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ScienceDirect
Neuropharmacology 52 (2007) 77–86
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

The role of NMDAR subtypes and charge transfer during hippocampal LTP induction

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Received 21 May 2006; received in revised form 10 July 2006; accepted 12 July 2006

Abstract

Activation of NMDA receptors (NMDARs) is a requirement for persistent synaptic alterations, such as long-term potentiation of synaptic transmission (LTP). NMDARs are composed of NR1 and NR2 subunits, and NR2 subunit-dependent gating properties of NMDAR subtypes cause dramatic differences in the timing of charge transfer. These postsynaptic temporal profiles are further influenced by the frequency of synaptic activation. Here, we investigated in the CA1 region of hippocampal slices from P28 mice, whether particular NMDAR subtypes are recruited based on NR2 subunit-specific gating following different induction protocols. For high frequency afferent stimulation (HFS), we found that genetic impairment of NR2A or pharmacological block of NR2A- or NR2B-type NMDARs can reduce field LTP. In contrast, when pairing low frequency synaptic stimulation with postsynaptic depolarization (LFS pairing) in single CA1 neurons, pharmacological antagonism of either subtype modestly reduced the charge transfer during LFS pairing without reducing the LTP magnitude. These results indicate that HFS-triggered LTP is induced by more than one NMDAR subtype, whereas a single subtype is sufficient during LFS pairing. Analysis of charge transfer during LFS pairing in 13 different conditions revealed a threshold for LTP induction, which was independent of the NR2 antagonist tested. Thus, at least for LFS pairing, the amount of charge transfer, and thus Ca^{2+} influx, during LTP induction is a factor more critical than the participation of a particular NMDAR subtype.
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Keywords: Frequency; Temporal signaling; Gene-targeted mouse; NVP-AAM077; Ro25-6981; CP-101,606

1. Introduction

The NMDA receptor (NMDAR) plays a major role in both physiological and pathophysiological processes in the brain (Cull-Candy and Leszkiewicz, 2004; Dingledine et al., 1999), and has been a long-time CNS therapeutic target (Chazot, 2004). The identity of the NR2 subunit (NR2A-D, or ε1–4) strongly influences the biophysical and pharmacological properties of NMDAR assemblies (Vicini et al., 1998). NR2 subunit-specific gating is known to control the kinetics of NMDAR channels, including activation, probability of opening and deactivation (Erreger et al., 2005). These properties produce at the postsynaptic site different temporal signaling profiles, which could contribute to synaptic transmission and plasticity.

Current studies characterizing NMDAR subtypes in synaptic plasticity aim at unmasking NR2 subtype-specific roles. A recent proposal that NR2A-containing NMDARs, also called NR2A-type NMDARs, exclusively induce LTP (for review, see Collingridge et al., 2004) was questioned by other reports showing that NR2B can induce LTP as well (Berberich et al., 2005; Weitlauf et al., 2005; Zhao et al., 2005). At postnatal day 28, hippocampal neurons express comparable amounts of NR2A and NR2B subunits (Sans et al., 2000), forming di- and triheteromeric NMDAR assemblies (NR1/NR2A, NR1/NR2A/NR2B and NR1/NR2B) (Sheng et al., 1994). Their relative contribution to LTP induction is difficult to resolve: i) The available competitive NMDARs antagonists such as D-AP5, D-AP7, D-CPP, Con G and NVP-AAM077 lack

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0028-3908/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2006.07.016

NR2A/B-selectivity (for review, see Köhr, in press). ii) Although non-competitive NMDAR antagonists have higher preferences for the corresponding NR2-subtypes and are NR2-selective (e.g. Zinc for NR2A; ifenprodil and its derivatives Ro25-6981 and CP-101,606 for NR2B), they maximally inhibit NMDARs by 70–80% (for review, see Neyton and Paoletti, 2006). iii) NMDAR antagonists inhibit diheteromeric NMDARs more than triheteromeric NMDARs (for review, see Neyton and Paoletti, 2006).

Therefore, in this study we investigated influences of distinct gating properties of the hippocampal NMDAR subtypes during LTP induction and focused on the synaptic charge transfer when pairing low frequency stimulation with postsynaptic depolarization (LFS pairing), which reflects the Ca^{2+} influx during LTP induction. Previous simulations of synaptic responses suggested that the more rapidly gating NR1/NR2A receptors are more effective at mediating charge transfer during high frequency stimulation (HFS), whereas the slower gating NR1/NR2B receptors are better suited during low frequency stimulation (LFS) (Erreger et al., 2005). As Mg^{2+} unblocks faster from NR1/NR2A than from NR1/NR2B receptors (Clarke and Johnson, 2006) induction protocols involving prolonged postsynaptic depolarizations (e.g., LFS pairing) should reduce the relative importance of the Mg^{2+} unblock kinetics. Hence, we compared LTP induced by LFS pairing in whole-cell recordings with LTP induced by HFS of afferent fibers in field recordings. Whole-cell recordings allow controlling the extent of postsynaptic depolarization (e.g., 0 mV), while HFS in field recordings can be assumed to depolarize postsynaptic cells less efficiently. These experiments were performed in presence of non-competitive and/or competitive NMDAR antagonists at increasing concentrations to compare selective with unselective NR2 antagonism. In addition, we analyzed two NR2A mutants (*NR2A*$^{-/-}$; Sakimura et al., 1995 and *NR2A*$^{\Delta C/\Delta C}$; Sprengel et al., 1998) which both make use of NR1/NR2B receptors for LTP induction, but differ in the kinetics of their NMDA EPSCs (Berberich et al., 2005; Kiyama et al., 1998; Köhr et al., 2003), which should affect the charge transfer during LTP induction.

The present study substantiates the evidence for involvement of both NR2A- and NR2B-type NMDA receptors in LTP induction at Schaffer collateral/commissural synapses onto hippocampal CA1 pyramidal neurons. Furthermore, the recruitment of NMDAR subtypes depends on the LTP induction protocols. Finally, the quantitative relationship between charge transfer during induction and magnitude of LTP reveals a critical level of charge transfer via any NMDAR subtype.

2. Methods

All experimental procedures were in accordance with the animal welfare guidelines of the Max Planck Society, and in accordance with the Norwegian Animal Welfare Act and the European Union's Directive 86/609/EEC.

2.1. Extracellular field experiments

Wild-type (C57Bl/6 strain) mice and mice lacking the carboxy-terminal intracellular domain of the NR2A subunit (*NR2A*$^{\Delta C/\Delta C}$; Sprengel et al., 1998) were at P28 killed with desflurane. Transverse slices (400 μm) from the middle portion of each hippocampus were cut with a vibroslicer in the following artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 2 KCl, 1.25 KH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$ and 12 D-glucose; 4 °C, bubbled with 95% O_2/5% CO_2 (pH 7.4). Slices were placed in an interface chamber at 28–32 °C and perfused with ACSF, which in some experiments contained the NR2B-selective antagonists Ro25-6981 (Sigma), CP-101,606 (Pfizer) and/or the NR2A-preferring antagonist NVP-AAM077 (Novartis) at concentrations indicated in the Result section. Orthodromic synaptic stimulation was delivered alternately through two tungsten electrodes, one in stratum radiatum, and the other in stratum oriens. Extracellular responses were monitored in the corresponding layers by two glass electrodes filled with ACSF. Assessment of synaptic efficacy and tetanization procedures were as earlier described (Köhr et al., 2003). Six consecutive responses (1 min) were averaged and normalized to the mean value recorded 4–7 min before tetanic stimulation. Data were pooled and presented as mean ± SEM, and statistical significance was evaluated using a two-tailed, Student's *t*-test.

2.2. Whole-cell experiments

The brain was removed from deeply anaesthetized (halothane) P28 mice (wild-type, *NR2A*$^{\Delta C/\Delta C}$ and *NR2A*$^{-/-}$, Sakimura et al., 1995). Transverse hippocampal slices (250 μm) were prepared and incubated for 30 min at 35 °C in ACSF containing (in mM): 125 NaCl, 25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 1 $MgCl_2$, 25 D-glucose, 2 $CaCl_2$; bubbled with 95% O_2/5% CO_2 (pH 7.4). Patch pipettes were pulled from borosilicate glass capillaries and had resistances of 4–6 MΩ when filled with (in mM): 125 Cs-gluconate, 20 CsCl, 10 NaCl, 10 HEPES, 0.2 EGTA, 4 MgATP, 0.3 Na_3GTP (pH 7.3, 290–305 mOsm). Series resistances (15 to 30 MΩ) and input resistances (100 to 300 MΩ) were continuously monitored at negative holding potentials by measuring the peak and the steady-state current of the instantaneous capacitive transient in response to hyperpolarizing pulses (−5 mV; 20 ms). All patch experiments were performed at room temperature (22 °C).

EPSCs were activated by stimulating the Schaffer collaterals about 150 μm distant from the CA1 cell body with a glass electrode filled with 1 M NaCl. NMDA EPSCs were recorded at −40 or +40 mV in ACSF (see above) containing 10 μM bicuculline methiodide (BMI), 5 μM NBQX and 10 μM glycine. For LTP recordings, patch pipettes were filled with (in mM): 120 Cs-gluconate, 10 CsCl, 10 HEPES, 8 NaCl, 0.2 EGTA, 2 MgATP, 0.3 Na_3GTP, 10 phosphocreatine. EPSCs were evoked in solutions containing (in mM): 124 NaCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 2.5 KCl, 4 $CaCl_2$, 4 $MgSO_4$, 10 D-glucose, 0.010 glycine and 0.010 BMI (pH 7.3, 290–305 mOsm). In some experiments, NR2-antagonists were present (see above). LTP was induced by pairing low frequency stimulation (120 pulses, 0.7 Hz) in the test (str. radiatum) but not in the control (str. oriens) pathway with postsynaptic depolarization to 0 mV for 3 min (LFS pairing; Chen et al., 1999). The actual holding potential during induction was rather −11 mV, since liquid junction potentials were not corrected. The charge of each EPSC evoked during LFS pairing was calculated and averaged. Six consecutive EPSCs at −70 mV were averaged and normalized to the averaged responses obtained 5 min before LTP induction. EPSC potentiation was assessed 30 min after induction. All data were pooled across animals of the same genotype and are presented as mean ± SEM. Statistical significance was evaluated by one-way analysis of variance (ANOVA), followed by Post-Hoc Fisher's LSD analysis, or by a two-tailed, Student's *t*-test whenever noted.

3. Results

3.1. Tetanic stimulation

In the CA1 region of hippocampal slices from P28 wild-type mice, high frequency stimulation (HFS) of the afferent fibers, in either stratum radiatum or stratum oriens, produced an NMDAR-dependent (data not shown), homosynaptic potentiation of the synaptic responses, characteristic of LTP. The

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