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Characteristics of A_1 and A_2 Adenosine Receptors upon the Acetylcholine Release in the Rat Hippocampus

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As it has been reported that the depolarization induced acetylcholine (ACh) release is modulated by activation of presynaptic A_1 adenosine heteroreceptor and various lines of evidence suggest the A_2 adenosine receptor is present in the hippocampus. The present study was undertaken to delineate the role of adenosine receptors on the hippocampal ACh release. Slices from the rat hippocampus were equilibrated with $[^3H]$choline and then the release amount of the labelled product, $[^3H]$ACh, which was evoked by electrical stimulation (rectangular pulses, 3 Hz, 2 ms, 24 mA, 5 V/cm^{-1}, 2 min), was measured, and the influence of various adenosine receptor-related agents on the evoked tritium outflow was investigated. And also, the drug-receptor binding assay was performed in order to confirm the presence of A_1 and A_2 adenosine receptors in the rat hippocampus. N-ethylcarboxamidoadenosine (NECA), a potent adenosine receptor agonist with nearly equal affinity at A_1 and A_2 adenosine receptors, in concentrations ranging from 1∼30 μM, decreased the electrically-evoked $[^3H]$ACh release in a concentration-dependent manner without affecting the basal rate of release. And the effect of NECA was significantly inhibited by 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 2 μM), a selective A_1 adenosine receptor antagonist, but was not influenced by 3,7-dimethyl-1-propargylxanthine (DMPX, 5 μM), a specific A_2 adenosine receptor antagonist. N^6-cyclopentyladenosine (CPA), a selective A_1 adenosine receptor agonist, in doses ranging from 0.1 to 10 μM, reduced evoked $[^3H]$ACh release in a dose-dependent manner without the change of the basal release. And the effect of CPA was significantly inhibited by 2 μM DPCPX treatment. 2-P-(2-carboxyethyl)-phenethylamino-5′-N-ethylcarboxamidoadenosine hydrochloride (CGS-21680C), a potent A_2 adenosine receptor agonist, in concentrations ranging from 0.1 to 10 μM, did not alter the evoked ACh release. In the drug-receptor binding assay, the binding of $[^3H]$2-chloro-N^6-cyclopentyladenosine ($[^3H]$CCPA) to the A_1 adenosine receptor of rat hippocampal membranes was inhibited by CPA (K_i = 1.22 nM), NECA (K_i=10.17 nM) and DPCPX (K_i=161.86 nM), but not by CGS-21680C (K_i=2,380 nM) and DMPX (K_i=22,367 nM). However, the specific binding of $[^3H]$CGS-21680C to the A_2 adenosine receptor was not observed. These results suggest that the A_1 adenosine heteroreceptor play an important role in evoked ACh release, but the presence of A_2 adenosine receptor is not confirmed in this study.

Key Words: Adenosine receptors, Acetylcholine, Hippocampus, Drug-receptor binding assay

INTRODUCTION

It is well known that adenosine and its analogues inhibit the release of several neurotransmitters in the central nervous system (CNS). Adenosine receptors are subdivided into A_1 and A_2 adenosine receptors for their ability to either inhibit or stimulate adenylate cyclase (Daly et al, 1983; Hamprecht and Van Calker, 1985). The inhibition by adenosine analogues of several neurotransmitters including ACh, norepinephrine, 5-hydroxytryptamine and glutamate is apparently mediated via A_1 adenosine receptors (Jackisch et al, 1985; Fredholm et al, 1986; Fredholm and

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Lindgren, 1987). In the hippocampus, the release of ACh could be inhibited both by presynaptic mus carinic receptor (Hertting et al, 1987; Choi et al, 1991) and by presynaptic A_1 adenosine receptor (Jac kisch et al, 1984; Choi et al, 1992).

On the other hand, the A_2 adenosine receptor existed in the CNS as well as in the peripheral organs such as heart (Stone et al, 1988; Williams, 1989; Bruns, 1990). However, there have been still controversy about existence of A_2 adenosine receptor in the hippocampus. Fredholm (1982) and Fredholm et al. (1983) showed that A_1 and A_2 adenosine receptors in the hippocampal tissue decreased cAMP concentration and increased it, respectively. Sebastião and Ribeiro (1992) reported that 2-p-(2-carboxyethyl) phenethylamino-5′-N-ethylcarboxamidoadenosine hydrochloride (CGS-21680C), a specific A_2 adenosine receptor agonist, increased excitability of the hippocampus in an electrophysiological study. Lee (1994) and Cunha et al (1995) suggested a possible existence of the excitatory effect of A_2 adenosine receptor in rat hippocampal neurons. On the contrary, Yeung and Green (1984) reported that only A_1 subtype was demonstrated in the hippocampus by the experiment with the rat hippocampal homogenate. And Jarvis and his colleagues (1989) assumed that both A_1 and A_2 adenosine receptors existed in the striatum but only A_1 adenosine receptor existed in the hippocampus. Therefore, the functional role of A_1 and A_2 adenosine receptors in many organs has been known well whereas the anatomical evidence and physiological role of adenosine receptor subtypes have not been clear in the hippocampus.

The purpose of this study was to compare the role of the A_1 and A_2 adenosine receptors for the electrical stimulation-induced ACh release and to examine the existence of the A_1 and A_2 adenosine receptors using the drug-receptor binding assay in the rat hippocampus.

METHODS

Superfusion experiments

Slices of 2.5～3.0 mg, 400 μm in thickness, were prepared from the hippocampus of Sprague-Dawley rats weighing 250～300 gm with a Balzers® tissue chopper (Balzers Union Aktiengesellschaft, England) and were incubated in 2 ml of modified Krebs-Henseleit medium containing 0.1 μmol/L [^{3}H]choline for 30 min at 37°C. Subsequently, the [^{3}H]choline-pretreated slices were superfused with medium containing hemicholinium-3 (10 μM) and atropine (30 nM) for 140 min at a rate of 0.5 ml/min. The composition (mM) of superfusion medium was 118 NaCl, 4.8 KCl, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 $NaHCO_3$, 0.57 ascorbic acid, 0.03 Na_2EDTA and 11 glucose, and the superfusate was continuously aerated with 95% O_2+5% CO_2; the pH adjusted to 7.4.

Collection of 5 min fraction of the superfusate began after 50 min of superfusion. Electrical stimulations (3 Hz, 5 VCm^{-1}, 2 ms, rectangular pulses) for 2 minutes were performed at 60 min (S_1) and 120 min (S_2). Drugs were added between S_1 and S_2 to the superfusion medium. At the end of superfusion, the slices were solubilized in 0.5 ml tissue solubilizer (0.5 N quaternary ammonium hydroxide in toluene). The radioactivities in the superfusates and solubilized tissues were determined by liquid scintillation counting (Beckman LS 6500TD). The fractional rate of tritium-outflow (5 min^{-1}) was calculated as trituim-outflow per 5 min divided by the total tritium content in the slice at the start of the respective 5 min period (Hertting et al, 1980). As reported previously, the electrical stimulation of brain slices incubated with [^{3}H]choline causes the release of [^{3}H]ACh only (Richardson & Szerb, 1974). Drug effects on the evoked tritium-outflows were evaluated by calculating the ratio of the outflow evoked by S_2 and by S_1 (S_2/S_1). The influence of drugs on the basal outflow are expressed as the ratio b_2/b_1 between fractional rates of outflow immediately before S_2 (115～120 min) and S_1 (55～60 min).

The following chemicals were used: [methyl-^{3}H] choline chloride (72～78 Ci $mmol^{-1}$, Amersham), N-ethylcarboxamidoadenosine (NECA, Sigma), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, RBI), atropine sulfate (Sigma), CGS-21680C (RBI), N^6-cyclopentyladenosine (CPA, RBI), hemicholinium-3 (Sigma), 3,7-dimethyl-1-propargylxanthine (DMPX, RBI). Drugs were dissolved in the medium except for DPCPX which were initially dissolved in DMSO and then diluted in the medium. The maximum concentration of DMSO used in the experiments (0.1%, vol/vol) did not affect basal tritium outflow or stimulation-evoked [^{3}H]ACh release.

Receptor binding assay

Rats were sacrificed by decapitation, and their brains rapidly removed to ice. The hippocampus was dissected from the remaining brain tissue, collected in Tris-buffer (50 mM Tris HCl, 10 mM $MgCl_2$, pH adjusted to 7.4) and chilled on ice. The tissues were homogenized in 10 volumes of Tris-buffer with up and down stroke of a motor driven glass-teflon homogenizer on ice. The hippocampal homogenate was filtered through two layers of gauze and the filtrate centrifuged at 40,000 × g for 10 min at 4°C. The resulting pellet was resuspended in 10 volumes of ice-cold buffer and stirred for 30 min at 4°C. After the homogenate was recentrifuged at 40,000 × g for 10 min at 4°C, the pellet was resuspended in 20 volumes of Tris-buffer. Adenosine deaminase (2 IU/ml) was added and the suspension was incubated at 37°C for 30 min to remove endogenous adenosine. The suspension was recentrifuged at 40,000 × g for 10 min at 4°C and the final pellet was resuspended in ice-cold Tris-buffer. The crude membranes were stored frozen at −70°C. Protein contents were determined by the method of Bradford (1976) using bovine serum albumin as a standard.

Membranes were thawed at room temperature and homogenized by 10 strokes in a glass-teflon homogenizer. For A_1 adenosine receptor assays, DMPX (3 μM) was added to each tube in order to block A_2 adenosine sites and for A_2 adenosine receptor assays, DPCPX (2 μM) was added to each tube in order to block A_1 adenosine sites. These concentrations of site selective displacing ligands were selected from an analysis of their potencies as displacers of each labeled ligand. After all additions, the final membrane concentration in the assay tube was 1% w/v, corresponding to about 300~500 μg protein per sample. All drug solutions were prepared in Tris-buffer, pH 7.4. Final volume in each tube was 500 μl. Triplicate samples of membrane suspension were preincubated with or without non-radioactive displacer at 25°C. Radiolabelled ligands, 1 nM [^{3}H]2-chloro-N^6-cyclopentyladenosine ([^{3}H] CCPA) or 16 nM [^{3}H] CGS-21680C, were then added and the incubation continued for 120 min at the same conditions. The incubation was terminated by addition of 4 ml ice-cold buffer and rapid filtration through glass fiber filters (Type G-7; Inotech, Zürich, Switzerland) under reduced pressure by using a cell harvester (Inotech). The filters were washed with additional 4 ml buffer, transferred to scintillation vials, soaked in 0.5 ml absolute ethanol, and counted in 3 ml scintillation cocktail by liquid scintillation counter (Beckman LS 6500TD). Non-specific binding was defined as the fraction of bound radioligand that remained in the presence of 20 μM NECA.

Saturation and displacement data were analyzed by the use of the computer program LIGAND (Munson and Rodbard, 1980). This program utilizes a non-linear least squares curve fitting algorithm, and assumes the simultaneous contribution of one or more independent binding sites.

The following chemicals were used: [^{3}H]CCPA (30 ~60 Ci mmol^{-1}, Dupont New England Nuclear), [^{3}H]CGS-21680C (30~80 Ci mmol^{-1}, Dupont New England Nuclear), adenosine deaminase (Type VI, Sigma).

Satistics

All results are given as Mean ± SEM. Significance of difference between the groups was determined by ANOVA and subsequently by Duncan test (Snedecor, 1980).

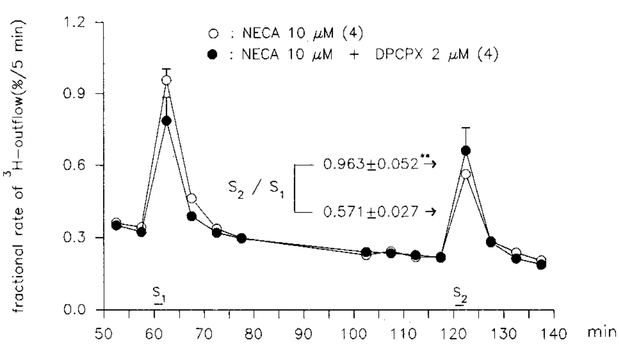

Fig. 1. A typical presentation of the tritium-outflow from the rat hippocampal slice preincubated with [^{3}H]choline. The slices were electrically stimulated twice for 2 min after 60 (S_1) and 120 min (S_2) of superfusion, respectively. The drug effect on the stimulation-evoked tritium outflow is expressed by the ratio S_2/S_1. N-ethylcarboxamidoadenosine (NECA) and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) were added 15 min before S_2. The tritium contents of the tissue at the start of experiments were 0.755 ± 0.009 (○) and 0.810 ± 0.046 (●) pmol. The mean ± SEM of the experiments (n) are given. Asterisks indicate the significant difference between groups (**; $p < 0.01$).

RESULTS

Effects of adenosine receptor agonists and antagonists on [^{3}H]ACh release

Hippocampal slices prelabelled with [^{3}H]choline, a [^{3}H]ACh precursor, were superfused with the medium containing hemicholinium-3 (10 μM), a choline uptake inhibitor. And in order to eliminate the inhibition of ACh release by activating muscarinic autoreceptor, atropine (30 nM) was added in the superfusion medium. During superfusion, the tissues were electrically stimulated twice.

As shown in Fig. 1, the example of the experiment observed the influence of NECA, a potent adenosine receptor agonist with nearly equal affinity at A_1 and A_2 adenosine receptors (Jackish et al, 1984), on [^{3}H]ACh release. NECA, in doses ranging from 1 to 30 μM, decreased the electrically-evoked [^{3}H]ACh release in a concentration-dependent manner (Table 1).

To ascertain the interaction between NECA and DPCPX, a selective A_1 adenosine receptor antagonist (Bruns et al, 1987), or DMPX, a selective A_2 adenosine receptor antagonist (Sebastião and Ribeiro, 1989), the effect of NECA was observed in the presence of the 2 μM DPCPX (control: b_2/b_1 = 0.6394 ±0.0273, S_2/S_1=1.0054±0.0215, n=7, 2 μM DPCPX: b_2/b_1=0.6605±0.0155, S_2/S_1=1.0351±0.0746, n=8) or 5 μM DMPX (control: b_2/b_1=0.6101±0.0293, S_2/S_1=0.9417±0.0447, n=5, 5 μM DMPX: b_2/b_1= 0.6715±0.0239, S_2/S_1=0.8223±0.0751, n=6). All drugs were added to the superfusion medium 15 min before S_2. The decrements of tritium outflow by NECA were significantly inhibited by DPCPX (Fig. 2), but were not influenced by DMPX (Fig. 3).

CPA, a selective A_1 adenosine receptor agonist (Williams et al, 1986), in doses ranging from 0.1 to 10 μM, decreased the electrically-evoked [^{3}H]ACh release in a dose-dependent manner without change of the basal release (Fig. 4). In addition, the effect of CPA was significantly inhibited by 2 μM DPCPX

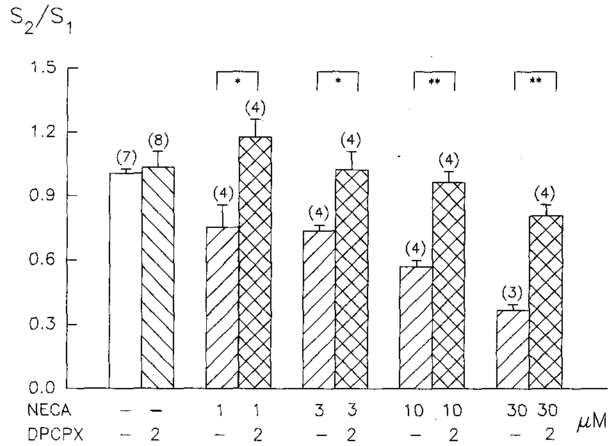

Fig. 2. Influence of DPCPX on the effect of NECA on the electrically-evoked tritium outflow from the rat hippocampus. In parentheses are the number of experiments. Asterisks indicate the significant difference between groups (*; p<0.05). Other legends are the same as in Fig. 1.

Table 1. Effect of N-ethylcarboxamidoadenosine (NECA) on the electrically-evoked and basal outflow of tritium from the rat hippocampal slices preincubated with [^{3}H]choline

	Drugs at S_2 (μM)	n	S_2/S_1	b_2/b_1
NECA	none	12	0.9789±0.0232	0.6272±0.0197
	1	4	0.7516±0.1057	0.6684±0.0241
	3	8	0.7073±0.0341***	0.5862±0.0224
	10	8	0.6036±0.0281***	0.6114±0.0177
	30	3	0.3650±0.0266***	0.5396±0.0109**

After preincubation, the slices were superfused with medium containing hemicholinium-3 (10 μM) and atropine (30 nM), and then stimulated twice (S_1, S_2). Drugs were present from 15 min before S_2 onwards at the concentrations indicated. Drug effects on basal outflow are expressed at the ratio b_2/b_1 between fractional rates of outflow immediately before S_2 (95 min~100 min) and before S_1 (55 min~60 min). Mean±SEMs from number (n) of observations are given. Significant differences from the drug-free control (none) are marked with asterisks (**; $p<0.01$ and ***; $p<0.001$).

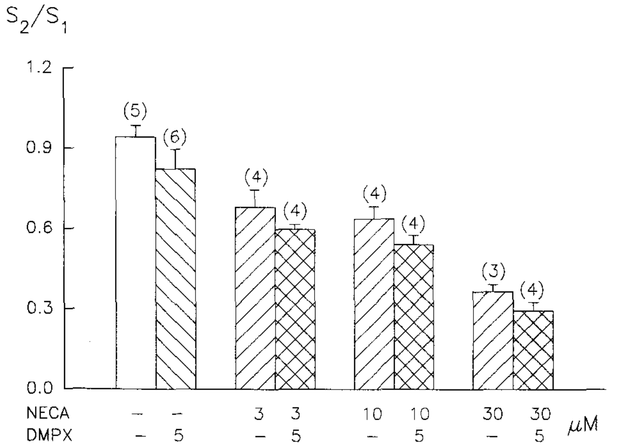

Fig. 3. Influence of 3,7-dimethyl-1-propargylxanthine (DMPX) on the effect of NECA on the electrically-evoked tritium outflow from the rat hippocampus. Legends are the same as in Fig. 2.

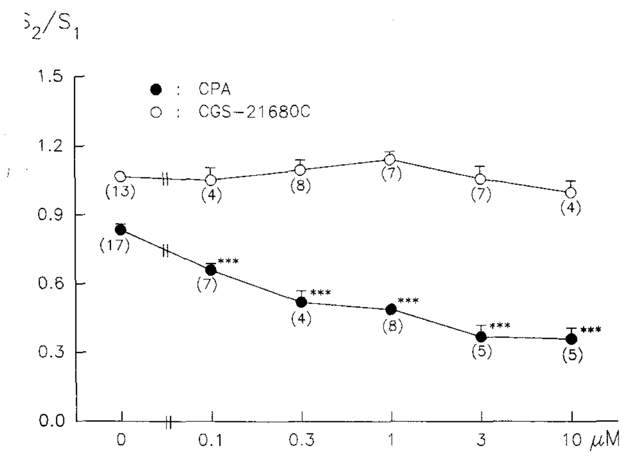

Fig. 4. Effects of N^6-cyclopentyladenosine (CPA) and 2-p-(2-carboxyethyl)phenethylamino- 5′-N-ethylcarboxamidoadenosine hydrochloride (CGS-21680C) on the electrically-evoked outflow of tritium from the rat hippocampal slices preincubated with [^{3}H]choline. Drugs were present from 15 min before S_2 onwards at the concentrations indicated. Each point denotes mean± SEM. In parentheses are the number of experiments. Significant differerces from the drug-free control (0 μM) are marked with asterisks (***; $p<0.001$). Other legends are the same as in Fig. 2.

(Fig. 5).

In order to study the role of A_2 adenosine receptor on ACh release in the rat hippocampus, the effect of CGS-21680C, an adenosine agonist which possesses a 170 fold selectivity for A_2 versus A_1 adenosine receptor (Hutchison et al, 1989), was examined. As

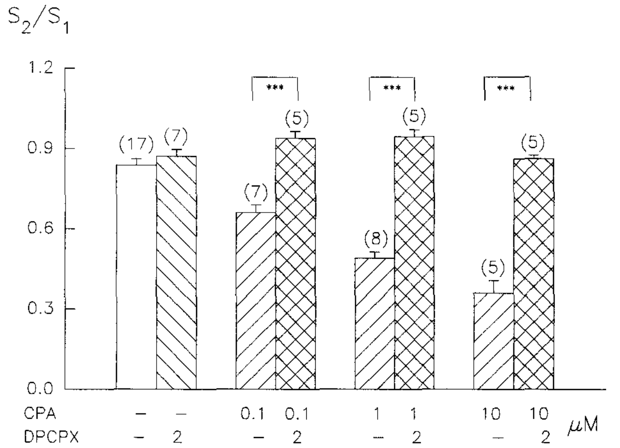

Fig. 5. Influence of DPCPX on the effect of CPA on the electrically-evoked tritium outflow from the rat hippocampus. Legends are the same as in Fig. 4.

shown in Fig. 4, CGS-21680C did not influenced the electrically-evoked [^{3}H]ACh release and basal release. On the other hand, as it was thought that if the stimuli of A_1 adenosine receptor had blocked the effect of A_2 adenosine receptor could have appeared, the effect of CGS-21680C observed in the presence of DPCPX, but CGS-21680C in the presence of the 2 μM DPCPX did not influence the ACh release (Table 2).

Receptor binding experiments

In the drug-receptor binding experiment with hippocampal membranes, after incubation for 120 min at 25°C, [^{3}H] CCPA (1 nM) was bound with specific binding amount approximately 85 % of total binding. However, the specific binding of [^{3}H]CGS- 21680C (16 nM) was not detected.

In rat hippocampal membrane preparation, when A_2 adenosine receptors were blocked by 3 μM DMPX, [^{3}H]CCPA binding was inhibited by CPA with a K_i value of 1.22 nM and a B_{max} of 219 fmol/mg protein (Fig. 6, Table 3). Also, competitions by several agonists and antagonists for [^{3}H]CCPA binding were measured to confirm the [^{3}H]CCPA binding to A_1 adenosine receptors. The specific binding of [^{3}H]CCPA was inhibited by CPA, NECA and DPCPX, the A_1 adenosine receptor-related drugs, but not by CGS-21680C and DMPX, the A_2 adenosine receptor-related drugs (Fig. 6, Table 3).

Table 2. Influence of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) upon the effect of 2-p-(2-carboxyethyl) phenethylamino-5′-N-ethylcarboxamidoadenosine hydrochloride (CGS-21680C) on the electrically-evoked tritium-outflows from the rat hippocampus

Drugs at S_2 (μM)		n	S_2/S_1	Net inhibition by CGS-21680C (%)
DPCPX	CGS-21680C			
–	–	13	1.067±0.019	
2	–	4	1.174±0.072	
–	0.1	4	1.052±0.053	– 0.015 (–1.4)
2	0.1	4	1.042±0.060	– 0.132 (–11.2)
–	0.3	4	1.096±0.089	+ 0.029 (+ 2.7)
2	0.3	4	1.294±0.060	+ 0.120 (+10.2)
–	1	4	1.101±0.015	+ 0.034 (+ 3.2)
2	1	4	1.112±0.031	– 0.062 (–5.3)
–	3	4	1.163±0.086	+ 0.096 (+ 9.0)
2	3	4	1.166±0.063	– 0.008 (–0.7)

Legends are the same as in Table 1.

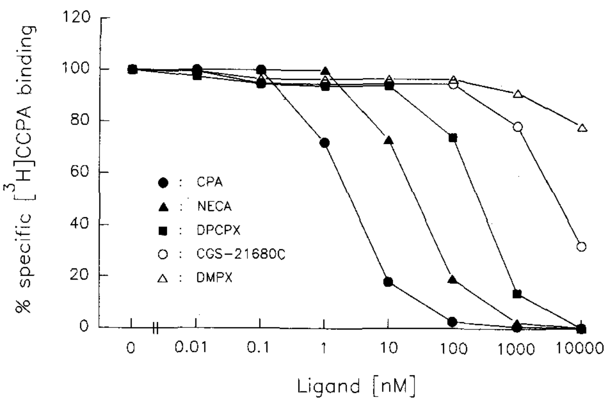

Fig. 6. Comparison of inhibitory potency of CPA, NECA, DPCPX, CGS-21680C and DMPX on the [^{3}H]2-chloro-N^6-cyclopentyladenosine ([^{3}H]CCPA) binding in the presence of 3 μM DMPX as a blocking ligands for A_2 adenosine receptors in the rat hippocampal membranes.

Table 3. Affinity constant (K_i, nM) of various adenosine receptor agonists and antagonists for specific binding site of [^{3}H]chloro-N^6-cyclopentyladenosine ([^{3}H]CCPA) in the presence of blocking ligands for A_2 adenosine site in the rat hippocampal membranes

Compound	K_i (nM)
CPA	1.22
NECA	10.17
DPCPX	161.86
CGS-21680C	2,380
DMPX	22,367

K_i Values were derived by the computer program LIGAND. Each value represented the mean of two or more independent experiment with varying SEMs less than ±10%. All experiments were carried out using Tris-buffer (50 mM Tris HCl, 10 mM $MgCl_2$, pH adjusted to 7.4). CPA: N^6-cyclopentyladenosine, NECA: N-ethylcarboxamidoadenosine, DPCPX: 8-cyclopentyl-1, 3-dipropylxanthine, CGS-21680C: 2-p-(2-carboxyethyl) phenethylamino-5′-N- ethylcarboxamidoadenosine hydrochloride, DMPX: 3,7-dimethyl-1-propargylxanthine.

DISCUSSION

In the present study, the electrically-evoked ACh release from the rat hippocampal slice was inhibited by NECA, a adenosine receptor agonist with nearly equal affinity at A_1 and A_2 adenosine receptor and CPA, a selective A_1 adenosine receptor agonist. And the inhibitory effects of CPA were far greater than those of NECA. This result is in accordance with other reports that CPA is more potent than NECA in ACh release experiment by using the hippocampal tissue (Londos et al, 1980; Jackish et al, 1984; Moos et al, 1985; Fredholm, 1990). Moreover, the decreasing effcet of ACh release by CPA or NECA was completely antagonized by DPCPX, a selective A_1 adenosine receptor antagonist, in this study. As these results correspond to the reports about the inhibition of ACh release by CPA (Dunér-Engström & Fredholm, 1988; Choi et al, 1992) or NECA (Jackish et al, 1984; Fredholm., 1990), it is suggested that the effects of CPA and NECA were mediated by A_1 adenosine receptor subtype.

Since it was known that the existence of A_2 adenosine receptor in the CNS, a large body of experimental evidences about the existence of A_2 adenosine receptor in the hippocampus have been accumulated. Sebastião and Ribeiro (1992) observed that CGS-21680C, a selective A_2 adenosine receptor agonist, increased the electrophysiological excitability of the rat hippocampal slices. Cunha et al (1995) reported that CGS-21680C increased the veratridine-induced ACh release in the experiments on the neurotransmitter release from the rat hippocampal homogenate, and they insisted the existence of A_2 adenosine receptor in the hippocampus. And, Bartrup and Stone (1988) observed that when the A_1 adenosine receptor was blocked, the effect of A_2 adenosine receptor could be expressed. In addition, Lee (1994) reported that when Mg^{++} was removed from the superfusion medium and then the inhibitory effect of A_1 adenosine receptor disappeared, the excitatory effect of A_2 adenosine receptor could be expressed, and also, we reported previously that the excitatory effect of A_2 adenosine receptor on neurotransmitter release from the rat hippocampus appeared when Mg^{++} was removed from the superfusion medium (Park et al, 1997). However, Yeung and Green (1984) described that both A_1 and A_2 adenosine receptors were present and functionally relevant in the rat striatum whereas only A_1 adenosine receptor was present in the rat hippocampus. Therefore, in this study, it was attemped to clarify whether A_2 adenosine receptor is involved in the evoked ACh release in the rat hippocampus. NECA decreased the electrically-evoked ACh release, and the inhibitory effect of NECA was antagonized by DPCPX but were not altered by DMPX, a selective A_2 adenosine receptor antagonist. Furthermore, CGS- 21680C which has about 170 fold selectivity for A_2 versus A_1 adenosine receptor did not influence on the electrically-evoked ACh release at all. Even in the presence of DPCPX, CGS-21680C did not alter the basal and evoked rates of ACh release. These findings, in conjunction with other report (Yeung & Green, 1984) that only A_1 adenosine receptor was present in the rat hippocampus, suggests that the A_1 adenosine receptor subtype can be related to the functional role of the cholinergic nerve terminal on the ACh release in the rat hippocampus.

Recently, Cunha et al (1995) observed the expression of A_{2A} mRNA in in situ hybridization in coronal rat brain section, and they proposed that A_{2A} adenosine receptors could be present in hippocampal cholinergic nerve terminals. Therefore, in this study, the drug-receptor binding assay using [^{3}H]CCPA and [^{3}H]CGS-21680C was performed in order to clarify the existence of A_1 and A_2 adenosine receptors and to examine the ligand selectivity of A_1 and A_2 adenosine receptors in rat hippocampal membranes. The specific binding of [^{3}H]CCPA was more than 85 % of total binding rate but the specific binding of [^{3}H]CGS-21680C was not observed. On the other hand, the binding of [^{3}H]CCPA was blocked by CPA, NECA and DPCPX, but was not blocked by CGS-21680C and DMPX. Also, in an our previous study using the buffer (including Mg^{2+} or not), the specific binding of [^{3}H]CGS-21680C to rat hippocampus was revealed not to be significant (Kim et al, 1997). These results were consistent with the report of Yeung and Green (1984) from the drug- receptor binding experiment, suggesting that the functionally relevant A_2 adenosine receptors are not exist in the rat hippocampus. However, our result and the results of others (Bartrup & Stone, 1988; Sebastião & Ribeiro, 1992; Lee, 1994; Cunha et al, 1995) about the presence and the role of A_2 adenosine receptor in hippocampal tissue are substantially inconsistent. The apparent discrepancy between the present result and the previous reports may be ascribed to the differences of experimental methods used, i.e. the hippocampal slices and synaptosomes, the drug-receptor binding assay and the in situ hybridization, the neurotransmitter release experiments and the electrophysiological experiments. Hence, further studies are required to understand the presence and the role of A_2 adenosine receptor in the rat hippocampus.

Overall, the results of the present study suggests that the decrement of the evoked ACh release is mediated by A_1 adenosine heteroreceptor in the rat hippocampal cholinergic neurons, and that the A_2 adenosine receptor seems not to exist in the rat hippocampus.

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