The Induction of Long-Term Potentiation at Amygdalo-Hippocampal Synapses in Vivo

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Electrical stimulation of the basolateral amygdala (BLA) evoked synaptic potentials in the dentate gyrus (DG) of the hippocampus in anesthetized rats. To determine if this pathway possesses synaptic plasticity, we investigated the impact of several conditions of high-frequency stimulation on BLA-DG synaptic potentials in these rats. Application of two trains of 100-pulse, 100-Hz stimulation or theta-burst stimulation to the BLA reproducibly induced long-term potentiation (LTP) of BLA-DG synaptic potentials. Paired-pulse facilitation was unchanged during LTP, suggesting that postsynaptic mechanisms are involved in the expression of LTP. In addition, the induction of LTP was not affected by the N-methyl-D-aspartate (NMDA) receptor antagonist 2-amino-5-phosphonovalerate, suggesting that activation of NMDA receptors is not required. This novel form of LTP should be a valuable model for elucidating neural mechanisms underlying the formation of emotional memory.

Key words amygdala; hippocampus; long-term potentiation; paired-pulse facilitation; N-methyl-D-aspartate (NMDA) receptor

Emotion and memory are closely related. Among brain regions, the amygdala is involved in emotional and motivational aspects of behavior, and the hippocampus is crucially involved in the formation of memory. The interaction between the amygdala and hippocampus may be a key to elucidating neural mechanisms linking emotion and memory. Behavioral experiments using animals have demonstrated that lesions of the amygdala or infusions of drugs into the amygdala impair or enhance hippocampal-dependent learning, suggesting that the amygdala modulates memory processing in the hippocampus. However, very little is known about the neural mechanisms by which the amygdala regulates hippocampal neuron activity.

Neural connection from the basolateral amygdala (BLA) to the dentate gyrus (DG) of the hippocampus has been demonstrated by several functional studies. Injection of the excitatory amino acid N-methyl-D-aspartate (NMDA) into the BLA induced c-fos expression in the ipsilateral DG. Furthermore, we have found that electrical stimulation of the BLA evoked field potentials in the DG in anesthetized rats. The BLA-evoked DG field potential was not recorded when the stimulating electrode was positioned outside the BLA, indicating that it originates from the BLA only. The evoked potential reversed polarity when the recording electrode was raised to the dendritic layer of the DG, indicating that it reflects the dendritic responses of DG granule cells. The evoked potential exhibited paired-pulse facilitation (PPF), and its latency was little shifted with increasing stimulus intensity, indicating that it represents monosynaptic responses. Our findings have been followed by those in another laboratory. Further analysis of the BLA-evoked DG field potential should give clues to elucidate neural mechanisms underlying memory formation associated with emotional experiences.

Long-term potentiation (LTP) of synaptic transmission is a form of activity-dependent synaptic plasticity. To determine if the BLA-DG pathway displays this plasticity, we investigated the impact of several conditions of high-frequency stimulation on BLA-DG synaptic potentials in anesthetized rats.

MATERIALS AND METHODS

Evoked potential in the BLA-DG pathway was recorded as described previously. Briefly, male Wistar rats, 7—9 weeks old, were anesthetized with urethane (1 g/kg, i.p.) and α-chloralose (25 mg/kg, i.p.) and fixed in a stereotaxic frame. A bipolar stimulating electrode was placed in the BLA (2.8 mm posterior to bregma, 5.2 mm lateral to midline, 7.6 mm ventral to dura), and the evoked potential was extracellularly recorded from the granule cell layer of the DG (3.5 mm posterior to bregma, 4.4 mm lateral to midline, 3.0 mm ventral to dura). Test stimulation (0.08 ms duration) was applied at intervals of 30 s. As previously reported, maximal stimulation of the BLA evoked biphasic field potentials in the DG, with early non-synaptic component and late synaptic component. Modest intensity (<40 μA) of stimulation evoked only the late synaptic component. Thus, the intensity of test stimulation was adjusted so that the early component was not elicited and the amplitude of the late component was approximately 50% of the maximum amplitude. After stable baseline responses were obtained for 20 min, several conditions of high-frequency stimulation were applied, and changes in the amplitude of evoked potentials were observed. In part of the experiments, PPF was observed by applying paired-pulse stimulation at an interpulse interval of 50 ms. For intracerebroventricular injection of a drug, a stainless steel cylindrical cannula was inserted into the contralateral ventricle (0.8 mm posterior to bregma, 1.5 mm lateral to midline, 3.7 mm ventral to dura), and the drug solution was injected over 2.5 min using a microsyringe. All efforts were made for the care and use of animals according to the Guidelines for Animal Experiment of Hoshi University.

RESULTS

Single-pulse test stimulation of the BLA evoked characteristic positive-going field potentials in the DG (Fig. 1A). Changes in synaptic transmission at the BLA-DG pathway were quantitated by measuring the amplitude of the BLA-evoked DG field potentials, as shown in Fig. 1A. When 100-
Pulse, 100-Hz tetanic stimulation was applied to the BLA, the subsequent responses were potentiated, but declined to the basal level within 40—60 min (Fig. 1B). Since this condition of tetanic stimulation seemed insufficient to induce LTP, a stronger stimulation was tested. When 100-pulse, 100-Hz tetanic stimulation was applied twice at an interval of 30 s, the subsequent responses were increased, and the potentiation lasted for 60 min or more (Figs. 1A, B). Since theta-burst stimulation has often been used to induce hippocampal LTP, the same stimulation was tested in the BLA-DG pathway. Application of theta-burst stimulation (10 trains of 4-pulse, 100-Hz bursts at intervals of 0.2 s) successfully produced LTP (Fig. 1B). In the following experiments, LTP was induced by two trains of 100-pulse, 100-Hz tetanic stimulation.

LTP could be expressed by an increase in transmitter release from presynaptic terminals or an increase in postsynaptic responses. To determine which is the case in LTP in the BLA-DG pathway, we examined PPF after the induction of LTP. PPF is an increase in the amplitude of the second of two synaptic potentials evoked by closely spaced stimuli; it is believed to result from an increase in presynaptic transmitter release. PPF should decrease if the probability of presynaptic transmitter release increases. As shown in Fig. 2, PPF 60 min after tetanic stimulation was not different from that before tetanic stimulation, indicating that this LTP involves an increase in postsynaptic responses.

The induction of LTP at the perforant path-DG granule cell synapses requires activation of the NMDA type of glutamate receptors. To determine if the induction of LTP in the BLA-DG pathway is dependent on NMDA receptors, the effect of the selective NMDA receptor antagonist 2-amino-5-phosphonovalerate (APV) was investigated. However, the induction of LTP in the BLA-DG pathway was not affected by intracerebroventricular injection of 50 nmol APV. We previously confirmed that this dose of APV is sufficient to block the induction of LTP at the perforant path-DG granule cell synapses in anesthetized rats.

**DISCUSSION**

One train of 100-pulse, 100-Hz stimulation has been reported to be sufficient to induce LTP in the perforant path-DG granule cell synapses in anesthetized rats. However, this stimulation condition was not sufficient to induce LTP in the BLA-DG pathway, indicating that the condition required for LTP induction differs among synaptic pathways. We have found that two trains of 100-pulse, 100-Hz stimulation or theta-burst stimulation successfully produces LTP in the BLA-DG pathway.

PPF was unchanged after LTP had been expressed in this
pathway, suggesting that postsynaptic mechanisms are involved in the expression of LTP. Furthermore, the induction of LTP in the BLA-DG pathway was not affected by APV, suggesting that it does not require activation of NMDA receptors. The NMDA-independent form of LTP has been reported in hippocampal mossy fiber-CA3 synapses, lateral amygdala synapses, thalamoamygdala synapses, corticothalamic synapses, etc. The induction of NMDA-independent LTP has been proposed to be triggered by activation of voltage-gated Ca$^{2+}$ channels, which is modulated by $\beta$-adrenoceptors, opioid receptors or muscarinic receptors. Further investigations are underway in our laboratory to elucidate molecular mechanisms of LTP in the BLA-DG pathway.

In conclusion, we have demonstrated for the first time that two trains of 100-Hz tetanic stimulation or theta-burst stimulation produces LTP in the BLA-DG synaptic pathway, which is NMDA independent and expressed by changes in postsynaptic mechanisms. LTP in the BLA-DG pathway may represent a synaptic mechanism by which emotional experiences induce changes in the strength of connections between the amygdala and the hippocampus. This novel form of LTP should be a valuable model for elucidating neural mechanisms underlying the formation of memory associated with emotional experiences.

REFERENCES