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THE MAGNITUDE OF HIPPOCAMPAL LONG TERM DEPRESSION DEPENDS ON THE SYNAPTIC LOCATION OF ACTIVATED NR2-CONTAINING *N*-METHYL-D-ASPARTATE RECEPTORS

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Abstract—Activation of *N*-methyl-D-aspartate receptors (NMDARs) is the first step in the induction of certain forms of synaptic plasticity in the hippocampus. In the adult rat hippocampus, NMDARs are composed almost exclusively of NR1 and NR2 subunits with NR1 subunits being mainly associated with either NR2A and/or NR2B subunits. The role played by the different subunits in synaptic plasticity is still controversial. In the present study, we used two different long term depression (LTD) –inducing protocols (electrical and chemical stimulation) to show that activation of NR2A-containing NMDAR subunits leads to the induction of LTD. We also demonstrated that extrasynaptic NR2B-containing NMDARs regulate the magnitude of LTD by exerting a control over the function of synaptic NR2A-containing NMDARs while having no effect on plasticity in the absence of synaptic receptor activation. Taken as a whole, these experiments demonstrate that NMDAR subunits play different roles according to their nature (NR2A or NR2B) and location (synaptic versus extrasynaptic). This sheds new light on the functional role of extrasynaptic NR2B containing-NMDARs. These results are particularly important for a better understanding of certain pathological disorders associated with glutamatergic overactivity.

Key words: hippocampus, synaptic plasticity, NR2A and NR2B subunits, glutamate uptake, rat.

Long term potentiation (LTP) and long term depression (LTD) are the principal forms of synaptic plasticity that are thought to be cellular mechanisms of learning and memory (Whitlock et al., 2006; Massey and Bashir, 2007). *N*-methyl-D-aspartate receptors (NMDARs) play a fundamental role in brain function and especially in certain forms of synaptic plasticity. They are heteromers, composed of NR1 subunits associated with NR2 (NR2A, NR2B, NR2C or NR2D) or NR3 subunits (Moriyoshi et al., 1991; Monyer et al., 1992; Hollmann and Heinemann, 1994). In the adult hippocampus, NMDARs are mostly composed of NR1/NR2A and/or NR1/NR2B associations (Sheng et al., 1994; Luo et al., 1997). There is also some evidence that both NR2A and NR2B subunits together can combine with the NR1 subunits to form triheteromeric receptors such as NR1/NR2A/NR2B (Kirson and Yaari, 1996; Dingledine et al., 1999; Tovar and Westbrook, 1999). It is well established that the specific subunit composition of NR2A- and NR2B-containing receptors determines their biophysical properties (Erreger et al., 2005). Furthermore, the physiological roles played by NR2A and NR2B subunits may differ for several reasons: i) NR2A- and NR2B-containing NMDARs are considered to be preferentially synaptic and extrasynaptic respectively, even if they are expressed in both synaptic and extrasynaptic sites (Stocca and Vicini, 1998; Tovar and Westbrook, 1999; Thomas et al., 2006); ii) the different intracellular C-termini of the NR2 subunits serve as critical scaffolds for different complex intracellular signal transduction cascades (Sprengel et al., 1998); iii) NR2 subunits display distinct expression patterns which are different over the course of lifespan (Monyer et al., 1994; Petralia et al., 2005).

In the last few years, the contribution of the different NMDAR subunits in hippocampal synaptic plasticity (LTD and LTP) has been extensively studied. Whereas early studies found a difference in the potency of NR2A/NR2B antagonists vs. NR2C/NR2D antagonists to affect LTP vs. LTD, respectively (Hrabetova and Sacktor, 1997; Hrabetova et al., 2000), more recent studies have emphasized the distinction between NR2A and NR2B. Nevertheless, their respective participation in these phenomena remains an issue of debate. Indeed, several studies had shown that activation of NR1/NR2A receptors induced LTP while activation of NR1/NR2B receptors induced LTD (Hrabetova et al., 2000; Liu et al., 2004; Massey et al., 2004) while other studies using transgenic mice (Weitlauf et al., 2005) or pharmacological approaches (Fox et al., 2006; Bartlett et al., 2007; Berberich et al., 2007; Morishita et al., 2007) confirmed or refuted these results. It is conceivable that differences in species, strains, age, brain structures and protocols might account for these discrepancies. Here we studied the role of NR2A- and NR2B-containing NMDARs in the induction of LTD in adult rat hippocampi.

Two main protocols can induce NMDARs-dependent LTD: low frequency stimulation (LFS) (Dudek and Bear, 1993; Malenka, 1994) or bath application of *N*-methyl-D-

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Abbreviations: ACSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; CP-101,606, (1S,2S)-1-(4-hydroxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol; D-APV, D-2-amino-5-phosphonovalerate; D,L-tBOA, D,L-threo-beta-benzyloxyaspartic acid; fEPSP, field excitatory postsynaptic potential; LFS, low frequency stimulation; LTD, long term depression; LTP, long term potentiation; NMDA, *N*-methyl-D-aspartate; NMDAR, NMDA receptor; NVP-AAM077, (R)-[(S)-1-(4-bromo-phenyl)-ethylamino]-[(2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-5yl)-methyl]-phosphonic acid.

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aspartate (NMDA) (Chemical-LTD or Chem-LTD) (Lee et al., 1998). Both protocols induce AMPA receptor internalization and a decrease in synaptic strength (Beattie et al., 2000; Man et al., 2000; Carroll et al., 2001; Malenka and Bear, 2004). We have previously shown that these two methods result in forms of LTD that do not share common mechanisms since they respond differently to phosphatase (PP1/PP2A and calcineurin) inhibitors (Jouvenceau et al., 2003; Jouvenceau and Dutar, 2006); see also (Mulkey et al., 1994; O'Dell and Kandel, 1994; Kameyama et al., 1998).

Given the established role of intracellular signaling pathways in these two means of inducing LTD, this raises the question of whether NR2A and NR2B subunits are differently involved in these LTDs due to their different synaptic locations. Here, we show that NR2A-containing NMDAR subunits are involved in hippocampal LTD induction regardless of the protocol used, while the NR2B-containing NMDARs seem to modulate the LTD induction only when extrasynaptic NMDARs are activated.

EXPERIMENTAL PROCEDURES

Slice preparation for electrophysiology

All experimental procedures involving animals and their care conformed with INSERM committee guidelines and to the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize pain and suffering and to reduce the number of animals used.

Young adult (2–4 months old) Sprague–Dawley rats were anesthetized with halothane and decapitated. The hippocampus was quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (mM) NaCl 124, KCl 3.5, $MgSO_4$ 1.5, $CaCl_2$ 2.5, $NaHCO_3$ 26.2, NaH_2PO_4 1.2, glucose 11. Transverse slices (300–400 μm thick) were cut and placed in a holding chamber (at 27 °C) at least 1 h before recording. Each slice was individually transferred to a submersion-type recording chamber between two nylon holding nets, submerged with ACSF, and continuously superfused (flow rate of approximately 1.5 ml/min) and equilibrated with 95% O_2, 5% CO_2.

Electrophysiological recordings

Extracellular recordings were obtained at room temperature from apical dendritic layers of hippocampal CA1 area using glass micropipettes filled with 2 M NaCl and with a resistance of 2–6 MΩ. Field excitatory postsynaptic potentials (fEPSPs), mostly resulting from activation of AMPA receptors, were evoked by electrical stimulation (bipolar electrode) of CA1 afferent Schaeffer's collaterals and commissural fibers in the stratum radiatum. The intensity of test stimuli (100 μs duration) was adjusted to 50% of the maximum fEPSP response and applied every 10 s. The magnitude of the fEPSP was determined by measuring its slope. To obtain NMDAR-mediated fEPSPs, bicuculline (10 μM) and CNQX (10 μM) were bath applied, and the stimulus intensity was increased.

Electrically-induced LTD (LFS-LTD) was induced by application of LFS (1200 pulses, 2 Hz at test intensity), and chemical form of plasticity was induced by bath application of NMDA (20 μM for 5 min). In this case, the CA3 area was cut. Plasticity was measured for 45 min after LFS or chemical stimulation.

For the pharmacological studies, drugs were superfused through the bath 10 min before and during LFS stimulation or 15 min before and during NMDA application. For each pharmacological study, control conditions were systematically carried out on the same day.

All results are expressed as mean±S.E.M. The mean values of the synaptic plasticity were measured between the 35th and 45th minute after the end of the conditioning stimulation. The degree of statistical significance was calculated using one factor analysis of variance (ANOVA) or ANOVAs for repeated measured data.

Drugs

Drugs used in this study include: the NMDA (Sigma-Aldrich, St. Quentin Fallavier, France); the NR2B antagonists ifenprodil hemitartrate (Tocris, Illkirch, France) and (1S,2S)-1-(4-hydroxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol CP-101,606 (1S,2S)-1-(4-hydroxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol (gift from Dr. F. Menniti, Pfizer, Inc, Groton, CT, USA), the NR2A non selective but preferential antagonist NVP-AAM077 ((R)-[(S)-1-(4-bromo-phenyl)-ethylamino]-[(2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-5yl)-methyl]-phosphonic acid, gift from Dr. Y. P. Auberson, Novartis Pharma AG, Basel, Switzerland), the selective non-competitive NMDAR antagonist MK801 hydrogen maleate (Tocris) acting through blockade of the associated channel, the glutamate uptake inhibitor D,L-tBOA (D,L-threo-beta-benzyloxyaspartic acid, Tocris), the NMDAR antagonist D-APV (D-2-amino-5-phosphonovalerate, Tocris), the $GABA_A$ receptor antagonist bicuculline (Sigma, St. Louis, MO, USA), the AMPA receptor antagonist CNQX (6-cyano-7-nitroquinoxaline-2,3-dione, Tocris, Cookson, Bristol, UK) and the metabotropic glutamate receptor antagonist (RS)-MCPG ((RS)-α-methyl-4-carboxyphenylglycine, Tocris Cookson). All pharmacological agents were diluted directly in the incubation or superfusion medium from stock solutions prepared in distilled water or in dimethylsulfoxide (DMSO).

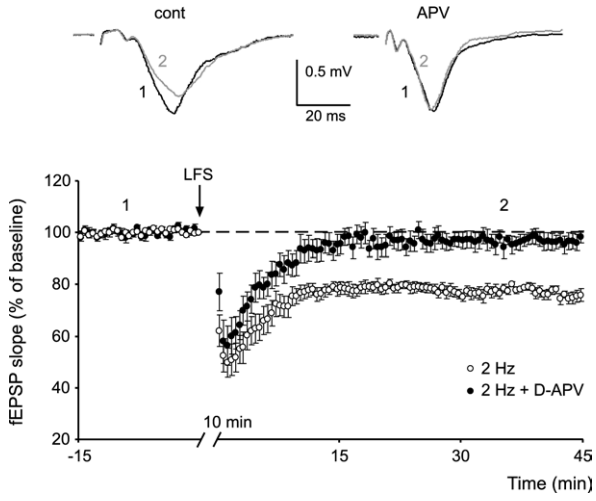

Fig. 1. Implication of NMDARs in LFS-LTD. Following a 15 min baseline period, LFS (LFS, 2 Hz for 10 min) was applied at the time indicated by the arrow. Recording is stopped during the 10 min conditioning stimulation and resumed after completion of LFS. LFS induced a depression of the fEPSP response indicated by the open circles (n=11 slices from nine rats). This LFS-LTD is antagonized by the NMDAR antagonist, D-APV (100 μM) (n=9 slices from seven rats, black circles). Top traces show superimposed individual fEPSP, recorded at time points 1 and 2 indicated on the response time course.