The Effect of Prior Prolonged Low Frequency Stimulation on the Further Synaptic Plasticity at Hippocampal CA1 Synapses

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Abstract

The objective of this study is to determine the role of prior prolonged low frequency stimulation (900 pulses at 1 Hz) on the further induced long-term potentiation (LTP) and depression (LTD) of synaptic activity in the rat hippocampal CA1 area. Hippocampal slices and standard extracellular field potential recording techniques were employed. LTP and LTD were induced using stimulation at 5 Hz (900 pulses) paired with or without simultaneous application of 1 μM isoproterenol respectively, at either normal CA1 synapses or CA1 synapses that were pre-conditioned with prolonged low frequency stimulation at 1 Hz. LTD could be successfully induced upon 900 pulses of stimulation given at 5 Hz at normal synapses (82.1 ± 2.9%; n = 5); it was, however, reduced to 96.5 ± 4.7% (n = 6) at the pre-conditioned synapses. When paired with application of isoproterenol, 900 pulses of stimulation given at 5 Hz produced LTP (139.9 ± 9.6%, n = 5) at normal synapses. The magnitude of LTP is decreased to (130±13.2%) (n = 6) at pre-conditioned synapses, though the difference is not significant. These results suggest that at a given CA1 synapses the expression of LTP and LTD is dependent on their history of use.

Key Words: LTP, LTD, isoproterenol, hippocampal slices, rats

Introduction

Long term potentiation (LTP) and long term depression (LTD) are use-dependent change in synaptic efficacy that have been attracted considerable attraction as a possible cellular mechanism underlying memory storage (2, 4, 17) and development of cortical architecture (15) in the central nervous system. The induction of LTP and LTD share some common mechanisms, for example, they both involve the activation of N-methyl-D-aspartate (NMDA) subtype of glutamate receptors (2). A crucial factor that determines whether the synaptic activity would be potentiated or depressed is the cytoplasm concentration of calcium elevated via activation of NMDA receptors during the conditioned stimulation for LTP and LTD induction (2). High level of calcium concentration elevation caused by a brief burst of high frequency stimulation, e.g. 100 Hz, activates calcium-calmodulin dependent protein kinase, which in turn facilitates the function of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) subtype of glutamate receptors by phosphorylating them and leads to expression of LTP (18). Low level of calcium concentration elevation caused by a prolonged low frequency stimulation, e.g. 1 Hz, activates pho-

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sphatases, which in turn inhibits the function of AMPA receptors by dephosphorylating them and leads to LTD (18). Therefore, a modification threshold, θ*m*, for synaptic response to undergoing potentiation or depression upon conditioned stimulation was proposed (2, 3).

The θ*m* of given synapses is not set to a fixed value, rather it can be changed in an activity-dependent manner (14). It is proposed that previous high level of synaptic activity decreases the likelihood of LTP and increases that of LTD (3). This argument has been recently supported by experimental data (14, 16). In this study, we wish to examine the effect of other possibility, i.e. previous decreased synaptic efficacy, on the likelihood of LTP and LTD at hippocampal CA1 synapses.

Materials and Methods

The use of animals in this study was accordance with the guidelines of the local ethical committee for animal research. Male Sprague-Dawley rats (100-125 g) were anesthetized with halothane, and decapitated. The brains were quickly removed and placed in ice-cold artificial cerebral spinal fluid (ACSF), containing (mM) NaCl 119, KCl 1.2, $NaHCO_3$ 26.2, NaH_2PO_4 1, $MgSO_4$ 1.3, $CaCl_2$ 2.5, glucose 11; pH were adjusted to 7.4 by gassed with 5% CO_2+95% O_2. Transverse hippocampal slices of 400 μm thickness were cut with a vibrating tissue slicer (Campden Instruments, UK), transferred to an interface type holding chamber at room temperature (28 °C). For extracellular field potential recording, slices were transferred to an immersion type recording chamber, perfused with ACSF containing 0.1 mM picrotoxin at 31-33 °C. The perfusion rate was controlled at 2 ml/min. To prevent epileptiform discharge of pyramidal neurons, a surgical cut was made at the border between CA1 and CA3 areas. Glass pipette filled with 3 M NaCl was positioned at stratum radiatum of CAl area to record field excitatory postsynaptic potential (fEPSP), which was elicited upon stimulation to Schaffer collateral branches by positioning one bipolar stainless steel (FHC, USA) electrodes near the recording pipette every 30 seconds. The intensity of stimulation was adjusted so that 40-50% of maximal response was elicited. LTP was induced by a train of 900 pulses stimulation at 5 Hz paired with simultaneous application of 1 μM isoproterenol. LTD was induced by long stimulus train comprising 900 pulses at 1Hz for pre-conditioned of CA1 synapses or at 5Hz for comparison with LTP. All signal were filtered at 2 kHz, and digitized at 5kHz using CED micro interface running Signal software provided by CED (Cambridge Electronic Design, UK). All drugs were bath applied. They were purchased from Sigma, except that the D, L-APV was from Tocris (UK). The initial fEPSP slope was measured; LTP and LTD were calculated as normalizing slope of fEPSP recorded 25-30 minutes after conditioned stimulation to the base line activity. All data given were Mean ± standard error, and were statistically compared by either paired t-test or one way ANOVA test. The criterion for significance was $p < 0.05$.

Results

Following a period of stable recording of the base line, the fEPSP slope was reduced to 80.1% of the baseline upon delivery of the low frequency stimulation (900 pulses at 5 Hz); this depression of fEPSP slope could last for at least 30 minutes (F1A; filled circles). Similar results were obtained from 5 out of 5 slices tested; the averaged results revealed a small but highly significant LTD (82.1 ± 2.9%, n = 5; $p < 0.005$, paired t-test; Fig. 1B, filled circle). Low frequency stimulation (5 Hz) paired with bath application of 1 μM isoproterenol, however, potentiated the slope of fEPSP that could last for at least 30 minutes (F1A, open circles). The averaged LTP determined from 5 identical experiments was 5k8.2 ± 6.2% ($p < 0.005$, paired t-test; Fig. 1C, open circles). LTP induced by this protocol was blocked when 50 μM D, L-APV was included in the bath to block the NMDA receptors (Fig. 1C). These results suggest that low frequency stimulation paired with activation β-adrenergic receptors can induce LTP that is NMDA dependent. Bath application of 1 μM isoproterenol alone could enhance fEPSP slope of the base line (139.9 ± 9.6%, $p < 0.05$); this effect of isoproterenol on synaptic transmission has also been previously reported at CA1 and many other CNS synapses (6, 8,12, 19).

In the next serial of experiments we first induced LTD using 1 Hz stimulation (900 pulses) at given CA1 synapses, then we examined the effect of this pre-conditioning procedure on LTP induced by 5 Hz stimulation paired with application of 1 μM isoproterenol afterward at the same synapses. Of all slices tested, LTD with averaged magnitude of 80.6 ± 5.4% (n = 5; $p < 0.01$, paired t-test) was induced after the pre-conditioning procedure. Once the LTD was successfully induced, i.e. a given population of synapses were successfully conditioned, 5Hz stimulation was applied with (Fig. 2B) or without (Fig. 2A) bath application of isoproterenol. In contrast to the above results, prolonged stimulation at 5 Hz produced no significant change in the slope of fEPSP (96.5 ± 4.7%; $p = 0.48$, paired t-test; Fig. 2A&C, filled circles). Figure 3A shows the superimposing of results that were obtained from normal (filled circle)

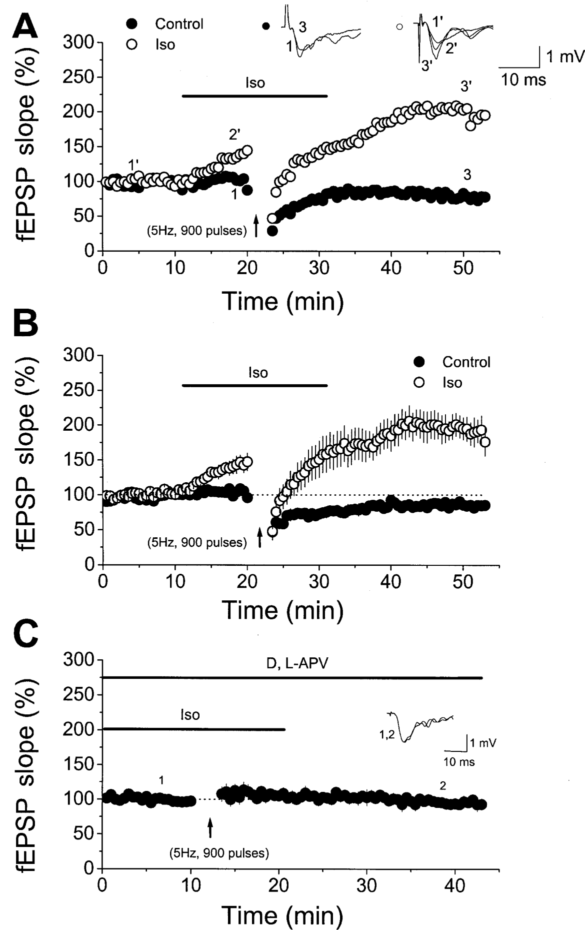

Fig. 1. Induction of LTD and LTP at normal synapses. **A**, Open circles show a typical experiment demonstrating the induction of LTD by using 900 pulses given at 5 Hz (arrow). The insert is the averaged responses of 10 fEPSP evoked before (1) and after (3) the induction of LTD. Filled circles show another typical experiment demonstrating the induction of LTP by using 900 pulses given at 5 Hz (arrow) paired with bath application of 1 μM isoproterenol (bar). The insert is the averaged responses of 10 fEPSP evoked before application of isoproterenol (1'), and before (2') and after (3') the induction of LTP. **B**, The summarized results from 5 identical experiments as in **A** (Filled circles for Control; open circles for Iso) are superimposed for comparison. **C**, LTP induced by using 900 pulses given at 5 Hz (arrow) paired with bath application of 1 μM isoproterenol (lower bar) was NMDA dependent. Note no change in fEPSP slope after the induction protocol when D,L-APV was included into ACSF (upper bar). The insert is the averaged responses of 10 fEPSP evoked before (1) and after (2) the induction of LTP.

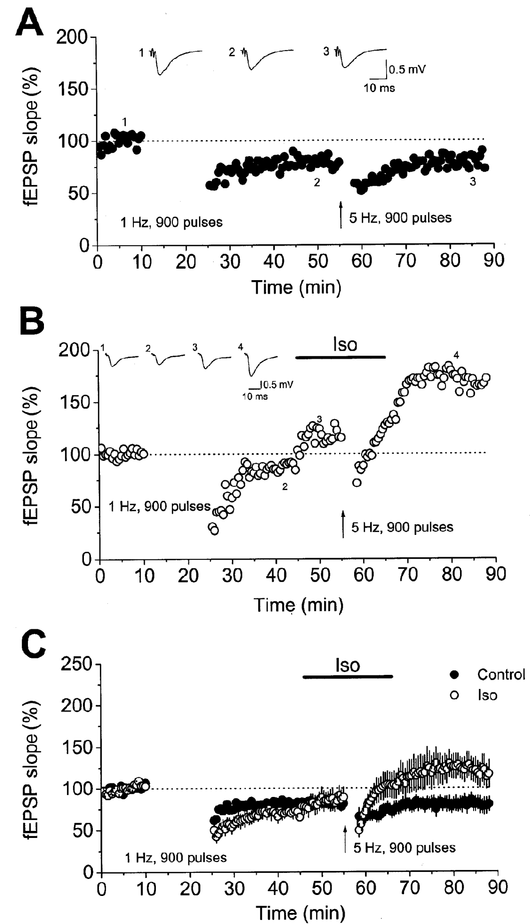

Fig. 2. Induction of LTD and LTP at pre-conditioned synapses. **A**, A typical experiment demonstrating the pre-conditioning of synapses using 900 pulses stimulation at 1 Hz, and the induction of LTD afterward by using 900 pulses at 5 Hz (arrow) at the same synapse. The insert is the averaged responses of 10 fEPSP evoked before pre-conditioned (1), and before (2) and after (3) the induction of LTD afterward. **B**, A typical experiment demonstrating the pre-conditioning of synapses using 900 pulses stimulation at 1 Hz, and the induction of LTP by using 900 pulses given at 5 Hz (arrow) paired with bath application of 1 μM isoproterenol (bar) afterward at the same synapses. The insert is the averaged responses of 10 fEPSP evoked before pre-conditioning (1), application of isoproterenol after pre-conditioning (2), and before (2) and after (4) the induction of LTP. **C**, The summarized results from 5 identical experiments as in **A** (Control, filled circle) and 6 in **B** (Iso, open circle) are superimposed for comparison.

and pre-conditioned synapses (open circle), with the base lines being normalized to 100%. As can be seen, no further LTD can be induced by prolonged stimulation at 5 Hz at synapses that were preconditioned by prolonged stimulation at 1 Hz (pre-conditioned synapses : control synapses = 96.5 ± 4.7% : 82.1 ± 2.9%; $p < 0.05$, one-way ANOVA test). Furthermore, at preconditioned synapses, prolonged stimulation at 5 Hz paired with bath application of isopropentenol could produce LTP afterward, with the magnitude of LTP being 130 ± 13.2% (n = 6; $p < 0.05$, paired t-test; Fig. 2B&C, open circles). It is, however, not significantly different from LTP induced at normal synapses using same induction paradigm (Fig. 3 B; pre-conditioned synapses : normal synapses : 130.5 ± 13.2% : 138.2 ± 6.3%; p = 0.6, one-way ANOVA test). At pre-conditioned synapses, bath application isoproterenol alone also enhances slope of fEPSP to a magnitude of 121.1 ± 11.5% (n = 6); this is consistent

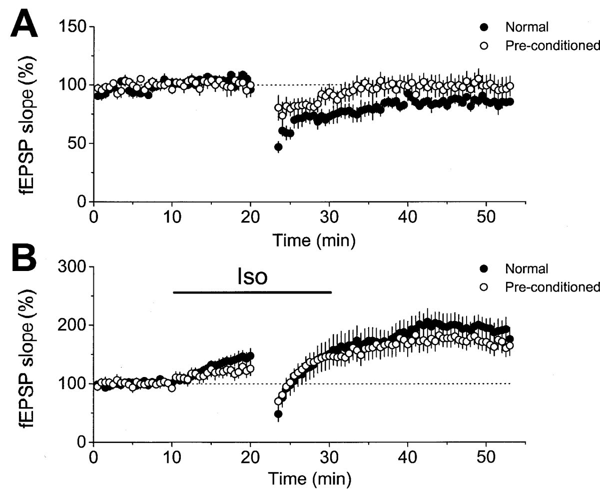

Fig. 3. **A**, The comparison of LTD induced at normal (filled circle) and preconditioned synapses (open circle). Note the base lines of the both cases were normalized to the same level (100%) **B**, The comparison of LTP induced at normal (filled circle) and pre-conditioned synapse (open circle). Note for the both cases the base lines before application of isoproterenol were normalized to 100%.

with results obtained in normal synapses (Fig. 3 B; pre-LTD pathway : control pathway = 121.1 ± 11.5% : 139.9 ± 9.6%; p = 0.25, one-way ANOVA test).

Discussion

The results of the present study suggest that at given hippocampal CA1 synapses prolonged low stimulation at 5 Hz could not induce LTD if they were pre-conditioned with prolonged low frequency stimulation at 1 Hz; the magnitude of LTP induced using 5 Hz stimulation paired with activation of β-adrenergic receptors was also reduced in these synapses. In our previous study, we reported that while conditioned stimulation at 1 Hz could induce LTD of 77.5 ± 16.7%, conditioned stimulation at 20 Hz and 100 Hz induced LTP with magnitude of 131.2 ± 13.7% and 151 ± 17.5% respectively (21). We therefore proposed that the modification threshold, (m, is about at 5 Hz to 10 Hz at CA1 synapses. The θ*m* is defined as a critical value that active synapses are potentiated when the total postsynaptic response exceed this value; in the contrast the active synapses will be depressed when the total postsynaptic response exceed zero but less than θ*m* (2, 3). Since we found conditioned stimulation at 5 Hz induced LTD with magnitude of 82.1 ± 2.9% in this study, with convolution of this results with our previous study, we suggest that at hippocampal CA1 synapses the θ*m* should be close to 10 Hz. This estimation is in agree with results of Katsuki et al (1997) (13).

It is now generally accepted that prolonged low frequency stimulation could only produce a relative small membrane depolarization, therefore activate a limit number of NMDA receptors which in turn cause a slight elevation of intracellular calcium concentration, and finally activate calcineurin (18). The calcineurin can activate many phosphatases via inhibiting the activity of inhibitor-I, a phosphatase inhibiting protein (5, 18). The activation of phosphatases then results in dephosphorylation of AMPA subtype of glutamate receptors, and finally leads to expression of LTD (1, 18). In the present study, pre-conditioning CA1 synapses using prolonged low frequency stimulation at 1 Hz maybe saturated all of the activity of phosphatases, as a result, no further LTD could be induced by prolonged low frequency stimulation at 5 Hz afterward at the same synapses.

The activation of β-adrenergic receptors can raise the concentration of adenosine cyclic monophosphate (c-AMP) in cytoplasm, which can activate protein kinase A (PKA) (7, 21). The PKA can inhibit phosphatases via activation of inhibitor-I (5), and promote the phosphorylation of AMPA receptor and CREB gene (9, 20). All of these effects could increase the expression of LTP. Therefore, in spite of the fact that prolonged stimulation at 5 Hz could induce small LTD, LTP could actually be induced by this stimulation paradigm if it was simultaneously couple with high level of PKA activity via activation of β-adrenergic receptors (10, 20).

Since we also found that LTP induced by prolonged low frequency stimulation at 5Hz paired with activation β-adrenergic receptors is a NMDA receptor dependent phenomenon, the β-adrenergic receptors must partially exert their role in facilitating the expression of LTP through direct modulation on the function of NMDA receptors, or activation of NMDA receptor could also mediate PKA activity (11). Because the activity of phosphatases might be saturated at synapses that were pre-conditioned with prolonged low frequency at 1 Hz, to induce LTP with the same magnitude as that induced at normal synapses, a stronger conditioned stimulation may be needed to overcome the activity of the phosphatases. Therefore, by using the stimulate paradigm, i.e. 900 pulses at 5 Hz paired with application of 1 μM isoproterenol, the magnitude of LTP induced at synapses that have previously been conditioned with prolonged low frequency stimulation should be smaller than LTP induced at normal synapses, though in the present study the difference was not significant.

In conclusion, our results support the idea that the θ*m* of a given pathway is not fixed at certain value, rather it could undergo an active-dependent sliding mechanism. The history of the use of synaptic activity might be an important factor underlying such a

mechanism, and the change in local biochemical environment according to the synaptic activity may provide a molecular account for the sliding of θm.

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