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The Influence of N^6-cyclopentyladenosine and Magnesium on Norepinephrine Release in the Rat Hippocampus

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As it has been reported that the depolarization-induced norepinephrine (NE) release is modulated by activation of presynaptic A_1-adenosine heteroreceptor and various lines of evidence indicate that A_2-adenosine receptor also presents in hippocampus, and that the adenosine effect is magnesium dependent, the present study was undertaken to delineate the role of adenosine receptors in the modulation of hippocampal NE release. Slices from the rat hippocampus were equilibrated with [^{3}H]-NE and the release of the labelled product, [^{3}H]-NE, was evoked by electrical stimulation (3 Hz, 5 V cm^{-1}, 2 ms, rectangular pulses), and the influence of various agents on the evoked tritium outflow was investigated. N^6-cyclopentyladenosine (CPA), in concentrations ranging from 0.1 to 10 μM, decreased the [^{3}H]-NE release in a dose-dependent manner without changing the basal rate of release, and these effects were significantly inhibited by 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 2 μM) treatment. When the magnesium concentration was reduced to 0.4 mM or completely removed, the evoked NE release increased along with decreased basal rate of release. In contrast, increasing the magnesium concentrations to 2.4 and 4 mM, decreased the evoked NE release. The CPA effects on evoked NE release were reduced by magnesium removal, but potentiated by 2.4 mM magnesium in the medium. 5-(N-cyclopropyl)-carboxamodiadenosine (CPCA, 1 & 10 μM), an A_2-agonist, decreased the evoked tritium outflow, and this effect was also abolished by DPCPX pretreatment. CGS, a powerful A_2-agonist, did not affect the evoked NE release. However, the effects of CPCA and CGS on evoked NE release were significantly increased by pretreatment of DPCPX in the magnesium-free medium. These results indicate that inhibitory effect of A_1-adenosine receptor on NE release is magnesium-dependent, and A_2-receptor may be present in the rat hippocampus.

Key Words: N^6-cyclopentyladenosine, Magnesium, Norepinephrine, Hippocampus

INTRODUCTION

Since it is known that adenosine and related nucleotides are endogenous modulators of neuronal activity in the peripheral and central nervous system (Fredholm & Hedqvist, 1980; Burnstock & Brown, 1981; Stone, 1981; Schubert et al, 1982), a large body of data on the adenosine receptors controlling the release of neurotransmitters has been accumulated (Dolphin & Archer, 1983; Jackisch et al, 1983 & 1984; Richardt et al, 1987; Fredholm & Lindgren, 1987).

Two adenosine receptor subtypes, termed A_1 and A_2, have been differentiated based on the pharmacological profiles of adenosine agonists and antagonists at each receptor subtype (Daly et al, 1983; Hamprecht & Van Calker, 1985). Inhibition by adenosine on release of various neurotransmitters including acetylcholine, norepinephrine, 5-hydroxytryptamine and glutamate in the central nervous system has been reported, and the receptor participated in the inhibitory effect was defined as A_1-subtype (Jackisch

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et al, 1985; Fredholm et al, 1986a; Fredholm & Lindgren, 1987).

In the hippocampus, NE release is modulated not only by presynaptic α-adrenergic receptor (Jackisch et al, 1984; Hertting et al, 1989) but also by adenosine receptor, and the presynaptic inhibitory effect of adenosine is mediated by A_1-subtype (Jonzon & Fredholm, 1984; Fredholm & Lindgren, 1988).

On the other hand, several workers reported the existence of A_2-subtype with heterogeneous distribution in central nervous system as well as in peripheral tissues (Stone et al, 1988; Bruns et al, 1990). Furthermore, Fredholm et al, (1986b) suggested that the A_2-receptor is present in the hippocampus. Recently, Bartrup & Stone (1988) observed that A_1-receptor-mediated inhibitory effect of adenosine is eliminated in magnesium-free medium, then suggesting the existence of the A_2-excitatory receptor in the rat hippocampus

The physiological significance of adenosine receptors in the central nervous system still remains unclear whereas functionally distinct roles of A_1- and A_2-receptors have been established for many peripheral tissue. This study was undertaken, therefore, to characterize the role of the A_1-adenosine receptor in the evoked NE release in the rat hippocampus, and to attempt to define, if possible, the role of A_2-receptors involved in controlling NE release.

METHODS

Slices of 2.5~3.0 mg, 400 μm in thickness, were prepared from the hippocampus of Sprague-Dawley rats of either sex weighing 250~300 gm with a Balzers® tissue chopper and were incubated in 2 ml of modified Krebs-Henseleit medium containing 0.1 μmol/L [^{3}H]-NE for 30 min at 37°C. Subsequently, the [^{3}H]-NE-pretreated slices were superfused with medium containing yohimbine HCl (1 μM) and desipramine (1 μM) for 150 min at a rate of 1 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 fractions (5 ml) 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 125 min (S_2). Drugs were added between S_1 and S_2 to the superfusion medium. Upon ending the superfusion, the slices were dissolved in 0.5 ml tissue solubilizer (0.5 N quaternary ammonium hydroxide in toluene). The radioactivity in the superfusates and solubilized tissues were determined by liquid scintillation counter (Beckman LS 5000TD). The fractional rate of tritium-outflow (5 min^{-1}) was calculated as tritium-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). Drug effects on the evoked tritium-outflows were evaluated by calculating the ratio of the outflows evoked by S_2 and by S_1 (S_2/S_1). And the influences on the basal outflow are expressed at the ratio b_2/b_1 between fractional rates of outflow immediately before S_2 (120~125 min) and S_1 (55~60 min).

The following chemicals were used: l-[7,8-^{3}H]-noradrenaline (30-50 Ci $mmol^{-1}$, Amersham), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, RBI), desipramine HCl (Sigma), yohimbine HCl (Sigma), CGS-21680 HCl (RBI), N^6-cyclopentyladenosine (CPA,

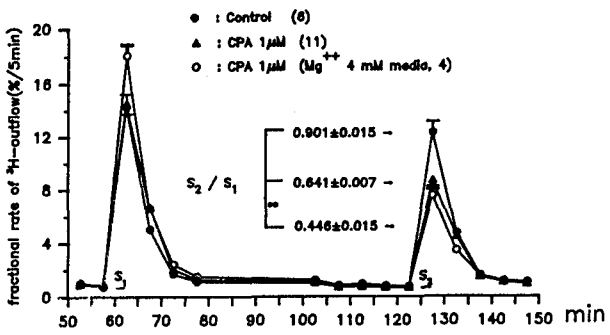

Fig. 1. Influence of high magnesium concentration on the effect of N^6-cyclopentyladenosine (CPA) on the tritium-outflow from the rat hippocampal slices preincubated with ^{3}H-norepinephrine. The slices were electrically stimulated twice for 2 min each, after 60 and 125 min of superfusion (S_1, S_2). The drug effect on the stimulation-evoked tritium outflow is expressed by the ratio S_2/S_1. CPA was presented 15 min and the concentration of magnesium was changed 45 min before S_2 onwards. The tritium contents of the tissues at the start of experiments were 2.46±0.10 (●), 2.69±0.27 (▲) and 1.88±0.08 (○) pmol. The mean±SEM of the experiments (n) are given. Asterisks indicate the significant difference between groups (**;$p<0.01$).

RBI) and 5'-(N-cyclopropyl)-carboxamidoadenosine (CPCA, RBI). Drugs were dissolved in the medium except for CPCA and DPCPX, which were initially dissolved in DMSO and then diluted in the medium.

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

RESULTS

Effects of N^6-cyclopentyladenosine (CPA) and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) on [^{3}H]-norepinephrine release evoked by electrical stimulation

Hippocampal slices prelabelled with [^{3}H]-NE were superfused with the medium containing a NE uptake inhibitor, desipramine (1 μM). And in order to eliminate the inhibition of NE release by activating α_2-adrenergic autoreceptor, yohimbine (1 μM) was added in the superfusion medium. During superfusion, the tissue was electrically stimulated twice.

As shown in Fig. 1, 1 μM CPA decreased the electrically-evoked outflow of tritium, but there was no change in the basal release. CPA in doses ranging from 0.1 to 10 μM decreased the electrically-evoked [^{3}H]-NE release in a concentration-dependent manner (Table 1).

To ascertain the interaction between adenosine and DPCPX, the effects of adenosine were observed in the presence of the DPCPX, a selective A_1-adenosine receptor antagonist (Bruns et al, 1987). Both drugs were added to the superfusion medium 15 min before

Table 1. Effect of N^6-cyclopentyladenosine (CPA) on the electrically-evoked and basal outflows of tritium from the rat hippocampal slices preincubated with ^{3}H-norepinephrine

Drug (μM)		n	S_2/S_1	b_2/b_1
	Control	6	0.902 ± 0.017	0.805 ± 0.034
CPA	0.1	10	0.735 ± 0.013***	0.730 ± 0.018*
	1	11	0.603 ± 0.038***	0.731 ± 0.021*
	3	14	0.569 ± 0.015***	0.698 ± 0.026*
	10	7	0.549 ± 0.030***	0.693 ± 0.018*

After preincubation, the slices were superfused with medium containing 1 μM desipramine and 1 μM yohimbine, and then stimulated twice (S_1, S_2). Drugs were presented from 15 min before S_2 onwards at the concentrations indicated. Drug effects on basal outflow are expressed as the ratio b_2/b_1 between fractional rates of outflow immediately before S_2 (120-125 min) and before S_1 (55-60 min). Mean ± SEM from number (n) of observation are given. Significant differences from the drug-free control are marked with asterisks (*;$p<0.05$ and ***;$p<0.001$). Other legends are the same as in Fig. 1.

Table 2. Effect of varying magnesium concentration on the electrically-evoked and basal tritium-outflow from the rat hippocampal slices preincubated with ^{3}H-norepinephrine

Mg^{2+} concentration (mM)		n	S_2/S_1	b_2/b_1
0		4	1.096 ± 0.016***	0.706 ± 0.220*
0.4		4	0.992 ± 0.007**	0.731 ± 0.014***
1.2	(normal)	6	0.902 ± 0.017	0.805 ± 0.034
2.4		4	0.797 ± 0.020***	0.824 ± 0.032
4.0		4	0.579 ± 0.045***	0.905 ± 0.049

Magnesium concentration were changed from 45 min before S_2 onwards. Significant differences from the drug-free control (normal Mg^{2+} concentration) are marked with asterisks (**;$p<0.01$). Other legends are the same as in Table 1.

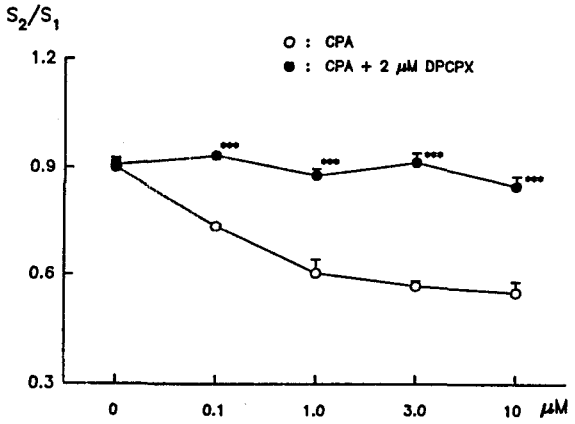

Fig. 2. Influence of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) on the effect of CPA. Each point denote mean and one standard error from 6 to 11 experiments, but the SEM smaller than the width of the points are not shown. Asterisks (***;$p<0.001$) indicate the significant difference between both groups. Legends are the same as in Fig. 1.

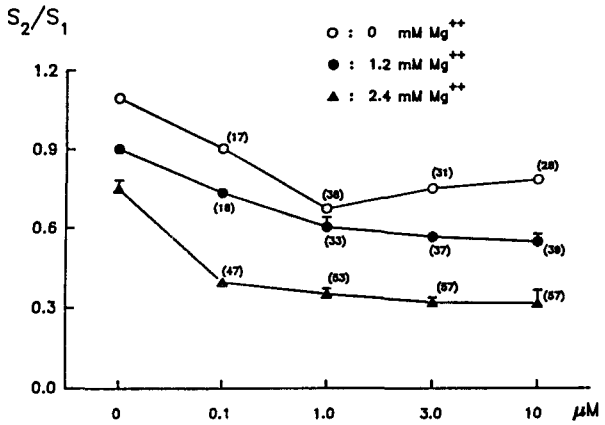

Fig. 3. Influence of the varying magnesium (Mg^{2+}) concentrations upon the effect of CPA. Each point denotes mean and one standard error from 4 to 12 experiments per each group. In the parentheses are the net inhibition(%) from control. Other legends are the same as in Fig. 2.

Table 3. Effect of CGS21680 on the evoked and basal outflows of tritium from the rat hippocampal slices preincubated with ^{3}H-norepinephrine

Drug (μM)		n	S_2/S_1	b_2/b_1
none		12	0.8692 ± 0.0391	0.8130 ± 0.0070
CGS21680	0.1	4	0.8850 ± 0.0103	0.8839 ± 0.0217
	1	4	0.8657 ± 0.0085	0.8912 ± 0.0355
	3	4	0.8900 ± 0.0227	0.7898 ± 0.0210
	10	4	0.8967 ± 0.0151	0.8231 ± 0.0444

Drugs were presented from 15 min before S_2 onwards at the concentrations indicated. Other legends are the same as in Table 2.

S_2. Fig. 2 depicts the effects of adenosine on DPCPX-treated slices as compared with those of non-treated group. The decrements of tritium-outflow were significantly inhibited by DPCPX.

Influence of magnesium ion on [^{3}H]-NE release

In order to see whether the CPA effects are modulated by magnesium ion, the effects of CPA were examined in the different magnesium concentration in superfusion medium. When the magnesium concentrations was decreased to 0.4 and 0 mM, the evoked NE release increased dose-dependently with decreased basal rate of release. But, magnesium in concentrations ranging from 1.2 to 4.0 mM decreased the evoked NE release in a dose-dependent manner without any change of basal release (Table 2). The CPA effect on [^{3}H]-NE release was inhibited by removing the Mg^{2+}. In contrast, increasing the magnesium concentration to 2.4 mM enhanced the adenosine effects of adenosine on the [^{3}H]-NE release (Fig. 3).

Interactions of 5-(N-cyclopropyl)-carboxamidoadenosine (CPCA), CGS 21680C (CGS), DPCPX and magnesium ion on [^{3}H]-norepinephrine release

Because, in the low concentrations of magnesium, evoked [^{3}H]-NE release was increased and the CPA

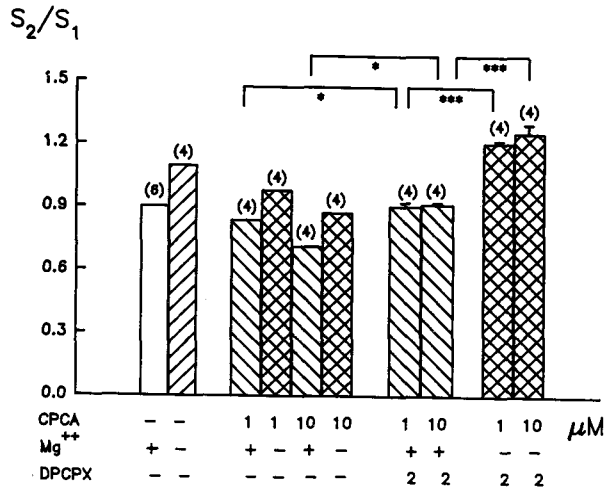

Fig. 4. Influence of Mg^{2+} removal and DPCPX on the effect of 5-(N-cyclopropyl)-carboxamidoadenosine (CPCA). Asterisks (*;p<0.05 and ***;p<0.001) indicate the significant difference between groups. Other legends are the same as in Fig. 2.

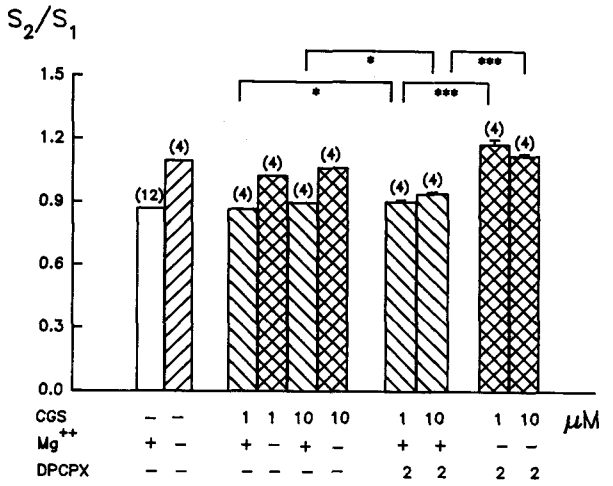

Fig. 5. Influence of Mg^{2+} removal and DPCPX on the effect of CGS21680 (CGS). Asterisks (*;p<0.05 and ***;p<0.001) indicate the significant difference between groups. Other legends are the same as in Fig. 2.

effect was significantly inhibited, the involvement of A_2-receptor in the [^{3}H]-NE release was hypothetized; and thus, the effect of CPCA, a specific A_2-agonist (Bruns et al, 1986), were investigated. As shown in Fig. 4, CPCA in doses 1 and 10 μM decreased evoked [^{3}H]-NE release in a dose-related fashion, but the basal rate of release increased. But, CGS, a recently introduced A_2-agonist (Hutchison et al, 1989), did neither alter the evoked outflow, nor basal tritium outflow (Table 3).

To ascertain whether the NE-release decreasing of CPCA effects are mediated by A_1-receptor, the effect of CPCA was examined in the presence of DPCPX. As depicted in Fig. 4, the decrements of tritium-outflow by CPCA were completely abolished by addition of 2 μM DPCPX. The CPCA and CGS effects on evoked NE release were significantly increased by the treatment of DPCPX in the magnesium free medium (Figs. 4 and 5).

DISCUSSION

It is well established that adenosine is one of the potent neuromodulators with multiple actions upon the physiology and biochemistry in the central nervous system, exerting mainly depressant action on neuronal excitement (Phillis & Wu, 1981; Dunwiddie, 1985). The effects of adenosine are mediated by specific receptors, which can be subdivided into A_1- or A_2-receptors according to their ability to either inhibit or stimulate adenylate cyclase (Van Calker et al, 1979; Londos et al, 1980), and both types are found to exist in the rat hippocampus (Fredholm et al, 1986b). Inhibition by adenosine of release of various neurotransmitters including acetylcholine, norepinephrine and glutamate in the hippocampus has been reported, and the presynaptic receptor participated in the inhibitory effect of adenosine is defined as A_1-subtype (Jackisch et al, 1983, 1985; Jonzon & Fredholm, 1984; Fredholm et al, 1986a).

The present study showed that CPA inhibited the electrically-evoked release of [^{3}H]-NE from the rat hippocampal slice. This result is in accordance with other reports that R-N^6-(2-phenylisopropyl)adenosine and adenosine decreased the electrically-evoked release of NE in the rat hippocampus (Fredholm & Lindgren, 1987; Kim et al, 1995). Moreover, DPCPX, a selective A_1-receptor antagonist, inhibited the effect of CPA. These facts indicate that the inhibitory effect of CPAis mediated by activation of A_1-receptor in rat hippocampus.

The existence of A_2-receptor in the brain tissue is now widely accepted (Williams, 1989; Bruns, 1990),

but the presence of A_2-receptor in the hippocampal tissue controversy still lingers. Fredholm et al. (1982; 1983) described both types of adenosine receptors mediated either increases or decreases of cAMP accumulation in the hippocampal slices. And Sebastião & Ribeiro (1992) observed that CGS21680, a specific A_2-agonist, enhanced hippocampal excitability and insisted the existance of A_2-adenosine receptor in the rat hippocampus. In contrast, Yeung & Green (1984), working with tissue homogenates, concluded that A_1-receptors only are present in the rat hippocampus, whereas in the striatum both A_1- and A_2- receptors are functionally relevant. In experiments on rat cortical slices, Spignoli et al. (1984) have shown that 5'-N-ethylcarboxamideadenosine, an A_2-specific agonist, increased, whereas N^6-cyclohexyladenosine, an A_1-specific agonist, decreased the electrically- evoked acetylcholine release. Thus, both receptor subtypes seem to be involved in the modulation of acetylcholine release in the rat cortex. In the present study, it was attempted to see whether A_2-receptors are involved in NE release in the rat hippocampus, by observing the effects of 5-(N-cyclopropyl)- carboxamidoadenosine (CPCA) and CGS21680C (CGS), recently introduced specific A_2-agonists, on the evoked tritium outflow. These findings that CGS did not increase but CPCA strongly inhibited the evoked hippocampal NE release, and that effects of CPCA were completely inhibited by DPCPX, a specific A_1-antagonist, may be considered to be in line with the view of Yeung and Green (1984) that in the hippocampus, at least at the level of the cholinergic nerve terminal, only A_1-adenosine receptor subtype seems to be functionally relevant.

On the other hand, since it is known that magnesium enhances the binding of cyclohexyladenosine, an adenosine analogue, to adenosine receptors, magnesium has been widely used as a tool for studying the involvement of adenosine receptors in physiological events (Goodman et al, 1981; Yeung & Green, 1984; Yeung et al, 1985). In electrophysiological experiments on the rat hippocampal slice, Bartrup & Stone (1988) have shown that high magnesium medium enhances, wheareas magnesium-free medium greatly attenuates, the potency of adenosine in reducing orthodromically evoked population potentials elicited in area CA_1, suggesting that magnesium is needed for the A_1-mediated inhibitory effects of adenosine. However, there is no reports so far about the influence of magnesium upon the role of adenosine receptor in controlling the neurotransmitter release. The present experiment shows that magnesium itself affects both basal and evoked releases of tritium significantly. In interaction experiments, the concentration- response relationship for CPA was observed in the presence of high or low magnesium. High magnesium (2.4 mM) significantly potentiated the inhibitory effects of CPA, but magnesium-free medium decreased the CPA effect. When magnesium was removed from the superfusion medium, the evoked NE release by CPCA and CGS was significantly increased by DPCPX pretreatment. These findings suggest that the inhibition of NE release by CPA is also magnesium-dependent much the same as the electrophysiological effects of adenosine. And A_2-adenosine receptor which is involved in NE release is present in rat hippocampus. There are, however, evidence that the NMDA receptors are also involved in the magnesium effects (Mayer et al, 1984; Nowak et al, 1984; Mody et al, 1987); hence, further studies need to be done.

Overall, the results of the present study suggest that the decrement of the evoked NE release by CPA is mediated by A_1-heteroreceptors and that the inhibitory effect of CPA is magnesium-dependent, and that A_2-receptor may be present in the rat hippocampus.

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