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Bidirectional regulations for glutamate and GABA release in the hippocampus by α7 and non-α7 ACh receptors

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Abstract

In the assay of glutamate and γ-aminobutyric acid (GABA) with a high-performance liquid chromatography, spontaneous release of glutamate and GABA from rat hippocampal slices was significantly enhanced by mecamylamine, an inhibitor of non-α7 ACh receptors, or α-bungarotoxin, an inhibitor of α7 ACh receptors in the absence of tetrodotoxin (TTX), but not in the presence of TTX. Nicotine significantly enhanced glutamate and GABA release in the absence of TTX, that is abolished by mecamylamine or α-bungarotoxin, while it had no effect on the release in the presence of TTX. In the recording of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor-mediated excitatory postsynaptic currents (AMPA-EPSCs) and $GABA_A$ receptor-mediated inhibitory postsynaptic currents ($GABA_A$-IPSCs) from CA1 pyramidal neurons of rat hippocampal slices, nicotine did not affect the rate and amplitude of AMPA-EPSCs and AMPA-miniature EPSCs. In contrast, nicotine significantly increased the rate of $GABA_A$-IPSCs, without affecting the amplitude, but such effect was not obtained with $GABA_A$-miniature IPSCs. The collective results suggest that α7 and non-α7 ACh receptors expressed in the hippocampus, activated under the basal conditions, inhibit release of glutamate and GABA controlled through multi-synaptic relays, but that otherwise, those receptors, highly activated by nicotine, stimulate both the release, with a part of GABA released from interneurons transmitting to CA1 pyramidal neurons. Furthermore, the results also suggest that α7 and non-α7 ACh receptors do not have potency sufficiently to modulate glutamate and GABA release controlled by single synapses.

Keywords: α7 Acetylcholine receptor; Non-α7 acetylcholine receptor; Glutamate release; γ-Aminobutyric acid release; Hippocampus

Nicotinic acetylcholine (ACh) receptors are a family of ligand-gated receptors containing non-selective cation channels. Neuronal nicotinic ACh receptor subunits include α subunit (α2–α9) and β subunit (β2–β4), and α7 and α4β2 ACh receptors are preferentially expressed in the brain [1–4]. Several avenues of evidence have confirmed that brain nicotinic ACh receptors regulate the release of a variety of neurotransmitters including the excitatory neurotransmitter glutamate and the inhibitory neurotransmitter γ-aminobutyric acid (GABA) [5,6]. Presynaptic α7 ACh receptors on the glutamatergic terminals regulate excitatory synaptic transmission through glutamate release [7,8]. α7 ACh receptor activity is recognized to be enhanced by the nootropic agent nefiracetam, *cis*-unsaturated free fatty acids such as arachidonic, linoleic, and linolenic acids, or the linoleic acid derivative DCP-LA (former name, FR236924), thereby causing a marked increase in glutamate release, responsible for a long-lasting facilitation of hippocampal synaptic transmission [9–17]. Non-α7 ACh receptors such as α3β4 and α4β2 ACh receptors as well as α7 ACh receptors, alternatively, are abundantly expressed on GABAergic interneurons in the hippocampus, and mediate inhibition and disinhibition of hippocampal neuronal networks [18]. Regulation for glutamate and GABA release by α7 and non-α7 ACh receptors expressed on interneurons in the hippocampus, however, is not fully understood.

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To address this question, we assayed glutamate and GABA released from rat hippocampal slices with a high-performance liquid chromatography (HPLC) and monitored α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor-mediated excitatory postsynaptic currents (EPSCs) (AMPA-EPSCs) or $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs) ($GABA_A$-IPSCs) from CA1 pyramidal neurons of rat hippocampal slices. We show here that α7 and non-α7 ACh receptors in the hippocampus exert their inhibitory and stimulatory bidirectional actions on glutamate and GABA release controlled through multi-synaptic relays.

Materials and methods

Assay of glutamate and GABA. Rat hippocampal slices (400 μm) (male Wistar rat, 6 weeks) were prepared and incubated in standard artificial cerebrospinal fluid (ACSF) (117 mM NaCl, 3.6 mM KCl, 1.2 mM NaH_2PO_4, 1.2 mM $MgCl_2$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, and 11.5 mM glucose) oxygenated with 95% O_2 and 5% CO_2 at room temperature for 1 h followed by at 34 °C for 50 min. Then, slices were transferred to a chamber filled with 1 ml TTX (0.5 μM)-free and -containing ACSF in the presence and absence of nicotine (1 μM) together with or without mecamylamine (3 μM) or α-bungarotoxin (100 nM), oxygenated with 95% O_2 and 5% CO_2 at 34 °C for 20 min. After treatment, external solution was collected, and glutamate and GABA released were labeled with 4-fluoro-7-nitrobenzofurazan (NBD-F). Twenty microliters of the solution was injected onto the column (150 × 4.6 mm), and loaded onto the HPLC system (Shimadzu, Japan). NBD-F was detected at an excitation of wavelength of 350 nm and an emission wavelength of 450 nm using a fluorescence detector (Shimadzu, Japan). Total amount of protein was determined with a Protein Quantification Kit (Dojindo, Japan).

Statistical analysis was carried out using unpaired *t* test.

Recording of EPSCs and IPSCs. Slice patches were made from CA1 pyramidal neurons of rat hippocampal slices (male Wistar rat, 6 weeks). AMPA-EPSCs and AMPA-miniature EPSCs (AMPA-mEPSCs) were monitored in standard ACSF containing DL-2-amino-5-phosphonovaleric acid (DL-AP5) (100 μM), a selective inhibitor of NMDA receptors, bicuculline (20 μM), a selective inhibitor of $GABA_A$ receptors, and CGP55845 (3 μM), a selective inhibitor of $GABA_B$ receptors, in the absence and presence of TTX (0.5 μM), respectively, oxygenated with 95% O_2 and 5% CO_2, at 34 °C, with an Axopatch-200 A amplifier (Axon Instruments, USA). $GABA_A$-IPSCs and $GABA_A$-miniature IPSCs ($GABA_A$-mIPSCs) were recorded in a standard ASCF containing DNQX (20 μM), a selective inhibitor of AMPA receptors, and DL-AP5 (100 μM) in the absence and presence of TTX (0.5 μM), respectively, oxygenated with 95% O_2 and 5% CO_2 at 34 °C, with an Axopatch-200 A amplifier (Axon Instruments, USA). A recording chamber was continuously perfused with ACSF at the flow rate of 2 ml/min, and nicotine (1 μM) was bath-applied by switching a three-way cock. The patch electrode-filling solution was (mM); $110Cs_2SO_4$, 5 tetraethylammonium chloride, 2 $MgCl_2$, 0.5 $CaCl_2$, 5 ethylene glycol-bis (β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetate (EGTA), 5 Hepes, and 5 MgATP for EPCS recording and that with 135 CsCl instead of 110 Cs_2SO_4 for IPSC recording.

Statistical analysis was carried out using Kolmogorov–Smirnov test.

Results

α7 and non-α7 ACh receptors exert their inhibitory and stimulatory bidirectional actions on glutamate release controlled through multi-synaptic relays

Spontaneous glutamate release from rat hippocampal slices was 0.08 ± 0.01 pmol/min/μg protein in the absence of TTX, and the release was significantly enhanced by mecamylamine (3 μM), an inhibitor of non-α7 ACh receptors, or α-bungarotoxin (100 nM), an inhibitor of α7 ACh receptors, each reaching approximately three times of basal levels ($P < 0.001$, unpaired *t* test) (Fig. 1A). In the experiments of *Xenopus* oocytes expressing rat α3β4, α4β2, and α7 ACh receptors, 3 μM of mecamylamine depressed α3β4 and α4β2 ACh receptor currents each to approximately 30% of original amplitude, without affecting α7 ACh receptor currents, while 100 nM of α-bungarotoxin abolished α7 ACh receptor currents, with no/little inhibition of α3β4 and α4β2 ACh receptor currents [17], confirming that mecamylamine and α-bungarotoxin can inhibit non-α7 and α7 ACh receptors at the doses used here, respectively. The result here, therefore, implies that α7 and non-α7 ACh receptors are still activated under the basal conditions and exert their inhibitory action on

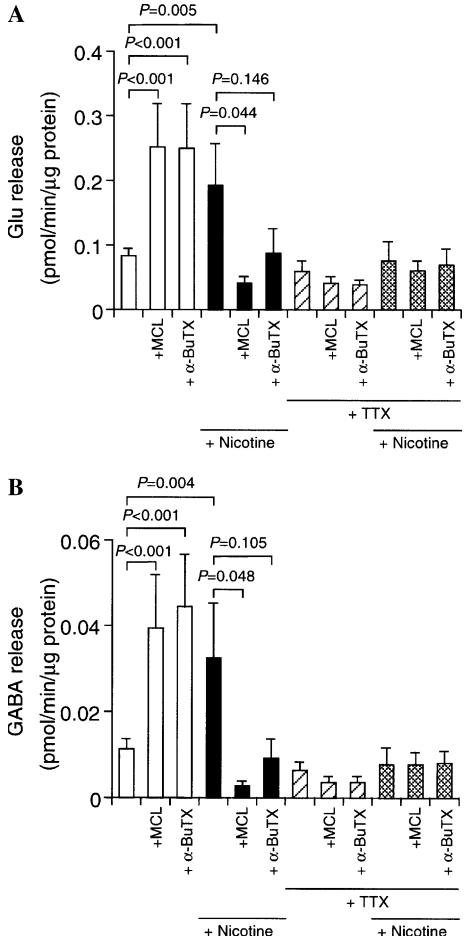

Fig. 1. Release of glutamate and GABA from hippocampal slices. Rat hippocampal slices were incubated in the presence and absence of TTX (0.5 μM) together with and without nicotine (1 μM), mecamylamine (MCL) (3 μM), or α-bungarotoxin (α-BuTX) (100 nM) for 20 min, and glutamate and GABA released were assayed with an HPLC. (A) In the graph, each point represents the mean (±SEM) glutamate concentrations ($n = 7$). (B) In the graph, each point represents the mean (±SEM) GABA concentrations ($n = 7$).