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Endogenous activation of nAChRs and NMDA receptors contributes to the excitability of CA1 stratum radiatum interneurons in rat hippocampal slices: Effects of kynurenic acid

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ABSTRACT

CA1 stratum radiatum interneurons (SRIs) express α7 nicotinic receptors (nAChRs) and receive inputs from glutamatergic neurons/axons that express α3β4β2 nAChRs. To test the hypothesis that endogenously active α7 and/or α3β4β2 nAChRs control the excitability of CA1 SRIs in the rat hippocampus, we examined the effects of selective receptor antagonists on spontaneous fast current transients (CTs) recorded from these interneurons under cell-attached configuration. The frequency of CTs, which represent action potentials, increased in the absence of extracellular Mg^{2+} and decreased in the presence of the α3β4β2 nAChR antagonist mecamylamine (3 μM) or the NMDA receptor antagonist APV (50 μM). However, it was unaffected by the α7 nAChR antagonist MLA (10 nM) or the AMPA receptor antagonist CNQX (10 μM). Thus, in addition to synaptically and tonically activated NMDA receptors, α3β4β2 nAChRs that are present on glutamatergic axons/neurons synapsing onto SRIs and are activated by basal levels of acetylcholine contribute to the maintenance of the excitability of these interneurons. Kynurenic acid (KYNA), an astrocyte-derived kynurenine metabolite whose levels are increased in the brains of patients with schizophrenia, also controls the excitability of SRIs. At high micromolar concentrations, KYNA, acting primarily as an NMDA receptor antagonist, decreased the CT frequency recorded from the interneurons. At 2 μM, KYNA reduced the CA1 SRI excitability via mechanisms independent of NMDA receptor block. KYNA-induced reduction of excitability of SRIs may contribute to sensory gating deficits that have been attributed to deficient hippocampal GABAergic transmission and high levels of KYNA in the brain of patients with schizophrenia.

1. Introduction

Cholinergic innervation of hippocampal neurons is known to play an important role in a variety of cognitive processes. In fact, several lines of evidence have suggested that impairment of hippocampal inhibitory neurotransmission due to deficits in cholinergic stimulation of hippocampal interneurons contributes to the over-inclusive thought processing of patients with schizophrenia [1]. In this disorder, impaired cholinergic stimulation of GABAergic neurons in the hippocampus may result from elevated levels of kynurenic acid (KYNA) [2], an astrocyte-derived kynurenine metabolite known to block both NMDA receptors and α7 nicotinic acetylcholine receptors (nAChRs) [3–6].

Interneurons in the hippocampus receive cholinergic inputs from the medial septal nucleus/diagonal band complex [7] and from cholinergic neurons intrinsic to the hippocampal formation [8,9]. It is well documented that the excitability of the interneurons is controlled by interactions of endogenously released acetylcholine (ACh) with different subtypes of muscarinic receptors present in the local interneuronal circuitry and on glutamatergic axons/neurons that synapse onto the interneurons [10,11]. By contrast, much less is known regarding control of neuronal excitability in the hippocampus by the actions of the endogenous neurotransmitter, ACh, on nicotinic receptors (nAChRs). Two independent studies reported the existence of nicotinic synaptic transmission mediated by α7 nAChRs on a small population of CA1 stratum radiatum interneurons (SRIs) [12,13]. Evoked release of ACh in hippocampal slices has also been shown to cause α7 nAChR-dependent heterosynaptic depression of GABAergic transmission in interneurons [14]. However, exogenous application of nicotinic agonists to hippocampal interneurons led to the identification of

Abbreviations: ACh, acetylcholine; ACSF, artificial cerebrospinal fluid; AMPA, (2R)-amino-5-phosphonovaleric acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; CT, current transient; EPSC, excitatory postsynaptic current; KYNA, kynurenic acid; MLA, methyllycaconitine; nAChR, nicotinic acetylcholine receptor; SRI, stratum radiatum interneuron.

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neuron-type specific expression of pharmacologically distinct nAChR subtypes [15]. For instance, exogenous application of ACh and other nicotinic agonists to the majority of CA1 SRIs in rat hippocampal slices induces responses that have the pharmacological profile of α7 nAChR [16–19]. However, these neurons also receive glutamatergic inputs whose activity is increased by α3β4β2 nAChRs [20] and GABAergic inputs whose activity is regulated by α7 nAChRs and α4β2 nAChRs [17]. Therefore, one can hypothesize that nicotinic cholinergic regulation of the excitability of CA1 SRIs is a result of the interactions of endogenous ACh with: (i) α7 nAChRs present on the somatodendritic region of SRIs, (ii) α3β4β2 nAChRs present on glutamatergic neurons/axons synapsing onto the SRIs, and (iii) α7 and α4β2 nAChRs present on interneurons that synapse onto the SRIs. The present study was designed to address specifically the contribution of α7 and α3β4β2 nAChR activation by endogenous ACh to the resting excitability of CA1 SRIs.

Action potentials, the output signal of neurons, are commonly studied in intracellular or whole-cell current-clamp recordings performed in brain slices [21,22]. In a few studies, action potentials have been detected as fast current transients (CTs) in single neurons under cell-attached voltage-clamped condition [23,24]. Under voltage-clamp, these fast CTs appear as inverted action potentials. A pharmacological analysis of spontaneous CTs in identified neuron types in brain slices can underpin the contribution of specific neurotransmitter systems to the regulation of neuronal excitability [21,22]. Thus, here, to identify how endogenously activated nAChRs and glutamate receptors control the excitability of CA1 SRIs, receptor-subtype selective antagonists were applied to rat hippocampal slices where fast CTs were recorded from the interneurons under voltage-clamp cell-attached configuration. Specifically, we analyzed the effects of the α7 nAChR antagonist methyllycaconitine (MLA), the NMDA receptor antagonist (2R)-amino-5-phosphonovaleric acid (APV), and the AMPA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) on the frequency of CTs. The effects of a concentration of mecamylamine that selectively inhibits α3β4β2 nAChRs were also analyzed. Finally, we examined whether the frequency of CTs recorded from SRIs is affected by endogenously produced and exogenously applied KYNA.

2. Methods

2.1. Animals

Timed pregnant rats (Sprague–Dawley, gestation day 16–18) were purchased from Charles River Laboratories (Wilmington, MA) and housed individually in a temperature- and light-controlled animal-care unit. Male pups were weaned at 21 days of age and housed in groups of three–four per cage and used for experiments on postnatal days 23–30. Animals were handled according to the regulations of the Association for Assessment and Accreditation of Laboratory Animal Care, in compliance with the standards of the Animal Welfare Act and in adherence to the principles of the '1996 Guide for the Care and Use of Laboratory Animals.'

2.2. Hippocampal slices

Rats were euthanized by asphyxiation in a CO_2 atmosphere followed by decapitation using a guillotine. To reduce cell swelling, removal of the brains as well as dissection and slicing of the hippocampi were performed in an ice-cold solution consisting of a mixture of equal parts of regular artificial cerebrospinal fluid (ACSF) and sucrose-containing ACSF. Regular ACSF (Mg-ACSF) was composed of (in mM): NaCl, 125; $NaHCO_3$, 26; KCl, 2.5; NaH_2PO_4, 1.25; $CaCl_2$, 2; $MgCl_2$, 1; and glucose, 25. Nominally Mg^{2+}-free ACSF had the same composition but $MgCl_2$ was excluded. Sucrose-containing ACSF was composed of (in mM): sucrose, 230; KCl, 2.5; NaH_2PO_4, 1.25; $NaHCO_3$, 26; $CaCl_2$, 0.5; $MgSO_4$, 10; and glucose, 10. Hippocampal slices of 300-μm thickness were cut using the vibratome Leica VT1000S (Leica Microsystems Inc., Bannockburn, IL) and transferred to an immersion chamber containing regular ACSF that was continuously bubbled with 95% O_2/5% CO_2. After 30 min incubation in regular ACSF, 12 hippocampal slices were transferred to a 50-ml chamber containing ACSF, with test compounds, that was continuously bubbled with 95% O_2/5% CO_2. The chambers were maintained in a water bath at 30 °C. Time of incubation of the slices with the test compounds ranged from 1 to 6 h.

2.3. Electrophysiological recordings

Hippocampal slices were transferred to a 1-ml recording chamber, where they were continuously superfused with ACSF at 2 ml/min at room temperature. In all experiments, ACSF used to superfuse the slices contained the muscarinic antagonist atropine (0.5 μM) and $GABA_A$ receptor antagonist bicuculline (10 μM). Cell-attached and whole-cell recordings were obtained from the soma of SRIs in hippocampal slices according to the standard patch-clamp technique using an EPC9 amplifier (HEKA Elektronik, Lambrecht, Germany).

Signals were filtered at 3 kHz and either videotape recorded for later analysis or directly sampled by a microcomputer using the PULSE software (ALA Scientific Instruments, Inc., Westbury, NY). Patch pipettes were pulled from a borosilicate glass capillary (1.2-mm OD) that, when filled with internal solution, had resistances between 3 and 5 MΩ. The internal pipette solution contained 0.5% biocytin in addition to ethylene-glycol bis (β-amino-ethyl ether)-N-N′-tetraacetic acid, 10 mM; 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 10 mM; Cs-methane sulfonate, 130 mM; CsCl, 10 mM; $MgCl_2$, 2 mM (pH adjusted to 7.3 with CsOH; 340 mOsm). To record AMPA or NMDA EPSCs from neurons under whole-cell configuration, 5 mM N-(2,6-dimethylphenyl-carbamoylmethyl)triethylammonium bromide (QX314) was included in the pipette solution. QX314 – free pipette solution was used for all cell-attached recordings, except those that preceded whole-cell recordings from a single neuron. CTs were recorded in the cell-attached mode after formation of the seal, which ranged between 1 and 8 GΩ in our recordings. During cell-attached recordings the patch pipette was held at −60 mV (i.e., transmembrane potential close to 0 mV) to inactivate voltage-dependent currents [23]. All experiments were carried out at room temperature (20–22 °C).

2.4. Data analysis

The frequency of CTs was analyzed using the pCLAMP9 software (Molecular Devices, Sunnyvale, CA). Frequency, peak amplitude, 10–90% rise time, and decay-time constants of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) excitatory PSCs (EPSCs) were analyzed using WinEDR V2.3 and WinWCP 4.2.8 (Strathclyde Electrophysiology Software, Glasgow, Scotland). Results are presented as mean ± SEM. Statistical significance was tested with the StatsDirect software v 2.7.7 (StatsDirect Ltd., Cheshire, UK) using unpaired t-test, Fisher's exact test, or one-way ANOVA followed by an appropriate post-hoc test.

2.5. Chemicals used

(−)Bicuculline methochloride was purchased from Tocris (Ellisville, MO). Atropine sulfate, L-kynurenine sulfate, KYNA, QX-314 bromide, and 2-amino-5-phosphonovaleric acid (APV)