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

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ABSTRACT

Since it was been reported that the depolarization-induced ACh 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 ACh 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 ACh release in this study.

Slices from rat hippocampus were equilibrated with ^{3}H-choline and the release of the labelled products was evoked by electrical stimulation (3 Hz, 5 VCm^{-1}, 2ms, rectangular pulses), and the influence of various agents on the evoked tritium-outflow was investigated.

Adenosine, in concentrations ranging from 0.3~300 μM, decreased the ACh release in a dose-dependent manner, without affecting the basal rate of release. The adenosine effects were significantly inhibited by DPCPX (2 μM), a selective A_1-receptor antagonist. The responses to N-ethylmaleimide (10 & 30 μM), a SH-alkylating agent of G-protein, were characterized by increments of the evoked ACh-release and the basal release, and the adenosine effects were completely abolished by NEM pretreatment. PDB (1~10 μM), a specific protein kinase C (PKC) activator, increased, whereas PMB (0.03~1 mg), a PKC inhibitor, decreased the evoked ACh-release, and the adenosine effects were not affected by these agents. Nifedipine (1 μM), a Ca^{2+}-channel blocker of dihydropyridine analogue, significantly inhibited the adenosine effect, but glibenclamide, a K^+-channel blocker, did not. Finally, 8-bromo cyclic AMP (100 & 300 μM), a membrane-permeable analogue of cAMP, did not alter the ACh release, but adenosine effects were inhibited by pretreatment with large dose of 8-br-cAMP (300 μM).

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

Key Words: Hippocampus, [^{3}H]-ACh release, Adenosine, cAMP, Ca^{++}-chnnel

INTRODUCTION

It is generally accepted that adenosine and re

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lated 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, 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 (Jakisch *et al.*, 1985; Fredholm *et al.*, 1986a; Fredholm and Lindgren, 1987).

In the hippocampus, acetylcholine (ACh) release is modulated not only by muscarinic ACh receptor (Hertting *et al.*, 1987; Choi *et al.*, 1991) but also by adenosine receptor, and the presynaptic inhibitory effect of adenosine is mediated by A_1-subtype (Jakisch *et al.*, 1984; Choi *et al.*, 1992), but the mechanisms underlie such presynaptic control of transmitter release remain unclear.

Although activation of A_1-adenosine receptor has been shown to reduce adenylate cyclase activity with decreased cAMP accumulation (Fredholm *et al.*, 1986b) and the decrement of evoked ACh release by adenosine analogues is apparently mediated via an N-ethylmaleimide-sensitive G-protein (Dunér-Engström and Fredholm, 1988; Choi *et al.*, 1992), the involvement of adenylate cyclase system in controlling ACh 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 reports that the inhibitory effects by adenosine have been attributed both to 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), and these actions are thought 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 due to stimulation of protein kinase C (Rane and Dunlap, 1986). Furthermore, there are reports suggesting the involvement of protein kinase C in neural function, i.e., effects of noradrenaline on neurons can be mimicked by protein kinase C stimulation (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.*, 1989; Fredholm and Lindgren, 1988; Ramidine *et al.*, 1989a, b). However, Fredholm (1990a, b) insisted that a dihydropyridine-sensitive L-type calcium channel, 4-aminopyridine-sensitive potassium channel or protein kinase C are not involved in inhibitory effects of ACh release by adenosine receptor.

After all, though a large body of experimental data has been accumulated, the Post-receptor mechanism controlling ACh 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 of the adenosine receptor in the evoked ACh release in the rat hippocampus.

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]-choline for 30 min at 37℃. 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 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 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 radioactivity in the superfusates and solubilized tissues were determined by liquid scintillation counting (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). As reported previously, the electrical stimulation of brain slices incubated with [^{3}H]-choline causes the release of [^{3}H]-acetylcholine only (Richardson and Szerb, 1974). 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).

The following chemicals were used: [methyl-^{3}H]-choline chloride (72~78 $Cimmol^{-1}$, Amersham), adenosine (Sigma), 8-cyclopentyl-1,3-dipropylxanthine (RBI), atropine sulfate (Sigma), N-ethylmaleimide (Sigma), verapamil HCl (Sigma), nifedipine (Sigma), glibenclamide (RBI) and hemicholinium-3 (Sigma). Drugs were dissolved in the medium except for glibenclamide, cyclopentyl-1, 3-dipropylxanthine and nifedipine which were initially dissolved in DMSO and then diluted in the medium. Nifedipine is 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 (DPCPX) on ^{3}H-acetylcholine release evoked by electrical stimulation

Hippocampal slices prelabelled with ^{3}H-choline, a ^{3}H-acetylcholine 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

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

Drugs befors S_2 (μM)	n	S_2/S_1	b_2/b_1
none	7	1.055±0.026	0.633±0.023
0.3	4	1.077±0.025	0.626±0.071
1.0	4	0.952±0.037	0.670±0.033
3.0	4	0.640±0.086**	0.623±0.066
10.0	4	0.526±0.069***	0.712±0.029
30.0	7	0.402±0.034***	0.687±0.035
100.0	7	0.323±0.021***	0.683±0.028
300.0	6	0.315±0.060***	0.774±0.060

After preincubation, the slices were superfused with medium containing 10 μM hemicholinium-3 & 30 nM atropine and 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 (115~120 min) and before S_1 (55~60 min). Means±SEM from number (n) of observation are given. Significant differences from the drug-free control are marked with asterisks (**=P<0.01 and ***=P<0.001). Other legends are the same as in Fig. 1.

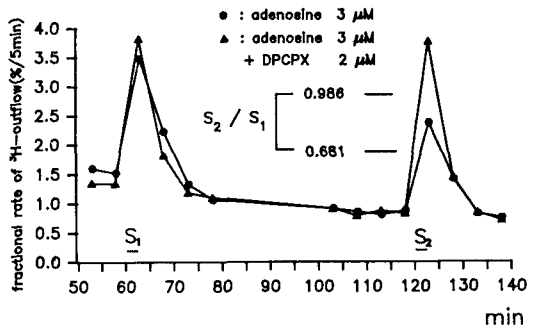

Fig. 1. A typical presentation of the tritium-outflow from the rat hippocampal slice preincubated with ^{3}H-choline. The slices were electrically stimulated twice for 2 min each, after 60 and 120 min of superfusion (S_1, S_2). The drug effect on the stimulation-evoked tritium outflow is expressed by the ratio S_2/S_1. The radioactivities of the tissues at the start of experiment were 0.751(●) and 0.819(▲) pmol. Adenosine and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) were added 15 min before S_2.

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			
–	–	4	1.052±0.048	
2	–	4	1.148±0.082	
–	1	4	1.058±0.061	+0.006(0) *
2	1	4	1.342±0.087	+0.194(+ 5.9)
–	3	4	0.756±0.028	−0.296(−28.1) ***
2	3	4	1.277±0.055	+0.129(+11.2)
–	10	4	0.591±0.042	−0.461(−43.8) ***
2	10	4	1.104±0.051	−0.044(− 3.8)

Asterisks (*=P<0.05 and ***=P<0.001) indicate significant difference between groups. Other legends are the same as in Table 1.

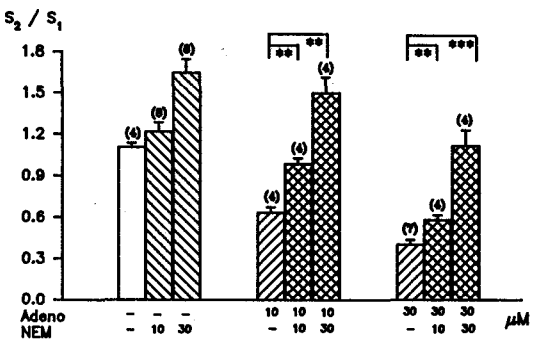

Fig. 2. Influence of the N-ethylmaleimide (NEM) on the effect of adenosine on the electrically-evoked tritium outflow from the rat hippocampus. NEM was added to the medium between the two stimulation for 30 min. In parentheses are the number of experiments. Asterisks indicate significant difference (**P<0.01 and ***P <0.001) between the NEM-free and NEM-treated groups. Other legends are the same as in Fig. 1.

nM), a muscarinic antagonist, was added in the superfusion medium. During superfusion, the tissue was electrically stimulated twice.

As shown in Fig. 1, 3 μM adenosine decreased the electrically-evoked outflow of tritium (S_2/S_1, 0.681), but there was no change in the basal release. Adenosine, in doses ranging from 0.3 to 300 μM decreased the electrically-evoked ^{3}H-ACh release 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 on DPCPX-treated slices as compared with those of non-treated group. The decrements of tritium-outflow were significantly inhibited by DPCPX.

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

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

As shown in Table 3, PDB, an activator of protein kinase C (Nishizuka, 1984), in doses ranging from 0.3 to 10 μM, increased the electrically evoked ^{3}H-ACh release in a dose-related fashion. The decrements of evoked ^{3}H-ACh release by 30 μM adenosine were not affected by pretreatment

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			
–	–	13	1.067±0.019	
0.3	–	4	2.073±0.199	
–	30.0	8	0.503±0.032	0.564(53)
0.3	30.0	4	0.803±0.013	1.270(61)
1.0	–	12	2.417±0.208	
1.0	30.0	8	1.232±0.085	1.185(49)
10.0	–	7	2.814±0.183	
10.0	30.0	7	1.245±0.107	1.569(56)

Legends are the same as in Table 1.

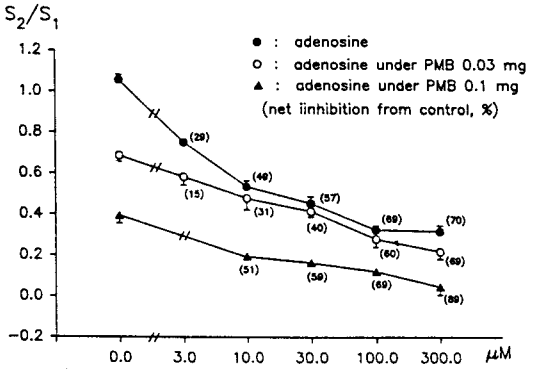

Fig. 3. Influence of the polymyxin B (PMB) upon the effect of adenosine on the electrically evoked tritium outflow from the rat hippocampal slices. PMB was added in 30 min before S_2 onwards. Each point denotes mean±SEM from 4 ~8 experiments per group. Other legends are the same as in previous figures.

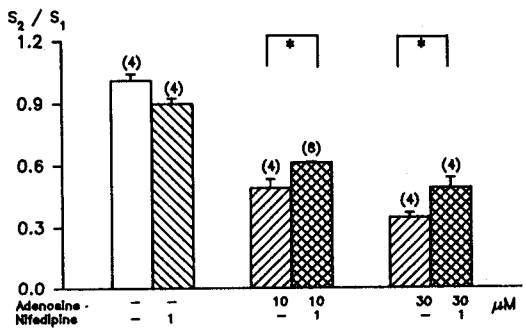

Fig. 4. Influence of nifedipine on the effect of adenosine on the electrically-evoked tritium outfolw from the rat hippocampus. Asterisks (*=P<0.05) indicate the significant difference between groups. Other legends are the same as in previous figures.

with PDB.

PMB, a protein kinase C inhibitor (Kuo *et al.*, 1983), in doses ranging from 0.03 to 1 mg, decresed the electrically evoked ^{3}H-ACh in a dose-dependent fashion, but the basal release of ^{3}H-ACh was slightly increased by 0.3 mg (1.691±0.125, n=4) and 1 mg (4.286±0.289, n=4) of PMB, compared with the control (0.657±0.028, n=6). Thus, 0.03 and 0.1 mg of PMB were employed in this study. As depicted in Fig. 3, in the presence of 0.03 and 0.1 mg PMB, the adenosine dose-dependently decreased the evoked tritium-outflow in similar fashion to those without PMB. To ascertain the interaction between PMB and adenosine, the slopes of three regression lines, adenosine ($y=-0.0015x+0.685$), adenosine under 0.03 mg PMB ($y=-0.0012x+0.532$) and adenosine under 0.1 mg PMB ($y=-0.008x+0.250$), were compared, but there were no significant differences among the slopes.

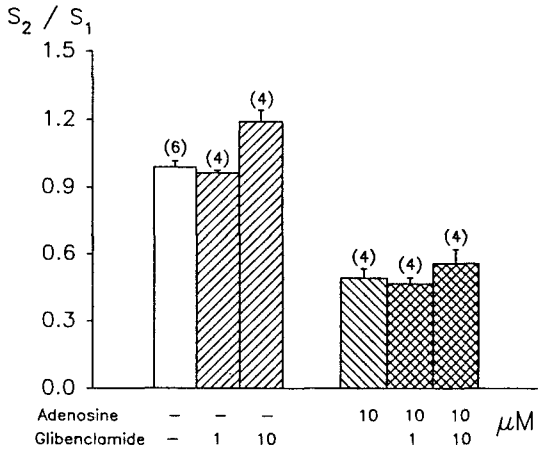

Fig. 5. Influence of glibenclamide on the effect of adenosine on the electrically evoked tritium outflow from the rat hippocampus. Legends are the same as in previous figures.

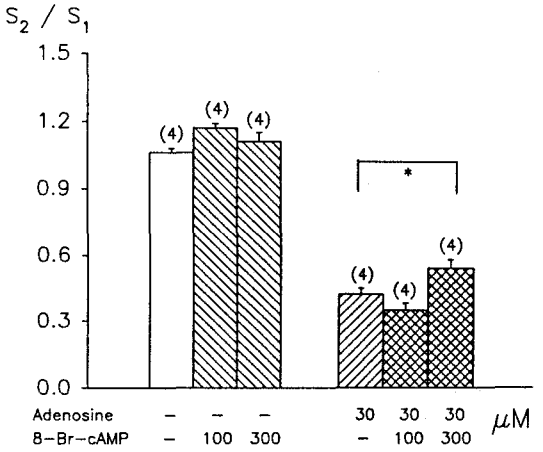

Fig. 6. Influence of 8-bromo-cAMP on the effect of adenosine. Legends are the same as in previous figures.

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

To ascertain whether the adenosine effects are mediated by Ca^{++}- and K^{+}-channel modulation, the effect of adenosine were examined in the presence of verapamil, nifedipine and glibenclamide.

Verapamil, a Ca^{++}-channel blocker, decreased the evoked ^{3}H-ACh, in a dose-related manner, but did not affect the adenosine effects (Data are not shown). However, the decrements of ^{3}H-ACh by adenosine were significantly inhibited by nifedipine (Fig. 4).

Next, the influence of glibenclamide, a K^{+}-channel blocker, on the adenosine effects was investigated. As shown in Fig. 5, adenosine effects were not affected by glibenclamide pretreatment.

Interaction of 8-bromo-cAMP and adenosine in ^{3}H-acetylcholine 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, with adenosine added at 15 min later.

As shown in Fig. 6, in the presence 300 μM 8-bromo-cAMP, the inhibitory effect of 30 μM adenosine was reduced significantly.

DISCUSSION

In the present study, th electrically evoked release of ^{3}H-ACh 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, N^{6}-cyclopentyladenosine and adenosine decreased the electrically-evoked release of ACh in the rat hippocampus (Dunér-Engström and Fredholm, 1988; Choi *et al.*, 1992, Choi and Yoon, 1993). Moreover, 8-cyclopentyl-1,3-dipropylxanthine, a selective A_1-receptor antagonist, inhibited the effect of adenosine. 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 *et 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 N-ethylmaleimide (Jacobs *et al.*,

1981; Smith and Harden, 1984). Therefore, in order to confirm whether the G_i-protein is involoved in the A_1-receptor-mediated modulation of ACh release, the influence of NEM upon the adenosine effects were investigated in this study. When the hippocampal slices were treated with NEM, the evoked ACh 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 ACh 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-ACh release, but significantly inhibited the adenosine effects. This result, in accordance with previous report that forskolin, an adenylate cyclase activator (Seamon and Daly, 1983), potentiated electrically evoked release of acetylcholine and inhibited the effects of N^6-cyclopentyladenosine in the rat hippocampus (Choi *et al.*, 1992), indicate that the modulation of ACh release by A_1-adenosine receptor is coupled to an intraneuronal adenylate cyclase system. Dunér-Engström and Fredholm (1988), 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 ACh release in rat hippocampus. Discrepancy between the present finding and theirs may not be easily reconciled, thus further studies are required to elucidate the involvement of adenylate cyclase system.

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, 1987). It seemed, therefore, reasonable to use protein kinase C modulators to examine the possible role of protein kinase C involved in the regulation of ACh release by adenosine. In the present study, PDB, a selective protein kinase C activator, markedly enhanced the release of ACh, whereas PMB, a protein kinase C inhibitor, decreased it. But adenosine effects on evoked ACh release were not affected by these agents. This result is in accordance with other report that PDB did not antagonize the presynaptic inhibitory effect of R-PIA and staurosporine, a PKC inhibitor, did not alter the effect of R-PIA on the evoked ACh release in the rat hippocampus (Fredholm, 1990), and indicates that protein kinase C might be participated in the ACh-releasing process in the rat hippocampus, but the A_1-receptor-mediated inhibition of evoked ACh release does not depend on protein kinase C.

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, by itself did not alter the basal and evoked rates of ACh release, but inhibited the adenosine effect significantly. But, glibenclamide did not affect 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. Also, in the present experiment, verapamil, a phenylalkylamine analogue of Ca^{2+}-channel blocker, did not affect the adenosine effect (data are not shown). 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 ACh release by A_1-adenosine receptor.

Overall, the present study has shown that the decrement of the evoked ACh release by A_1-adenosine receptor is mediated by the G-protein. It seems also that nifedipine-sensitive Ca^{2+}-channel and adenylate cyclase system are coupled partly to this effect, and that protein kinase C and glibenclamide-sensitive K^+-channel are no involved in this process.

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=국문초록=

흰쥐 해마에서 Acetylcholine 유리에 관여하는 Adenosine Receptor의 Post-Receptor 기전에 관한 연구

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흰쥐 해마(hippocampus)에서 acetylcholine (ACh) 유리에 미치는 A_1-adenosine 수용체의 post-receptor 기전에 관한 지견을 얻고자 하여 ^{3}H-choline으로 평형시킨 해마 slice를 사용하여 ^{3}H-ACh 유리에 미치는 여러가지 약물들의 영향을 관찰하였다.

Adenosine (0.3~300 μM)은 전기자극(3Hz, 2 ms, 5 VCm^{-1}, 구형파)에 의한 ACh 유리를 용량의존적으로 감소 시켰으며, 이러한 효과는 A_1-adenosine 수용체의 선택적 차단제인 8-cyclopentyl-1, 3-dipropylxanthine (2 μM)에 의해 차단됨을 볼 수 있었다. G-단백 억제제인 N-ethylmaleimide (NEM, 10과 30 μM)는 그 자체에 의하여 자극유발성 ACh 유리를 증가시켰으며, adenosine의 효과는 NEM 전처리에 의하여 완전히 소실되었다.

Protein kinase C 활성화제인 4β-phorbol 12, 13-dibutyrate (PDB, 1~10 μM)는 ACh 유리 증가를 일으켰으며 억제제인 polymyxin B (PMB, 0.03~1 mg)는 감소를 일으켰으나, adenosine에 의한 ACh 유리 감소효과는 PDB 및 PMB에 의해 영향을 받지 않았다.

Ca^{++}-통로 차단제인 nifedipine (1 μM)은 adenosine의 효과를 길항함을 볼 수 있었으나 K^+-통로 차단제인 glibenclamide는 adenosine의 효과에 영향을 미치지 못하였다.

8-Bromo-cAMP (100과 300 μM) 그 자체로는 ACh 유리에 별다른 영향을 미치지 못하였으나 300 μM 8-bromo-cAMP 전처리에 의하여 30 μM adenosine의 효과가 억제됨을 볼 수 있었다.

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