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Short communication

Requirement of basolateral amygdala neuron activity for the induction of long-term potentiation in the dentate gyrus in vivo

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Abstract

We investigated the effect of injection of a local anesthetic, tetracaine, into the ipsilateral basolateral amygdala (BLA) on long-term potentiation (LTP) in the medial perforant path-dentate gyrus granule cell synapses of anesthetized rats. The dentate gyrus synaptic potential evoked by low frequency test stimulation did not change following tetracaine injection into BLA. However, when tetanic stimulation (30 pulses at 60 Hz) was applied 10 min after tetracaine injection, the magnitude of LTP was significantly attenuated in a dose-dependent manner. Injection of tetracaine after tetanic stimulation did not affect the established LTP. The small LTP observed in the BLA-lesioned rats was regarded as the BLA-independent component of LTP, which was not affected by tetracaine injection. These results suggest that neuron activities in the ipsilateral BLA are partly required for the induction of LTP in the dentate gyrus in vivo.

Keywords: Long-term potentiation; Hippocampus; Basolateral amygdala; Tetracaine; Neuron activity

Long-term potentiation (LTP) of evoked potentials in the hippocampus is a form of activity-dependent synaptic plasticity which may underlie learning and memory [1,2,7]. LTP has been studied extensively on synaptic mechanisms between intrinsic hippocampal neurons, but modulation of LTP by extrinsic inputs is also an important subject to elucidate the nature of LTP in vivo. We have very recently found that the magnitude of LTP of population spikes in the medial perforant path–dentate granule cell synapses in vivo was attenuated by acute or chronic lesions of the ipsilateral, but not contralateral, basolateral amygdala (BLA) [4]. Since functional neural connections from the amygdala to the entorhinal cortex or the dentate gyrus have been demonstrated [3,6,8], it is possible that the ipsilateral BLA participates in the generation of LTP at the dentate gyrus synapses in vivo. However, in our previous study, the amygdaloid nuclei were lesioned by heat, and possible influence of tissue damage caused by the lesioning procedure could not be completely ruled out. Therefore, in the present study, in order to prove the involvement of the BLA in hippocampal LTP, we attempted to test how the dentate gyrus LTP in vivo is affected when the BLA neuron activity is inactivated by injection of tetracaine, a local anesthetic.

Recording of evoked potential was made as described in our previous paper [5]. Briefly, male Wistar rats, 7–11 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 left entorhinal cortex to stimulate the medial perforant path and evoked potential was extracellularly recorded from the granule cell layer of the ipsilateral dentate gyrus. A single test stimulus (0.08 ms duration) was applied at intervals of 30 s and the stimulus intensity was set to a level which evoked a population spike of about 50% of the maximum. For local injection of tetracaine, 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 BLA (5.2 mm lateral to midline, 2.8 mm posterior to bregma, 7.6 mm ventral to dura). After the response became stable, 1 μl of saline or tetracaine dissolved in saline was injected into the BLA through the cannula (injection time: 2.5 min). Ten min after the drug injection, brief

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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 basolateral amygdaloid lesions resulted in attenuation of LTP of population spikes [4], the effect of injection of tetracaine on tetanus-induced LTP was also evaluated by employing the amplitude of population spike as a measure of overall changes in cellular responses. The way of measuring the population spike amplitude is described in Fig. 1A.

When tetanic stimulation (30 pulses at 60 Hz) was applied in the intact rats that were not inserted the cannula for injection, the amplitude of population spike was greatly potentiated and LTP was generated in all of 6 cases tested (Fig. 1B and C). Injection of saline into the ipsilateral BLA did not affect the basal responses before application of tetanus, and the magnitude of LTP induced by tetanus in the saline-injected group was not different from that in the intact group (Fig. 1B and C). Therefore, it can be ruled out that implantation of cannula or local injection of saline may non-specifically influence the normal synaptic transmission or the induction of LTP. Injection of 2–200 nmol tetracaine (2–200 mM, 1 μl) into the ipsilateral BLA did not affect the basal evoked potential before tetanus, but significantly attenuated the magnitude of LTP induced by tetanus in a dose-dependent manner (Fig. 1B and C). The magnitude of LTP of population spikes in the 200 nmol tetracaine-injected rats was about 50% of that in the intact or saline-injected rats, very similar to our previous observation that the magnitude of LTP in the BLA-lesioned rats was about half of that in the intact or sham-operated rats [4].

In order to check the reversibility of the effect of tetracaine injection, tetanic stimulation (30 pulses at 60 Hz) was applied 80 min after injection of 200 nmol tetracaine. The magnitude of LTP in the group which received injection of tetracaine 80 min prior to tetanus was not significantly different from that in the control group which received saline injection 80 min prior to tetanus: average population spike amplitudes 30 to 60 min after tetanus were $153.1 \pm 6.5\%$ ($n = 5$) in the tetracaine group and $171.6 \pm 8.3\%$ ($n = 5$) in the control group.

The effect of tetracaine injection into the ipsilateral BLA on the maintenance phase of LTP was also investigated. LTP was induced by application of tetanus (30 pulses at 60 Hz) and 200 nmol tetracaine was injected 20 min after tetanus. As shown in Fig. 2, the post-tetanus injection of tetracaine into the BLA showed no influence on the established LTP.

Finally, we examined the effect of tetracaine injection in the BLA-lesioned rats. The aim of this experiment is to check whether locally-injected tetracaine acted on the BLA neurons. If tetracaine spread over the brain region other than the amygdala and affected

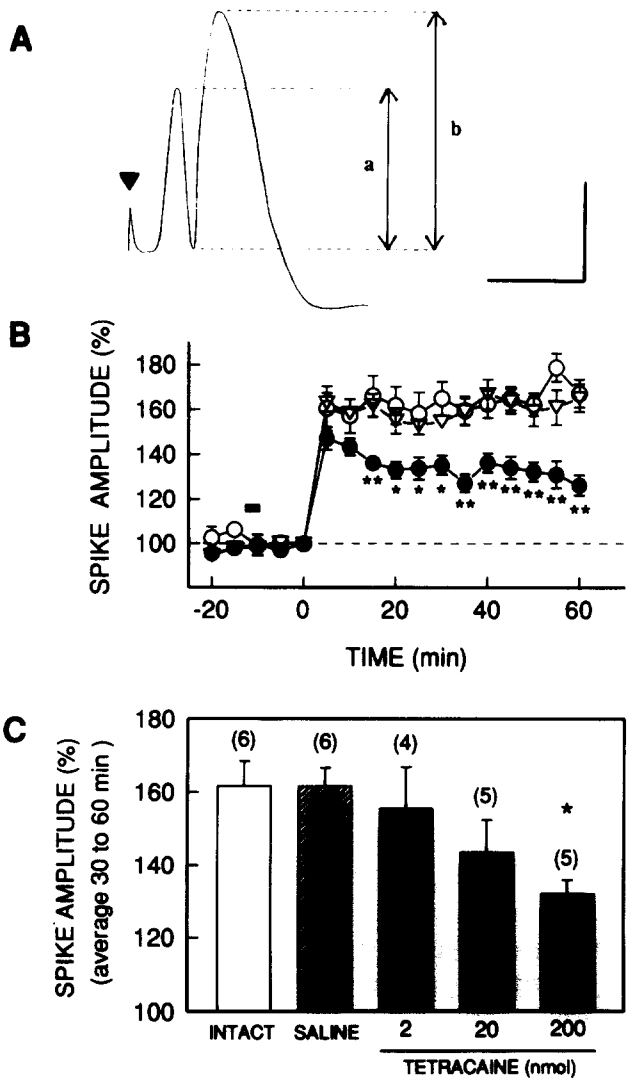

Fig. 1. The effect of tetracaine injection into the ipsilateral BLA on the induction of LTP in the dentate gyrus in vivo. A: a typical evoked potential recorded from the dentate granule cell layer of anesthetized rats. Test stimulation was delivered at time indicated by an arrowhead. Calibration bars: vertical 5 mV, horizontal 10 ms. The amplitude of 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 second positive peak (b), i.e. $(a + b)/2$. B: time-course of LTP induced by tetanus (30 pulses at 60 Hz) in the intact group (○, $n = 6$), in the saline-injected group (▽, $n = 6$) and in the 200 nmol tetracaine-injected group (●, $n = 5$). Saline or tetracaine was injected into the BLA during the time indicated by black bars (12.5–10 min prior to application of tetanus), and then tetanus was applied at time 0 min. Population spike amplitude is expressed as a percentage of baseline value immediately before tetanus. C: a dose-dependent effect of tetracaine on the magnitude of LTP. The average of percent amplitude of population spikes 30 to 60 min after tetanus was calculated to compare the magnitude of LTP produced in each group. All data are represented as the means ± S.E.M. In panel C, the numbers of cases in each group are shown in parentheses. Asterisks indicate significant differences from the saline-injected group: * $P < 0.05$; ** $P < 0.01$; Duncan's multiple range test following analysis of variance (ANOVA).

the induction of hippocampal LTP, it might affect the LTP in the BLA-lesioned rats also. If tetracaine suppressed only the BLA neuron activity, there should be no additivity between the effect of tetracaine injection and lesions of BLA. The ipsilateral BLA was destroyed 60 min prior to application of tetanus by the same procedure as in our previous study [4]. Briefly, the electrode was stereotaxically inserted into the left BLA, and lesions of the BLA were caused by keeping the temperature of the electrode tip at 80°C for 10 s with a lesion generator. After the electrode used for the lesioning was pulled out, the cannula for drug injection was inserted in the same region. Consistent with our previous observation [4], the magnitude of LTP in the ipsilateral BLA-lesioned rats was significantly smaller than that in the intact rats, but the small LTP still remained in the lesioned rats (Fig. 3). The small LTP observed in the lesioned rats was regarded as the BLA-independent component of LTP. When 200 nmol tetracaine was injected 10 min prior to tetanus in the BLA-lesioned rats, it did not significantly affect the BLA-independent component of LTP (Fig. 3).

We have shown that the injection of tetracaine into the ipsilateral BLA attenuated the magnitude of LTP in the medial perforant path-dentate gyrus. This effect of tetracaine was dose dependent and reversible. Furthermore, no additivity of effects of surgical lesions of the BLA and injection of tetracaine into the BLA was observed, confirming that tetracaine indeed inactivated the BLA neurons only. Therefore, these data indicate that a temporal inactivation of the BLA neurons results in an attenuation of the dentate gyrus LTP. In addition, since injection of tetracaine, when made after application of tetanus, did not affect the established LTP, activities of BLA neurons are required for the induction of LTP but not for the maintenance of LTP.

Injection of tetracaine into the BLA did not affect the baseline synaptic responses in the dentate gyrus.

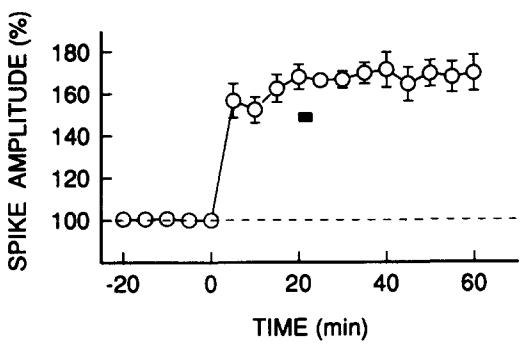

Fig. 2. No effect of tetracaine injection into the BLA on the maintenance phase of LTP. LTP was induced by application of tetanus (30 pulses at 60 Hz), and then 200 nmol tetracaine was injected into the ipsilateral BLA during time indicated by black bars (20–22.5 min after application of tetanus). The data are shown as the means ± S.E.M. of 4 cases.

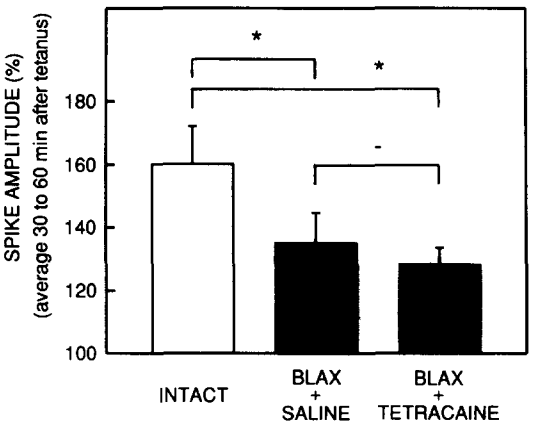

Fig. 3. No additivity of effects of surgical lesions of the BLA and injection of tetracaine into the BLA. The ipsilateral BLA was lesioned 60 min prior to application of tetanus, and saline (a hatched column, $n = 6$) or 200 nmol tetracaine (a solid column, $n = 5$) was injected into the lesioned area 10 min prior to tetanus (30 pulses at 60 Hz). The average of percent amplitude of population spikes 30–60 min after tetanus was calculated to compare the magnitude of LTP produced in each group. All data are represented as means ± S.E.M. For comparison, the data for LTP in the intact group, which was already shown in Fig. 1B, was superimposed are shown again (a white column). * $P < 0.05$; Duncan's multiple range test following analysis of variance (ANOVA).

Furthermore, we have previously observed that the baseline synaptic response in the dentate gyrus are not changed by chronic lesions of BLA [4]. It is therefore unlikely that the BLA neurons modulate the excitability of dentate granule cells under normal conditions. It is probable that the BLA neurons regulate specifically the event during or following tetanic stimulation. For example, the amygdaloid inputs may enhance the activity of the perforant path in response to tetanic stimulation, or the dentate granule cells may be in a state of heightened plasticity in the presence of amygdaloid inputs. To further clarify the cellular mechanisms, we are planning to investigate the effect of BLA stimulation on the dentate gyrus LTP.

We previously observed a partial attenuation of the dentate gyrus LTP by surgical lesions of the BLA, and now could reproduce similar effects by local injection of tetracaine into the BLA. The pharmacological approach employed in the present study has the following advantages over the lesion experiment: (1) non-specific influence due to tissue damage can be ruled out; and (2) effects of drug injections can be repeatedly tested using the same animal. This technique will be useful to further specify the interactions between the BLA neurons and dentate gyrus synapses in vivo.

In conclusion, the present data strongly support the idea that the BLA neuron activity participates in the formation of LTP in the dentate gyrus in vivo. Further investigations on amygdalo-hippocampal interactions will definitely give a new insight for elucidating the nature of hippocampal LTP in vivo.

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