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Amygdala *N*-methyl-D-aspartate receptors participate in the induction of long-term potentiation in the dentate gyrus in vivo

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Abstract

We investigated the effects of injection of *N*-methyl-D-aspartate (NMDA) receptor antagonists, 2-amino-5-phosphonovalerate (APV) and 7-chlorokynurenate (7-Cl-Kyn), into the basolateral amygdala (BLA) on long-term potentiation (LTP) in the medial perforant path-dentate gyrus granule cell synapses of anesthetized rats. Injection of APV or 7-Cl-Kyn into the ipsilateral BLA did not affect the baseline synaptic responses, but significantly attenuated the dentate gyrus LTP induced by tetanic stimulation. Injection of APV into the contralateral BLA did not affect the induction of LTP. When APV was injected after tetanic stimulation, it did not affect the maintenance phase of LTP. These results suggest that NMDA receptors in the ipsilateral BLA partly participate in the induction of LTP in the dentate gyrus in vivo.

Keywords: Long-term potentiation; Hippocampus; Basolateral amygdala; *N*-methyl-D-aspartate receptor; Amygdalo-hippocampal interaction; Synaptic plasticity

The amygdala is thought to be involved in certain types of learning and memory besides emotional and motivational aspects of behavior [7,8,18]. With respect to the role of amygdala in memory processes, the following controversial hypotheses have been proposed: (1) the amygdala itself plays a crucial role in memory; or (2) the amygdala serves to modulate the function of other brain regions (e.g. the hippocampal system) that are critically involved in memory [14,22]. Supporting the latter hypothesis, recent behavioral studies have demonstrated that the amygdala modulates hippocampal-dependent memory processes [17,20]. Neural projections from the amygdala to the hippocampus have been confirmed by anatomical and physiological studies [1,3,23,25]. Furthermore, we have recently found that hippocampal long-term potentiation (LTP), a synaptic basis of memory [2], is attenuated by lesions of the basolateral amygdala (BLA) [10] and is facilitated by electrical stimulation of the BLA [12]. These observations suggest the importance of amygdalo-hippocampal interaction in memory processes.

Several laboratories have reported that intraamygdala injections of *N*-methyl-D-aspartate (NMDA) receptor antagonists impair memory processes [6,13,15,19]. The authors seem to have supposed only that NMDA receptor antagonists block neural changes within the amygdala. However, as mentioned above, the amygdala modulates hippocampal function; thus the effects of NMDA receptor antagonists injected into the amygdala may possibly involve changes of hippocampal function. To test this possibility, we examined the effects of intraamygdala injections of NMDA receptor antagonists, 2-amino-5-phosphonovalerate (APV) and 7-chlorokynurenate (7-Cl-Kyn), on the induction of hippocampal LTP in anesthetized rats.

Recording of evoked potential was made as described in our previous paper [10]. Briefly, male Wistar rats 8–9 weeks old were anesthetized with a combination of 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 medial perforant path, and the evoked potential was extracellularly recorded from the granule cell layer of the ipsilateral dentate gyrus. A single test stimulation (0.08 ms duration) was applied at intervals of 30 s, and the stimulus intensity was set to a level

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which evoked a population spike of about 50% of the maximum. For local injection of NMDA receptor antagonists, a stainless steel cylindrical cannula (0.5 mm o.d., 0.35 mm i.d.) connected to a micrometer syringe was inserted so that the tip of the cannula was set in the left (ipsilateral) or right (contralateral) BLA (2.8 mm posterior to bregma, 5.2 mm lateral to midline, 7.6 mm ventral to dura). After the response became stable, 1 μl of saline or NMDA receptor antagonists dissolved in saline was injected into the BLA through the cannula (injection time 1 min). Tetanic stimulation (30 pulses at 60 Hz) was applied at the same stimulus intensity through the same electrode as used for test stimulation. Since we have previously observed that the BLA lesions attenuated the magnitude of LTP of population spikes [10], the effect of injection of NMDA receptor antagonists on LTP were also evaluated by measuring changes in the population spike amplitude. The population spike amplitude was measured as described in Fig. 1A.

When tetanic stimulation (30 pulses at 60 Hz) was applied in the intact rats that did not receive the cannula implantation, LTP was induced in all of the 6 cases tested (Figs. 1B,C). Injection of saline into the BLA did not affect the baseline responses before tetanic stimulation, and the magnitude of LTP in the saline-injected group was not different from that in the intact group (Figs. 1B,C). Therefore, it is unlikely that cannula implantation or saline injection alone affects the induction of LTP. Injection of 2–50 nmol APV (2–50 mM, 1 μl) into the ipsilateral BLA did not affect the baseline response before tetanic stimulation, but significantly attenuated the magnitude of LTP induced by tetanic stimulation in a dose-dependent manner (Fig. 1B,C). It should be noted that the small LTP remained unblocked by injection of APV into the BLA. Similarly, injection of 7-Cl-Kyn into the BLA attenuated the dentate gyrus LTP in a dose-dependent manner (Fig. 1C). APV and 7-Cl-Kyn block the glutamate recognition site and the glycine binding site, respectively, on the NMDA receptor channel complex [16]. The two agents which inhibit NMDA receptors in different ways showed similar effects, making it probable that their effects observed here are due to blockade of NMDA receptors.

Since we have previously observed that ipsilateral, but not contralateral, BLA lesions attenuated the dentate gyrus LTP [10], the effect of injection of APV into the contralateral BLA was also investigated. As shown in Fig. 2, injection of 20 nmol APV into the contralateral BLA did not affect the dentate gyrus LTP. This result supports the idea that BLA neurons unilaterally regulates the medial perforant path-dentate granule cell synapses, consistent with our previous finding [10].

The effect of APV injection on the maintenance phase of LTP was also investigated. LTP was induced by application of tetanic stimulation, and 20 nmol APV was injected into the ipsilateral BLA 20 min after tetanic stimulation. APV injection showed no influence on the established LTP. The population spike amplitude immediately before APV injection (i.e. 20 min after tetanic stimulation) was 162.8 ± 7.1% ($n = 4$), and that 30 min after

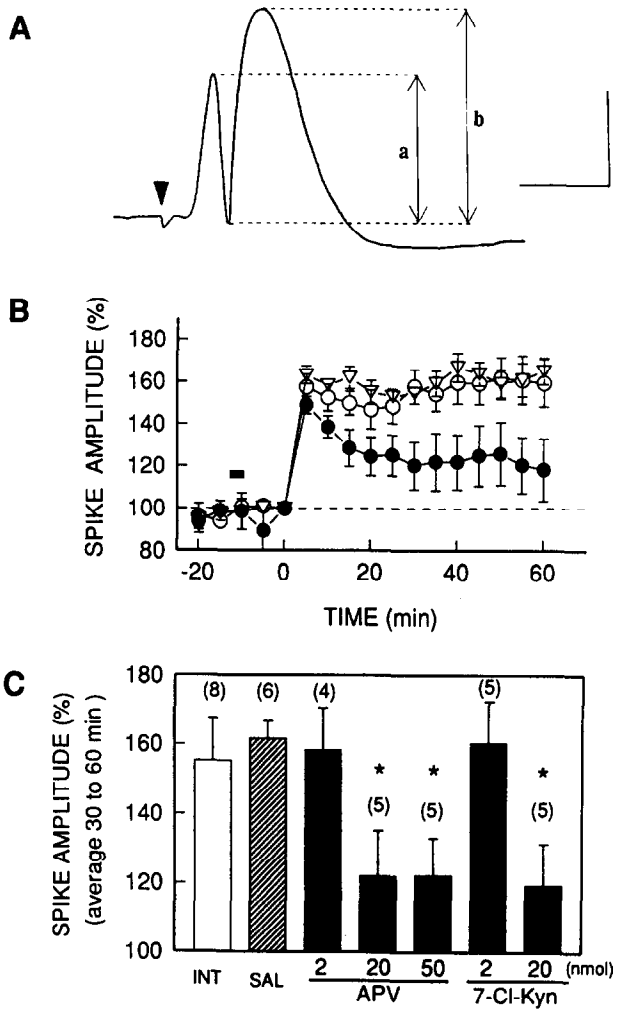

Fig. 1. The effects of NMDA receptor antagonists, APV and 7-Cl-Kyn, injected into the ipsilateral BLA on the induction of LTP in the dentate gyrus in vivo. (A) A typical evoked potential recorded from the dentate granule cell layer of an anesthetized rat. Test stimulation was delivered at time indicated by an arrowhead. Calibration bars: vertical 5 mV, horizontal 10 ms. The amplitude of the population spike was defined as the average of the amplitude from the first positive peak to the succeeding negative peak (a) and the amplitude from the negative peak to the second positive peak (b), i.e. $(a + b)/2$. (B) Time-course of the dentate gyrus LTP induced by tetanic stimulation (30 pulses at 60 Hz) in the intact group (O, $n = 8$), in the saline-injected group (▽, $n = 6$) and in the 20 nmol APV-injected group (●, $n = 5$). Saline or APV was injected into the ipsilateral BLA during the time indicated by black bars (11–10 min prior to tetanic stimulation), and then tetanic stimulation was applied at time 0 min. Population spike amplitude is expressed as a percentage of baseline value immediately before tetanus. (C) Dose-dependent effects of APV and 7-Cl-Kyn on the magnitude of LTP. The average of percent amplitude of population spikes 30–60 min after tetanus was calculated to compare the magnitude of LTP in each group. INT, intact group; SAL, saline-injected group. All data are represented as the means ± SEM. In (C), the numbers of cases in each group are shown in parentheses. Asterisks indicate significant differences from the saline-injected group: $^*P < 0.05$; Duncan's multiple range test following analysis of variance (ANOVA).

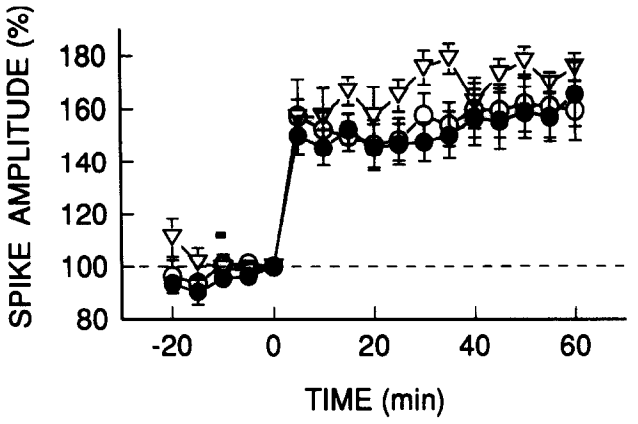

Fig. 2. Injection of APV into the contralateral BLA did not affect the induction of the dentate gyrus LTP. Saline (▽) or 20 nmol APV (●) was injected into the contralateral BLA during the time indicated by black bars (11–10 min prior to application of tetanic stimulation (30 pulses at 60 Hz)), and then tetanic stimulation was applied at time 0 min. For comparison, the data for LTP in the intact group, which was already shown in Fig. 1B, are shown again in this figure (○). Average population spike amplitude 30–60 min after tetanus in the APV-injected group (155.5 ± 9.0%, $n = 6$) is not significantly different from that in the saline-injected group (172.9 ± 3.2%, $n = 6$).

APV injection was 160.1 ± 9.1% ($n = 4$). This result is consistent with our previous observation that BLA lesions did not affect the maintenance phase of hippocampal LTP [10].

Furthermore, we conducted several experiments to check if the locally injected NMDA receptor antagonists actually act on the BLA neurons. First, we injected trypan blue through the cannula used for drug injection, and observed that the trypan blue remained within the BLA 5 or 30 min after injection. Secondly, we examined the effect of 20 nmol APV injected into the caudate putamen, the region just above the BLA (2.8 mm posterior to bregma, 5.2 mm lateral to midline, 6.4 mm ventral to dura). However, it did not affect the induction of LTP in the dentate gyrus. Average population spike amplitude 30–60 min after tetanus in the group injected with APV into the caudate putamen (149.9 ± 6.4%, $n = 5$) was not significantly different from that in the group injected with saline into the same region (145.9 ± 13.3%, $n = 5$). Thirdly, we examined the effect of APV injection in the BLA-lesioned rats. If APV had affected the dentate gyrus LTP by spreading over brain regions other than the amygdala, it might also affect the LTP in the amygdala-lesioned rats. The ipsilateral BLA was destroyed 60 min prior to application of tetanus by the same procedure as in our previous study [10]. After the electrode used for lesioning was pulled out, the cannula for drug injection was inserted in the same region. Consistent with our previous observation [10], the magnitude of the dentate gyrus LTP was significantly attenuated by the BLA lesions (average population spike amplitude 30–60 min after tetanus, 128.7 ± 6.2%, $n = 6$). The small LTP observed in the lesioned rats was regarded as the BLA-independent component of LTP. When 20 nmol APV was injected 10 min prior to tetanus in the BLA-lesioned rats, the BLA-independent component of LTP was still observed (average population spike amplitude 30–60 min after tetanus, 131.7 ± 19.6%, $n = 6$). Taken together, there is no doubt that APV injected into the BLA suppressed the dentate gyrus LTP by blocking the NMDA receptors in the BLA.

We demonstrated that blockade of amygdala NMDA receptors result in attenuation of hippocampal LTP. This fact suggests that some of the behavioral effects of intraamygdala injections of NMDA receptor antagonists result from attenuated hippocampal synaptic plasticity. To what degree the drug effects involve the amygdalo-hippocampal interaction would depend on the type of learning tasks used.

We previously observed that hippocampal LTP is attenuated by BLA lesion [10] or by injection of a local anesthetic tetracaine into the BLA [11], indicating that BLA neuron activity modulates the induction of hippocampal LTP. However, since it has been generally thought that NMDA receptors contribute very little to excitatory synaptic transmission under normal physiological conditions [5], the present results are somewhat surprising.

How NMDA receptors regulate the BLA neuron activity is an interesting subject. Rainnie et al. [21] have reported that (1) stimulation of the stria terminalis (ST), a major afferent pathway of the amygdala, evoked the excitatory postsynaptic potential (EPSP) in the BLA, (2) the slow component of the ST-evoked EPSP was blocked by APV, and (3) spontaneous EPSPs in the BLA were completely blocked by APV. These data indicate that under normal physiological conditions postsynaptic NMDA receptors are activated on BLA neurons. In addition, NMDA receptors are involved in the induction of LTP in the amygdala synapses [4,9,24,26]. NMDA receptors may play a role in regulating neuron excitability and synaptic plasticity in the BLA. Further investigations are underway in our laboratory.

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