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ACTIVATION OF INOSITOL 1,4,5-TRISPHOSPHATE RECEPTORS DURING PRECONDITIONING LOW-FREQUENCY STIMULATION SUPPRESSES SUBSEQUENT INDUCTION OF LONG-TERM POTENTIATION IN HIPPOCAMPAL CA1 NEURONS

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Abstract—We investigated the role of inositol 1,4,5-trisphosphate receptors (IP_3Rs) activated during preconditioning low-frequency stimulation (LFS) in the subsequent high-frequency stimulation (HFS)-induced induction of long-term potentiation (LTP) in CA1 neurons in hippocampal slices from mature guinea pigs. Induction of LTP in the field excitatory postsynaptic potential (EPSP) or the population spike (PS) by delivery of HFS (a tetanus of 100 pulses at 100 Hz) to the Schaffer collateral–commissural pathway to CA1 neuron synapses was suppressed when the CA1 synapses were preconditioned by LFS of 1000 pulses at 1 Hz. This effect was inhibited when the preconditioning LFS was applied in the presence of an *N*-methyl-D-aspartate receptors (NMDARs) antagonist, a metabotropic glutamate receptor (mGluR) antagonist, IP_3R antagonist, a calmodulin-dependent kinase II inhibitor or a calcineurin inhibitor. Furthermore, blockade of group I mGluRs immediately before the delivery of HFS blocked the inhibitory effect of the preconditioning LFS on subsequent induction of LTP by HFS. These results suggest that, in hippocampal CA1 neuron synapses, co-activation of NMDARs and IP_3Rs during a preconditioning LFS results in both phosphorylation and dephosphorylation events that lead to prolonged activation of group I mGluRs that is responsible for the failure of LTP induction.

Key words: preconditioning, IP_3 receptors, CA1 synapses, LTP, LTP suppression, calcineurin.

INTRODUCTION

Activity-dependent modification of synaptic efficacy is fundamental to the storage of information in the brain. Long-term potentiation (LTP) in the hippocampus is a long-lasting change in synaptic efficacy and is thought to play an important role in learning and memory (Bliss and Collingridge, 1993). The high-frequency stimulation (HFS)-induced LTP at CA1 synapses is generally believed to be triggered by the influx, during HFS, of Ca^{2+} into the postsynaptic neuron through channels coupled to *N*-methyl-D-aspartate glutamate receptors (NMDARs) and this increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) activates Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) to phosphorylate the GluA1 subunit of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) in postsynaptic neurons (Alford et al., 1993; Bliss and Collingridge, 1993).

Activation of metabotropic glutamate receptors (mGluRs) can facilitate the HFS-induced LTP in CA1 neurons (Behnisch and Reymann, 1993) and is, therefore, believed to be a significant functional component of the cellular mechanisms of LTP formation in hippocampal CA1 neurons (Bashir et al., 1993). Stimulation of group I mGluRs on hippocampal neurons activates phospholipase C, which hydrolyzes the inositol lipid precursor in the postsynaptic plasma membrane into inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol, the former opening IP_3 receptors (IP_3Rs) and the latter activating protein kinase C (PKC) (Ben-Ari et al., 1992; Nakanishi, 1992). IP_3Rs act as IP_3-gated Ca^{2+} release channels in a variety of cells (Berridge, 1993; Mikoshiba, 1993).

The type 1 IP_3R (IP_3R1) is the major IP_3R family member in the central nervous system and is predominantly enriched in cerebellar Purkinje cells and hippocampal neurons (Furuichi et al., 1989; Nakanishi et al., 1991). Activation of IP_3Rs during, or after, HFS is involved in the mechanism of LTP induction in hippocampal CA1 neurons (Nagase et al., 2003; Yoshioka et al., 2010). We previously demonstrated that LTP induction in hippocampal CA1 neurons is facilitated in IP_3R1-lacking mice (Fujii et al., 2000b). In addition, bath

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Abbreviations: 2-APB, 2-aminoethoxydiphenyl borate; 4-CPG, S-4-carboxyphenylglycine; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; AP5, D,L-2-amino-5-phosphonovalerate; A-PS, amplitude of the PS; CaMKII, calmodulin-dependent protein kinase II; EPSP, excitatory postsynaptic potential; HFS, high-frequency stimulation; IP_3Rs, inositol 1,4,5-trisphosphate receptors; IRBIT, IP_3R binding protein released with IP_3; LFS, low-frequency stimulation; LTP, long-term potentiation; MCPG, (RS)-α-methyl-4-carboxyphenylglycine; mGluR, metabotropic glutamate receptor; NMDARs, *N*-methyl-D-aspartate receptors; PKC, protein kinase C; PPF, paired-pulse facilitation; PPI, paired-pulse inhibition; PPS, paired-pulse stimulation; PS, population spike; S-EPSP, slope of the field EPSP.

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application of 2-aminoethoxydiphenyl borate (2-APB), an IP_3R antagonist (Peppiatt et al., 2003), or of α-methyl-4-carboxyphenylglycine (MCPG), a wide-spectrum mGluR antagonist (Pin and Duvoisin, 1995), during brief HFS (10 or 15 pulses at 100 Hz) significantly increases the magnitude of the LTP in hippocampal CA1 neurons (Taufiq et al., 2005). These results suggest that IP_3R1 plays an important role in suppressing LTP induction in hippocampal CA1 neurons.

In our previous studies (Fujii et al., 1991, 1996, 2000a), we reported another type of synaptic plasticity in hippocampal CA1 neurons that we refer to here as "LTP suppression", in which a train of low-frequency stimulation (LFS) given prior to the delivery of HFS (100 pulses at 100 Hz) suppresses LTP induction. In one of our studies on LTP suppression in hippocampal CA1 neurons (Fujii et al., 1996), we demonstrated a suppressive effect of a preconditioning LFS of 200 or 1000 pulses at 1 Hz on HFS-induced LTP in the field responses and that this suppressive effect was significantly reduced when the LFS at 1 Hz consisted of fewer pulses (80 pulses) or when the time lag between LFS and HFS was shorter (20 min) or longer (100 min). We also demonstrated that the suppression of LTP induction by a preconditioning LFS (1000 pulses at 1 Hz) given 60 min before HFS was attenuated in hippocampal CA1 neurons in IP_3R1-deficient mice (Fujii et al., 2000b). Since LFS of more than 200 pulses at 1 Hz activates IP_3Rs at CA1 synapses (Fujii et al., 2010), these results suggest that activation of IP_3Rs during LFS of 200 or 1000 pulses at 1 Hz is involved in the mechanism responsible for the LTP suppression. Although activation of IP_3Rs during a preconditioning LFS results in dephosphorylation events that lead to a failure of HFS to induce subsequent LTP in mossy fiber-CA3 synapses (Yamazaki et al., 2012), it is not known how activation of IP_3R during LFS contributes to LTP suppression at Schaffer colaterals-CA1 synapses. In the present study, we investigated the role of IP_3Rs in the induction of LTP suppression in the hippocampal CA1 region of mature guinea pigs.

EXPERIMENTAL PROCEDURES

Slice preparation

The animals used were maintained and handled following the guidelines of the Animal Care and Use Committee of the Yamagata University School of Medicine. Male Hartley guinea pigs (4- to 6-week-old, Funabashi Farm Co., Tokyo, Japan) were decapitated and the hippocampi rapidly removed and cut into 500-μm thick transverse slices using a tissue slicer with a round rotary blade (Dosaka DK-7700, Kyoto, Japan). The slices were preincubated for a minimum of 1 h at 30 °C in a 95% O_2/5% CO_2 atmosphere in standard perfusion solution [(mM) NaCl, 124; KCl, 5.0; NaH_2PO_4, 1.25; $MgSO_4$, 2.0; $CaCl_2$, 2.5; $NaHCO_3$, 22.0; and D-glucose, 10.0] before being placed in a 1-ml recording chamber and completely submerged in standard solution and perfused continuously at a rate of 2–3 ml/min, the temperature in the recording chamber being maintained at 30 °C.

Electrophysiology

A bipolar stimulating electrode was placed in the stratum radiatum to stimulate the input pathways to the CA1 neurons. One recording electrode was positioned in the stratum radiatum and another in the pyramidal cell body layer of the CA1 region to record, respectively, the field EPSP and the PS and a test stimulus was applied every 20 s with a pulse duration of 0.1 ms. The slope of the field EPSP (S-EPSP) and the amplitude of the PS (A-PS) were measured and plotted automatically. The experimental protocol used in this study was as follows. At the beginning of each experiment, the strength of the stimulus pulse was adjusted to elicit a field EPSP with an initial slope 40–60% of maximal and was then fixed at this level, then, after checking the stability of the S-EPSP for more than 15 min, a conditioning stimulus of tetanus or LFS was delivered to induce synaptic plasticity at CA1 neurons.

To induce LTP, HFS consisting of one train of 100 pulses at 100 Hz (tetanus) was used (Fig. 1A), while, to suppress the induction of LTP, LFS consisting of 1000 pulses at 1 Hz was applied 60 min before HFS (Fig. 1B). The mean value of the S-EPSP or the A-PS during the 10 min immediately before delivery of LFS or HFS was defined as the 100% level and other responses expressed as mean percentage ± S.E.M. of this level with n, the number of slices tested. To evaluate the suppressive effects of the preconditioning LFS on LTP induced by subsequent HFS delivery, the mean value of the S-EPSP or the A-PS in the 5-min period before the delivery of HFS was defined as the 100% control level and the mean magnitude of LTP of the S-EPSP and A-PS was measured over the period 55–60 min after the HFS.

In paired-pulse stimulation (PPS) studies, at 10 min before, or 45–50 min after, delivery of LFS or at 45–50 min after delivery of HFS, PPS was applied to the input pathway of the CA1 neurons at an interval of 10 or 30 ms to induce, respectively, paired-pulse inhibition (PPI) or paired-pulse facilitation (PPF) of the A-PS elicited by the second of the paired stimuli. In this study, the PPI or PPF was expressed as the A-PS produced by the second pulse expressed as a ratio of that produced by the first pulse.

D,L-2-amino-5-phosphonovalerate (AP5), (RS)-MCPG, and S-4-carboxyphenylglycine (4-CPG) were purchased from Tocris Cookson Ltd. (Bristol, UK), while 2-APB, picrotoxin, FK506, and KN62 were obtained from Sigma (St. Louis, MO, USA). In most studies, these drugs were applied from 5 min before, and during, the preconditioning LFS, but, in one study, 4-CPG was applied from 55 min to 60 min after the preconditioning LFS (i.e. during the 5 min immediately before HFS). Results were analyzed for statistical significance using Mann–Whitney taking a p value < 0.05 as significant.

RESULTS

Effects of a preconditioning LFS on the HFS-induced LTP

In hippocampal CA1 neurons perfused with standard perfusion solution containing 2.5 mM Ca^{2+}, LTP was