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OPPOSITE REGULATION BY THE β-ADRENOCEPTOR–CYCLIC AMP SYSTEM OF SYNAPTIC PLASTICITY IN THE MEDIAL AND LATERAL AMYGDALA *IN VITRO*

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Abstract—The effects of β-adrenoceptor activation on short-term potentiation in the medial and lateral amygdala were investigated using rat brain slice preparations *in vitro*. Application of tetanic stimulation (100 pulses at 100 Hz) induced only short-term potentiation under normal recording conditions. In the medial amygdala, when the same tetanic stimulation was applied in the presence of a β-adrenoceptor agonist, isoproterenol, short-term potentiation was significantly enhanced and long-term potentiation was induced. Phenylephrine, an α-adrenoceptor agonist, did not affect short-term potentiation. The short-term potentiation-enhancing effect of isoproterenol was mimicked by forskolin, an adenylate cyclase activator, and was blocked by Rp-adenosine-3′,5′-cyclic-monophosphothioate, an inhibitor of cyclic AMP-dependent protein kinase. On the other hand, in the lateral amygdala, isoproterenol suppressed short-term potentiation. The short-term potentiation-suppressing effect of isoproterenol was mimicked by forskolin, and was blocked by Rp-adenosine-3′,5′-cyclic-monophosphothioate.

These results suggest that the β-adrenoceptor–cyclic AMP system plays a role in facilitating the induction of long-term potentiation in the medial amygdala, but suppresses synaptic plasticity in the lateral amygdala.

Key words: β-adrenoceptors, cyclic AMP, short-term potentiation, long-term potentiation, medial amygdala, lateral amygdala.

The amygdala is thought to be involved in certain types of learning and memory, as well as emotional and motivational aspects of behavior.[17,21] It has been reported that the synapses of the amygdala display long-term potentiation (LTP),[3,4,8,24,29] a long-lasting increase of synaptic strength that is widely believed to be a cellular basis of learning and memory.[1] However, the cellular mechanisms of amygdala LTP are not well understood. The amygdaloid complex consists of several subnuclei, and recent studies have shown that the mechanism underlying LTP is different among amygdaloid subnuclei. For example, the induction of LTP in the medial amygdala requires the activation of *N*-methyl-D-aspartate receptors,[3,24,29] whereas the LTP in the lateral amygdala is independent of *N*-methyl-D-aspartate receptors.[4,29] Furthermore, we have recently found that the lateral amygdala LTP is blocked by muscarinic antagonists, suggesting that the muscarinic receptors are involved in the lateral amygdala LTP.[29] Investigations about regional differences in the mechanism of synaptic plasticity will provide a better understanding of the roles of the amygdaloid subnuclei in processes of learning.

Noradrenergic neurons send projections from the locus coeruleus to the amygdala via the dorsal noradrenergic bundle,[14,26,27] and β-adrenoceptors are present throughout the amygdaloid complex.[18,19,27] Behavioral studies have demonstrated that β-adrenoceptors in the amygdala play a role in memory processing.[13,16] Furthermore, in the hippocampus, where LTP has been best characterized to date, the induction of LTP is regulated by the noradrenergic system through β-adrenoceptors.[6,7,10,25] It is of great interest whether or not β-adrenoceptors participate in amygdaloid LTP. Therefore, in the present study, we investigated possible roles of β-adrenoceptors in synaptic plasticity in the medial and lateral amygdala by using brain slice preparations *in vitro*.

EXPERIMENTAL PROCEDURES

Male Wistar rats (seven to nine weeks old, Charles River) were decapitated under mild anesthesia with ether and the whole brain was isolated. Preparation of brain slices and recording of evoked potentials were made as described in our previous paper.[29] Briefly, the brain was trimmed to a block containing the amygdala and cut into coronal slices (400–500 μm) with a Vibratome. To reduce the number of animals used, slices obtained from both hemispheres were

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Abbreviations: ACSF, artificial cerebrospinal fluid; LTP, long-term potentiation; PKA, cyclic AMP-dependent protein kinase; Rp-cAMPS, Rp-adenosine-3′,5′-cyclic-monophosphothioate; STP, short-term potentiation.

used. The slices were allowed to recover for more than 1 h in an incubation chamber containing artificial cerebrospinal fluid (ACSF) which was maintained at 34°C and continuously oxygenated with 95% O_2–5% CO_2. The composition of ACSF was as follows (mM): 124.0 NaCl, 5.0 KCl, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 1.24 KH_2PO_4, 26.0 $NaHCO_3$ and 10.0 glucose. Each slice was transferred into a submersion chamber (3 ml) where warmed (34°C) and oxygenated (95% O_2–5% CO_2) ACSF was continuously perfused at a rate of 1 ml/min. A bipolar tungsten electrode was placed on the stria terminalis or the external capsule to stimulate afferent fibers, and the evoked potential was recorded extracellularly from the medial amygdaloid nucleus or the lateral amygdaloid nucleus, respectively (Fig. 1A). A glass capillary microelectrode filled with 0.9% NaCl (tip resistance 5 [gmOM]) was used for the recording. A single test stimulation (0.05 ms duration) was applied at intervals of 20 s. As

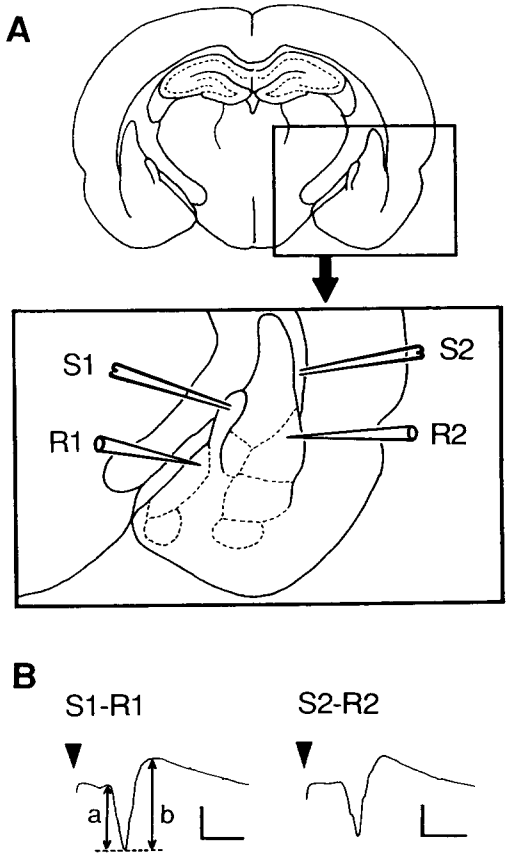

Fig. 1. Recording of synaptic potentials in the medial and lateral amygdala of a rat brain slice. (A) Schematic illustrations of a coronal brain slice. In the medial amygdala, a stimulating electrode (S1) was placed in the stria terminalis, and the evoked potential was recorded from the medial amygdaloid nucleus (R1). In the lateral amygdala, a stimulating electrode (S2) was placed in the external capsule, and the evoked potential was recorded from the lateral amygdaloid nucleus (R2). (B) Sample records of evoked potentials in the medial (S1-R1) and lateral amygdala (S2-R2). Test stimulation was delivered at time indicated by arrowheads. Calibration bars: vertical 0.5 mV, horizontal 5 ms. The voltage difference between the sharp negative onset and the negative peak (a), and that between the negative peak and subsequent positive peak (b) were measured, and the amplitude of the population spike was calculated as $(a + b)/2$.

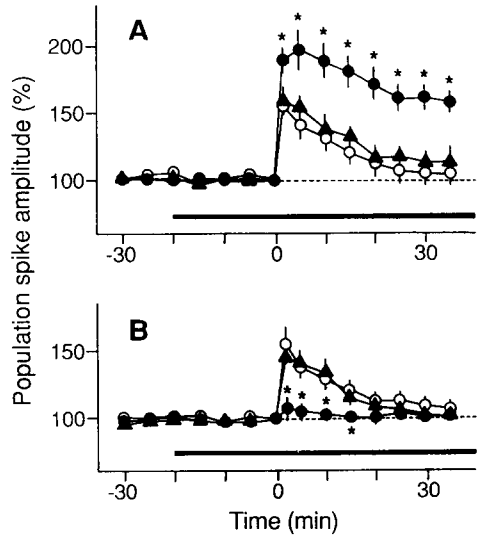

Fig. 2. Effects of isoproterenol and timolol on STP in the medial (A) and lateral amygdala (B). In control slices (open circles), tetanic stimulation (100 pulses at 100 Hz) was applied at time 0 in normal ACSF. In the drug-treated group, slices were perfused with 1 μM isoproterenol (filled circles) or 1 μM isoproterenol plus 10 μM timolol (filled triangles) during the time indicated by black bars, and tetanic stimulation (100 pulses at 100 Hz) was applied at time 0. Ordinate indicates population spike amplitude expressed as a percentage of baseline values immediately before tetanus. All data are represented as means ± S.E.M. ($n = 5$). Asterisks indicate significant differences from the control data: $^*P < 0.05$, $^{**}P < 0.01$; Mann–Whitney U-test.

reported in our previous paper,[29] the negative-going field potentials recorded from the amygdaloid nuclei are population spikes. The population spike amplitude was measured as shown in Fig. 1B. The stimulus intensity was adjusted to produce a population spike of about 50% of the maximum amplitude. Tetanic stimulation (100 pulses at 100 Hz) was applied at the same stimulus intensity through the same electrode as used for test stimulation. As described in our previous paper,[29] LTP was considered to have occurred if the potentiated spike amplitude remained at least 20% higher than the baseline value 30 min after tetanus. All drugs were delivered by perfusion.

RESULTS

In control experiments, application of tetanic stimulation (100 pulses at 100 Hz) to the stria terminalis increased the subsequent synaptic responses in the medial amygdala, but the potentiation declined to the baseline level within 20 min, which was regarded as short-term potentiation (STP) but not LTP (Fig. 2A). Similarly, application of a 100-pulse, 100-Hz tetanic stimulation to the external capsule produced only STP in the lateral amygdala (Fig. 2B).

In the medial amygdala, 1 μM isoproterenol, a β-adrenoceptor agonist, did not affect the baseline synaptic potentials evoked by test stimulation, but significantly enhanced the tetanus-induced STP and facilitated the induction of LTP (Fig. 2A). The STP-enhancing effect of isoproterenol was blocked by the concomitant presence of 10 μM timolol, a

β-adrenoceptor antagonist[12] (Fig. 2A). On the other hand, in the lateral amygdala, isoproterenol (1 μM) suppressed STP, and this effect of isoproterenol was blocked by timolol (Fig. 2B). Unlike isoproterenol, the α-adrenoceptor agonist phenylephrine (1 μM) showed no effect on the tetanus-induced STP in the lateral and medial amygdala (Fig. 3A, B).

It is generally known that stimulation of β-adrenoceptors activates adenylate cyclase and increases intracellular levels of cyclic AMP. To examine if adenylate cyclase is involved in the modulation of synaptic plasticity in the amygdala, we tested the effect of forskolin, an adenylate cyclase activator.[11,23] In the medial amygdala, 20 μM forskolin enhanced the tetanus-induced STP and facilitated the induction of LTP (Fig. 4A). On the other hand, in the lateral amygdala, 20 μM forskolin suppressed STP (Fig. 4B). The effects of forskolin were very similar to those of isoproterenol in both medial and lateral amygdala.

Furthermore, it is generally presumed that β-adrenoceptor-mediated increases in intracellular cyclic AMP are followed by activation of cyclic AMP-dependent protein kinase (PKA). To test if PKA mediates the STP-modulating effects of isoproterenol, we examined the influence of Rp-adenosine-3′,5′-cyclic-monophosphothioate (Rp-cAMPS), a specific membrane-permeable inhibitor of PKA.[11,20,28] In the medial amygdala, 10 μM Rp-cAMPS alone did not affect the baseline synaptic potentials nor the tetanus-induced STP. However, when 1 μM isoproterenol and 10 μM Rp-cAMPS were applied together, only STP was induced, indicating that the STP-enhancing effect of isoproterenol was blocked by the presence of Rp-cAMPS (Fig. 5A). Similarly, in the lateral amygdala, the STP-suppressing

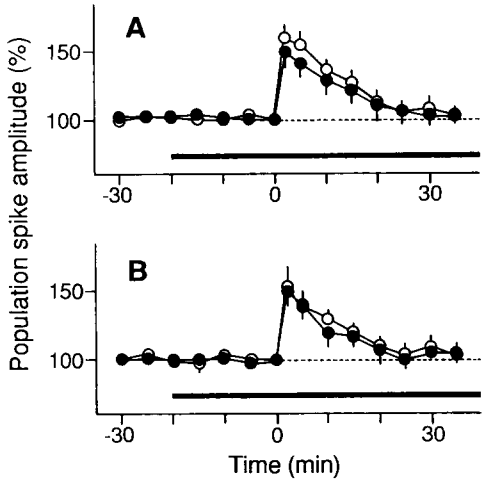

Fig. 3. Effects of phenylephrine on STP in the medial (A) and lateral amygdala (B). During the time indicated by black bars, 1 μM phenylephrine (filled circles) was perfused and tetanic stimulation (100 pulses at 100 Hz) was applied at time 0. The STP in the presence of phenylephrine was not significantly different from the control STP in normal ACSF (open circles). Abscissa and ordinate are as in Fig. 2. All data are represented as means ± S.E.M. ($n = 5$).

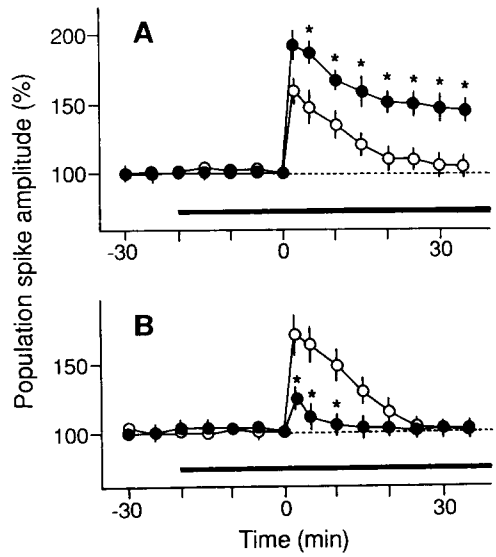

Fig. 4. Forskolin mimicked the effects of isoproterenol on STP in the medial (A) and lateral amygdala (B). During the time indicated by black bars, 20 μM forskolin (filled circles) was perfused and tetanic stimulation (100 pulses at 100 Hz) was applied at time 0. Open circles represent control STP in normal ACSF. Abscissa and ordinate are as in Fig. 2. All data are represented as means ± S.E.M. ($n = 6$). $^*P < 0.05$ vs control; Mann–Whitney U-test.

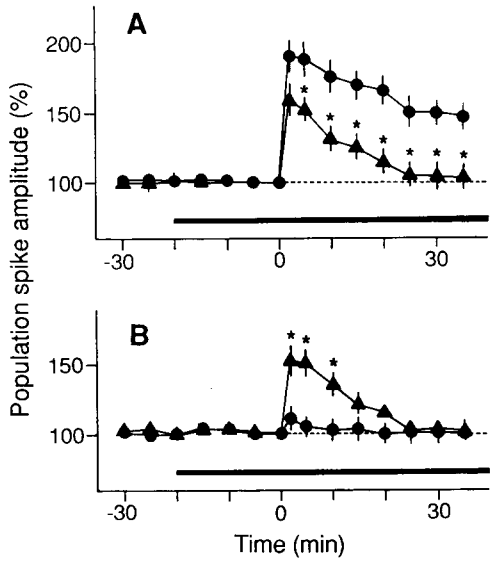

Fig. 5. Rp-cAMPS blocked the STP-enhancing and -suppressing effects of isoproterenol in the medial (A) and lateral amygdala (B), respectively. During the time indicated by black bars, 1 μM isoproterenol alone (filled circles) or 1 μM isoproterenol plus 10 μM Rp-cAMPS (filled triangles) was perfused and tetanic stimulation (100 pulses at 100 Hz) was applied at time 0. Abscissa and ordinate are as in Fig. 2. All data are represented as means ± S.E.M. ($n = 5$). $^*P < 0.05$ vs isoproterenol alone; Mann–Whitney U-test.

effect of 1 μM isoproterenol was blocked by 10 μM Rp-cAMPS (Fig. 5B).

DISCUSSION

The main finding in the present study was that isoproterenol enhanced STP in the medial amygdala but suppressed STP in the lateral amygdala. Both effects of isoproterenol were blocked by timolol, and phenylephrine did not affect STP. These results suggest that activation of β-adrenoceptors facilitates synaptic plasticity in the medial amygdala and suppresses synaptic plasticity in the lateral amygdala. Since the STP-enhancing and -suppressing effects of isoproterenol were mimicked by forskolin and blocked by Rp-cAMPS, it is probable that both effects are mediated by the adenylate cyclase–cyclic AMP–PKA signal transduction pathway.

Although it is not clear why the β-adrenoceptor–cyclic AMP–PKA signal exerts opposite effects on synaptic plasticity in the medial and lateral amygdala, several possibilities may be considered. In the hippocampus, norepinephrine facilitates the induction of LTP through β-adrenoceptors and cyclic AMP.[7,10] The LTP-facilitating effect of norepinephrine may be associated with a depolarizing action[15,22] or with potentiation of *N*-methyl-D-aspartate receptor-mediated responses[2,11] or with enhancement of voltage-gated Ca^{2+} currents.[9] The role of β-adrenoceptors in the medial amygdala may be similar to that in the hippocampus. On the other hand, in cerebellar Purkinje cells, norepinephrine enhances GABA-mediated inhibitory synaptic mechanisms via β-adrenoceptors and cyclic AMP.[5,23] Since synaptic plasticity in the amygdala is suppressed by GABA receptor-mediated inhibition,[29] it is likely that the β-adrenoceptor–cyclic AMP system in the lateral amygdala suppresses STP by enhancing GABAergic inhibition. In addition, it is possible that the β-adrenoceptor agonist and forskolin act on multiple cell types or pathways within the slice. The differential effects observed in the medial and lateral amygdala may reflect the difference in neural circuit connections.

Several behavioral studies have shown that β-adrenoceptors in the amygdala are involved in learning and memory in intact animals.[13,16] For example, Liang *et al.*[13] have reported that intra-amygdala injection of norepinephrine shows memory-enhancing effects in rats and that the effects are blocked by simultaneous administration of a β-adrenoceptor antagonist, propranolol. However, there are no reports addressing the possibility that β-adrenoceptors in the medial and lateral amygdala play different roles in memory processing. It would be interesting to test if β-adrenoceptor agonist injected into the medial and lateral amygdala would show differential effects on learning behaviors. Such data will provide further information about the role of amygdaloid LTP in learning and memory.

CONCLUSION

These studies demonstrate for the first time that β-adrenoceptor stimulation facilitates and suppresses synaptic plasticity in the medial and lateral amygdala, respectively. Both effects are mediated by the cyclic AMP–PKA signal transduction pathway. Differential regulation by β-adrenoceptors of synaptic plasticity in the medial and lateral amygdala may be important for selective information processing in the amygdaloid complex.

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