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A Study on the Post-Receptor Mechanism of Adenosine Receptor on Norepinephrine Release in the Rat Hippocampus

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ABSTRACT

Since it has been reported that the depolarization-induced norepinephrine (NE) release is inhibited by activation of presynaptic A_1-adenosine heteroreceptor in hippocampus, a large body of experimental data on the post-receptor mechanism of this process has been accumulated. But, the post-receptor mechanism of presynaptic A_1-adenosine receptor on the NE release has not been clearly elucidated yet. Therefore, it was attempted to clarify the post-receptor mechanisms of the A_1-adenosine receptor-mediated control of NE release in this study.

Slices from rat hippocampus were equilibrated with ^{3}H-norepinephrine and the release of the labelled products was evoked by electrical stimulation (3 Hz, 5 VCm^{-1}, 2 ms, rectangular pulses), and the influence of various agents on the evoked tritium-outflow was investigated. Adenosine, in concentrations ranging from 1~30 μM, decreased the NE release in a dose-dependent manner, without affecting the basal rate of release. The adenosine effects were significantly inhibited by 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 2 μM), a selective A_1-receptor antagonist. The responses to N-ethylmaleimide (NEM, 10 & 30 μM), a SH-alkylating agent of G-protein, were characterized by increments of the evoked NE-release and the basal release, and the adenosine effects were completely abolished by NEM pretreatment. 4β-Phorbol 12,13-dibutyrate (PDB, 1 μM), a specific protein kinase C (PKC) activator, increased the evoked NE release, whereas polymyxin B sulfate (PMB, 0.1 mg), a PKC inhibitor, decreased the release, and the adenosine effects were inhibited by these agents. Nifedipine (1 μM), a Ca^{2+}-channel blocker of dihydropyridine analogue, did not affect the adenosine effect. Tetraethylammonium (TEA, 3 mM) increased the evoked NE release, and inhibited the adenosine effects, but glibenclamide, a ATP dependent K^+-channel blocker, did not. Finally, 8-bromo cyclic AMP (100 & 300 μM), a membrane-permeable analogue of cAMP, did not alter the NE release, but adenosine effects were inhibited by pretreatment with 8-br-cAMP.

These results suggest that the decrement of the evoked NE-release by A_1-adenosine receptor is mediated by the G-protein, which is coupled to protein kinase C, adenylate cyclase system and TEA sensitive K^+-channel, and that nifedipine-sensitive Ca^{2+}-channel and glibenclamide-sensitive K^+-channel are not involved in this process.

Key Words: Hippocampus, [^{3}H]-NE release, Adenosine, Receptor

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INTRODUCTION

It is generally accepted that adenosine and its related nucleotides are endogenous modulators of neuronal activity in the peripheral and central nervous systems (Fredholm and Hedqvist, 1980; Burnstock and Brown, 1981; Schubert *et al.*, 1982). 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 subtypes (Daly *et al.*, 1983; Hamprecht and Van Calker, 1985). Inhibition by adenosine on the release of various neurotransmitters including acetylcholine (Ach), norepinephrine (NE), 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 (Jakisch *et al.*, 1985; Fredholm *et al.*, 1986; Fredholm and Lindgren, 1987).

In the hippocampus, norepinephrine release is modulated not only by presynaptic-adrenergic receptor (Jackish et al., 1984, 1985; Hertting *et al.*, 1987) but also by adenosine receptor, and the presynaptic inhibitory effect of adenosine is mediated by A_1-subtype (Jonzon and Fredholm, 1984; Fredholn and Lindgren, 1988) but the mechanisms underlying mechanisms of such presynaptic control of transmitter release remain unclear until now.

Although activation of A_1-adenosine receptor has been shown to reduce adenylate cyclase activity with decreased cAMP accumulation (Fredholm *et al.*, 1986) and the decrement of evoked NE release by adenosine analogues is apparently mediated via an N-ethylmaleimide-sensitive G-protein (Fredholm and Lindgren, 1987), the involvement of adenylate cyclase system in controlling NE release by the presynaptic A_1-adenosine receptor was controversial.

On the other hand, adenosine has been shown to modulate ion-fluxes through the membrane and second messenger system as well as transmitter release through a variety of receptor-mediated mechanisms (Williams, 1989). There are other reports that the inhibitory effects by adenosine have been attributed to both inhibition of calcium conductance (Proctor and Dunwiddie, 1983; Madison *et al.*, 1986; Dolphin *et al.*, 1986) and activation of potassium channels (Dunwiddie, 1985; Trussell and Jackson, 1985), which seems to be mediated by G-protein (Pfaffinger *et al.*, 1985; Hescheler *et al.*, 1987; Trusell and Jackson, 1987). And also, it has been suggested that the effect of presynaptic agonists on calcium channels is indirect and is due to stimulation of protein kinase C (Rane and Dunlap, 1986). Furthermore, there are reports suggesting that the involvement of protein kinase C in neural function, i.e., effects of noradrenaline on neurons can be mimicked by activation of protein kinase C (Rane and Dunlap, 1986) and abolished by selective inhibition of protein kinase C (Rane *et al.*, 1989). Conversely, there is evidence that activation of protein kinase C may reduce the presynaptic activity of several agonists (Allgaier *et al.*, 1986; Fredholm and Lindgren, 1988; Ramdine *et al.*, 1989a, b). However, Hu and Fredholm (1989, 1991) insisted that 4-aminopyridine-sensitive potassium channels are not involved in inhibitory effects of NE release by adenosine receptor.

After all, though a large body of experimental data has been accumulated, the post-receptor mechanism controlling NE release by the presynaptic A_1-adenosine receptor still remains to be elucidated.

The present study, therefore, was designed to delineate the post-receptor mechanisms on the adenosine receptor in the evoked NE release in the rat hippocampus.

METHODS

Slices of 2.5~3.0 mg, 400 μM 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]-norepinephrine for 30 min at 37℃. Subsequently, the [^{3}H]-norepnepihrine-pretreated slices were superfused with medium containing desipramine (1 μM) and yohimbine (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. At the end of superfusion, the slices were solubilized 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]-noradrelanine (30~50 Ci $mmol^{-1}$, Amersham), adeno-sine (RBI), 8-cyclopentyl-1,3-dipropylxanthine (RBI), desipramine HCl (Sigma), yohimbine HCl (Sigma), N-ethylmaleimide (Sigma), polymyxin B sulfate (PMB, Sigma), 4β-phorbol 12,13-dibutyrate (PDB, Sigma), nifedipine (Sigma), tetraethylammonium bromide (Sigma), glibenclamide (RBI) and 8-bromo-cAMP (Sigma). Drugs were dissolved in the medium except glibenclamide, cyclopentyl-1,3-dipropylxanthine, PMB, PDB and nifedipine which were initially dissolved in DMSO and then diluted with the medium. Nifedipine, 8-bromo-cAMP and PMB are protected from exposure to light.

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 adenosine and 8-cyclopentyl-1,3-dipropylxanthine (CPCPX) on ^{3}H-norepinephrine release evoked by electrical stimulation

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

Table 1. Effect of adenodine on the electrically-evoked and basal outflows of tritium from the rat hippocampal slices preincubated with ^{3}H-norepinephrine

Drugs before S_2(μM)		n	S_2/S_1	b_2/b_1
none		12	0.8692±0.0391	0.8130±0.0070
Adenosine	1	12	0.7933±0.0145	0.7756±0.0233
	3	12	0.7591±0.0170*	0.8035±0.0293
	10	11	0.6366±0.0217***	0.7507±0.0209*
	30	12	0.6326±0.0178***	0.7522±0.0243*

After preincubation, the slices were superfused with medium containing 1 μM desipramine & 1 μM yohimbine, and then stimulated twice (S_1, S_2). Drugs were presented 15 min before S_2 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.

As shown in Fig. 1, 10 μM adenosine decreased the electrically-evoked outflow of tritium (S_2/S_1, 0.711±0.019), but there was no change in the basal release. Adenosine, in doses ranging from 1 to 30 μM decreased the electrically-evoked ^{3}H-NE release (adenosine effects) in a concentration-dependent manner (Table 1).

To ascertain the interaction between adenosine and DPCPX, a selective A_1 antagonist (Bruns *et al.*, 1987), the effects of adenosine were observed in the presence of the DPCPX (Fig. 1). Both drugs were added to the superfusion medium 15 min before S_2. Table 2 summarize the effects of adenosine in control and DPCPX-treated slices. The decrements of tritium-outflow were significantly inhibited by DPCPX.

Interactions of N-ethylmaleimide (NEM) and adenosine on ^{3}H-norepinephrine release

In order to study whether the adenosine effects are mediated by G-protein, the effects of adenosine were examined in the presence of the NEM, a SH-alkylating agent. NEM (10 & 30 μM) increased the tritium-outflow in a dose-dependent manner. The decrements of tritium outflow by 10 and 30 μM adenosine were completely inhibited by pretreatment with NEM (Fig. 2).

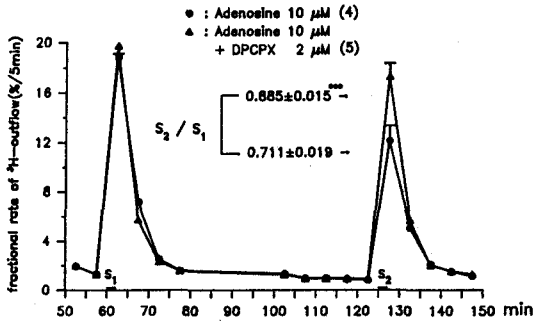

Fig. 1. A typical presentation of the tritium-outflow from the rat hippocampal slice preincubated with ^{3}H-norepinephrine. The slices were electrically stimulated twice for 2 min each, after 60 and 125 min of superfusion (S_1, S_2). The drug effect on the stimulation-evoked tritium outflow is expressed by the ratio S_2/S_1. The radioactivities of the tissues at the start of experiment were 1.292±0.052 (●) and 1.244±0.094 (▲) pmol. Adenosine and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) were added 15 min before S_2. Asterisks indicate significant difference (***; $p<0.001$) between the adenosine and adenosine plus DPCPX-treated groups.

Table 2. Influence of DPCPX upon the effect of adenosine on the electrically-evoked tritium-outflows from the rat hippocampus

Drugs at S_2(μM)		n	S_2/S_1	Net inhibition by adenosine(%)	
DPCPX	Adenosine				
–	–	5	0.770±0.035		
2	–	5	0.943±0.010		
–	1	5	0.623±0.063	−0.147(−19.1)	**
2	1	5	0.885±0.015	−0.058(− 6.2)	
–	3	5	0.453±0.047	−0.317(−41.2)	***
2	3	5	0.900±0.018	−0.043(− 4.6)	
–	10	5	0.429±0.072	−0.341(−44.3)	***
2	10	5	0.969±0.041	+0.026(+ 2.8)	

Asterisks (**;$p<0.01$) indicate significant difference between groups. Other legends are the same as in Table 1.

Interactions of adenosine and 4β-phorbol-12,13-dibutyrate (PDB) or polymyxin B (PMB) on ^{3}H-norepinephrine release

As shown in Table 3, an activator of protein kinase C (Nishizuka, 1984), 1 μM PDB increased the electrically evoked ^{3}H-NE release significantly. The decrements of evoked ^{3}H-NE release by 1 to 10 μM adenosine were completely blocked by pretreatment with PDB.

As depicted in Fig. 3, a protein kinase C inhibitor (Kuo *et al.*, 1983), 0.1 mg of PMB decreased the electrically evoked ^{3}H-NE, and in the presence of 0.1 mg PMB, the decrements of ^{3}H-NE by adenosine were not appeared.

Influences of the Ca^{2+}-channel and K^+-channel blockers on the adenosine effect

To ascertain whether the adenosine effects

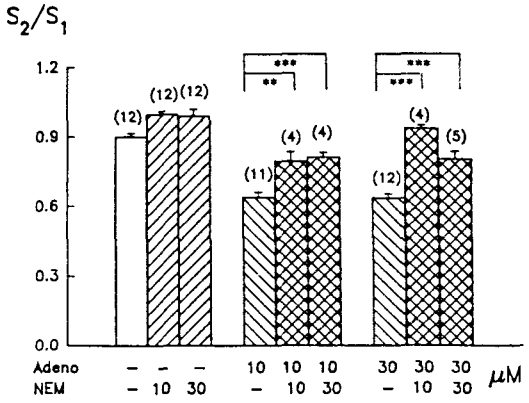

Fig. 2. Influence of the N-ethylmaleimide (NEM) upon the adenosine effects. NEM was added to the medium between the two stimulation for 30 min. In parentheses are the number of experiments. Legends are the same as in Table 1.

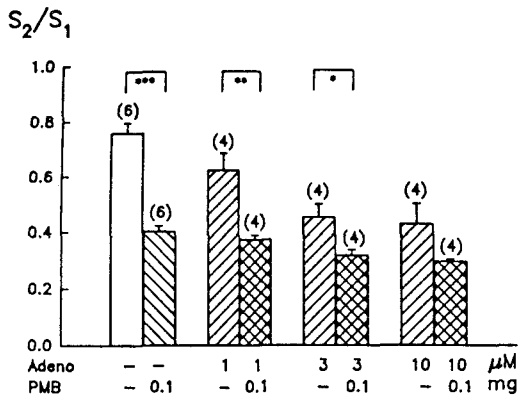

Fig. 3. Influence of the polymyxin B (PMB) upon the effect of adenosine on the electrically evoked tritium outflow from the rat hippocampal slices. PMB was added in 30 min before S_2. Asterisks indicate significant difference (*; $p<0.05$) between groups. Other legends are the same as in Fig. 2.

Table 3. Influence of PDB upon the effect of adenosine on the electrically evoked tritium outflows from the rat hippocampus

Drugs at S_2(μM)		n	S_2/S_1	Net inhibition by adenosine(%)	
PDB	Adenosine				
–	–	5	0.759±0.037		
1	–	5	1.300±0.040		
–	1	5	0.623±0.063	−0.136(−17.9)	***
1	1	5	1.316±0.011	+0.016(+ 1.2)	
–	3	5	0.453±0.047	−0.306(−40.3)	***
1	3	5	1.340±0.023	+0.040(+ 3.1)	
–	10	5	0.429±0.072	−0.330(−43.5)	***
1	10	5	1.324±0.032	+0.024(+ 1.8)	

Legends are the same as in Table 1.

are mediated by Ca^{2+}-and K^+-channel modulation, the effect of adenosine were examined in the presence of nifedipine, glibenclamide or tetraethyl ammonium (TEA). Nifedipine, a calcium channel blocker, did not affect the adenosine effects (Fig. 4).

Next, the influence of TEA, a delayed rectifier potassium channel blocker, on the adenosine was investigated. As depicted in Fig. 5, in the presence of 3 mM TEA slightly inhibited the adenosine effects. To ascertain the interaction between TEA and adenosine, the slopes of the two regression lines, adenosine ($y=-0.0070x+1.1358$, $r=0.906$), and adenosine under TEA 3 mM ($y=-0.0065x+0.7956$, $r=0.794$), were compared, but there were no significant difference between the slopes. However, glibenclamide, a ATP-dependent K^+-channel blocker, did not affect adenosine effects (Fig. 6).

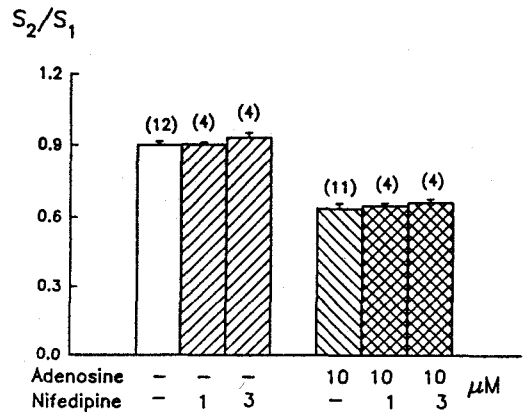

Fig. 4. Influence of nifedipine on the adenosine effects. Legends are the same as in Fig. 2.

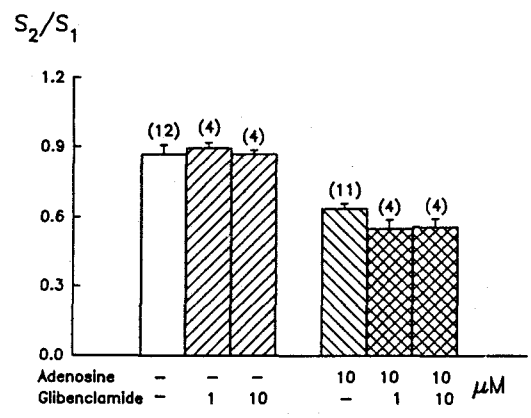

Fig. 6. Influence of glibenclamide on the effect of adenosine on the electrically evoked tritium outflow from the rat hippocampus. Legends are the same as in Fig. 2.

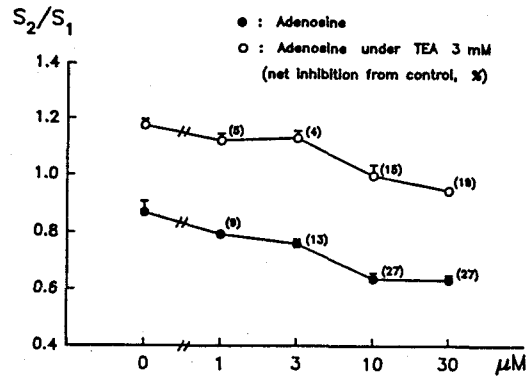

Fig. 5. Influence of the tetraethylammonium (TEA) upon the effect of adenosine on the electrically evoked tritium outflow from the rat hippocampal slices. Each point denotes mean ±SEM from 4~8 experiments per group.

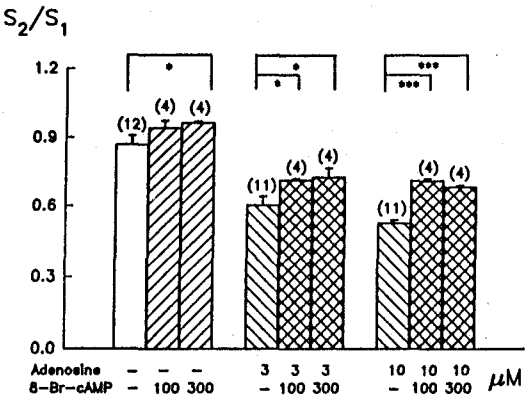

Fig. 7. Influence of 8-bromo(Br)-cAMP on the effect of adenosine. Legends are the same as in Fig. 2.

Interaction of 8-bromo-cAMP and adenosine in ^{3}H-norepinephrine release

To clarify if the adenylate cyclase system was involved in adenosine effects, the effect of adenosine was examined in the presence of the 8-bromo-cAMP. 8-bromo-cAMP was infused from 30 min before S_2 and adenosine was added at 15 min later.

As shown in Fig. 7, in the presence of 8-bromo-cAMP, the inhibitory effects of 3 and 30 μM adenosine were significantly reduced.

DISCUSSION

In the present study, the electrically evoked release of ^{3}H-NE from the rat hippocampal slices was inhibited by adenosine. This result is in accordance with other reports that the R-N^6-(2-phenylisopropyl)adenosine and adenosine decreased the electrically-evoked release of NE in the rat (Hu and Fredholm, 1991; Kim *et al.*, 1995) and rabbit (Jackisch *et al.*, 1985; Hertting *et al.*, 1987) hippocampus. Moreover, in this study, 8-cyclopentyl-1,3-dipropylxanthine, a selective A_1-receptor antagonist, inhibited the adenosine effect. These facts indicate that the adenosine effect is mediated by A_1-receptor in rat hippocampus.

It has repeatedly been found that effects of A_1-adenosine receptor coupled to adenylate cyclase are mediated by guanine nucleotide-binding protein (Cooper *at al.*, 1980; Yeung and Green, 1984), which was defined as G_i (Katada and Ui, 1982) and can be irreversibly inhibited by sulfhydryl alkylating agent, NEM (Jacobs *et al.*, 1981; Smith and Harden, 1984). Therefore, in order to confirm whether the G_i-protein is involved in the A_1-receptor-mediated modulation of NE release, the influence of NEM upon the adenosine effects was investigated in this study. When the hippocampal slices were treated with NEM, the evoked NE release was significantly enhanced, and the adenosine effects were abolished by NEM-pretreatment. This finding agrees well with those of others that the various receptor-coupled inhibition of neurotransmitter release is mediated by G protein (Allgaier *et al.*, 1987; Fredholm *et al.*, 1986b; Hertting *et al.*, 1987). And also, because it is thought that A_1-receptor activation generally inhibits production of cAMP (Fredholm *et al.*, 1986a), the possibility was examined that the A_1-adenosine receptor mediated inhibition of NE release is a consequence of inhibition of adenylate cyclase. The present study shows that 8-bromo-cAMP, a membrane-permeable analogue of cAMP, did not affect the evoked ^{3}H-NE release, but significantly inhibited the adenosine effects. This result, in accordance with previous reports that forskolin, an adenylate cyclase activator (Seamon and Daly, 1983) potentiated the electrically evoked release of NE in rat cerebrocortical slices (Markstein *et al.*, 1984) and inhibited the effects of N^6-cyclopentyladenosine in the rat hippocampus (Yang, 1995), indicates that the modulation of NE release by A_1-adenosine receptor is coupled to an intraneuronal adenylate cyclase system. Fredholm and Lindgren (1987), however, observed that effects of forskolin and rolipram (phosphodiesterase inhibitor) were completely antagonized by N^6-R-phenylisopropyladenosine (R-PIA), and thus, proposed that the adenylate cyclase system is not involved in the A_1-receptor-regulated NE release in rat hippocampus. Discrepancy between the present finding and this reports may not be easily reconciled. Thus further studies are required to clearly elucidate the involvement of adenylate cyclase system on the adenosine effects.

On the other hand, there are impressive evidences that protein kinase C is involved in the neurotransmitter releasing process in many types of preparations (Tanaka *et al.*, 1984; Grega *et al.*, 1987; Nichols *et al.*, 1987) including the hippocampus (Malenka *et al.*, 1986; Allgaier and Hertting, 1986; Allgaier *et al.*, 1986, 1987a). It is likely to be, therefore, reasonable to use protein kinase C modulators to examine the possible role of protein kinase C involved in the regulation of NE release by adenosine. In the present study, PDB, a selective protein kinase C activator (Nishizuka, 1984), markedly enhanced the release of NE whereas PMB, a protein kinase C inhibitor (Kuo *et al.*, 1983), decreased it. But the adenosine effects on evoked NE release could not be observed under PDB and PMB treatment. This result is in ac-

cordance with other reports that adenosine effects in the central nervous system are due to the K^+-currents activation (Trussel and Jackson, 1985) and phorbol esters and protein kinase C activators inhibiting the adenosine-induced K^+-current in oocyte (Dascal *et al.*, 1985) indicates that protein kinase C might participated in the A_1-receptor-mediated inhibition of evoked NE release in the rat hippocampus. But, there are the reports that protein kinase C is not involved in A_1-receptor mediated ACh release (Fredholm, 1990a) and NE release (Fredholm and Lindgren, 1988) in the rat hippocampus.

There are reports that calcium and potassium channels are involved in the adenosine effects, and that the GTP-binding protein can also couple to them (see introduction). Therefore, in order to confirm whether calcium and potassium channels are involved in the adenosine effect, the influence of calcium and potassium channel blockers upon the adenosine effects was investigated in this study. Nifedipine, a dihydropyridine analogue of Ca^{2+} channel blocker, did not alter the basal and evoked rates of NE release by itself, but significantly inhibited the adenosine effect. This finding, in conjunction with the report of Adamson *et al.*, (1989) which presented the existence of functioning L-type Ca^{2+}-channel in rat brain, indicates clearly that the inhibitory adenosine effect is coupled to nifedipine-sensitive Ca^{2+}-channel. Fredholm (1990b), however, observed that the evoked ACh release was unaffected by nifedipine in the presence or the absence of phorbol esters, and insisted that a dihydropyridine-sensitive L-type Ca^{2+}-channel is probably not involved in the A_1-receptor mediated effect in the rat hippocampus. The difference between the present finding and Fredholm's may not be easily explained, and thus, further study should be needed in the involvement of Ca^{2+}-channel in post-receptor mechanism of NE release by A_1-adenosine receptor.

Recently, Allgaier *et al.*, (1993) demonstrated the TEA increased the evoked NE release in rat and rabbit hippocampus. The present study confirms this result and shows, in addition, that TEA inhibited the inhibitory effects of A_1-adenosine agonist, and these indicate that TEA-sensitive K^+-channel might be participated in the A_1-adenosine effect in rat hippocampus.

Overall, the present study has shown that the decrement of the evoked NE release by A_1-adenosine receptor is mediated by the G-protein. It seems also that protein kinase C, adenylate cyclase system and TEA sensitive K^+-channel are coupled partly to this effect, and that nifedipine-sensitive Ca^{2+}-channel and glibenclamide-sensitive K^+-channel are not involved in this process.

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=국문초록=

흰쥐 해마에서 Norepinephrine 유리에 미치는 Adenosine Receptor의 Post-Receptor 기전에 관한 연구

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흰쥐 해마(hippocampus)에서 norepinephrine(NE) 유리에 미치는 A_1-adenosine 수용체의 post-receptor 기전에 관한 지견을 얻고자 하여 ^{3}H-norepinephrine으로 평형시킨 해마 절편을 사용하여 adenosine의 ^{3}H-NE 유리에 미치는 여러가지 약물의 영향을 관찰하였다.

Adenosine(1~30 μM)은 전기자극(3 Hz, 2 ms, 5 VCm-1, 구형파)에 의한 NE 유리를 용량 의존적으로 감소시켰고, 이 효과는 선택적인 A_1-adenosine 수용체 차단제인 8-cyclopentyl-1,3-dipropylxanthine(2 μM)에 의해 차단되었다. G-단백 억제제인 N-ethylmaleimide(NEM, 10과 30 μM)는 그 자체로써 전기자극으로 유발시킨 NE 유리를 증가시켰으며, adenosine의 NE 유리 억제효과는 NEM 전처리에 의하여 완전히 소실되었다. Protein kinase C 활성화제인 4β-phorbol 12,13-dibutyrate(PDB, 1 μM)는 NE 유리 증가를 일으켰고, 이 효소 억제제인 4β-polymyxin B(PMB, 0.1 mg)는 NE 유리감소를 일으켰으며, adenosine에 의한 NE 유리 감소효과는 PDB에 의해 억제되었고, PMB 전처리하에서는 감소효과가 출현하지 않았다. Ca^{2+}-통로 차단제인 nifedipine(1 μM)은 adenosine의 NE 유리 억제효과에 영향을 미치지 못하고, ATP에 의존적인 K^+-통로 차단제인 glibenclamide역시 adenosine의 NE 유리 억제효과에 영향을 미치지 못하였다. 그러나 delayed rectifier K^+-통로 차단제인 tetraethylammonium(TEA, 3 mM)은 그 자체로 NE 유리를 증가 시켰으며, adenosine의 NE 유리 억제효과를 차단함을 볼 수 있었다. 8-bromo-cAMP(100과 300 μM) 그 자체로는 NE 유리에 별다른 영향을 미치지 못하였으나 8-bromo-cAMP 전처리에 의하여 adenosine의 NE 유리 억제효과가 억제됨을 볼 수 있었다.

이상의 실험결과로 흰쥐 해마에서 A_1-adenosine 수용체를 통한 adenosine의 NE 유리 감소는 G-단백에 의존적이며, 이러한 효과에 protein kinase C, TEA에 예민한 K^+-통로 및 adenylate cyclase계가 복합적으로 관여하고 nifedipine에 예민한 Ca^{2+}-통로와 glibenclamide에 예민한 K^+-통로는 관여하지 않는 것으로 사료된다.