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ScienceDirect
Neuroscience Letters 432 (2008) 212–216
Neuroscience Letters
www.elsevier.com/locate/neulet

Tonic facilitation of glutamate release by glycine binding sites on presynaptic NR2B-containing NMDA autoreceptors in the rat visual cortex

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Received 13 July 2007; received in revised form 28 November 2007; accepted 5 December 2007

Abstract

We have previously shown that glycine binding sites on presynaptic NMDA receptors (NMDA-Rs) can tonically regulate glutamate release in the rat visual cortex. In the present study, we investigated the subunit composition of these presynaptic NMDA-Rs. We recorded miniature *a*-amino-3-hydroxy-5-methyl-4-*iso*xazolepropionic acid (AMPA) receptor-mediated excitatory postsynaptic currents (mEPSCs) using whole-cell voltage clamp in layer II/III pyramidal neurons of the rat visual cortex with the open-channel NMDA receptor blocker, MK-801, in the recording pipette. We found that the frequency of mEPSCs is significantly reduced by 7-chloro-kynurenic acid (7-Cl KYNA) an NMDA-R glycine binding site antagonist, and glycine reverses this effect. Using a specific antagonist for NR2B-NMDA-Rs, Ro 25-6981 [(αR,βS)-α-(4-hydroxyphenyl)-β-methyl-4-(phenylmethyl)-1-piperidinepropanol hydrochloride], instead of 7-Cl KYNA, we found that the frequency of mEPSCs is also significantly reduced but glycine cannot reverse this effect. Moreover, Zn^{2+}, an NR2A-NMDA-R antagonist, did not affect mEPSC frequency. These results suggest that presynaptic NR2B-containing NMDA-Rs are located in layer II/III pyramidal neurons of the rat visual cortex, and that the glycine binding site of these type NMDA-Rs tonically regulates glutamate release.

Keywords: NMDA receptors; NR2B; NR2A; Glycine; Presynaptic regulation; mEPSCs

Recent studies have shown that *N*-methyl-D-aspartate receptors (NMDA-Rs) are present on presynaptic as well as postsynaptic cells. There is evidence that presynaptic NMDA-Rs enhance neurotransmitter release in different regions of the central nervous system (CNS) including the spinal cord, cerebellum, entorhinal cortex, and neocortex [2,4,9,25,26] and also mediate synaptic plasticity (i.e., long term potentiation and long term depression) [8,24]. Immunocytochemical studies have demonstrated that presynaptic NR1 and NR2B-containing NMDA-Rs are present in rat visual cortex [1]. Moreover, presynaptic NMDA-Rs enhance neurotransmitter release in layer V visual cortex neurons [24]. We have previously shown that glycine binding sites on presynaptic NMDA-Rs can tonically regulate glutamate release in layer II/III pyramidal neurons of the rat visual cortex [13].

NMDA-Rs are composed of the obligatory NR1 subunit in combination with NR2A–D and NR3A–B subunits that confer distinct receptor properties [15,16]. Of the four NR2 subunits, rodent cortex contains primarily NR2A and NR2B. Developmentally, NR2B subunits are already expressed at birth and increase to near a plateau level within the first 2 weeks, whereas NR2A subunits are not expressed until the second week and increase gradually in subsequent weeks. Therefore, the ratio of NR2A- to NR2B-containing NMDA-Rs increases with maturation [23]. Additionally, the four NR2 subunits have distinct distributions in the CNS, with patterns of expression that change drastically during development. For example, only NR2B and NR2D subunits are expressed embryonically, while NR2A is ubiquitously expressed in the adult brain. Moreover, NR2B expression is restricted to the forebrain while NR2C is highly enriched in the cerebellum [18]. Because NR2A-containing NMDA-Rs possess shorter current durations than NR2B-containing NMDA-Rs, NMDA-R-mediated current durations shorten over development [11]. The developmental increase in NR2A in the visual cortex is delayed by dark-rearing and is thus thought to be experience-dependent [17,21].

However, we do not know yet the subunit composition of presynaptic NMDA-Rs in layer II/III pyramidal neurons of the

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doi:10.1016/j.neulet.2007.12.023

rat visual cortex and subunit of these presynaptic NMDA-Rs whose glycine binding site regulates glutamate release. In this study, we have utilized specific antagonists for NR2B-NMDA-R (Ro 25-6981) and NR2A-NMDA-R antagonist (Zn^{2+}) to probe for the subunit composition of presynaptic NMDA-Rs. Our results show that presynaptic NMDA-Rs are NR2B-containing and that the glycine binding site on these presynaptic NR2B-containing NMDA-Rs tonically facilitates glutamate release.

The use and care of animals in this study follow the guidelines of the Xi'an Jiaotong University Animal Research Advisory Committee. Visual cortex slices were prepared from Sprague–Dawley rats aged 13–15 days. All animals were housed in a standard environment on a 12/12 h light/dark cycle with lights on at 07:00, and had *ad libitum* access to water and food. Rats were initially anesthetized with ether and then immersed in ice-cold water, with the nose exposed, for 3 min to reduce brain temperature. Immediately after decapitation, the brain was dissected out and placed in ice-cold artificial cerebrospinal fluid (ACSF) bubbled with 95% O_2 and 5% CO_2 (carbogen) at a pH of 7.4. The ACSF consisted of 124 mM NaCl, 5 mM KCl, 1.2 mM KH_2PO_4, 1.3 mM $MgSO_4$, 2.4 mM $CaCl_2$, 26 mM $NaHCO_3$, 10 mM glucose, and it had an osmolality of 305–310 mOsm/kg H_2O. A block of tissue containing the primary visual cortex was cut into 350 μm slices with a vibratome (Campden Instruments, London, UK). Slices were transferred to an incubating chamber containing ACSF equilibrated with carbogen and incubated for at least 1.5 h at room temperature (20 °C) prior to electrophysiological recording.

For electrophysiological recordings, slices were individually transferred to a recording chamber where they were perfused (2.5–3 ml/min) with oxygenated Mg-free ACSF at 31 ± 0.5 °C. The temperature of the recording chamber was continuously monitored and controlled by a custom-made temperature controller. The slices were placed on an upright infrared video microscope with differential interference contrast (DIC) optics (OLYMPUS BX51WI) which was mounted on a Gibraltar X–Y table. Slices were observed through a 40× water immersion objective using an infrared-sensitive camera (DAGE-MTI, IR-1000). Layer II/III pyramidal neurons of the visual cortex were visually selected as previously described [14]. Patch clamp recordings were performed in the whole cell configuration. Unpolished and un-coated patch pipettes (1.5 mm/1.1 mm; Sutter Instruments, Novato, CA) with a resistance of 4–6 MΩ were pulled using a horizontal puller (MODEL P-97, Sutter Instruments, Novato, CA). The pipette solution contained 124 mM cesium methanesulfonate, 5 mM QX-314, 2 mM $MgCl_2$, 10 mM BAPTA, 1 mM (+)-MK-801, 10 mM HEPES, 2 mM Na_2ATP, and 0.25 mM Na_3GTP (pH 7.3–7.4, adjusted with CsOH, 280–290 mOsm/kg H_2O). To facilitate the blockade of the open channel blocker MK-801, neurons were depolarized to −10 mV for 10 s at intervals during the 10 min period after gaining whole-cell access [26]. Cells were held at −60 mV and current recorded using an Axopatch 700B amplifier (Axon Instruments, USA) and digitized using a data acquisition board (Digidata 1322A) operated by pCLAMP 9.2 software (Axon Instruments). Currents were filtered at 2 kHz and digitized at 10 kHz. Compensation for the series resistance was not employed. Statistical comparisons were performed only when series resistance was lower than 20 MΩ and did not change by more than 10%.

Glycine, strychnine, picrotoxin, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), (+)-MK-801, QX-314, BAPTA, 7-chlorokynurenic acid (7-Cl KYNA), *N*-tris (hydroxymethyl) methylglycine (tricine), zinc chloride and Ro 25-6981 were purchased from Sigma. Tetrodotoxin (TTX) was obtained from Hebei Province Marine Science and Technology CO., LTD, PR China. The 30 nM free Zn^{2+} was made by adding 7.8 mM Zn^{2+} to 10 mM tricine [20]. TTX (0.5 μM) strychnine (1 μM) and picrotoxin (100 μM) were added to Mg-free ACSF to suppress the sodium currents, glycinergic and GABAergic transmission, respectively. Mg-free ACSF was obtained by omitting $MgSO_4$ (1.3 mM) from the ACSF without compensation for the loss of osmolarity or for the amount of divalent ions. All drugs were applied at known concentrations by changing the perfusion line.

Miniature EPSCs were detected and analyzed with Mini-Analysis software (Version 6.0, Synaptosoft, USA) using a threshold-crossing criterion. Although, the threshold level varied from neuron to neuron, it was always the same before and after drug application [5]. Averaged mEPSCs were obtained by aligning events on the rising phase. Events occurring during 120 s were averaged and analyzed in every group. Comparisons of mEPSC distributions were performed using the Kolmogorov–Smirnov test, and values of $P < 0.01$ were accepted as significant [4]. Group means were compared using a paired Student's *t* test, and $P < 0.05$ was considered significant. All data are expressed as mean ± S.E.M.

Whole-cell mEPSCs recordings were performed on 46 pyramidal neurons in layer II/III of rat visual cortex slices. The mEPSCs were recorded in the presence of TTX (0.5 μM) strychnine (1 μM) and picrotoxin (100 μM) and were abolished by application of CNQX (10 μM in Mg-free ACSF) with the open-channel NMDA-R blocker, dizocilpine maleate (MK-801; 1 mM) in the recording pipette previously described by Berretta and Jones [4] (data not shown). This indicates that the mEPSCs were comprised of non-NMDA glutamatergic components [3,19]. In juvenile animals, we have shown previously that the nonspecific antagonist D-APV and 7-Cl KYNA reduce the frequency of mEPSCs in layer II/III pyramidal neurons of the rat visual cortex by blocking tonically active presynaptic NMDA receptors [13]. We now report a similar effect with the highly specific NR2B antagonist, Ro 25-6981. Application of exogenous Ro 25-6981 (1 μM) inhibited non-NMDA-R-mediated mEPSC frequency (Fig. 1A). Fig. 1A shows a representative example of Ro 25-6981-induced decrease in mEPSC frequency. Cumulative probability distributions for inter-event intervals from the same neuron as recorded in 1A (Fig. 1C) show that in the presence of Ro 25-6981, the distributions are shifted to the right (KS, $P < 0.001$) reflecting a decreased frequency of mEPSCs. The frequency of mEPSCs under control conditions was 2.25 ± 0.58 Hz. During perfusion with Ro 25-6981 (1 μM) the frequency reduced to 1.52 ± 0.52 Hz ($n = 12$, $P < 0.001$; shown in Fig. 1D). Mean amplitudes were unaltered (Control, 38.2 ± 8.22 *versus* Treated, 39.0 ± 8.23, $n = 12$, $P > 0.05$; Fig. 1E). Taken together, the data indicate that Ro 25-6981 decreased the frequency but not amplitude of mEPSCs, sug-