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AN N-METHYL-D-ASPARTATE-RECEPTOR DEPENDENT, LATE-PHASE LONG-TERM DEPRESSION IN MIDDLE-AGED MICE IDENTIFIES NO GLUN2-SUBUNIT BIAS

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Abstract—Late-phase long-term depression (L-LTD) in middle-aged mice has been difficult to achieve and maintain. Here we report an electrically induced, homosynaptic, input-specific form of LTD that could be stably maintained for at least 4 h in the CA1 area of hippocampal slices of 10–14 months old mice. This form of L-LTD was similar in magnitude in aged, middle-aged and young mice and was blocked by high concentrations of broad-spectrum N-methyl-D-aspartate receptor (NMDAR) antagonists such as D(−)-2-amino-5-phospho-pentanoic acid (D-AP5) and (R)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP). Extracellular and whole cell recordings revealed a decreased sensitivity to D-AP5 with age, without any differences in NMDAR conductance between the age groups tested. This L-LTD could be inhibited neither by common doses of NMDA-subunit specific antagonists like zinc, ifenprodil and Ro-25-6981, nor by various co-applications of these compounds. In addition to the lack of any GluN2 subunit bias, L-LTD did not show any discernible involvement of L-type voltage-gated calcium channels. In conclusion, our results do not support any specific role of NMDAR subunits in LTD.

Key words: long term depression, synaptic plasticity, hippocampal CA1-region, *in vitro*.

Most forms of long-term potentiation (LTP) and depression (LTD) in area CA1 of the rodent hippocampus depend on activation of the N-methyl-D-aspartate receptor (NMDAR). NMDAR-dependent forms of LTP and LTD share many of the characteristics associated with learning and memory, and play a role in developmental neuroplasticity (Martin et al., 2000; Cooke and Bliss, 2006). NMDAR is a glutamate-gated ion channel consisting of GluN1 and GluN2 subunits (for nomenclature see Collingridge et al., 2009). Alternative splicing can result in up to eight different GluN1 subunits from a single gene (Zukin and Bennett, 1995), whereas four different GluN2 genes yield at least four subunits (GluN2A–D) (Monyer et al., 1994; Dingledine et al., 1999). The functional NMDAR heteromer has been postulated to include two obligatory GluN1 subunits complexed with two or three GluN2 subunits. Since each of the GluN2 subunits has a unique sequence, particular NMDAR heteromeric assemblies have distinct electrophysiological properties and interactions with synaptic proteins and enzymes. For example, GluN2B but not GluN2A is coupled to the Ras signaling pathway (Cull-Candy et al., 2001; Krapivinsky et al., 2003; Kim et al., 2005; Ryan et al., 2008; Cull-Candy and Leszkiewicz, 2004; Köhr, 2006; Paoletti and Neyton, 2007) and GluN2B phosphorylation enhances NMDAR-dependent calcium influx (Carroll and Zukin, 2002).

The GluN2B subunit is the predominant GluN2 subunit in juvenile animals, whereas the GluN2A subunit dominate the NMDAR complex in adult hippocampus (Cull-Candy et al., 2001; Cull-Candy and Leszkiewicz, 2004; Köhr, 2006; Paoletti and Neyton, 2007). A primary example of this is in the hippocampus of older rats, where there is a two-fold ratio of GluN2A to GluN2B (Al-Hallaq et al., 2007). These developmental changes in NMDAR structure might explain the reported difficulties to achieve LTD in older relative to younger rodents using standard induction protocols (Bear and Abraham, 1996; Dudek and Bear, 1993; Kamal et al., 1998; Wagner and Alger, 1995; Kemp et al., 2000; Monyer et al., 1994; Magnusson, 1998; Dingledine et al., 1999; Kemp and Bashir, 2001; Milner et al., 2004; Billard and Rouaud, 2007). Studies with subunit specific pharmacological antagonists have led to the hypothesis that LTP is mediated by GluN2A containing NMDARs and LTD by GluN2B containing receptors (Liu et al., 2004; Massey et al., 2004; Fox et al., 2006; Bartlett et al., 2007). However, recent studies in young animals failed to produce conclusive evidence of subunit bias for one or the other form of synaptic plasticity (Izumi et al., 2006; Morishita et al., 2007).

In the present report, we describe a robust input-specific and NMDAR-dependent L-LTD in the hippocampal CA1 region of mice beyond 10 months of age. Using different GluN2 subunit-specific antagonists we found no evidence that L-LTD depends on the activation of specific GluNR2 subunits in aged mice.

EXPERIMENTAL PROCEDURES

In the studies detailed here, middle-aged C57/BL6 mice (10–14 months-old) of both sexes were tested for synaptic plasticity in the hippocampal CA1-region. Mice 6–8 weeks and 6–8 months old served as "young adult" and "adult," respectively, control groups.

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Abbreviations: ACSF, artificial cerebrospinal fluid; CPP, (R)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid; D-AP5, D(−)-2-amino-5-phospho-pentanoic acid; EPSCs, excitatory postsynaptic currents; fEPSP, field excitatory postsynaptic potentials; LFS, low frequency stimulation; L-LTD, late-phase long-term depression; LTD, long-term depression; LTP, long-term potentiation; NMDAR, N-methyl-D-aspartate receptor.

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The animals were maintained and experiments were in accordance with KUL Institutional, State and Government regulations.

Extracellular recordings

Animals were killed by cervical dislocation and the hippocampus was rapidly dissected out into ice-cold (4 °C) artificial cerebrospinal fluid (ACSF), oxygen saturated with carbogen (95% O_2 / 5% CO_2). ACSF consisted of (in mM): 124 NaCl, 4.9 KCl, 24.6 $NaHCO_3$, 1.20 KH_2PO_4, 2.0 $CaCl_2$, 2.0 $MgCl_2$, 10.0 glucose, pH 7.4. In a separate group of studies KH_2PO_4 was replaced with NaH_2PO_4, and the KCl reduced to 3.0 mM (see text). Transverse slices (400 μm thick) were prepared from the dorsal area of the right hippocampus with a tissue chopper and placed into a submerged-type chamber, where they were kept at 32 °C and continuously perfused with ACSF at a flow-rate of 2.2 ml/min. After about 90 min incubation, one slice was arbitrarily selected and two lacquer-coated monopolar stainless steel or tungsten electrodes were placed in CA1 stratum radiatum opposite one another to stimulate two independent inputs to the same neuronal population. For recording of field excitatory postsynaptic potentials (fEPSPs), a glass electrode (filled with ACSF, 3–7 MΩ) was placed in the stratum radiatum between the two stimulating electrodes (see Fig. 1A for schematic illustration). The time course of the field EPSP was measured as the descending slope function for all sets of experiments. The second independent input (termed S2) was to monitor basal synaptic transmission (Balschun et al., 2003; Ahmed and Frey, 2005). Its independence was tested by crossed paired-pulse facilitation (PPF) where paired pulses to S1 and S2 were applied at an interpulse interval of 50 ms, that is the first pulse was delivered to S1, the second to S2 and vice versa. After input/output curves had been established, the stimulation strength was adjusted to elicit a fEPSP-slope of 35% of the maximum and kept constant throughout the experiment. During baseline recording, three single stimuli (0.1 ms pulse width; 10 s interval) were measured every 5 min and averaged. For LTD induction, low frequency stimulation (LFS) consisting of 1500 pulses at 2 Hz (0.2 ms pulse-width) was applied for 10 min and repeated three times with a 10-min interval between completion of one LFS-train and the start of the successive one. This repetition of LFS protocol has been documented to be successful for the induction and maintenance of late-phase LTD in rats (Kerr and Abraham, 1995; Mockett et al., 2002) and mice (Balschun et al., 2003). LTP was induced by theta burst stimulation (TBS; 10 pulses of four stimuli at 100 Hz separated by 200 ms, 0.2 ms pulse-width; Larson and Lynch, 1986; Larson et al., 1986) repeated three times at 10 min intervals. Immediately after each conditioning stimulus, evoked responses were monitored at 2, 5 and 8 min and then subsequently every 5 min recording up to 4 h after the first conditioning stimulus.

Whole-cell recordings

Brains were isolated as above and transverse (400 μm thick) hippocampal slices were prepared from young (1 month) and middle-aged mice (12 months) with a vibratome (MIKROM HM 650V Microm Instruments GmbH, Wallsdorf, Germany) and stored at room temperature in a holding bath containing oxygenated ACSF. After a recovery period of at least 1 h, an individual slice was transferred to the recording chamber where it was continuously superfused with oxygenated ACSF at a rate of 2.5 ml/min. Whole-cell recordings from CA1 pyramidal neurons were measured with a two-channel patch-clamp amplifier (MultiClamp 700B, Axon Instruments, Molecular Devices, Inc., Sunnyvale, CA, USA). Excitatory postsynaptic currents (EPSCs) were evoked by stimulating the Schaffer collateral fibres through 150 μs constant-current test pulses delivered by a bipolar Pl-Ir electrode. Neuronal patching was under visual control by an infrared differential interference contrast optics system (Axioskop2 FC Plus, Zeiss Instruments, Jena, Germany). EPSCs were recorded from CA1 pyramidal cells in whole-cell mode at a holding potential of −60 mV with glass microelectrodes (3–6 MΩ) filled with a solution containing (in mM): 135.0 $CsMeSO_4$, 4.0 NaCl, 0.3 Na-GTP, 4.0 Mg-ATP, 0.5 EGTA, 10.0 K-HEPES, 5.0 QX-314; pH 7.24, osmolarity 284.0 mOsm. Since this intracellular solution contained Cs^+ to block most K^+ channels and QX-314 to block Na^+ channels, measurements were delayed for 10 min after the whole-cell configuration had been obtained to ensure that these blockers had time to reach distal dendrites. Signals were low-pass filtered at 2 kHz and

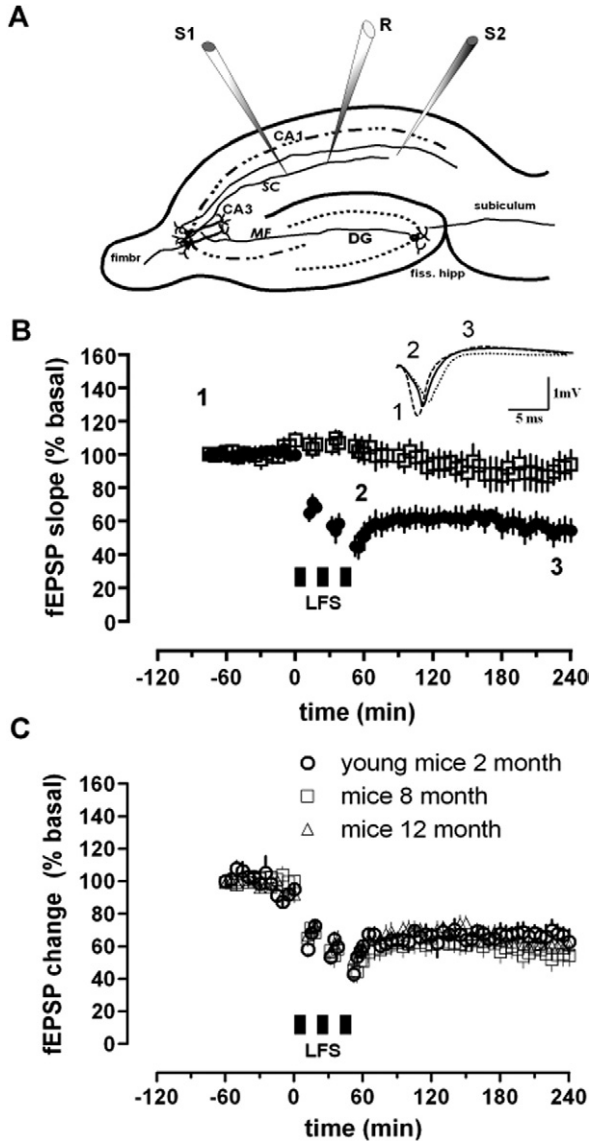

Fig. 1. Input-specific late-phase LTD in hippocampal slices of middle-aged mice. A. Scheme of electrode placement in the hippocampal slice, R = recording electrode (glass); S1, S2 = stimulating electrodes (metal). Two independent inputs (pathways) are stimulated. B. Late-phase LTD (L-LTD) in the conditioned pathway (filled circles), as compared with recordings in the control pathway (open squares). Insets give representative analogue traces from the conditioned pathway, numbers indicate when traces were sampled: (1) during baseline (full line), (2) 2 min after LTD induction (hatched line) and, (3) from the last 30 min of the experiment (hatched-dot line). C. Comparison of the magnitude of depression of L-LTD in 6–8 week-old (open circle), 6–8 month-old (open square) and 10–14 month-old mice (open triangle). Filled rectangles indicate trains of low-frequency stimulation (LFS).