Korean J Physiol Pharmacol
Vol 8: 301–305, December, 2004

Effects of NMDA, AMPA and Kainate on the Release of Acetylcholine in Rat Hippocampal and Striatal Slices

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This study examined the effects of N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate on basal and electrically-evoked release of acetylcholine (ACh) from the rat hippocampal and striatal slices which were preincubated with [^{3}H]choline. Unexpectedly, the basal and evoked ACh release were not affected at all by the treatment with NMDA (3~100 μM), AMPA (1~100 μM) or kainate (1~100 μM) in hippocampal slices. However, in striatal slices, under the Mg^{2+}-free medium, 30 μM NMDA increased the basal ACh release with significant decrease of the electrically-evoked releases. The treatment with 1 μM MK-801 not only reversed the 30 μM NMDA-induced decrease of the evoked ACh release, but also attenuated the facilitatory effect of 30 μM NMDA on the basal ACh release. The treatment with either 30 μM AMPA or 100 μM kainate increased the basal ACh release without any effects on the evoked release. The treatment with 10 μM NBQX abolished the AMPA- or kainate-induced increase of the basal ACh release. Interestingly, NBQX significantly attenuated the evoked release when it was treated with AMPA, although it did not affect the evoked release alone without AMPA. These observations demonstrate that in hippocampal slices, ionotropic glutamate receptors do not modulate the ACh release in cholinergic terminals, whereas in striatal slices, activations of ionotropic glutamate receptors increase the basal ACh release though NMDA may decrease the electrically-evoked ACh release.

Key Words: NMDA, AMPA, Kainate, Acetylcholine release, Hippocampus, Striatum

INTRODUCTION

Glutamate, the major excitatory neurotransmitter in the mammalian central nervous system, plays an important role in a variety of normal and abnormal neuronal processes (Cotman et al, 1981). Glutamate activates two different classes of receptors, ie, metabotropic and ionotropic glutamate receptors (Gasic & Hollmann, 1992). The latter are subdivided into N-methyl-D-aspartic acid (NMDA), α- amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate receptors based on the relative selectivity of agonists and antagonists (Zorumski & Thio, 1992; Ruzicka & Jhamandas, 1993).

Rat hippocampal slices have been used for investigating the influences of presynaptic modulation on the basal and evoked releases of Acetylcholine (ACh) or norepinephrine (NE) (Milusheva et al, 1994; Monnet et al, 1995; Hadjiivanova et al, 1997; Leslie et al, 2002) because hippicampus receives massive cholinergic and noradrenergic innervations and it has no intrinsic cholinergic or noradrenergic neuronal somata (Lewis et al, 1967; Hortnagl et al, 1991). It has been well known that the activation of ionotropic glutamate receptors presynaptically regulates the release of NE in hippocampal slice and synaptosomes (Pittaluga & Raiteri, 1992a, b; Raiteri et al, 1992; Monnet et al, 1995). On the contrary, there were few reports which demonstrated the effects of the activation of ionotropic glutamate receptors on the release of ACh in hippocampal slices. It has also been demonstrated that glutamate exerts a facilitatory control of the release of ACh via pre- and postsynaptic ionotropic glutamate receptors in rat striatal slices which have cholinergic neuronal somata differently from hippocampal slices (Lehman & Scatton, 1982; Jin & Fredholm, 1994; Nankai et al, 1995; Morari et al, 1998).

The present study, therefore, was undertaken to investigate the role of glutamatergic modulation of hippocampal and striatal cholinergic transmission in vitro by comparing the effects of NMDA, AMPA and kainate on the basal and electrically-evoked ACh releases in rat hippocampal and striatal slices.

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ABBREVIATIONS: NMDA, N-methyl-D-aspartic acid; AMPA, a-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; Ach, acetylcholine; NE, norepinephrine; NBQX, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide.

METHODS

Slice preparation

Slices of 2.5~3.0 mg (wet weight, 400 μm in thickness) were prepared from the hippocampus or striatum of male Sprague-Dawley rats weighing 250~300 gm with a Balzers® tissue chopper (Balzer Union, England). The slices were labelled by incubation for 30 min at 37°C with 0.1 μmol/L [^{3}H]choline. Subsequently, the [^{3}H]choline-pretreated slices were superfused with modified Krebs-Henseleit medium containing 10 μM hemicholinium-3 and 30 nM atropine at 37°C for 140 min at a rate of 0.5 ml/min. The composition (mM) of superfusion medium (with or without $MgSO_4$ 1.2 mM) was 118 NaCl, 4.8 KCl, 1.3 $CaCl_2$, 1.2 KH_2PO_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 and 5% CO_2, and the pH adjusted to 7.4.

Release experiment

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 min were performed at 60 min (S_1) and 120 min (S_2). Drugs were added between S_1 and S_2 to the superfusion medium. At the end of superfusion, the slices were solubilized in 0.5 ml tissue solubilizer (0.5 N quaternary ammonium hydroxide in toluene). The radioactivities in the superfusates and solubilized tissues were determined by liquid scintillation counter (Beckman® LS6500, U.S.A.). 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 or basal tritium-outflow were evaluated by calculating the ratio of the outflow evoked by S_2 and by S_1 (S_2/S_1) or the ratio b_2/b_1 between fractional rates of outflow immediately before S_2 (115~120 min) and S_1 (55~60 min), respectively (Fig. 1).

The following chemicals were used: [methyl-^{3}H]choline chloride (specific activity 60~85 Ci mmol^{-1}) was obtained from the Radiochemical Centre (Amersham, U.K.). Hemicholinium-3, atropine sulfate, kainate, NMDA and MK-801 HCl were obtained from Sigma-Aldrich Co. (St. Louis, Mo, U.S.A.). AMPA and 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo [f]quinoxaline-7-sulfonamide (NBQX) were obtained from Tocris Cookson Ltd. (Avonmouth, U.K.). Drugs were dissolved in the medium except AMPA and NBQX which were initially dissolved in dimethyl sulfoxide and then diluted with the medium. All the other chemicals were reagent grade and obtained from commercial sources.

All results are given as mean±SEM. Statistical significance among experimental groups was determined by ANOVA and subsequently by Tukey-Kramer multiple comparison test.

RESULTS

In this study, the hippocampal and striatal slices prelabelled with [^{3}H]choline, a [^{3}H]ACh precursor, were superfused with the medium containing 10 μM hemicholinium, a choline uptake inhibitor. And in order to eliminate the inhibition of ACh release by activating muscarinic autoreceptors, atropine (30 nM), a muscarinic receptor antag-

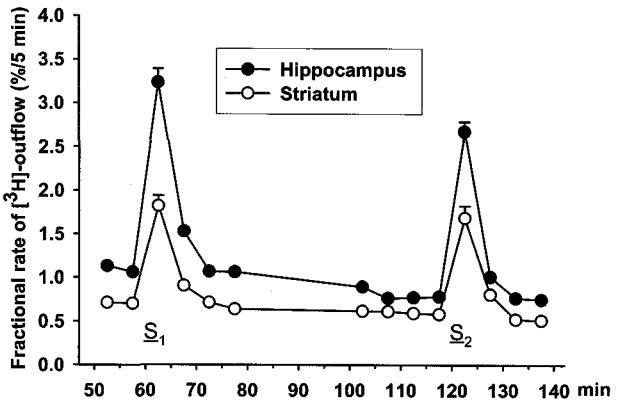

Fig. 1. The electrically-evoked and basal outflows of tritium from the rat hippocampal (●) and striatal (○) slices preincubated with [^{3}H]choline. The fractional rate of tritium-outflow was calculated as tritium-outflow per 5 min divided by the total tritium content in the slice at the start of the respective 5-min period. The tritium contents of hippocampal and striatal slices at the start of experiment were 0.3642±0.0275 and 1.595±0.184 pmol, respectively. The slices were electrically stimulated twice for 2 min each, after 60 and 120 min of superfusion (S_1, S_2). When the drug effects were examined, the drugs were presented 15 min before S_2 onwards. Drug effects on the evoked or basal tritium-outflow were evaluated by calculating the ratio of the outflow evoked by S_2 and by S_1 (S_2/S_1) or the ratio b_2/b_1 between fractional rates of outflow immediately before S_2 (115~120 min) and S_1 (55~60 min), respectively. Each point represents the mean±SEM of the experiments (n=6~9).

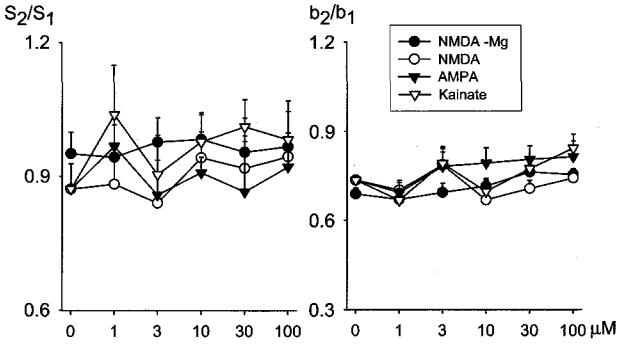

Fig. 2. Effects of NMDA in Mg^{+2} containing medium (3-100 μM, ○, NMDA), NMDA in Mg^{+2} free medium (3-100 μM, ●, NMDA-Mg^{+2}), AMPA (1-100 μM, ▼) and kainate (1-100 μM, ▽) on the electrically-evoked (left panel) and the basal outflows of tritium (right panel) from the rat hippocampal slices preincubated with [^{3}H]choline. After preincubation, the slices were superfused with medium containing 10 μM hemicholinium-3 and 30 nM atropine, and then electrically stimulated twice for 2 min each, after 60 and 120 min of superfusion (S_1, S_2). All drugs were presented from 15 min before S_2 onwards at the concentrations indicated. The drug effect of the stimulation-evoked tritium-outflow is expressed by the ratio S_2/S_1. 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). Number of each experimental group were 4~10. Data are expressed as mean± SEM.

Table 1. Effect of NMDA, AMPA and kainate on the electrically-evoked and basal outflows of tritium from the rat striatal slices preincubated with [^{3}H]-choline

Drugs before S_2 (μM)	n	S_2/S_1	b_2/b_1
NMDA (30)	9	0.892±0.022	0.889±0.031
AMPA (30)	6	0.951±0.043	0.992±0.043†
Kainate (100)	6	0.909±0.012	0.974±0.027†
Control (Mg^{2+})	10	0.946±0.033	0.827±0.030
Control (Mg^{2+} −)	9	0.850±0.032	0.794±0.031
NMDA(30, Mg^{2+} −)	9	0.717±0.029*	1.342±0.036*

After preincubation, the slices were superfused with medium containing 10 μM hemicholinium-3 and 30 nM atropine, 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 (115~120 min) and before S_1 (55~60 min). Mean±SEM from number (n) of observations are given. Asterisk and sharp indicate significant differences from the control in Mg^{2+} free (Mg^{2+} −) and Mg^{2+} containing (Mg^{2+}) medium, repectively (*:$p<0.05$, †:$p<0.05$).

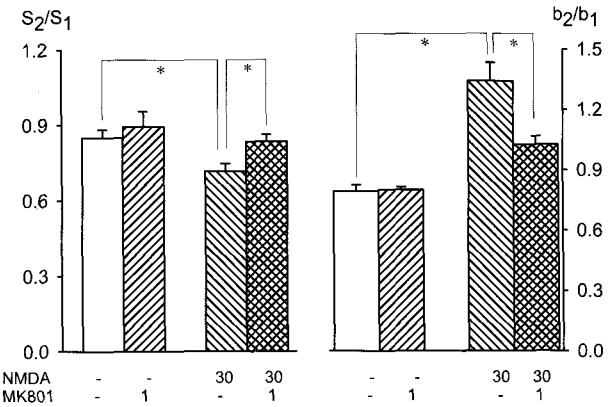

Fig. 3. Influence of 1 μM MK-801 on the effect of 30 μM NMDA on the electrically-evoked (left panel) and the basal outflows of tritium (right panel) in the Mg^{2+} free medium from the rat striatal slices preincubated with [^{3}H]choline. Number of each experimental group were 6~9. Asterisk indicates the significant difference (*:$p<0.05$) between groups. Other legends are the same as in Fig. 2.

onist, was added in the superfusion medium in all experiments. During superfusion, the tissue was electrically stimulated twice. As shown in Fig. 1, electrical stimulation induced evoked ACh release from both preparations.

Effect of NMDA, AMPA and kainate on the release of [^{3}H]ACh from rat hippocmapus

Unexpectedly, the treatments with NMDA (3~100 μM) in Mg^{2+}-containing or Mg^{2+}-free medium, AMPA (1~100 μM) and kainate (1~100 μM) had no effect on the electrically-evoked and basal ACh releases (Fig. 2).

Effect of NMDA, AMPA and kainate on the release of [^{3}H]ACh from rat striatum

Because the ionotropic glutamate ligands failed to modulate the ACh release in hippocampal slices, we examined the effects of NMDA, AMPA and kainate on the electrically-evoked and basal ACh releases in striatal slices prelabelled with [^{3}H]choline.

Treatment with 30 μM NMDA did not affect the ACh release from striatal slices in Mg^{2+}-containing medium. However, under the Mg^{2+}-free medium, 30 μM NMDA increased the basal ACh release with significant decrease of the electrically-evoked release. Moreover, the treatment with either 30 μM AMPA or 100 μM kainate increased the basal ACh release without any effects on the evoked release (Table 1).

To determine whether the effect of NMDA on ACh release is NMDA receptor-mediated or not, the effect of MK-801, a selective NMDA receptor antagonist, was examined. Treatment with 1 μM MK-801 itself did not affect the ACh releases, while it not only reversed the 30 μM NMDA-induced decrease of the evoked ACh release, but also attenuated the facilatory effect of 30 μM NMDA on the basal ACh release (Fig. 3). Next the effect of NBQX, a selective antagonist of the AMPA receptor, on the facilitatory effects of 30 μM AMPA or 100 μM kainate on the basal ACh release was examined. The treatment with 10 μM NBQX itself had no effect on the basal ACh release, and abolished the AMPA- or kainate-induced increase of basal ACh release. Interestingly, 10 μM NBQX itself, though statistically not significant, showed tendency inhibiting the electrically-evoked ACh release, and NBQX significantly attenuated the evoked release when it was treated with AMPA, although it did not affect the evoked release alone without AMPA (Fig. 4).

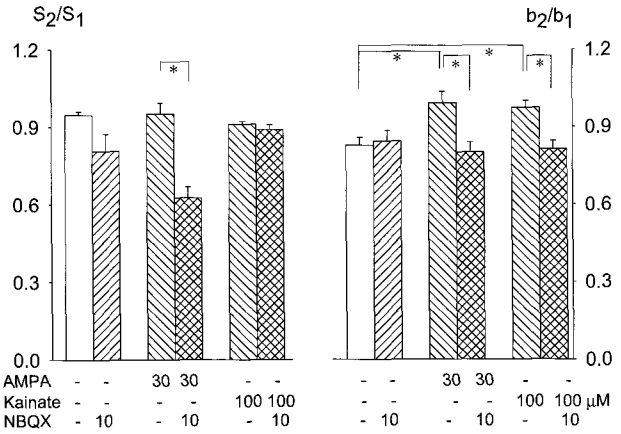

Fig. 4. Influence of 10 μM 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo [f]quinoxaline-7- sulfonamide (NBQX) on the effect of 30 μM AMPA and 100 μM kainate on the electrically-evoked (left panel) and the basal outflows of tritium (right panel) from the rat striatal slices preincubated with [^{3}H]choline. Number of each experimental group were 4~9. Asterisk indicates the significant difference (*:$p<0.05$) between groups. Other legends are the same as in Fig. 2.

DISCUSSION

In this study, we examined glutamatergic control of the ACh release from hippocampus and striatum. ACh is implicated in the regulation of learning, memory and

control of movement. The loss of memory function associated with Alzheimer disease has been attributed partly to the degeneration of cholinergic neurons of basal forebrain which project to hippocampus and cerebral cortex, while the hyperactivity of cholinergic interneuron at striatum causes the movement impairment in Parkinson disease. So the control of ACh release in hippocampus or striatum may affect the clinical consequences of Alzheimer or Parkinson Disease, respectively.

In this study, NMDA, AMPA and kainate did not affect not only the electrically-evoked but also the basal ACh release from hippocampal slices. These results are consistent with the report that NMDA augments the basal ACh release from cortical but not hippocampal slices (Ulus et al, 1992). However, on the contrary to our data, there were reports that the activation of non-NMDA receptors in the hippocampus regulates hippocampal ACh release (Giovannini et al, 1998) and a positive modulator of AMPA receptor with cognitive-enhancing properties increases hippocampal ACh release (Rosi et al, 2004). It is difficult to explain this discrepancy at this point Anyway these results suggest that the functionally relevant NMDA, AMPA and kainate receptors may not exist in the rat hippocampal cholinergic nerve ending.

In striatal slices, NMDA did not affect the electrically-evoked and basal ACh release in the Mg^{2+}-containing medium, but induced the increase of the basal release and the decrease of evoked release in the Mg^{2+}-free medium. These results show one characteristic of NMDA receptor which are the voltage-dependent channel block by Mg^{2+}. And a host of studies about the Mg^{2+}-dependent NMDA receptor activation were reported (Pittaluga and Raiteri, 1992a; 1992b; Ulus et al, 1992; Jin & Fredholm, 1994; Jin & Fredholm, 1997; Arruda Paes et al, 2004). NMDA increased the basal release but decreased the electrically-evoked release. And, the dual effects of NMDA were completely blocked by MK-801, indicating that the NMDA effect is generated by the action of NMDA receptor. There are some reports that prolonged activation of NMDA receptor inhibits ACh release from striatal slices (Badini et al, 1997; Morari et al, 1998; Marti et al, 1999). However, it is difficult to explain the mechanism of decrease of electrically-evoked ACh release by NMDA.

The finding that AMPA (30 μM) or kainate (100 μM) increased the basal ACh release and the treatment with 10 μM NBQX abolished the AMPA- or kainate-induced increase of basal ACh release, indicate the involvement of non-NMDA receptors in the control of ACh release in striatum, and there are many reports that show similar results (Jin & Fredholm, 1994; Kendrick et al, 1996; Jin & Fredholm, 1997; Morari et al, 1998). Interestingly, in present study, NBQX itself, though statistically not significant, showed tendency inhibiting the electrically-evoked ACh release, and NBQX significantly attenuated the evoked release when it was treated with AMPA, although it did not affect the evoked release alone without AMPA. These findings suggest that the activation of AMPA receptor may involve in the electrically-evoked ACh release.

Overall, These observations demonstrate that in hippocampal slices, ionotropic glutamate receptors do not modulate the ACh release in cholinergic terminals, whereas in striatal slices, activations of ionotropic glutamate receptors increase the basal ACh release though NMDA may decrease the electrically-evoked ACh release.

ACKNOWLEDGEMENT

This paper was supported by Wonkwang University in 2003.

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