes) ranged from 15 to 22% (data not shown). The basal striatal extracellular dopamine level was 51.82 ± 6.93 fmol/sample (in 20 min). The rate at which carbamazepine diffused from the dialysis tube ranged from 18 to 22% of the amount of carbamazepine that was perfused in 120 min

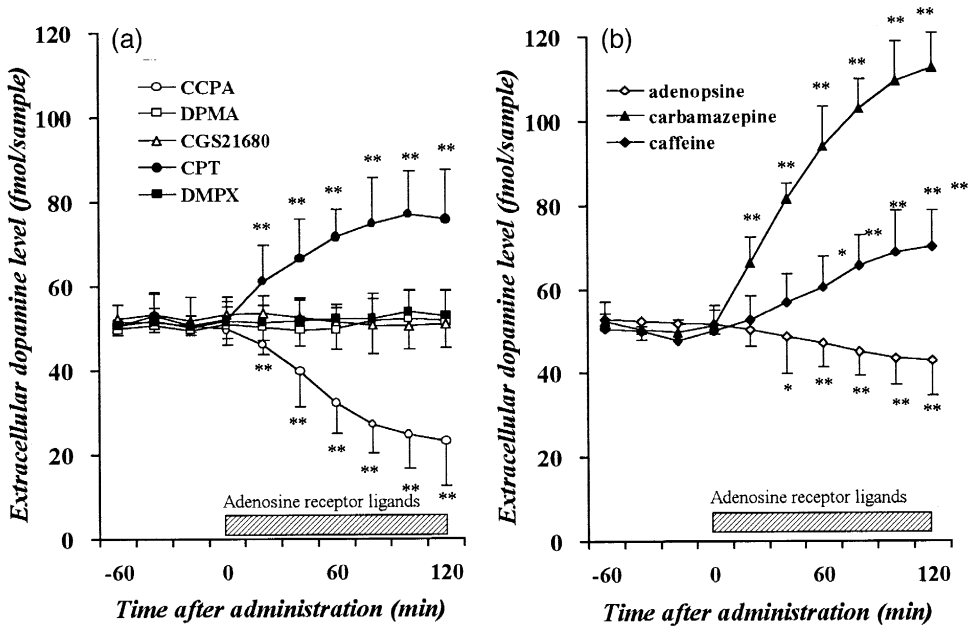

Fig. 1. Effects of adenosine receptor ligands on striatal extracellular dopamine level. The extracellular dopamine level was measured in striatal perfusate for 60 min during the pre-drug period (control), and for 120 min during perfusion with 1 μM of 2-chloro-N^6-cyclopentyladenosine (CCPA: ○), 10 μM of 2-[4-(2-carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamideadenosine (CGS21680: △), 5 μM of N^6-[2-(3,5-dimethoxyphenyl)-2-(methylphenyl)ethyl]adenosine (DPMA: □), 50 μM of 8-cyclopentyl-1,3-dimethylxanthine (CPT: ●) and 10 μM of 3,7-dimethyl-1-propargylxanthine (DMPX: ■), 50 μM of adenosine (◇), 50 μM of caffeine (◆) and 100 μM of carbamazepine (▲). Fig. 1a shows the effects of selective adenosine receptor ligands (CCPA, CGS21680, DPMA, CPT and DMPX), and Fig. 1b shows the effects of adenosine, caffeine and carbamazepine on the striatal extracellular dopamine level. The mean ± S.E.M ($n = 6$) extracellular dopamine level (fmol/sample) is shown on the ordinate and the time in minutes on the abscissa. The mean values obtained before and during perfusion with adenosine receptor ligands were compared by using repeated measurements one-way analysis of variance with a randomized blocked design and Dunnett's multiple comparison test (* $P < 0.05$, ** $P < 0.01$). The striatal extracellular dopamine level was reduced significantly by perfusates containing CCPA and adenosine ($P < 0.01$). CPT, caffeine and carbamazepine increased the striatal extracellular dopamine level significantly ($P < 0.01$), whereas CGS21680, DPMA and DMPX had no effect.

(data not shown), indicating that the level of carbamazepine in the brain tissue ranged from 18 to 22 μM. These concentrations of carbamazepine are the lowest in the therapeutic range (Masuda et al., 1979).

3.1. Experiment 1

3.1.1. Effects of selective adenosine receptor ligands on striatal extracellular dopamine level

The extracellular dopamine level was measured ($n = 6$) in striatal perfusates for 60 min during the pre-drug period (control) and for 120 min during perfusion with CCPA, CGS21680, DPMA, CPT and DMPX. Fig. 1a shows the results of the effects of CCPA (1 μM), CPT (50 μM), DPMA (5 μM), CGS21680 (10 μM) and DMPX (10 μM) on the striatal extracellular dopamine level. CCPA (1 μM) reduced the striatal extracellular dopamine level significantly ($P < 0.01$), and CPT (50 μM) increased it significantly ($P < 0.01$), whereas neither DPMA (5 μM), CGS21680 (10 μM) nor DMPX (10 μM) affected it.

3.1.2. Effects of adenosine, caffeine and carbamazepine on striatal extracellular dopamine level

The extracellular dopamine level was measured ($n = 6$) in striatal perfusates for 60 min during the pre-drug period (control) and for 120 min during perfusion with adenosine, caffeine and carbamazepine. Fig. 1b shows the results of the effects of adenosine (50 μM), caffeine (50 μM), and carbamazepine (100 μM) on the striatal extracellular dopamine level. Caffeine (50 μM) and carbamazepine (100 μM) increased the striatal extracellular dopamine level significantly ($P < 0.01$), and adenosine (50 μM) reduced it significantly ($P < 0.01$).

3.2. Experiment 2

3.2.1. Effects of selective adenosine A_2 receptor ligands on striatal extracellular dopamine level under conditions of adenosine A_1 receptor blockade

The extracellular dopamine level was measured ($n = 6$) in striatal perfusates for 60 min during perfusion with CPT (50 μM) (control) and for 120 min during perfusion with CPT (50 μM) plus CGS21680, DPMA or DMPX. Fig. 2a shows the effects of DPMA (5 μM), CGS21680 (10 μM) and DMPX (10 μM) on the striatal extracellular dopamine level under conditions of adenosine A_1 receptor blockade by CPT. Under conditions of adenosine A_1 receptor blockade by CPT, DPMA (5 μM) increased, whereas DMPX (10 μM) reduced the striatal extracellular dopamine level significantly ($P < 0.01$), and CGS21680 (10 μM) had no effect.

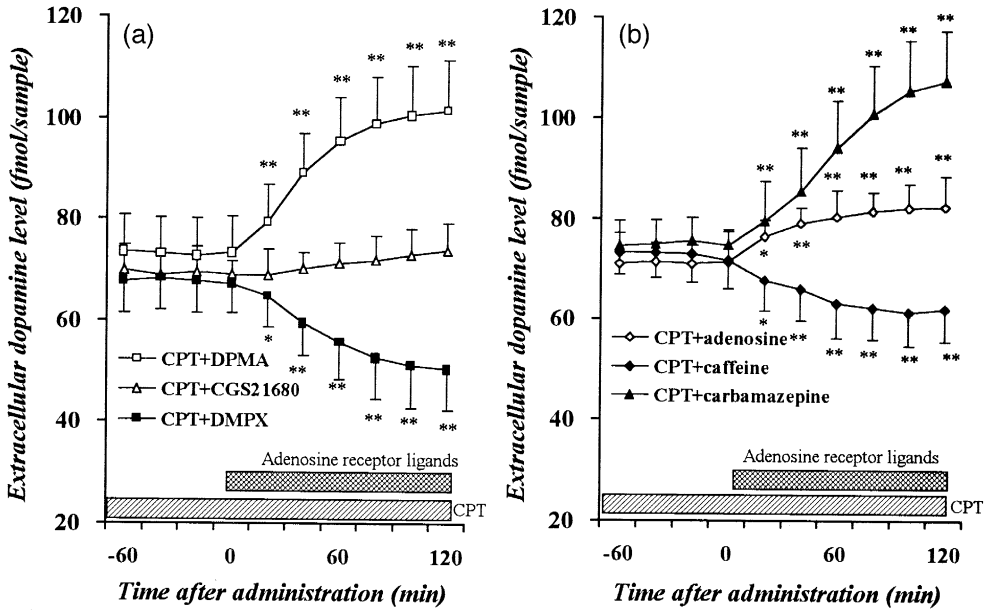

Fig. 2. Effects of adenosine receptor ligands on striatal extracellular dopamine level under conditions of adenosine A_1 receptor blockade. The extracellular dopamine level was measured in striatal perfusate for 60 min during perfusion with 50 μM of 8-cyclopentyl-1,3-dimethylxanthine (CPT) (control) and for 120 min during perfusion with CPT plus adenosine receptor ligands, 10 μM of 2-[4-(2-carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamideadenosine (CGS21680: △), 5 μM of N^6-[2-(3,5-dimethoxyphenyl)-2-(methyl-phenyl)ethyl]adenosine (DPMA: □) and 10 μM of 3,7-dimethyl-1-propargylxanthine (DMPX: ■), 50 μM of adenosine (◇), 50 μM of caffeine (◆) and 100 μM of carbamazepine (▲). Fig. 2a shows the effects of selective adenosine A_2 receptor ligands (CGS21680, DPMA and DMPX), and Fig. 2b shows the effects of adenosine, caffeine and carbamazepine on the striatal extracellular dopamine level. The mean ± S.E.M ($n = 6$) extracellular dopamine level (fmol/sample) is shown on the ordinate and the time after the start of perfusion with CPT plus adenosine receptor ligands in modified Ringer's solution on the abscissa. The mean values obtained under control conditions and during perfusion of adenosine receptor ligands were compared by using repeated measurements one-way analysis of variance with a randomized blocked design and Dunnett's multiple comparison test (* $P < 0.05$, ** $P < 0.01$). Under conditions of adenosine A_1 receptor blockade, DPMA, adenosine and carbamazepine increased the striatal extracellular dopamine level significantly ($P < 0.01$), DMPX and caffeine reduced it significantly ($P < 0.01$), and CGS21680 had no effect.

3.2.2. Effects of adenosine, caffeine and carbamazepine on striatal extracellular dopamine level under conditions of adenosine A_1 receptor blockade

The extracellular dopamine level was measured ($n = 6$) in striatal perfusates for 60 min during perfusion with CPT (50 μM) (control) and for 120 min during perfusion with CPT (50 μM) plus adenosine, caffeine or carbamazepine. Fig. 2b shows the results of the effects of adenosine (50 μM), caffeine (50 μM) and carbamazepine (100 μM) on the striatal extracellular dopamine level, under conditions of adenosine A_1 receptor blockade by CPT. Under conditions of adenosine A_1 receptor blockade by CPT, caffeine (50 μM) reduced, whereas carbamazepine (100 μM) and adenosine (50 μM) increased, the striatal extracellular dopamine level significantly ($P < 0.01$).

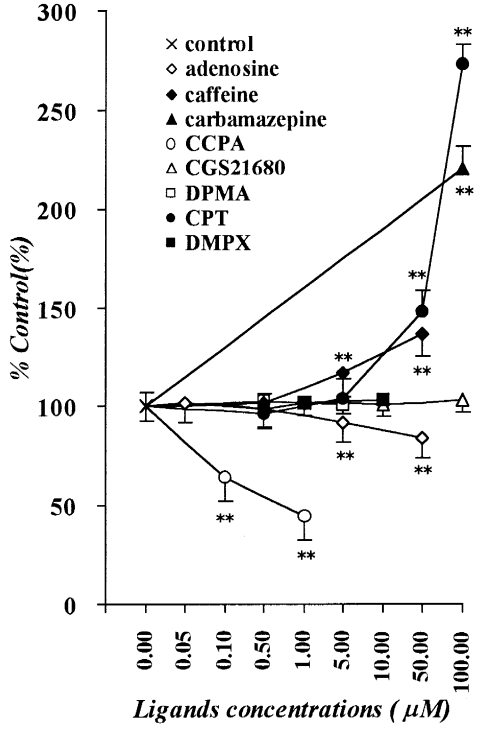

Fig. 3. Effects of adenosine receptor ligands on striatal extracellular dopamine level. The extracellular dopamine level was measured in perfusates for 60 min during the pre-drug period (control: ×), and for 120 min during perfusion with 2-chloro-N^6-cyclopentyladenosine (CCPA: ○), 2-[4-(2-carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamideadenosine (CGS21680: △), N^6-[2-(3,5-dimethoxyphenyl)-2-(methylphenyl)ethyl]adenosine (DPMA: □), 8-cyclopentyl-1,3-dimethylxanthine (CPT: ●), 3,7-dimethyl-1-propargylxanthine (DMPX: ■), adenosine (◇), caffeine (◆) and carbamazepine (▲). The mean ± S.E.M ($n = 6$) extracellular dopamine level (% control), which was determined from 100 to 120 min after addition of adenosine receptor ligands to the perfusion medium, is shown on the ordinate and the drug concentration on the abscissa. The mean values obtained before and during perfusion with adenosine receptor ligands were compared by using repeated measurements one-way analysis of variance with a randomized blocked design and Dunnett's multiple comparison test (* $P < 0.05$, ** $P < 0.01$). The striatal extracellular dopamine level was reduced significantly by perfusates containing adenosine and CCPA, in a concentration-dependent manner ($P < 0.01$). Caffeine and CPT increased the striatal extracellular dopamine level significantly ($P < 0.01$), in a concentration-dependent manner, whereas CGS21680, DPMA and DMPX had no effect.

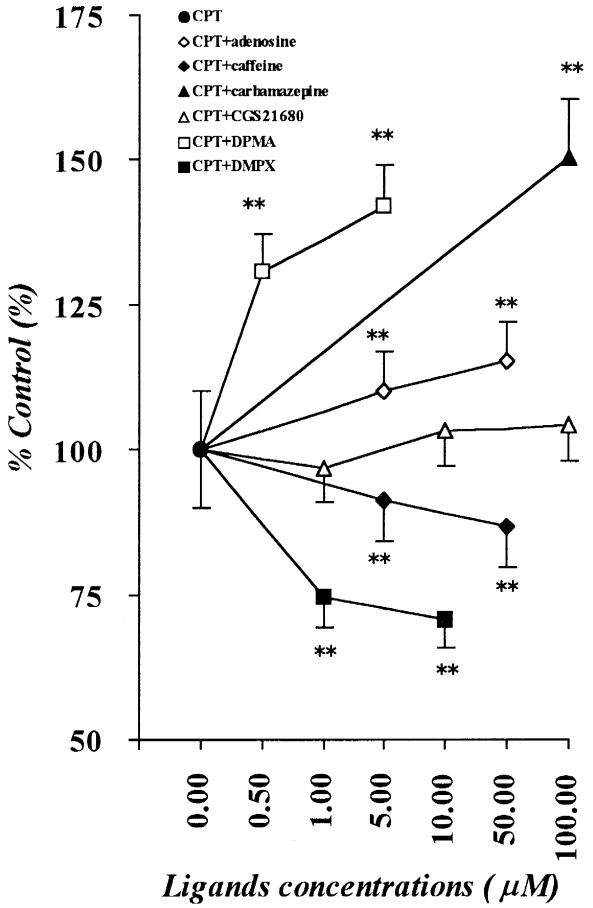

Fig. 4. Effects of adenosine receptor ligands on striatal extracellular dopamine level under conditions of adenosine A_1 receptor blockade. The extracellular dopamine level was measured in striatal perfusate during perfusion of 50 μM of 8-cyclopentyl-1,3-dimethylxanthine (CPT) (control: ●) and for 120 min during perfusion of CPT plus adenosine receptor ligands 2-[4-(2-carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamideadenosine (CGS21680: △), N^6-[2-(3,5-dimethoxyphenyl)-2-(methylphenyl)ethyl]adenosine (DPMA: □), 3,7-dimethyl-1-propargylxanthine (DMPX: ■), adenosine (◇), caffeine (◆) and carbamazepine (▲). The mean ± S.E.M ($n = 6$) extracellular dopamine level (% control), which was determined from 100 to 120 min after addition of adenosine receptor ligands to the perfusion medium is shown on the ordinate and the drug concentrations on the abscissa. The mean values obtained under control conditions and during perfusion with adenosine receptor ligands were compared by using repeated measurements one-way analysis of variance with a randomized blocked design and Dunnett's multiple comparison test (* $P < 0.05$, ** $P < 0.01$). Under conditions of adenosine A_1 receptor blockade, adenosine and DPMA increased the striatal extracellular dopamine level significantly in a concentration-dependent manner ($P < 0.01$), caffeine and DMPX reduced it significantly in a concentration-dependent manner ($P < 0.01$), and CGS21680 had no effect.

3.3. Dose-effect relationship of the different adenosine receptor ligands

3.3.1. Effects of adenosine receptor ligands on striatal extracellular dopamine level

A summary of the effects of adenosine receptor ligands on the striatal extracellular dopamine level 120 min after addition of adenosine receptor ligands to the perfusate medium (MRS) is shown in Fig. 3. Adenosine (5 and 50 μM) and CCPA (0.1 and 1 μM) reduced the striatal extracellular dopamine level significantly, in a concentration-dependent manner ($P < 0.01$), and caffeine (5 and 50 μM) as well as CPT (50 and 100 μM) increased it significantly, in a concentration-dependent manner ($P < 0.01$). However, adenosine (0.05 and 0.5 μM), caffeine (0.5 μM), CPT (0.5 and 5 μM), DPMA (0.5 and 5 μM), CGS21680 (1, 10 and 100 μM) and DMPX (1 and 10 μM) had no effect. Carbamazepine (100 μM) increased the striatal extracellular dopamine level significantly ($P < 0.01$).

3.3.2. Effects of adenosine receptor ligands on striatal extracellular dopamine level under conditions of adenosine A_1 receptor blockade

A summary of the effects of adenosine receptor ligands on the striatal extracellular dopamine level under conditions of adenosine A_1 receptor blockade 120 min after addition of adenosine receptor ligands to the perfusate medium (MRS) is shown in Fig. 4. Under conditions of adenosine A_1 receptor blockade by CPT (50 μM: control), adenosine (5 and 50 μM) and DPMA (0.5 and 5 μM) increased the extracellular dopamine level significantly, in a concentration-dependent manner, whereas caffeine (5 and 50 μM) and DMPX (1 and 10 μM) produced a significant concentration-dependent reduction ($P < 0.01$). CGS21680 (1, 10 and 100 μM) had no effect. Carbamazepine (100 μM) increased the striatal extracellular dopamine level significantly ($P < 0.01$).

4. Discussion

The results of this study, summarized in Table 2, show clearly that striatal dopaminergic neurotransmission is modulated by striatal adenosine receptor subtypes. Various studies have shown that adenosine A_1 receptor stimulation inhibits adenylate cyclase, N-type (Mogul et al., 1993) and Q-type (Wheeler et al., 1994) Ca^{2+} channels and opens K^+ channels (Stone and Bartrup, 1991), suggesting that adenosine A_1 receptors can depress neuronal transmission by a combination of these effects (Okada et al., 1996). In our previous study (Okada et al., 1996) and in the present one, a selective adenosine A_1 receptor agonist, CCPA, reduced the striatal extracellular dopamine level, whereas a selective adenosine A_1 antagonist, CPT, increased it, indicating that adenosine A_1 receptor stimulation reduces striatal extracellular dopamine levels. The previous study was unable to demonstrate the stimulatory effects of CPT (from 10 to 100 μM) on the striatal extracellular dopamine level by in vivo microdialysis during perfusion with Ringer's solution adjusted to pH 6.4 (Zetterström and Fillenz, 1990). However, in this study, 50 μM CPT increased the striatal extracellular dopamine level during perfusion with MRS adjusted to pH 7.4 by phosphate and Tris buffer. These results indicate that physiological pH is needed in order to observe the functions of the adenosine receptor in in vivo microdialysis preparations.

Neither an adenosine A_2 receptor agonist, DPMA, nor an antagonist, DMPX, alone affected the striatal extracellular dopamine level, but a stimulatory effect of DPMA and an inhibitory effect of DMPX on the striatal extracellular dopamine level were manifested under conditions of adenosine A_1 receptor blockade by a selective adenosine A_1 receptor antagonist CPT, indicating that the stimulatory effects of adenosine A_2 receptors were masked by the inhibitory effects of adenosine A_1 receptors. In addition, an adenosine A_{2A} receptor agonist, CGS21680, did not affect the striatal extracellular dopamine level, irrespective of whether the adenosine A_1 receptors were functional.

Table 2
Summary of effects of adenosine receptor ligands on striatal dopamine levels

	Single administration	Combined administration with CPT
Adenosine A_1 receptor agonist (CCPA)	↓	
Adenosine A_1 receptor antagonist (CPT)	↑	
Adenosine A_2 receptor agonist (DPMA)	→	↑
Adenosine A_{2A} receptor agonist (CGS21680)	→	→
Adenosine A_2 receptor antagonist (DMPX)	→	↓
Adenosine	↓	↑
Caffeine	↑	↓
Carbamazepine	↑	↑

The arrows indicate significant increase (↑), decrease (↓) or no change (→) in striatal extracellular dopamine level.

Similarly, it has already been reported that 1 μM CGS21680 does not affect endogenous basal or stimulation-evoked dopamine release in striatal slices (Lupica et al., 1990). These results are explained by the fact that adenosine A_{2A} receptors are localized on striatal GABAergic neurons (Kirk and Richardson, 1994) and that they become rapidly desensitized in response to prolonged agonist stimulation (Barraco et al., 1995). Therefore, in order to observe the stimulatory effects of adenosine A_2 receptors on neurotransmission, activation of adenosine A_{2B} receptors or both the adenosine A_{2A} and A_{2B} receptor subtypes under conditions of adenosine A_1 receptor blockade is needed. Support for this conclusion was provided by Mogul et al. (1993), who demonstrated that adenosine A_{2B} receptor activation resulted in significant potentiation of P-type Ca^{2+} channel activity, which can stimulate neurotransmission (Takahashi and Momiyama, 1993), when the adenosine A_1 receptors were blocked. However, other possibilities, such as an increase in adenosine release by the adenosine A_1 receptor antagonist, cannot be ruled out.

The adenosine A_1 receptor is sensitive to low concentrations, in the nanomolar range, of adenosine derivatives, whereas micromolar concentrations are required for adenosine A_2 receptor activation (Reddington and Lee, 1991). However, in our previous study (Okada et al., 1996) and in the present one, we demonstrated no stimulatory effects of adenosine (from 50 nM to 50 μM) on striatal extracellular dopamine levels, indicating that the stimulatory effect of adenosine A_2 receptors is masked by the inhibitory effect of adenosine A_1 receptors activated by adenosine, which stimulates both adenosine A_1 and A_2 receptors.

Whether carbamazepine acts as an agonist or antagonist at adenosine receptors is controversial. A strong interaction between carbamazepine and adenosine receptors has been reported, and it has been suggested that carbamazepine has agonistic, antagonistic and mixed agonistic/antagonistic properties at adenosine A_1 as well as adenosine A_2 receptors (Marangos et al., 1983; Skerritt et al., 1983; Fujiwara et al., 1986; Daval et al., 1989; Elphick et al., 1990). Biochemical (Fujiwara et al., 1986) and behavioral (Hornfeldt and Larson, 1994) experiments have indicated that carbamazepine is an agonist at adenosine A_1 receptors and an antagonist at adenosine A_2 receptors, but other reports suggest the reverse. Daval et al. (1989) suggested that carbamazepine and caffeine both act as antagonists at adenosine A_1 receptors, since in autoradiographic experiments, adenosine A_1 receptors and forskolin binding sites were found to be upregulated by chronic caffeine and carbamazepine treatment. Biochemical (cAMP synthesis assay) and behavioral experiments (Elphick et al., 1990) indicated that chronic carbamazepine treatment functionally down-regulates adenosine A_2 receptors. Our present study suggested that carbamazepine appears to be an adenosine A_2 receptor agonist and adenosine A_1 receptor antagonist, as it increased striatal dopamine release, irrespective of whether the adenosine A_1 receptors were functional. To confirm this, future study is needed when a selective adenosine A_{2B} receptor antagonist is available. Furthermore, our results indicate that caffeine has antagonistic effects at both adenosine A_1 and A_2 receptors, because caffeine increased striatal dopamine release, like a selective adenosine A_1 receptor antagonist, and reduced it under conditions of adenosine A_1 receptor blockade, like a selective adenosine A_2 receptor antagonist.

Adenosine receptor agonists have generally been considered to exert anticonvulsive activity, and inhibition of adenosine function reduces the seizure threshold (Wood et al., 1989). It should, however, be noted that the anticonvulsive dose of an adenosine A_1 receptor agonist could induce hypothermia, hypotension and sedation (Dunwiddie and Worth, 1982), and a proconvulsive dose of an adenosine receptor antagonist possibly induces cardiac arrhythmia (Chu, 1981). Surprisingly, at low doses caffeine and CPT reduce the incidence of seizures (Kostopoulos et al., 1987; Klitgaard and Knutsen, 1992). Therefore, our present results indicate that the antagonistic action of carbamazepine at adenosine A_1 receptors does not rule out the anticonvulsive action of carbamazepine at therapeutic doses and throw some light on the mechanisms responsible for the 'paradoxical intoxication' (Troupin and Ojemann, 1975; Weaver et al., 1988) induced by supratherapeutic doses of carbamazepine. This hypothesis may explain the fact that carbamazepine pretreatment failed to inhibit theophylline-induced tonic seizures (Hornfeldt and Larson, 1994). Adenosine A_2 receptor agonists show potent anticonvulsive effects against adenosine A_1 receptor antagonist-induced seizures (Klitgaard et al., 1993). Therefore, the combination of the adenosine A_1 receptor antagonistic and adenosine A_2 receptor agonistic actions of carbamazepine, or the latter alone, at least partially mediate the mechanism responsible for the anticonvulsant effects of carbamazepine.

The psychotropic actions of carbamazepine and caffeine are opposite: carbamazepine shows mood-stabilizing effects (Okuma et al., 1990) and reduces the frequency of panic attacks (Tondo et al., 1989), whereas caffeine induces anxiety with depression as well as panic attacks (Apfeldorf and Shear, 1993). These different psychotropic actions can be explained, at least partially, by the different effects on adenosine A_2 receptors, i.e. carbamazepine is an agonist and caffeine an antagonist. This, therefore, raises the possibility that agonist action at adenosine A_2 receptors may reduce the frequency of panic attacks.

In summary, the present results indicate that adenosine A_2 (or A_{2B}) receptor function can be exhibited when adenosine A_1 receptors are blocked. In other words, the function of adenosine A_2 (or A_{2B}) receptors is masked by that of adenosine A_1 receptors. The balance between the effects of adenosine A_1 and adenosine A_2 receptors accounts for the different psychotropic and neurological efficacies of carbamazepine and caffeine in convulsive, mood and panic disorders.

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