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ACTIVATION OF INOSITOL 1, 4, 5-TRISPHOSPHATE RECEPTORS DURING PRECONDITIONING LOW-FREQUENCY STIMULATION LEADS TO REVERSAL OF LONG-TERM POTENTIATION IN HIPPOCAMPAL CA1 NEURONS

Y. YAMAZAKI,[a] S. FUJII,[a]* T. AIHARA[b] AND K. MIKOSHIBA[c]

[a]*Department of Physiology, Yamagata University School of Medicine, Yamagata 990-9585, Japan*

[b]*Department of Software Science, College of Engineering, Tamagawa University, Machida, Tokyo 194-8610, Japan*

[c]*Laboratory for Developmental Neurobiology, Riken Brain Science Institute, Wako, Saitama 351-0198, Japan*

Abstract—We investigated the role of inositol 1, 4, 5-trisphosphate receptors (IP3Rs) that were activated during preconditioning low-frequency afferent stimulation (LFS) in the subsequent induction of synaptic plasticity in CA1 neurons in hippocampal slices from mature guinea pigs. In standard perfusate, long-term potentiation (LTP) was induced in the field excitatory postsynaptic potential (EPSP) by the delivery of LFS (80 pulses at 1 Hz), and was reversed by an identical LFS applied 20 min later. However, when CA1 synapses were preconditioned in the presence of an IP3R antagonist and stimulated by the second LFS in the absence of the antagonist, LTP was not reversed, but was increased, by the second LFS. In addition, when CA1 synapses were preconditioned in standard solution, but stimulated by the second LFS in the presence of an *N*-methyl-D-aspartate receptor (NMDAR) antagonist, LTP was again not reversed, but increased. The excitatory postsynaptic current (EPSC) through NMDARs recorded from CA1 pyramidal neurons increased significantly 20 min after a single LFS and this increase was inhibited when the LFS was delivered in the presence of an IP3R antagonist or a Ca^{2+}/calmodulin-dependent protein kinase II inhibitor. These results suggest that activation of IP3Rs by a preconditioning LFS results in postsynaptic protein phosphorylation and/or enhancement of NMDAR activation during a subsequent LFS, leading to reversal of LTP in the field EPSP in hippocampal CA1 neurons.

Key words: LTP, depotentiation, IP3 receptors, metabotropic glutamate receptors, hippocampus.

*Corresponding author. Tel: +81-236-28-5219; fax: +81-236-28-5221.
E-mail address: sfujii@med.id.yamagata-u.ac.jp (S. Fujii).

Abbreviations: AIDA, 1-aminoindan-1,5-dicarboxylic acid; AMPARs, α-amino-hydroxy-5-methyl-4-isoxazolepropionic acid type glutamate receptors; AP5, 2-amino-5-phosphonopropionic acid; A-PS, amplitude of the PS; DMSO, dimethylsulfoxide; EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; HFS, high-frequency stimulation; IP3, inositol 1, 4, 5-trisphosphate; IP3Rs, inositol 1, 4, 5-trisphosphate receptors; LFS, low-frequency afferent stimulation; LTP, long-term potentiation; MCPG, (RS)-α-methyl-4-carboxyphenylglycine; NMDAR, *N*-methyl-D-aspartate receptor; PLC, phospholipase C; PPF, pulse facilitation; PPI, paired-pulse inhibition; PPS, paired-pulse stimulation; PS, population spike; PTX, picrotoxin; S-EPSP, slope of the field EPSP; 2-APB, 2-aminoethoxydiphenyl borate; 4-CPG, (S)-4-carboxyphenylglycine.

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Prior synaptic activity can influence the subsequent induction of synaptic plasticity in the hippocampus. In our previous studies, we reported two types of synaptic plasticity in hippocampal CA1 neurons, which we named "depotentiation" in which low-frequency afferent stimulation (LFS) at 1–2 Hz reverses a pre-established long-term potentiation (LTP) (Fujii et al., 1991, 1999), and "LTP suppression" in which LFS at 1 Hz given before the delivery of high-frequency stimulation (HFS) suppresses LTP induction (Fujii et al., 1991, 1996, 2000a).

Activation of N-methyl-D-aspartate receptors (NMDARs) before delivery of HFS inhibits LTP induction at CA1 synapses, whereas activation of NMDARs before delivery of LFS facilitates formation of long-term depression (LTD) (Huang et al., 1992; Mockett et al., 2002). Bortolotto et al. (1994) reported that activation of metabotropic glutamate receptors (mGluRs) during preconditioning HFS overcomes the need for mGluR stimulation during subsequent LTP induction in hippocampal CA1 neurons. Wu et al. (2004) showed that depotentiation induced by LFS is dependent on the activation of group 1 mGluRs during induction of LTP at CA1 synapses. Thus, activation of NMDARs or mGluRs is suggested to be required for priming stimuli to exert their influence during the subsequent induction of synaptic plasticity at CA1 synapses, but the mechanism is still unknown.

Stimulation of group 1 mGluRs in hippocampal CA1 neurons activates phospholipase C (PLC), which hydrolyzes the inositol lipid precursor in the postsynaptic plasma membrane to inositol 1, 4, 5-trisphosphate (IP3), which opens IP3 receptor channels (IP3Rs), and diacylglycerol, which activates protein kinase C (Ben-Ari et al., 1992; Nakanishi, 1992). IP3Rs act as IP3-gated Ca^{2+} release channels in a variety of cells (Berridge, 1993; Mikoshiba, 1993). The type 1 IP3R1 is the major neuronal member of the IP3R family in the central nervous system and is predominantly enriched in cerebellar Purkinje cells and hippocampal CA1 neurons (Furuichi et al., 1993; Mikoshiba et al., 2003). Using IP3R1-deficient mice produced by gene targeting (Matsumoto et al., 1996), we investigated the role of the IP3R1 in depotentiation or LTP suppression in hippocampal CA1 neurons and demonstrated that both effects were attenuated in the mutant mice in which the mean magnitude of the responses after delivery of LFS or HFS was significantly greater than in wild-type mice (Fujii et al., 2000b; Mikoshiba et al., 2003). Recently, we found that the lack of IP3R1 in these mice causes a slower decay

in the transient intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in CA1 pyramidal neurons and attenuates the activity of inhibitory interneurons, resulting in enhancement of LTP induction (Yoshioka et al., 2010).

The results of these studies on depotentiation or LTP suppression in CA1 neurons suggest that the activation of group 1 mGluRs and/or IP3R1s during preconditioning stimulation is involved in the three suggested mechanisms for the induction of subsequent synaptic plasticity in hippocampal CA1 neurons, namely (i) reversal of LTP or suppression of LTP induction because of a reduction in presynaptic neurotransmitter release, (ii) enhancement of local inhibitory circuits in the hippocampal CA1 region after delivery of preconditioning stimulation, and (iii) increased postsynaptic dephosphorylation of α-amino-hydroxy-5-methyl-4-isoxazolepropionic acid type glutamate receptors (AMPARs) after LFS or HFS, all three leading to reversal, or suppression, of LTP in CA1 neurons.

Recently, we reported that, in hippocampal CA1 neurons, LFS of 80 pulses at 1 Hz induces LTP in hippocampal CA1 neurons, which is independent of mGluR activation, but involves NMDAR activation, whereas an identical LFS combined with activation of mGluRs induces LTD (Fujii et al., 2010). We also found that LTP induced at CA1 synapses by the delivery of LFS of 80 pulses at 1 Hz is reversed by an identical LFS applied 20 min later. In the present study, we examined the three possible mechanisms by which a priming LFS of 80 pulses at 1 Hz could affect the subsequent induction of synaptic plasticity at CA1 synapses.

In the present study, two stimuli were applied to the input pathway of CA1 synapses at intervals of 10–35 ms (paired-pulse stimulation, PPS), inducing paired-pulse inhibition (PPI) or paired-pulse facilitation (PPF) of the population spike (PS) elicited by the second of the paired stimuli; in the case of PPI, the amplitude of the second PS is smaller than that of the first, whereas, in the case of PPF, it is larger. We investigated whether there was a change in PPF or PPI after induction of depotentiation, as an increase in PPF would indicate a decrease in the probability of release of neurotransmitter from presynaptic terminals (Manabe et al., 1993; Gerges et al., 2003), whereas an increase in PPI would indicate increased inhibitory synaptic transmission in the local inhibitory circuit in the hippocampal CA1 region (Davies et al., 1991; Grunze et al., 1996; Freund and Busaki, 1996). We used a pharmacological approach to study the role of group 1 mGluRs, IP3Rs, some protein kinases, and a protein phosphatase activated during the preconditioning LFS or the second LFS in the depotentiation of CA1 neurons. Based on the results, the mechanism of the preconditioning-dependent modification of synaptic plasticity is discussed.

EXPERIMENTAL PROCEDURES

Slice preparation

Adult male Hartley guinea pigs (4- to 6-weeks-old, SLC Inc., Hamamatsu, Japan) were killed by decapitation. The hippocampi were quickly removed and cut into transverse slices 500-μm thick for extracellular field recordings or 400-μm thick for whole-cell recordings using a rotor slicer (Dosaka DK-7700, Kyoto, Japan). The slices were placed in a 100 ml chamber and preincubated at 30–32 °C for a minimum of 1 h in a 95% O_2/5% CO_2 atmosphere in artificial cerebrospinal fluid (ACSF) containing, in mM, the following: NaCl 124, KCl 5, NaH_2PO_4 1.25, $MgSO_4$ 2, $CaCl_2$ 2.5, $NaHCO_3$ 22, glucose 10, oxygenated with 95% O_2 and 5% CO_2.

Extracellular recordings

The slices were placed in a recording chamber (0.3–0.4 ml capacity) and submerged in oxygenated ACSF and continuously perfused at 2–3 ml/min with the same solution at 30 °C. A bipolar tungsten stimulating electrode was placed in the stratum radiatum to stimulate the input pathways to the CA1 neurons. A glass pipette recording electrode was positioned in the stratum radiatum and another in the pyramidal cell body layer of the CA1 region to record the field excitatory postsynaptic potential (EPSP) and the PS, respectively, and a biphasic test stimulus (100 μs duration) was applied every 20 s with a pulse duration of 0.1 ms. The initial slope of the field EPSP (S-EPSP) or the amplitude of the PS (A-PS) was measured and plotted automatically to evaluate changes in these parameters. At the beginning of each experiment, the strength of the stimulus pulse was adjusted to elicit a field EPSP or a PS with an initial slope or amplitude 40–60% of maximal and was then fixed at this level.

After checking the stability of the responses to a test stimulus given at 20 s intervals, an LFS consisting of 80 pulses at 1 Hz was delivered either once or twice at an interval of 20 min. To evaluate changes in the response, the mean value of the S-EPSP or the A-PS in the 10-min period before the delivery of the first LFS to naive slices was defined as the control level (100%) and the mean response at 60 min after the end of the first LFS expressed as a percentage of the control level. LTP in the S-EPSP or the A-PS was defined as a value greater than 120% of the baseline value.

At 10 min before, or at 15–20 min after, delivery of the preconditioning LFS or at 35–40 min after delivery of the second LFS, PPS was applied to the input pathway of the CA1 neurons at an interval of 10, 15, 20, 25, 30, or 35 ms in the absence or presence of 2 μM picrotoxin (Fig. 2A, B). PPI or PPF is presented as the second A-PS expressed as a percentage of the first A-PS (Fig. 2C).

When used, mGluR or IP3R inhibitors were applied from 5 min before the start of the first LFS to the end of the first LFS, then the medium was replaced with standard solution, and the effect of the inhibitor evaluated by comparing the S-EPSPs at 60 min after the end of the first LFS in the presence or absence of the drug. In some experiments, an NMDAR inhibitor, an IP3R inhibitor, or a Ca^{2+}/calmodulin-dependent protein kinase II inhibitor was applied from 5 min before the start of the second LFS to the end of the second LFS. When a phosphatase inhibitor was used, it was applied from 10 min before the start of the second LFS to 10 min after the end of the second LFS.

Whole-cell recordings

For whole-cell recordings, pyramidal cells were visualized using a 40× water-immersion objective and differential interference contrast system under infrared light (Nikon, Tokyo, Japan). Patch electrodes were pulled from borosilicate glass using a micropipette puller. The pipettes had a resistance of 3–7 MΩ when filled with pipette solution (140 mM K-gluconate, 10 mM HEPES, 0.5 mM EGTA, 10 mM NaCl, 1 mM $MgCl_2$, 2 mM Mg-ATP, 0.2 mM Na-GTP, and 5 mM QX-314, adjusted to pH 7.3 with KOH). NMDAR-mediated synaptic currents were recorded following delivery of test stimuli at 0.033 Hz in the presence of 20 μM 6, 7-dinitro-quinoxaline-2, 3-dione (DNQX), a non-NMDAR inhibitor, and 10 μM bicuculline, a $GABA_A$ receptor inhibitor. The recorded