Korean J Physiol Pharmacol
Vol 2: 147–154, April, 1998

Effect of K^+-channel Blockers on the Muscarinic- and A_1-adenosine-Receptor Coupled Regulation of Electrically Evoked Acetylcholine Release in the Rat Hippocampus

Byung Sik Yu[1], Do Kyung Kim, and Bong Kyu Choi[2]

[2]Department of Pharmacology, Wonkwang University School of Medicine and Medicinal Resources Research Center of Wonkwang University, Iksan 570–749, Korea; [1]Department of Anesthesiology, College of Medicine, Chosun University, Kwangju 501–140, Korea

It was attempted to clarify the participation of K^+-channels in the post-receptor mechanisms of the muscarinic and A_1-adenosine receptor- mediated control of acetylcholine (ACh) release in the present study. Slices from the rat hippocampus were equilibrated with [^{3}H]choline and the release of the labelled products was evoked by electrical stimulation (3 Hz, 5 V/cm, 2 ms, rectangular pulses), and the influence of various agents on the evoked tritium-outflow was investigated. Oxotremorine (Oxo, 0.1~10 μM), a muscarinic agonist, and N^6-cyclopentyladenosine (CPA, 1~30 μM), a specific A_1-adenosine agonist, decreased the ACh release in a dose-dependent manner, without affecting the basal rate of release. 4-aminopyridine (4AP), a specific A-type K^+-channel blocker (1~100 μM), increased the evoked ACh release in a dose-related fashion, and the basal rate of release is increased by 3 and 100 μM. Tetraethylammonium (TEA), a non-specific K^+-channel blocker (0.1~10 mM), increased the evoked ACh release in a dose-dependent manner without affecting the basal release. The effects of Oxo and CPA were not affected by 3 μM 4AP co-treatment, but 10 mM TEA significantly inhibited the effects of Oxo and CPA. 4AP (10 μM)- and TEA (10 mM)-induced increments of evoked ACh release were completely abolished in Ca^{2+}-free medium, but these were recoverd in low Ca^{2+} medium. And the effects of K^+-channel blockers in low Ca^{2+} medium were inhibited by Mg^{2+} (4 mM) and abolished by 0.3 μM tetrodotoxin (TTX). These results suggest that the changes in TEA-sensitive potassium channel permeability and the consequent limitation of Ca^{2+} influx are partly involved in the presynaptic modulation of the evoked ACh-release by muscarinic and A_1-adenosine receptors of the rat hippocampus.

Key Words: Tetraethylammonium, 4-aminopyridine, Muscarinic Receptor, A_1-adenosine Receptor, Acetylcholine, Hippocampus

INTRODUCTION

It is well known that ACh release is inhibited by stimulating the presynaptic muscarinic (Szerb & Somogyi, 1973; Kilbinger & Wessler, 1980; Briggs & Cooper, 1982) and A_1-adenosine receptors (Vizi., 1984; Choi et al, 1992), but the underlying mechanisms of such presynaptic control of transmitter release are incompletely understood (Starke, 1987; Fredholm & Dunwiddie, 1988; Choi & Oh, 1994).

On the other hand, there are reports suggesting that the K^+- and Ca^{2+}-channels are involved in the synaptic transmission elicited by muscarinic (Fredholm, 1990; Clos et al, 1994) and A_1-adenosine (Proctor & Dunwiddie, 1983; Dunwiddie, 1985; Trussell & Jackson, 1985; Madison et al, 1986; Dolphine et al, 1986) receptors activation in the central nervous system (CNS). However, the involvement of ionic

Corresponding to: Bong Kyu Choi, Department of Pharmacology, Wonkwang University School of Medicine and Medicinal Resources Research Center of Wonkwang University, Iksan 570-749, Korea

conductance system in controlling ACh release by the presynaptic muscarinic and A_1-adenosine receptors still remains unclear.

Recently, K^+-channels may exist in as many as twenty or more separate types in the CNS, and their pharmacologic and therapeutic significance of which are elucidated (Rudy, 1988; Robertson & Steinberg, 1990). And, numerous studies were undertaken to clarify the influence of K^+-channel modulators upon the release of neurotransmitters in the CNS. The evoked and basal releases of ACh from the hippocampus were increased by 4AP (Drukarch et al, 1989; Fredholm, 1990) and, TEA (Drukarch et al, 1989), and the presynaptic effects of α_2-, muscarinic- and A_1-adenosine receptors stimulation are reduced by this drug (Okada & Ozawa, 1980; Schoffelmer & Mulder, 1983; Fredholm, 1990). Moreover, Zoltay & Cooper (1990) proposed that the rat cortical muscarinic autoreceptor activation lead to opening of presysnaptic K^+-channel, Fredholm (1990) observed that 4AP blocked the ability of carbachol to decrease the evoked release of ACh from hippocampal slices, and Törocsik & Vizi (1990) found that 4AP prevents the Oxo-induced decrease in evoked release of ACh from cortical slices. On the other hand, however, there are conflicting reports that the hippocampal presynaptic A_1- and muscarinic receptors do not primarily regulate 4AP-dependent K^+-channels (Fredholm, 1990; Hu & Fredholm, 1991; Dolezal & Wecker, 1991).

The present study, therefore, was designed to delineate the role of K^+-channel on the evoked ACh release in the rat hippocampus, and to define the involvement of K^+-channels in post-receptor mechanisms of muscarinic and A_1-adenosine receptors.

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 (Balzers Union Aktiengesellschaft, England) and were incubated in 2 ml of modified Krebs-Henseleit medium containing 0.1 μ mol/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) for 140 min at a rate of 0.5 ml/min. The composition (mM) of superfusion medium was 118 NaCl, 4.8 KCl, 1.3 $CaCl_2$, 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 (2.5 ml) of the superfusate began 50 min after the superfusion. Electrical stimulations (3 Hz, 5 V/cm, 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 was determined by liquid scintillation counter (Beckman LS 5000TD). The fractional rate of tritium-outflow 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-outflow 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: [^{3}H]choline (72-78 Ci/mmol, Amersham), oxotremorine sesquifumarate (RBI), N^6-cyclopentyladenosine (RBI) tetraethylammonium bromide (Sigma), 4-aminopyridine (Nakarai), atropine sulfate (Sigma), hemicholinium-3 (Sigma) and tetrodotoxin (RBI). Drugs were dissolved in the medium except tetrodotoxin which was initially dissolved in DMSO and then diluted with 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 and Cochran, 1980).

RESULTS

Effect of Oxo and CPA on [^{3}H]ACh release evoked by electrical stimulation

Hippocampal slices prelabelled with [^{3}H]choline, a [^{3}H]ACh precursor, were superfused with the medium containing 10 μM hemicholinium-3, a choline uptake inhibitor. And in order to eliminate the inhibition of ACh release by activating muscarinic auto receptors,

Table 1. Effect of oxotremorine (Oxo) on the electrically-evoked and basal tritrium-outflows from the rat hippocampal slices preincubated with [^{3}H]choline

Oxo before S_2 (μM)	n	S_2/S_1	b_2/b_1
none	18	0.888±0.062	0.632±0.023
0.1	7	0.736±0.042	0.623±0.093
0.3	10	0.502±0.041***	0.680±0.019
1.0	7	0.230±0.038***	0.625±0.034
3.0	4	0.135±0.046***	0.662±0.058
10.0	4	0.097±0.038***	0.735±0.018

After preincubation, the slices were superfused with medium containing 10 μM hemicholinium-3 and then stimulated twice (S_1, S_2). Oxo was presented 15 min before S_2. Drug effects on basal outflow are expressed as b_2/b_1 between fractional rates of outflows immediately before S_2 (115~120 min) and before S_1 (55~60 min). Mean±SEM from the number (n) of observation are given. Significant differences from the drug-free control (none) are marked with asterisks(***$p<0.001$).

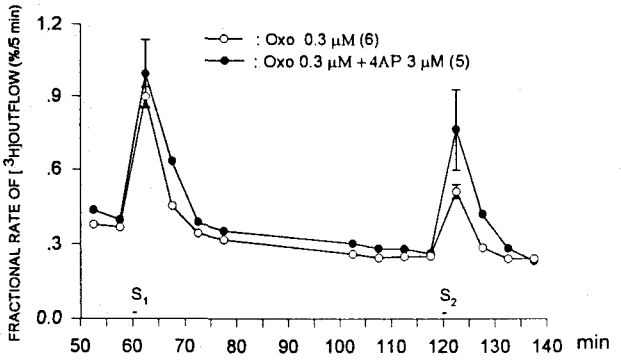

Fig. 1. A typical presentation of the tritium-outflow from the rat hippocampal slices preincubated with [^{3}H]choline. The slices were electrically stimulated twice for 2 min each, after 60 and 120 min of superfusion (S_1, S_2). The drug effect on the stimulation-evoked tritium outflow is expressed by the ratio S_2/S_1 (○; 0.479±0.060 and ●; 0.890±0.128). The radioactivities of the tissues at the start of experiment were 0.656±0.037 (○) and 0.657±0.023 (●) pmol. Oxotremorine (Oxo) and 4-aminopyridine (4AP) were added 15 min before S_2. In the parentheses are the number of experiments.

atropine (30 nM), a muscarinic antagonist, was added in the superfusion medium in the CPA experiments. During superfusion, the tissue was electrically stimulated twice.

As shown in Fig. 1, 0.3 μM Oxo decreased the electrically-evoked outflow of tritium without any change of the basal release. Oxo (0.1~10 μM) and CPA (1~30 μM) decreased the electrically-evoked ACh release in a concentration-dependent manner (Table 1 and 2).

Effect of K^+-channel blockers on ACh release

As shown in Table 3, 4AP, a specific A-type K^+-channel blocker, in doses ranging from 1 to 100 μM, increased the evoked ACh release in a dose-related fashion. Moreover, basal outflow of ACh is significantly increased by 3 and 100 μM 4AP. TEA, a nonspecific K^+-channel blocker, in doses ranging from 0.03 to 10 mM, increased the evoked ACh release in a dose-dependent manner, but did not change the basal release (Table 4).

Interaction of K^+-channel blockers and Oxo & CPA on ACh release

To ascertain whether the Oxo and CPA effects are mediated by K^+-channel modulation, the effects of K^+-channel blockers upon the Oxo and CPA were then studied.

As shown in Figs. 1 and 2A, when the slices were treated with combination of Oxo and 4AP, 4AP did not affect the inhibitory effects of Oxo effect, except for a slight inhibition of the effect of 1 μM Oxo. And also, the inhibitory effects of CPA were not affected by 4AP co-treatment (Fig. 3A).

Next, the influence of TEA on the Oxo and CPA effects were investigated. As depicted in Figs. 2B and 3B, the Oxo and CPA effects are significantly inhibited by 3 mM TEA.

Table 2. Effect of N^6-cyclopentyladenosine (CPA) on the electrically-evoked and basal tritium-outflows from the rat hippocampal slices preincubated with [^{3}H]choline

CPA before S_2 (μM)	n	S_2/S_1	b_2/b_1
none	7	0.975±0.026	0.682±0.025
1	5	0.682±0.096*	0.670±0.026
3	5	0.607±0.039***	0.690±0.013
10	5	0.434±0.042***	0.680±0.025
30	5	0.326±0.058***	0.645±0.019

Significant differences from the drug-free control are marked with asterisks (*$p<0.05$). Other legends are the same as in Table 1.

Table 3. Effect of 4-aminopyridine (4AP) on the electrically-evoked and basal outflows of tritium from the rat hippocampal slices preincubated with [^{3}H]choline

4AP before S_2 (μM)	n	S_2/S_1	b_2/b_1
none	4	0.953±0.064	0.673±0.020
1	5	1.114±0.035	0.749±0.053
3	5	1.349±0.067**	0.763±0.015**
10	5	1.612±0.071***	0.712±0.034
30	5	2.269±0.205***	0.758±0.036
100	5	3.316±0.433***	0.960±0.019***

Significant differences from the drug-free control are marked with asterisks (**$p<0.01$). Other legends are the same as in Table 1.

Table 4. Effect of tetraethylammonium (TEA) on the electrically-evoked and basal outflows of tritium from the rat hippocampal slices preincubated with [^{3}H]choline

TEA before S_2 (mM)	n	S_2/S_1	b_2/b_1
none	4	0.811±0.065	0.696±0.059
0.03	5	1.007±0.029*	0.714±0.026
0.10	5	0.998±0.031*	0.697±0.022
1	5	1.317±0.056**	0.642±0.027*
3	5	1.599±0.049	0.712±0.035
10	5	2.052±0.478	0.716±0.021

Legends are the same as in Table 2.

To clarify the mechanism of the K^+-channel blockers on the evoked ACh release, the effects of K^+-channel blockers were investigated in another sets of experiments in which either the concentration of Ca^{2+} was lowered 1.3 to 0.325 or 0 mM, and Mg^{2+} increased from 1.2 to 4 mM. In the preliminary experiments, the electrically-evoked ACh release was significantly decreased by lowered external Ca^{2+} from 1.3 to 0.325 mM (S_2/S_1; 0.120±0.008, n=4) compared with the control (S_2/S_1; 0.996±0.078, n=8). Adding magnesium (4 mM) to the lowered Ca^{2+} medium 45 min before S_2 onwards, inhibited further

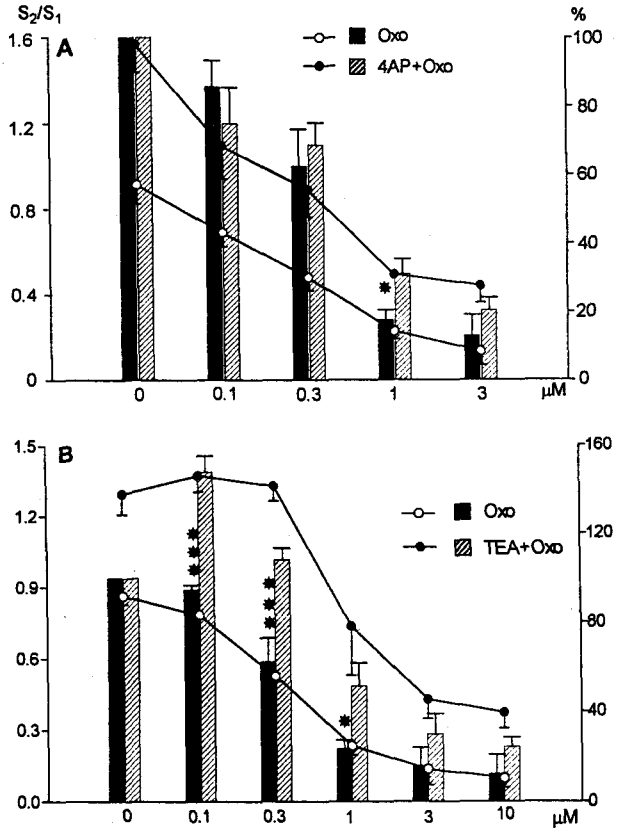

Fig. 2. Influence of the 4-aminopyridine (4AP) 3 μM (A) and tetraethylammonium (TEA) 3 mM (B) upon the effect of oxotremorine (Oxo) on the electrically evoked ACh release from the rat hippocampal slices. Line plots indicate S_2/S_1 ratios. Each point represents mean value from 5-7 experiments, with one SEM as bar. Histograms show percentages of the corresponding controls. Statistically significant difference is indicated with asterisks (*p <0.05 and ***p <0.001).

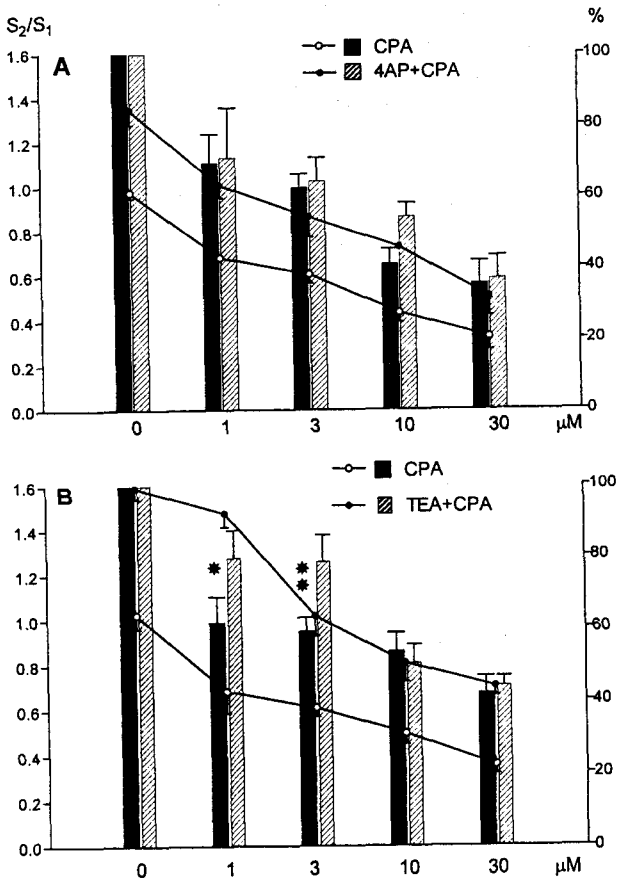

Fig. 3. Influence of the 4-aminopyridine (4AP) 3 μM (A) and tetraethylammonium (TEA) 3 mM (B) upon the effect of N^6-cyclopentyladenosine (CPA) on the electrically evoked ACh release from the rat hippocampal slices. Statistically significant difference is indicated with **p $<$ 0.01. Other legends are the same as in preceding figures.

the evoked ACh (S_2/S_1; 0.024 ± 0.009). As shown in Fig. 4, 4AP-induced increments of the evoked ACh release was completely abolished in Ca^{2+}-free medium, and this inhibition was lessened in low Ca^{2+} medium with increased basal rate of release. And, increasing effects of evoked and basal rate of release by 4AP in low Ca^{2+} medium were significantly inhibited by Mg^{2+} or completely abolished by TTX added to the medium.

Next, the effects of TEA on the evoked ACh release were investigated in the Ca^{2+} and Mg^{2+} modified media. The TEA-induced increase in evoked ACh release was completely abolished in Ca^{2+}-free medium, but significantly recoverd in low Ca^{2+} medium. And, this inhibitory effect was also attenuated by adding Mg^{2+} or abolished by TTX in medium, likewise in 4AP experiments (Fig. 5).

DISCUSSION

A major aim of the present study was to investigate the effects of K^+-channel blockers to gain more information about the mechanism underlying the presynaptic inhibitory effect of muscarinic and A_1-adenosine receptors. In the present study, the electrically evoked release of ACh from the rat hippocampal slice was inhibited by Oxo and CPA. These results are in accordance with other reports that muscarinic agonists such as carbachol & Oxo (Allgaier et al, 1988, 1993a; Choi et al, 1991), and adenosine agosists such as R-N^6-(2-phenylisopropyl)adenosine, N^6-cyclopen-

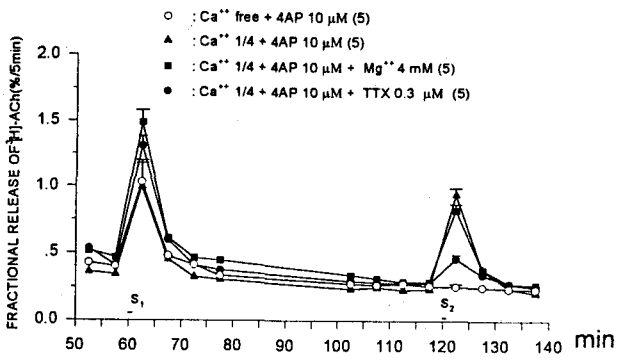

Fig. 4. The innfluence of Mg^{2+} and tetrodotoxin (TTX) upon the 4AP-induced tritium outflow in lowered or zero external Ca^{2+} concentration from rat hippocampal slices prelabelled with [^{3}H]choline. The concentration of external Ca^{2+} was lowered from 1.3 to 0.325 (1/4) or 0 (Ca^{2+}-free) mmol/l from 15 min and Mg^{2+} was increased 1.2 to 4 mM from 45 min before S_2 onwards, respectively. In some experiments either 4AP or TTX was present from 15 min before S_2 onwards. The S_2/S_1 ratios were: 0.015 ± 0.026 in Ca^{2+}- free; 1.098 ± 0.024 in low Ca^{2+}; 0.552 ± 0.022 in low Ca^{2+}/high Mg^{2+}; 0.251 ± 0.034 in low Ca^{2+}/TTX. In the parentheses are the number of experiments.

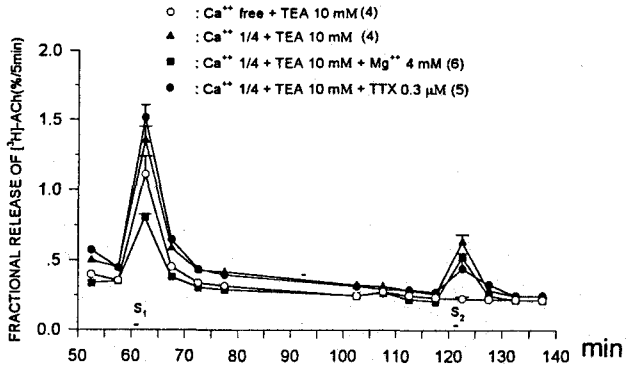

Fig. 5. The influence of Mg^{2+} and TTX upon the TEA-induced tritium outflow in lowered or zero external Ca^{2+} concentration from rat hippocampal slices prelabelled with [^{3}H]choline. The concentration of external Ca^{2+} was lowered from 1.3 to 0.325 (1/4) or 0 (Ca^{2+}-free) mM from 15 min and Mg^{2+} was increased 1.2 to 4 mM from 45 min before S_2 onwards, respectively. In some experiments either TEA or TTX was present from 15 min before S_2 onwards. The S_2/S_1 ratios were: -0.005 ± 0.014 in Ca^{2+} free; 0.682 ± 0.012 in low Ca^{2+}; 0.390 ± 0.043 in low Ca^{2+}/high Mg^{2+}; 0.216 ± 0.019 in low Ca^{2+}/TTX. In the parentheses are the number of experiments.

tyladenosine & adenosine (Duńer-Engström & Fredholm, 1988; Choi et al, 1992; Choi & Yoon, 1993; Choi & Oh, 1994) decreased the electrically- evoked release of ACh in the hippocampus.

On the other hand, there are reports that various types of voltage-dependent K^+-channels exist in the CNS (Rudy, 1988; Storm, 1990) and are evidence has been presented indicating that K^+-channels are involved in the releasing process of neurotransmitters in striatum (Drukarch et al, 1989) and hippocampus (Jackisch et al, 1992; Allgaier et al, 1993b). Therefore, in order to confirm the effects of voltage-dependent K^+-channel blockers on the evoked ACh release were investigated in this study.

4AP and TEA substantially increased ACh release evoked by electrical stimulation. The increasing effect was abolished by removing Ca^{2+} in the medium, but was significantly recovered in low Ca^{2+} medium. And the inhibitory effect was attenuated by Mg^{2+} and abolished by TTX, indicating that it reflects nerve impulse-triggered and calcium-dependent release. These facts, in conjunction with the evidence that K^+-channel blockers inhibit the outward K^+-currents, indicate that the increment of the transmitter release is probably due to increase of Ca^{2+} influx into the axon terminal followed by a prolongation of the duration of action potential. There are, however, several points not to be overlooked in the action of 4AP. In contrast to the effects of TEA, large doses of 4AP induce by itself significant increase of the spontaneous ACh release in normal and even in low Ca^{2+}medium. And also, 4AP significantly increased the evoked ACh release (S_2/S_1 : 0.552 ± 0.022, n=5, $p < 0.001$) compared with the drug-free control in low Ca^{2+}/high Mg^{2+} medium (S_2/S_1 : 0.016 ± 0.026, n=5). In view of the findings that the action site of 4AP is intracellular (Rudy, 1988) and that magnesium ion block the ionic channel in rat hippocampal slices (Mody et al, 1987), the possibility that the increments of spontaneous and evoked ACh release by 4AP may result from intracellular Ca^{2+} release from the storage sites could not be ruled out. But, 4AP dependent increments of evoked and spontaneous release were completely abolished by TTX-treatment and by removal of Ca^{2+} in the medium, indicating that these effects are TTX-sensitive and extracellular Ca^{2+}-dependent. On the basis of these findings, in conjunction with other reports that TEA blocks a wide variety of K^+-channels, whereas 4AP is supposed to possess some selectivity for the A current and delayed rectifier (for review see: Cook, 1988; Rudy, 1988; Castle et al, 1989), it is most likely that the

TEA- and 4AP-induced ACh release is mediated through different K^+-channels.

It is generally known that the presynaptic potassium conductance is involved in the feedback regulation of ACh release of the hippocampal ACh release (Zoltay & Cooper, 1990). Therefore, in order to confirm the involvement of voltage dependent K^+-channels in muscarinic- and A_1-adenosine-receptor-mediated decrease of ACh release, the influence of K^+-channel blockers upon the Oxo and CPA effects were investigated in this study.

In the interaction experiments, the concentration-response relations for Oxo and CPA observed in the presence of 4AP and TEA. The fact that TEA significantly attenuated the inhibitory effects of Oxo and CPA, but 4AP did not affect them. These results were quite in agrement with the reports that the K^+-channels are coupled to evoked-ACh release in the rat hippocampus (Fredholm, 1990) and cerebral cortex (Törocsik & Vizi, 1990), suggesting that Oxo and CPA binding to their specipic receptors induces the opening of TEA-sensitive K^+-channels and limits the Ca^{2+} influx. However Fredholm (1990) found that the presynaptic effect of carbachol on muscarinic autoreceptors was abolished by 4AP and proposed that the inhibitory effects of carbachol on hippocampal ACh release are coupled to 4AP sensitive K^+-channels. Furthermore, Törocsik & Vizi (1990) reported that 4AP interrupts the modulation of ACh release mediated by muscarinic and opiate receptors. In addition, there are reports that depressant effects of adenosine is blocked by 4AP in olfactory cortex (Scholfield and Steal, 1988) and hippocampus (Okada & Ozawa, 1980; Schubert et al, 1986). Discrepancies between the present results and the above reports may not be easily reconciled, but one of the possibilities accounting for the difference is that they did not observe the dose-response relationships of agonists and of agonists under K^+-channel blockers. And also, they clearly indicated that there are differences in the mechanism underlying different receptor effects and in species. Hence, further studies are required to determine the exact mechanism in the ACh release mediated by presynaptic muscarinic and A_1-adenosine receptors in the rat hippocampus.

Taken together, the present study showed that the decrements of the evoked ACh release by muscarinic and A_1-adenosine receptors is related to the TEA-sensitive K^+-channels, but not to the 4AP-sensitive K^+-channels, and that the changes of Ca^{2+} influx are consequential to this.

ACKNOWLEDGEMENT

This study is supported by Korean Ministry of Education through Research Fund in 1996 (96-200).

REFERENCES

Allgaier C, Choi BK, Hertting G: Muscarine receptors regulating electrically evoked release of acetylcholine in hippocampus are linked to pertussis toxin- sensitive G proteins but not to adenylate cyclase. *J Neurochem* 61: 1043−1049, 1993a

Allgaier C, Daschmann B, Huang HY, Hertting G: Protein kinase C and presynaptic modulation of acetylcholine release in rabbit hippocampus. *Br J Pharmacol* 93: 525−534, 1988

Allgaier C, Repp H, Hertting G: Effect of K^+ channel blockers on the α_2- adrenoceptor-coupled regulation of electrically evoked noradrenaline release in hippocampus. *Naunyn-Schmiedeberg's Arch Pharmacol* 347: 14−20, 1993b

Briggs CA, Cooper JR: Cholinergic modulation of the release of ^{3}H-acetylcholine from synaptosomes of the myenteric plexus. *J Neurochem* 38: 501−508, 1982

Castle NA, Haylett DG, Jenkinson DH: Toxins in the characterization of potassium channel. *Trends Neurosci* 12: 59−65, 1989

Choi BK, Oh JH: A study on the post-receptor mechanism of adenosine receptor on acetylcholine release in the rat hippocampus. *Kor J Pharmacol* 30: 263−272, 1994

Choi BK, Yoon YB: Influence of adenosine and magnesium on acetylcholine release in the rat hippocampus. *Kor J Pharmacol* 29: 175−182, 1993

Choi BK, Kim CS, Yoon YB, Kook YJ: Interaction of forskolin with the effects of oxotremorine on [^{3}H]-acetylcholine release in rabbit hippocampus. *Kor J Pharmacol* 27: 89−97, 1991

Choi BK, Park HM, Kang YW, Kook YJ: Interaction of forskolin with the effect of N^6-cyclopentyladenosine on [^{3}H]-acetylcholine release in rat hippocampus. *Kor J Pharmacol* 28: 129−136, 1992

Clos MV, Sanz AG, Sabria J, Pastor C, Bardia A: Diifferential contribution of L- and N-type calcium channels on rat hippocampal acetylcholine release. *Neuroscience* 182: 125−128, 1994

Cook NS: The pharmacology of potassium channels and their therapeutic potential. *Trends Pharmacol Sci* 9: 21−28, 1988

Dolezal V, Wecker L: Modulation of acetylcholine re-

lease from rat striatal slices: interaction between 4-aminopyridine and atropine. *J Pharmacol Exp Ther* 258: 762−766, 1991

Dolphin AC, Forda SR, Scott RH: Calcium-dependent currents in cultured dorsal root neurons are inhibited by an adenosine analogue. *J Physiol* 373: 47−62, 1986

Drukarch B, Kits KS, Leysen JE, Schepens E, Stoof JC: Restricted usefulness of tetraethylammonium and 4-aminopyridine for the characterization of receptor-operated K^+ channels. *Br J Pharmacol* 98: 113−118, 1989

Dunwiddie TV: The physiological role of adenosine in the central nervous system. *Int Rev Neurobiol* 27: 63−139, 1985

Dunér-Engström M, Fredholm BB: Evidence that prejunctional adenosine receptors regulating acetylcholine release from rat hippocampal slices are linked to a N-ethylmaleimide-sensitive G-protein, but not to adenylate cyclase or dihydropyridine-sensitive Ca^{2+}-channels. *Acta Physiol Scand* 134: 119−126, 1988

Fredholm BB, Dunwiddie TV: How does adenosine inhibit transmitter release? *Trends Pharmacol Sci* 9: 130−134, 1988

Fredholm BB: Differential sensitivity to blockade by 4-aminopyridine of presynaptic receptors regulating [^{3}H]acetylcholine release from rat hippocampus. *J Neurochem* 54: 1386−1390, 1990

Hertting G, Zumstein A, Jackisch R, Hoffmann I, Starke K: Modulation of endogenous dopamine of the release of acetylcholine in the caudate nucleus of the rabbit. *Naunyn-Schmiedeberg's Arch Pharmacol* 315: 111−117, 1980

Hu PS, Fredholm BB: 4-Aminopyridine-induced increase in basal and stimulation-evoked [^{3}H]-NA release in slices from rat hippocampus: Ca^{2+} sensitivity and presynaptic control. *Br J Pharmacol* 102: 764−768, 1991

Jackisch R, Huang HY, Rensing H, Lauth D, Allgaier C, Hertting C: α_2-Adrenoceptor mediated inhibition of exocytotic noradrenaline release in the absence of extracellular Ca^{2+}. *Eur J Pharmacol* 226: 245−252, 1992

Kilbinger H, Wessler I: Inhibition by acetylcholine of the stimulation-evoked release of ^{3}H-acetylcholine from the guinea pig myenteric plexus. *Neuroscience* 5: 1331−1340, 1980

Madison DV, Fox AP, Tsien RW: Adenosine reduces an inactivating component of calcium current in hippocampal Ca3-neurons in cell culture. *J physiol (Lond)* 370: 75−90, 1986

Mody I, Lambert JDC, Heinemann V: Low extracellular magnesium induces epileptiform activity and spreading depression in rat hippocampal slices. *J Neurophysiol* 57: 869−888, 1987

Okada Y, Ozawa S: Inhibitory action of adenosine on synaptic transmission in the hippocampus of the guinea-pig in vitro. *Eur J Pharmacol* 68: 483−492, 1980

Proctor WR, Dunwiddie: Adenosine inhibits calcium spikes in hippocampal pyramidal neurons. *Neurosci Lett* 35: 197−201, 1983

Robertson DW, Steinberg MI: Potassium channel Modulators: scientific applications and therapeutic promise. *J Med Chem* 33: 1529−1541, 1990

Rudy B: Diversity and ubiquity of K channels. *Neuroscience* 25: 729−749, 1988

Schoffelmer ANM, Mulder AH: ^{3}H-Noradrenaline release rat neocortical slices in the absence of extracellular Ca^{2+} and its presynaptic alpha-adrenergic modulation. A study on the possible role of cyclic AMP. *Naunyn-Schmiedeberg's Arch Pharmacol* 323: 188−192, 1983

Scholfifeld CN, Steel L: Presynaptic potassium channel blockade counteracts the depressant effect of adenosine in olfactory cortex. *Neuroscience* 24: 81−91, 1988

Schubert P, Heinemann U, Kolb R: Differential effect of adenosine on pre- and postsynaptic calcium fluxes. *Brain Res* 376: 382−386, 1986

Snedecor GW, Cochran WG: Statistical Methods, Iowa State Univ 7th ed, 1980

Starke K: Presynaptic α-autoreceptors. *Rev Physiol Biochem Pharmacol* 107: 73−146, 1987

Storm JF: Potassium currents in hippocampal pyramidal cells. *Prog Brain Res* 83: 161−187, 1990

Szerb JC, Somogyi GT: Depression of acetylcholine release from cerebral corticalslices by cholinesterase inhibition and by oxotremorine. *Nature* 241: 121−122, 1973

Trussell LO, Jackson MB: Adenosine-activated potassium conductance in cultured striatal neurons. *Proc Nat Acad Sci USA* 82: 4857−4861, 1985

Törocsik A, Vizi ES: 4-Aminopyridine interrupts the modulation of acetylcholine release mediated by muscarinic and opiate receptor. *J Neurosci Res* 27: 228−232, 1990

Vizi S: Non-synaptic interactions between neurons: Modulation of neurochemical transmission-Pharmacological and Clinical Aspects. *John Wiley and Sons, New York,* 1984

Zoltay G, Cooper JR: Ionic basis of inhibitory presynaptic modulation in rat cortical synaptosomes. *J Neurochem* 55: 1008−1012, 1990