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Research report

Repetition of mGluR-dependent LTD causes slowly developing persistent reduction in synaptic strength accompanied by synapse elimination

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Abstract

Synaptic plasticity, the cellular basis of memory, operates in a bidirectional manner. LTP (long-term potentiation) is followed by structural changes that may lead to the formation of new synapses. However, little is known whether LTD (long-term depression) is followed by morphological changes. Here we show that the repetitive induction of metabotropic glutamate receptor (mGluR)-dependent LTD in stable cultures of rat hippocampal slices led to a slowly developing persistent (ranging over weeks) reduction in synaptic strength that was accompanied by the loss of synaptic structures. LTD was induced pharmacologically 1–3 times at 24-h intervals by applying aseptically ACPD (1-aminocyclopentane-1,3-dicarboxylic acid), an agonist of group I/II mGluR, and APV (2-amino-5-phosphonovalerate), an antagonist of the NMDA (*N*-methyl-D-aspartate) receptor. One ACPD/APV application induced LTD that lasted less than 24 h. After three LTD inductions, however, a gradual attenuation of the fEPSP (field excitatory postsynaptic potential) amplitude and a decrease in the number of pre- and postsynaptic structures were observed. The blockade of LTD by an mGluR antagonist or a protein phosphatase 2B inhibitor abolished the development of the synaptic attenuation. In contrast to our previous finding that the repetitive LTP induction triggered a slowly developing persistent synaptic enhancement, the incremental and decremental forms of synaptic plasticity appeared to occur symmetrically not only on the minutes–hours time order but also on the days–weeks time order.

Theme: Neural basis of behaviour
Topic: Neural plasticity

Keywords: Electrophysiology; Hippocampus; LTD; Morphology; Synaptic elimination; Synaptic plasticity

1. Introduction

Synaptic plasticity, which involves activity-dependent changes in synaptic strength, is surmised to be the cellular basis of memory [6,13,16], and is generally classified into two phases, short and long term, corresponding to short- and long-term memory [2,13]. Short- and long-term plasticity is assumed to differ in terms of not only the rate of establishment (immediate vs. slow) and the period of maintenance (minutes–hours vs. days–weeks or even longer), but also the site of occurrence (change in strength of existing synapse vs. formation/elimination of synaptic structure per se) and the subcellular mechanism (posttranslational modification of existing molecules vs. transcriptional/translational regulation of genes). Although it is believed that the short-term plasticity is converted into the long-term one, the mechanism underlying the conversion is unknown. LTP (long-term potentiation), which involves the enhancement of synaptic strength induced by high-frequency stimulation of the mammalian hippocampus [3], includes both early posttranslational and late translational phases; thus, that analysis of the late LTP phase is expected to reveal the conversion process. Recently, it has been reported that some morphological changes take place during the establishment and maintenance of LTP, which may represent the conversion process [7,15,27].

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In contrast to LTP, there exists another form of synaptic plasticity in the hippocampus, LTD (long-term depression), which involves the reduction of synaptic strength induced by low-frequency stimulation [4]. It remains unclear, however, whether this form of plasticity is accompanied by morphological changes. It is only recently that the immediate withdrawal of dendritic spines after low-frequency stimulation of an input pathway has been reported [20,22,29]. Nevertheless, it is not known whether such long-lasting morphological changes as synaptic elimination take place after LTD induction.

For the long-term examination of such a long-lasting phenomenon, an experimental model that allows physiological, pharmacological, biochemical, and morphological analyses is required, as freshly prepared hippocampal slice that is used widely in synaptic plasticity analysis permits examinations for only several hours. The hippocampus in vivo allows for long-term examination but not a laboratory-designed manipulation of the neuronal environment or a detailed observation of the ongoing processes. We have recently used a stable culture of rat hippocampal slice for monitoring long-term physiological and morphological changes after LTP induction [23,26]. Repeated inductions, but not a single induction, of LTP in the cultured hippocampal slice evoked a slowly developing (i.e., requiring several days for development) and persistent (i.e., lasting more than 3 weeks) synaptic enhancement that was accompanied by an increase in the number of synaptic structures. It should be noted that this synaptic enhancement was not an extension of LTP: the LTP disappeared within 24 h, after which the novel synaptic enhancement developed gradually. The aim of the present study is to determine whether LTD induction in the cultured hippocampal slice evokes long-term synaptic changes both physiologically and morphologically. If the changes indeed occur, in what time course do they occur? Are they an extension of LTD or phenomena distinct from LTD? We show here a slowly developing persistent reduction in synaptic strength that is accompanied by a decrease in the number of synapses after repeated LTD inductions by metabotropic glutamate receptor (mGluR) activation, which is quite similar to the synaptic enhancement mentioned above, although the direction is opposite. A part of this study has been presented in an abstract form [24].

2. Materials and methods

2.1. Slice culture

Rat hippocampal slice culture was prepared following conventional methodology [5,25,26]. Briefly, whole brains were excised from ether-anesthetized Wistar/ST rats at postnatal day 7 and the hippocampi were isolated. Slices (400 μm thickness) were obtained from the central region of the hippocampi. The slices were placed on a polytetrafluoroethylene membrane filter (Millicell-CM®, Millipore) and culture medium (see below for composition) was added up to the bottom surface of the filter. The thus-prepared cultures were maintained at 34 °C in a humidified atmosphere for at least 2 weeks before an LTD induction (see Section 2.2), during which the thickness of the culture and viable cell number within became constant and voluntary synapse formation was settled [5,18,19,26]. After the LTD induction, the cultures were further maintained for 3 weeks, during which electrophysiological and morphological examinations were carried out. The culture medium contained 50% minimal essential medium supplemented with Hanks' salts, 25% Hanks' buffer and 25% heat-inactivated horse serum. The old culture medium was replaced twice a week with a new one during the entire culture period.

2.2. Electrophysiology

Extracellular recording of field excitatory postsynaptic potential (fEPSP) was performed in a conventional manner [10,11,18]. Briefly, a tungsten bipolar electrode was placed in the CA3 pyramidal cell layer. A glass microcapillary electrode filled with artificial cerebrospinal fluid (ACSF, see below for composition) for recording (5–10 MΩ in input resistance) was placed in the CA1 pyramidal cell layer, unless otherwise noted. The recorded potential was fed to a WPI amplifier (Model 707), digitized and stored. During examination, ACSF bubbled with 95% O_2/5% CO_2 was perfused continuously. To estimate the synaptic strength of a cultured slice, the maximal fEPSP amplitude was used, according to Muller et al. [18]. It is conventional in researches of acutely established synaptic plasticity to adopt the slope of the fEPSP recorded by an electrode placed in the hippocampal dendritic layer as the index of synaptic strength. In the case of long-term cultured slices, however, this index is not applicable. The slope varies markedly among the specimens, presumably due to the loosening of the input fibers. Comparison of the fEPSP slopes led us to postulate a priori the coherent onset of individual neurons' EPSPs (in other words, the uniformity of the conduction velocities of input fiber); however, this postulation does not hold in the cultured slices. As Muller et al. emphasized [18], the maximal fEPSP amplitude recorded by an electrode placed in the neuronal somatic layer is a better measure of the synaptic strength in the cultured slices than the slope. The maximal fEPSP amplitude was obtained at one recording site (in the central CA1 region) by gradually increasing the stimulus current until the fEPSP reached saturation (typically 0.2 to 0.3 mA). Then, the recording electrode was transferred to another site to obtain the maximal fEPSP amplitude there. This procedure was repeated more than three times for each slice, and the largest of the maximal fEPSPs was taken as the index of the synaptic strength of that slice. The test stimuli were delivered at a frequency as low as $\leq$0.05 Hz to avoid influencing the fEPSP. In the case