Ajou Medical Journal
Vol. 2, No. 1, 1997

Suppression of IPSP by Priming Stimulation in Rat Piriform Cortex

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High frequency burst stimulation repeated at theta (about 5Hz) frequency is optimal for induction of LTP in hippocampus. TBS is effective since fast IPSP is suppressed by priming stimulation maximal at around 200 ms interval, which results in a large voltage summation and hence activation of NMDA receptors in the postsynaptic neuron. LTP is reliably induced by TBS in piriform cortex, suggesting that the same cellular process (IPSP suppression) may operate in piriform cortex. To test this possibility, effects of priming stimulation on IPSP suppression were examined in the rostral piriform cortex. Intracellular recordings were made from layer II pyramidal neurons, and electrical stimulation was delivered to the intrinsic associational fibers. Both heterosynaptic and homosynaptic priming stimulation induced prolongation of subsequent synaptic responses evoked 200 ms later. However, heterosynaptic stimulation did not affect the peak amplitude of the subsequent synaptic responses, indicating that the effect is not due to other forms of synaptic plasticity such as paired-pulse facilitation. When the same experiments were repeated while the postsynaptic neurons were depolarized by current injection, suppression of fast IPSP by priming stimulation was clearly discernable. Burst stimulation resulted in a large temporal summation of EPSPs for primed burst responses, an ideal condition for activation of NMDA receptors. These results indicate that cellular mechanisms for IPSP suppression by priming stimulation indeed exist in piriform cortex as in hippocampus, and this is probably the reason why TBS is effective for induction of LTP in piriform cortex. Unlike hippocampus, however, homosynaptic stimulation was more effective than heterosynaptic stimulation for suppression of fast IPSP, and maximal effect was around 100 ms interval, suggesting that the optimal frequency and operational modes are different in the two structures.

Key Words: Piriform cortex, IPSP, Priming, Theta, LTP

Abbreviations: ASSN; associational, EEG; electroencephalogram, EPSP; excitatory postsynaptic potential, IPSP; inhibitory postsynaptic potential, LOT; lateral olfactory tract, LTP; long-term potentiation, NMDA; N-methyl-D-aspartate, TBS; theta burst stimulation

INTRODUCTION

The olfactory system uses highly rhythmic sampling patterns. During exploratory sniffing rats inhale at 4-7 Hz and these respirations become synchronized with hippocampal EEG theta rhythms and presumably activity in other olfactory structures[1]. The functional significance of the rhythmic sampling is not clear; however, induction of LTP in hippocampus is induced most efficiently by high frequency bursts repeated at the theta (about 5Hz) frequency[2~4]. In hippocampus, TBS is effective for inducing LTP because it results in suppression of feedforward IPSPs ("priming") and a concomitant enhancement of the evoked depolarization such that the NMDA receptor threshold is crossed[5~7]. This raises a possibility that rhythmic sampling and IPSP suppression have evolved to distinguish "learning mode" versus "performance mode"; When an animal is required to acquire new information, such

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as during explorative sniffing, theta frequency sampling is initiated which results in NMDA receptor activation and synaptic weight changes.

There is good reason to suspect that similar cellular mechanisms for IPSP suppression and NMDA receptor activation exist in olfactory cortex. First, the olfactory system uses highly rhythmic sampling when an animal is exploring a new environment[1]. Second, there is now overwhelming evidence that LTP can be induced in olfactory piriform cortex by rhythmic stimulation[8~13]. Both afferent and associational systems display NMDA receptor-dependent LTP, and induction and expression characteristics are quite similar to those of hippocampal LTP[10,11]. Interestingly, almost all the studies that successfully induced LTP in piriform cortex used TBS. Third, hippocampus and piriform cortex have similar physiological and pharmacological characteristics. Neurons in piriform cortex and hippocampus show similar synaptic response patterns to electrical stimulation; EPSP is followed by fast and slow IPSPs[14]. Pharmacological profiles are also quite similar[15]. It is therefore of interest to investigate whether or not the same cellular process (i.e., IPSP suppression) operates in piriform cortex during induction of LTP by TBS. To test this possibility intracellular recordings were made from layer II pyramidal cells of rat piriform cortex, and the effect of priming stimulation on subsequent IPSP was examined. Results indicate that piriform fast IPSP is indeed suppressed by priming stimulation as in hippocampus, demonstrating another similarity between these two evolutionary old cortices.

MATERIALS AND METHODS

Experiments were conducted on slices of olfactory cortex prepared from adult male Sprague Dawley rats and maintained *in vitro*[10]. Slices were obtained by cutting the rostral half of piriform cortex at an angle perpendicular to the cortical surface at a thickness of approximately 400 μm. Identification of the rostral piriform cortex was made by visually examining the LOT which is readily visible in the anterior piriform cortex as a thick band of massive myelinated axons. Slices were cut only from the region of piriform cortex underlying the LOT. Tissue was maintained at 35 ± 1°C and constantly perfused with medium in an interface chamber with the upper surface of the slices exposed to an atmosphere of humidified 95% O_2/5% CO_2. The standard perfusion medium contained (in mM): NaCl 124, KCl 3, KH_2PO_4 1.2, $CaCl_2$ 3.4, $MgSO_4$ 2.5, $NaHCO_3$ 26, D-glucose 10, and L-ascorbate 2.

Intracellular recordings were made by impaling layer II pyramidal cells of the anterior piriform cortex with microelectrodes filled with 2 M potassium methylsulfate (50~150 MOhms). Microelectrodes were connected to a high input impedance preamplifier equipped with a bridge circuit for passing current (WPI model M707A). Impalements were considered satisfactory if the membrane potential remained greater than 65 mV and stable for at least 30 minutes, input resistance was at least 15 MOhms, excitatory responses to orthodromic stimulation were larger than 10 mV and stable, and action potentials evoked by orthodromic stimulation or depolarizing current injection exhibited overshoot.

Bipolar stimulation electrodes were constructed of twisted strands of nichrome wire (64 μm diameter) insulated except at the cut ends. Synaptic responses evoked by the intrinsic ASSN fibers were identified as described elsewhere[10]. Criteria included different degrees of paired-pulse facilitation for LOT and ASSN synapses at 50 msec inter-pulse intervals, distinct laminar profiles, and lack of paired-pulse interaction between independent pathways. All of the experiments reported here were performed in the ASSN system. Test pulses (0.1 msec) were delivered every 20-40 seconds at currents ranging from 10~200 μA to obtain baseline responses (at least 10 minutes) about half-maximal. Evoked responses were amplified and displayed on an oscilloscope as well as digitized on-line with an IBM compatible personal computer. Measurements of relevant parameters of digitized responses were displayed on-line and stored with the records for off-line analysis. The suppression of fast IPSP was quantified by measuring increase in half-width of evoked responses. Records for the figures were produced on a digital plotter. Quantitative results are expressed as means with standard errors.

RESULTS

Basic physiological characteristics

Total of 21 layer II pyramidal cells were recorded in the present study. The average resting membrane potential of the recorded pyramidal cells was -71.7 ± 0.6 mV, membrane

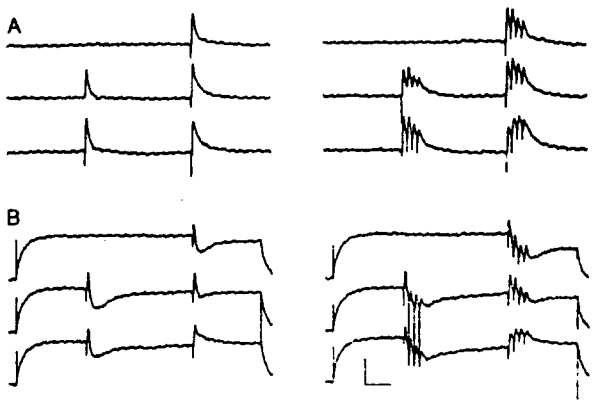

Fig. 1. Fast IPSP in the ASSN system is suppressed by priming stimulation. ASSN axons were stimulated to evoke about 10 mV response in a layer II cell at resting potential of -72 mV. The first row of each panel shows unprimed (control) responses to a pulse (left column) and burst (right column) stimulation. The second row shows responses to heterosynaptic priming stimulation (the first response) and primed responses (the second response) with 200 ms interval. The third row shows responses to homosynaptic stimulation with 200 ms interval. (A) Experiments were performed at the resting membrane potential (-72 mV). (B) 0.4 nA current was injected to depolarize the neuron and reveal fast IPSP. Calibration bars: 10 mV and 50 ms.

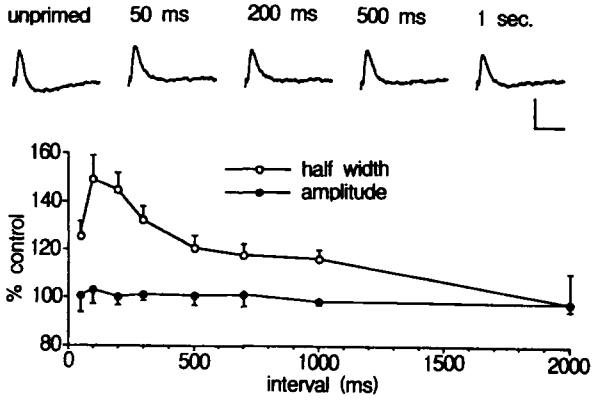

Fig. 2. Time course of IPSP suppression by priming stimulation. Top: Records show unprimed (control) and primed responses with 50 ms - 1 sec intervals. A heterosynaptic pulse was used for priming stimulation. Depolarizing current was injected to maintain membrane potential at -63 mV during stimulation. Calibration bars: 10 mV and 20 ms. Bottom: Grouped data showing the averaged half-width and peak amplitude of primed responses with 50 ms - 2 sec intervals following heterosynaptic priming stimulation (as a percentage of the control response, n=21).

resistance was 33±2.8 MOhm (measured with hyperpolarizing current injection), spike threshold was −41.3±2.6 mV, and spike overshoot was 25.4±2.5 mV. Stimulation of the ASSN fibers induced EPSP, followed by fast and slow IPSPs. Since the reversal potential of the fast IPSP is toward the depolarizing direction[14], the fast IPSP was not clearly distinguished at the resting membrane potential. It was clearly visible, however, when the membrane potential was depolarized by current injection (Fig. 1).

Suppression of IPSP

Priming stimulation of the ASSN system induced prolongation of evoked synaptic responses at the resting membrane potential. Depolarizing current injection revealed that this effect is due to suppression of fast IPSP. As shown in Fig. 1, priming stimulation at 200 ms interval suppressed fast IPSP and hence prolonged the evoked responses. This effect was more effective for homosynaptic priming stimulation than heterosynaptic one. When burst stimulation was used instead of pulses, primed burst stimulation (the second burst) resulted in a large voltage summation due to inactivation of fast IPSP (Fig. 1).

Time course of IPSP suppression

The time course of priming effect (IPSP suppression) was assessed by measuring the half-width of synaptic responses at 50 ms - 2 sec priming intervals. As expected, heterosynaptic priming stimulation induced prolongation of evoked responses in a time-dependent manner. The peak amplitude of the primed response was not changed by heterosynaptic stimulation, however, confirming that the effect was not due to other forms of synaptic plasticity such as paired-pulse facilitation[16]. A representative example and grouped data from 21 experiments are summarized in Fig. 2. As shown, suppression of fast IPSP was the most effective around 100 ms interval, and lasted at least for 1 sec.

DISCUSSION

The present study demonstrates that priming stimulation induces suppression of fast IPSP in rat piriform cortex. First,

priming stimulation induced prolongation, thus an increase in half-width, of synaptic responses. Second, priming effect was observed with heterosynaptic stimulation, therefore it is not due to homosynaptic plasticity such as paired-pulse facilitation or depression. This was confirmed by the result that the peak amplitude of the primed response was not changed. Third, suppression of fast IPSP was clearly discernable when fast IPSP was revealed by depolarizing current injection. The present results therefore suggest that IPSP suppression by priming stimulation is a rather general phenomenon. Considering the evidence that neocortex has evolved from hippocampus and olfactory cortex[17], IPSP suppression by rhythmic activity may well be a general principle in the entire cerebral cortex. Further investigations are necessary to confirm this proposition.

Basic characteristics of IPSP suppression by priming stimulation appear to be similar in hippocampus and piriform cortex; Shunting current (fast IPSP) is suppressed by both heterosynaptic and homosynaptic stimulation, and priming effect is dependent on time intervals so that there is an optimal time window for the maximal suppression of fast IPSP. Thus, maximal depolarization is induced by rhythmic stimulation at the right frequency in hippocampus and piriform cortex. When burst stimulation was used instead of pulses, primed burst stimulation (the second burst) resulted in a large voltage summation due to inactivation of IPSP as in hippocampus[5,6]. This is an ideal condition for activation of NMDA receptors, and hence an influx of calcium ions into the postsynaptic neuron that leads to development of LTP[18]. This is probably the reason why TBS is effective for inducing LTP in hippocampus as well as piriform cortex.

Two differences in the priming effect were noted between hippocampus and piriform cortex, however. First the time courses were somewhat different. One hundred ms priming interval induced the maximal IPSP suppression in piriform cortex whereas maximal LTP is induced by burst stimulation at 200 ms interval in hippocampus[3]. Experiments with a smaller time step are required to determine exact time courses and the optimal frequency for a maximal activation of the postsynaptic neuron. The present results suggest, however, that the optimal frequency for induction of LTP is somewhat different for hippocampus and piriform cortex. Second, homosynaptic priming stimulation was far more effective than heterosynaptic one for suppression of fast IPSP in piriform cortex. Homo- and heterosynaptic stimulation produces a similar degree of IPSP suppression in hippocampus (unpublished data). The reason for the difference is not clear. One possibility is that piriform interneurons have more limited dendritic branching patterns than hippocampal interneurons so that separate sets of ASSN axons activate overlapping but somewhat different sets of interneurons. Then homosynaptic stimulation would be more effective since only the activated interneurons are suppressed. Another possible explanation is an activation of a slow depolarizing current by priming stimulation at homosynaptic junctions; this current was observed in some cases in piriform cortex (data not shown). A residual degree of depolarization produced by the priming stimulus could add to the enhanced "primed" responses. More studies are required to test these speculative suggestions.

ACKNOWLEDGEMENT

The authors thank Drs. Gary Lynch and John Larson for many helpful discussions and support throughout the course of this studies. This research was supported by Korean Ministry of Health and Welfare grant HMP-96-M-3-1044 and HMP-96-M-22-1046 to M.W.J.

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