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Activation of group I metabotropic glutamate receptors induces long-term depression in the hippocampal CA1 region of adult rats in vitro

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Abstract

Previous studies have implicated that long-term depression (LTD) was developmentally regulated since LTD can be readily induced by low frequency stimulation (LFS) in acute hippocampal slices prepared from juvenile but not adult animals. Here, we have examined the LTD induced by LFS (1 Hz, 900 pulses) paired with a certain pattern at the Schaffer collateral-CAl synapse in adult hippocampal slices. We found that, in the 90-day-old rat hippocampus, LTD could be induced reliably by LFS paired with stronger stimulus intensity than that used during baseline recording. However, this synaptic depression could be completely abolished by application of metabotropic glutamate receptor (mGluR) antagonist (*S*)-amethyl-4-carboxyphenylglycine (MCPG) which had no effect on that induced by the same protocol in the 16-day-old rat hippocampus. Furthermore, preincubation with group I mGluR antagonist, 2-methyl-6-(phenylethynyl)-pyridine (MPEP) and (*S*)-2-methyl-4-carboxyphenyl-glycine (LY367385), also completely prevented the LFS-induced LTD. In contrast, group II mGluR antagonist (2*S*)-a-ethylglutamic acid (EGLU), *N*-methyl-D-aspartate (NMDA) receptor antagonist APV and voltage-gated calcium channel antagonist nimodipine had no effect on the LFS-induced LTD. Taken together, these observations suggest that LFS paired with strong stimulus strength can efficiently induce group I mGluR-dependent LTD in the adult hippocampal CA1 region, proving insight into the functional significance of hippocampal mGluR-mediated LTD in learning and memory.

Keywords: Long-term depression; Developmentally; Group I metabotropic glutamate receptors; Adult rats; Hippocampus

1. Introduction

Long-term depression (LTD) and long-term potentiation (LTP) are the subject of intense investigation since it is believed that these forms of synaptic plasticity are involved in a variety of physiological functions, including learning and memory (Bliss and Collingridge, 1993; Bear and Abraham, 1996). In the centre nervous system (CNS), LTD is a type of synaptic plasticity defined as a long-term depression in synaptic transmission, which is usually induced by low frequency stimulation (LFS) protocols (Bear and Abraham, 1996). It is well known that LFS (typically 1–5 Hz) can induce de novo LTD of glutamatergic transmission in slices prepared from young (14–21 days old) rats (Dudek and Bear, 1992; Mulkey and Malenka, 1992). This form of LTD is generally dependent on activation of ionotropic *N*-methyl-D-aspartate receptors (NMDARs) (Dudek and Bear, 1992; Mulkey and Malenka, 1992), but not dependent on metabotropic glutamate receptors (mGluRs) (Selig et al., 1995; Fitzjohn et al., 1998). Moreover, the plasticity is developmentally regulated because homo-synaptic LTD cannot be reliably induced in acute hippocampal slices prepared from adult animals or in vivo preparations (Errington et al., 1995). That is, the stimulus protocols generally used to generate LTD in young animals are relatively ineffective in adult animals (Bashir and Collingridge, 1994; Errington et al., 1995; Abraham et al., 1996).

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Recently, however, several studies demonstrate mGluR-dependent LTD can be induced by LFS with a substantial improvement in the adult CA1 region in vitro and in vivo, such as a paired-pulse LFS protocol (Doyere et al., 1996; Kemp and Bashir, 1999; Kemp et al., 2000). Meanwhile, other protocols have also recently been demonstrated to induce LTD in the adult hippocampus (Kemp and Bashir, 1997a,b; Berretta and Cherubini, 1998; Kemp et al., 2000; Lante et al., 2006). In addition to LTD induced by electrical stimulation, long-lasting decreases in excitatory synaptic transmission in the adult hippocampus can also be induced by pharmacological activation of mGluRs by selective agonist, such as (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD), (*RS*)-3,5-dihydroxyphenylglycine (DHPG) and (*S*)-4-carboxy-3-hydroxyphenylglycine (4C3HPG) (O'Mara et al., 1995; Schnabel et al., 1999; Poschel and Manahan-Vaughan, 2005; Naie et al., 2007). Based on these findings a developmental down-regulation of LTD has been inferred, which also suggests that information can be stored as mGluR-mediated LTD-relevant synaptic changes in the adult hippocampus (Manahan-Vaughan and Braunewell, 1999).

On the other hand, a means of studying mGluR-dependent LTD is just to transiently activate mGluRs with a group I mGluR agonist, such as DHPG (Palmer et al., 1997; Camodeca et al., 1999; Fitzjohn et al., 2001; Mellentin and Abraham, 2001). This, so-called, DHPG-induced LTD occludes with synaptically induced mGluR-dependent LTD and is being used increasingly to investigate signaling and expression mechanisms of this type of synaptic plasticity (Huber et al., 2001). However, mGluR-dependent LTD still coexists with NMDAR-dependent LTD in many brain areas before researchers develop a new protocol to induce the LTD depending on mGluRs exclusively, which is restricted to investigate the molecular mechanisms and functions of mGluR-dependent LTD in deferent brain areas. In the present study, electrophysiological recordings of LTD of excitatory synaptic transmission were induced by a simple protocol in the adult hippocampal Schaffer-CA1 pathway. We demonstrate that, similar to DHPG-induced LTD, group I mGluR-dependent LTD, but not NMDAR-dependent LTD, can be readily induced when the stimulus intensity used during LFS (1 Hz, but not 3 Hz) is increased enough to evoke more than 80% of the maximum synaptic response.

2. Materials and methods

2.1. Slice preparation

We prepared hippocampal slices as described previously (Sun et al., 2005). In brief, 90- or 16-day-old rats (30 days and 45 days in some experiments) were decapitated under deep halothane anesthesia. Hippocampal formation was dissected rapidly and placed in ice-cold artificial cerebral spinal fluid (ACSF). Horizontally sectioned 400 μm thick hippocampal slices were cut and then transferred into a submersion-type chamber with ACSF for at least 1 h to allow their energetic and functional recovery. Then, the slice was gently transferred into a recording chamber and held submerged between two nylon nets and maintained at stable temperature (32 °C). The recording chamber consisted of a circular well of low volume (1–2 ml) and was perfused with ACSF at a flow rate of 4–5 ml/min. The ACSF which was saturated and continuously bubbled with the gas mixture (95% O_2 and 5% CO_2) contained (in mM): NaCl, 120; KCl, 2.5; $NaHCO_3$, 26; NaH_2PO_4, 1.25; $CaCl_2$, 2; $MgSO_4$, 1 and D-glucose, 10. The experimental protocols were approved by the Care and Use of Animals Committee of China Pharmaceutical University.

2.2. Stimulation and recording

The recording chamber was placed on the stage of an upright Nikon microscope equipped with a 10× objective and a 10× ocular which was used to identify the CA1 region of the hippocampus. For extracellular recordings, the pipette (2–3 MΩ resistance) filled with 3 M NaCl was placed at the stratum radiatum of the hippocampal CA1 region to record field excitatory postsynaptic potentials (fEPSPs), in response to the stimulation of Commissural/Schaffer fibers. Input/output curves were measured and the stimulus intensity used during baseline recordings was adjusted to evoke approximately 50% of the maximum fEPSP amplitude. After baseline recordings (10 min, one sweep per 30 s), LFS (1 Hz, 900 pulses), during which the stimulus intensity was adjusted to evoke more than 80% of the maximum fEPSP amplitude, was applied to induce LTD. Then, synaptic responses were recorded for 40 min with the same stimulus intensity as baseline.

Stimulating electrode was made by gluing together a pair of twisted Teflon-coated 90% platinum/10% iridium wires (50 μm inner diameter, 75 μm outer diameter), and recording pipette was pulled from borosilicate glass tubing (1.5 mm outer diameter, 0.84 mm inner diameter; World Precision Instruments, USA) with a Brown–Flaming micropipette puller (P-87, Sutter Instruments Company, USA).

2.3. Drugs

All reagents in the present experiments were obtained from Sigma Chemical Co. (St. Louis, MO, USA). In the preparation of stock solutions, 2-amino-5-phosphonovaleric acid (APV), 2-methyl-6-(phenylethynyl)-pyridine (MPEP) and DHPG were dissolved in distilled water, (*S*)-amethyl-4-carboxyphenylglycine (MCPG), L(*S*)-2-methyl-4-carboxyphenylglycine (LY367385) and (2*S*)-*a*-ethylglutamic acid (EGLU) in NaOH. The receptor antagonists APV, MCPG, EGLU, LY367385, MPEP and nimodipine were perfused for at least 1 h prior to application of LFS.

2.4. Data analysis and statistics

Recordings were made by using HEKA amplifier and signals were filtered at 5 kHz and digitized at 20 kHz and stored on computer. All slices tested were included in the average, regardless of whether or not they exhibited robust LTD. The average magnitudes of LTD were the last 5 min (35–40 min after LFS) fEPSPs slope values and expressed as mean ± S.E.M.% of the baseline fEPSPs levels. Each point in the figures represents the average slope value of three successive fEPSPs responses with Igor software. All values are presented as the mean ± S.E.M. and *n* refers to the number of slices. Statistical significance was determined as $P < 0.05$ and $P < 0.01$ using either Student's *t*-test or ANOVA.

3. Results

3.1. LTD could be induced readily in the adult hippocampal CA1 region

In the adult hippocampal Schaffer-CA1 pathway, the typical LFS (1 Hz, 900 pulses), during which the stimulus intensity is adjusted to evoke approximately 50% of the maximum fEPSP amplitude same to that used during baseline recording, failed to induce LTD of fEPSPs (97.9 ± 7.4% of baseline, $n = 7$; Fig. 1B). However, LTD could be reliably induced when the stimulus intensity during LFS was adjusted to evoke more than 80% of the maximum fEPSP response, although the stimulus intensity used during the baseline