The role of basolateral amygdala adrenergic receptors in hippocampus dependent spatial memory in rat

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Received 10 Dec 2006; Revised 1 Sep 2007; Accepted 12 Sep 2007

ABSTRACT

Background and the purpose of the study: There are extensive evidences indicating that the noradrenergic system of the basolateral nucleus of the amygdala (BLA) is involved in memory processes. The present study investigated the role of the BLA adrenergic receptors (ARs) in hippocampus dependent spatial memory in place avoidance task in male rat.

Material and Methods: Long Evans rats (n=150) were trained to avoid footshock in a 60° segment while foraging for scattered food on a circular (80-cm diameter) arena. The rats were injected bilaterally in the BLA specific ARS (Adrenergic receptors) agonist norepinephrine (NE, 0.5 and 1 μg/μl) and specific β-ARs antagonist propranolol (PRO, 0.5 and 1 μg/μl) before acquisition, after training or before retrieval of the place avoidance task. Control rats received vehicle at the same volume. The learning in a single 30-min session was assessed 24h later by a 30-min extinction trial in which the time to first entrance and the number of entrances to the shocked area measured the avoidance memory.

Results: Acquisition and consolidation were enhanced and impaired significantly by NE and PRO when the drugs were injected 10 min before or immediately after training, respectively. In contrast, neither NE nor PRO influenced animal performances when injected before retention testing.

Conclusion: Findings of this study indicates that adrenergic system of the BLA plays an important role in regulation of memory storage and show further evidences for the opinion that the BLA plays an important role in integrating hormonal and neurotransmitter influences on memory storage.

Keywords: Basolateral amygdala, Place avoidance learning, Consolidation, Retrieval

INTRODUCTION

Adrenal hormones (catecholamines and glucocorticoids) together and with other components which are secreted during stressful events from adrenal glands have influence on the organism's ability to cope with stress. These hormones also affect memory function by influences on limbic brain structures (1). Also there are many evidences which support the view that specific hormonal and brain systems are activated by emotional arousal and regulate long-term memory storage (2). Extensive evidences have shown that the amygdala especially the basolateral amygdala (BLA) plays an important role in the integration of hormonal and neurotransmitter influences on memory formation. This area is critically involved in regulation of processes of acquisition, consolidation and retrieval of different forms of memory, including explicit and declarative (3).

It is well established that adrenergic drugs that readily enter to the brain activate adrenergic receptors of the brain, and regulate a variety of functions including memory processing (4, 5). Considerable evidences indicate that BLA plays a critical role in mediating influences of adrenergic drugs on memory storage. For example, lesions of the amygdala or the stria terminalis, a major amygdala pathway, block epinephrine-induced enhancing effects on memory (6). Also post-training infusions of NE or the β-adrenoreceptor agonist clenbuterol into the amygdala enhance memory storage (7-8). Systemic post-training administration of the agonist and antagonist of adrenergic receptors enhance and impair memory for inhibitory avoidance training and this effect is blocked in animals with excitotoxic lesions of the BLA (9). BLA lesions selectively blocked deficits in the water-maze escape training induced by adrenalectomy and/or by intracerebroventricular infusions of a specific adrenergic receptor antagonist (10). While post-training intra-BLA administration of NE or propranolol as β-adrenoreceptor agonist enhance inhibitory

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avoidance retention, post-training infusions of β-adrenoreceptor antagonists, propranolol into the BLA produce retention deficits attenuated by concurrent infusions of NE (5). Microinfusions of the β-adrenergic antagonist PRO into the amygdala blocks the memory-modulatory effects of epinephrine. Finally, post-training activation of amygdala adrenoreceptors (both α_1 and β) enhance memory for several tasks including the water-maze. These reports indicate that the α_1 and β-adrenoreceptor subtypes within the BLA plays an important role in emotional memory (4, 5) but their role in place avoidance memory, a form of hippocampus-dependent memory, is not clear. Thus, this study was designed to investigate the role of α and β-adrenoreceptor of the BLA in various phases of memory processing in place avoidance task in male rats.

MATERIAL AND METHODS

Apparatus
Place avoidance Setup (Germany) **(**Consisting of Circular metal arena, Infrared television camera, light-emitting diode, Computer and appendixes).

Surgery and injection equipments
Stereotaxic apparatus (USA), Guide cannulae, Dental acrylic, Stylets, Hamilton syringe, Polyethylene tube, T-shaped anchors (Czech Republic).

Drugs
Norepinephrine (Sigma), Propranolol (Sigma), Atropine (Sigma), Thiopental, Penicillin G

Subjects
Adult male rats of Long-Evans strain (weighting between 250-300 grams) were obtained from the Institute breeding colony of academy of sciences of Czech Republic. They were housed in groups of four in plastic cages in a laboratory vivarium at constant temperature (21°C) and natural lighting conditions. Water was freely available but food was only available for 1 h at the end of experiments in such a way that to keep 90% of their free feeding weights. A total of 150 rats (120 treatment and 30 vehicle) in 15 groups (n =10) were used in experiments.

Surgical Procedure
For surgery, rats were premeditated by intraperitoneal injection of atropine sulfate (0.5 mg/kg) and ten min later anaesthetized with thiopental (50-mg/kg IP). When the surgical stage of anesthesia was reached, rats were fixed in the stereotaxic apparatus and a midline incision of the skin in cranial region was made. The skull was dried and cleaned of fascias, and two slits were drilled bilaterally over the parietal region. Two stainless steel T-shaped anchors were put epidurally. Then a 4-cm long, uninstalled silver wire 200 μm in diameter was implanted subcutaneously at the back of neck and attached to connector cemented to the rat's skull. Permanent stainless steel guide cannulae (22 gauge, 12mm) aimed at both sides of the BLA (anterior–posterior =AP, -3 mm from bregma, medial-lateral= ML, ± 4.9 mm from midline, dorsal-ventral= DV, -6.4 mm from the surface of skull, (11) nose bar= -3.30 mm from interaural line were implanted bilaterally (Fig 1). The cannulae were affixed to the skull with dental acrylic; stylets were inserted into the cannulae to keep them patent. After surgery, each rat received immediately penicillin G (15000 - 30000 units IM) and was placed in a temperature-controlled chamber until recovery from anesthesia (12).

Place avoidance training

Apparatus
An elevated (50 cm) circular metal arena 80-cm in diameter was used. It was centered in a 5m x 4m room with many visual landmarks surrounding the maze. A feeder mounted 2 m above the arena dropped 20-mg pasta pellets to random places in the arena at 10 s intervals. Over 3 days, the rats were trained in a daily 20-min session to forage for scattered food (12, 13).

Tracking system
An infrared television camera mounted on the ceiling above the arena was used to record the position of the rat by tracking an infrared LED that was held between the rat's shoulders by a latex harness. A custom tracker in a PC was used to analyze the television signal. Position was recorded with a spatial resolution of 0.4 cm and a temporal resolution of 100 Ms (12).

Behavioral Training
Avoidance began after a week of recovery. Whenever the rat entered the prohibited sector for >0.5 s, 50 Hz current (<0.6 mA) was delivered for 0.5 s between the implanted wire and the high impedance contact between the rats feet and the grounded arena floor. The shock was repeated after 3 s if the animal did not leave the prohibited area. The shock condition was only intended to be unpleasant, and, once trained, the rats continued to forage over the unpunished surface of the arena without having signs of fear. Retention test of the place avoidance training was assessed 24h later by a 30-min extinction trial in which the latency time to first entrance (LT) and the number of entrances (NOE) to shocked area were used to measure the avoidance memory (12 -13).

Drugs and injection procedures
The specific ARs agonist (NE 0.5 and 1 μg/μl) or ARs antagonist (PRO, 0.5 and 1 μg/μl) or vehicle

was injected into the cannulae bilaterally through injection needles (30 gauge, 14 mm) attached to 10 µl Hamilton syringes via polyethylene tubing. The infusion was delivered at a rate of 1µl/min for 1 min. The injection needles remained in the cannulae for 1-min following the infusion in order to maximize diffusion from the needle tip and to minimize back flow along the injection track. Norepinephrine (Sigma, Germany) or Propranolol (Sigma, Germany) were dissolved in 0.9% saline and infused into either the left or right BLA 10 min prior or immediately after or 10 min prior of retrieval test. Drug solutions were freshly prepared and the doses were selected on the basis of references (4-5).

Histology

After completion of the behavioral tests, the rats were anaesthetized with an overdose of thiopental sodium (100-mg/kg IP). The brains were removed and placed in a 10% formalin solution for approximately 1 week, then sectioned into 40 um slices with a freezing microtome, and stained with thionin. Cannulae location was determined using a light microscope and standardized atlas plates (11) by an observer blind to the behavioral results. If the cannulae tip was not located in BLA, the rat was not included in the statistical analyses (Fig. 1).

Statistics

Retention data were analyzed by using one-way ANOVA. Turkey's post hoc test was performed to determine the source of detected significances. Values of $P<0.05$ were considered as significant.

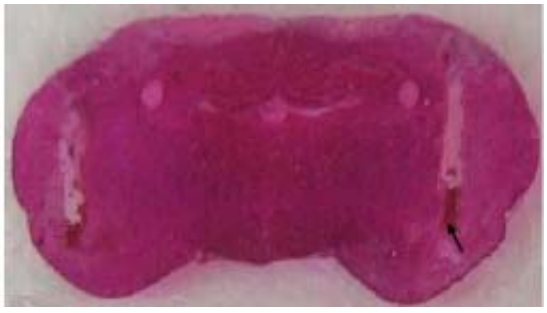

Figure 1. Representative photomicrographs illustrating placement of cannulae and needle tip in the basolateral amygdala. Arrow points the needle tip.

RESULTS

Intra-BLA NE infusions enhance acquisition, consolidation, but not retrieval of place avoidance memory

Figure 2 shows the effects of intra-BLA infusions of NE on memory processing in place avoidance memory. One -way ANOVA on retention data of acquisition experiment revealed significant effects of NE on both LT ($F2, 27 = 22.86$; $P = 0.001$), and NOE ($F2, 27 = 3.005$; $P = 0.001$). Also analysis of retention data of consolidation experiment revealed significant effects of NE on both LT ($F2, 27 = 30.54$; $P = 0.001$), and NOE ($F2, 27 = 13.04$; $P = 0.001$). Post-hoc analysis showed that the NE at the dose of 0.5 µg but not 1µg significantly increased LT and decreased NOE in comparison to those of control groups, respectively ($P<0.01$ in both cases) Analysis of retrieval experiment data revealed no significant effects of NE on LT ($F2, 27 = 1.566$; $P = 0.227$), and NOE ($F2, 27 = 0.981$; $P = 0.3$).

Intra-BLA PRO infusions impairs acquisition, consolidation, but not retrieval of place avoidance memory

Fig.3 shows the effects of intra-BLA infusions of PRO on memory processing in place avoidance memory. One -way ANOVA on retention data of acquisition experiment revealed significant effects of PRO on both LT ($F2, 27 = 17.34$; $P = 0.001$), and NOE ($F2, 27 = 20.40$; $P = 0.001$). Also analysis of retention data of consolidation experiment revealed significant effects of NE on both LT ($F2, 27 = 21.03$; $P = 0.001$), and NOE ($F2, 27 = 27.84$; $P = 0.001$). Post-hoc analysis showed that the PRO at both doses decreased LT and increased NOE significantly in comparison to those of control groups, ($P<0.01$ in both cases). Analysis of data of retrieval experiment revealed no significant effects of PRO on LT ($F2, 27 = 1.006$; $P = 0.3$), and NOE ($F2, 27 = 0.772$; $P = 0.47$).

DISCUSSION

The major findings of the present study are that infusions of NE and PRO into the BLA enhance and impair acquisition and consolidation of place avoidance memory, respectively. Retrieval was not affected by the treatments. The findings of this study confirm this view that an intact BLA is necessary for place avoidance memory as it was reported recently by our group (12).

Epinephrine and nor-epinephrine are released from the adrenal gland in a variety of stressful conditions. Several findings suggest that epinephrine activates β-adrenergic receptors located on vagal afferents that project to the nucleus of the solitary tract (NTS) and projection from the NTS releases NE in the amygdala (6). Strong evidences suggest that NE released in the BLA by arousing stimuli is involved in regulation of memory storage (5, 14-15). These findings support the view that BLA has a role in modulation of memory storage by interaction with other brain regions (16). As far as it is known, there is no evidence suggesting that the adrenergic

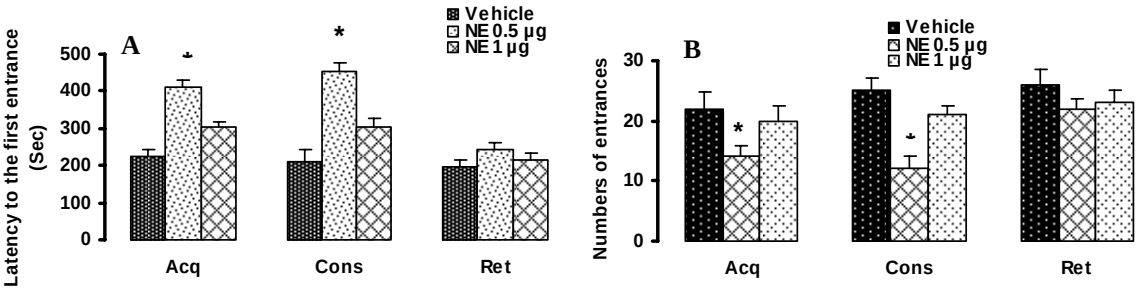

Figure 2. The effects of 10 min before and immediately after training or extinction of injections of ARs agonist (NE) into the BLA on acquisition, consolidation and retrieval of spatial memory in place avoidance learning. (A) Mean (± SEM) latency time (the time of the first entry to the zone shock) and (B) Mean (± SEM) of numbers of entrances during extinction at the 30 min. *P < 0.01 compared with control group.

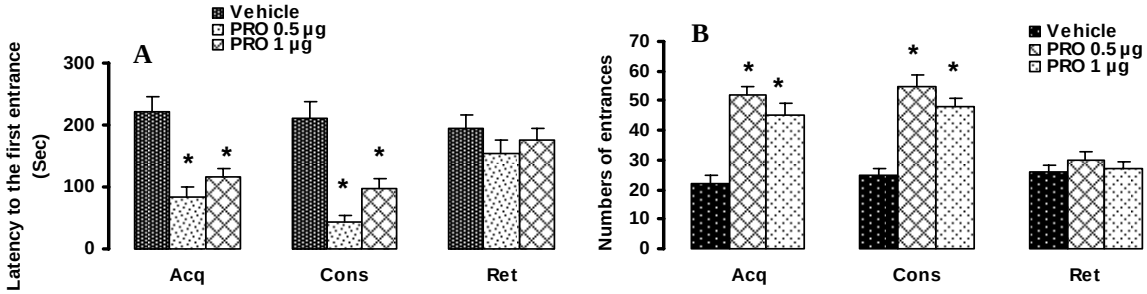

Figure 3. The effects of 10 min before and immediately after training or extinction injections of β-ARs antagonist (PRO) into the BLA on acquisition, consolidation and retrieval of spatial memory in place avoidance learning. (A) Mean (± SEM) latency time (the time of the first entry to the zone shock) and (B) Mean (± SEM) of numbers of entrances during extinction at the 30 min. *P < 0.01 compared with control group.

system of the BLA is necessary for place avoidance memory. However, in this study it was found that manipulation of the adrenergic system in the BLA has a profound effect on both acquisition and consolidation, but not on retrieval of place avoidance memory. Infusions of NE dose-dependently enhanced acquisition and consolidation of place avoidance memory. In contrast, propranolol as a β-adrenergic antagonist produced a dramatic impairment in the place avoidance memory. These results are highly similar to those previously found with the passive avoidance (14) and the water maze (8) tasks. In the study of Liang and colleagues, it was shown that the enhancing effect of NE was attenuated by concurrent administration of propranolol (14), which may indicates that NE via β-adrenergic receptors enhanced memory retention. Additionally, it was shown that levels of cAMP (as a second messenger in β-adrenergic receptor signaling) in the BLA are increased by NE (17). Also the β-adrenergic receptors of the BLA mediate the effects of other drugs on memory. For example, it has been shown that intra-BLA infusions of the β-adrenoreceptor antagonist atenolol blocked the memory enhancement induced by intrahippocampal infusions of glucocorticoids (6). Thus, it is more likely that the memory enhancing effects of NE in the present study is mediated by activation of β-adrenergic receptors. Results of this investigation are consistent with other studies indicating that infusions of amphetamine (18) and NE (8) into the amygdala resulted in enhanced memory in the water maze and passive avoidance tasks. Since amphetamine has been found to release both dopamine and NE in specific brain regions in certain conditions (14), it has been suggested that amphetamine enhances memory through release of NE (19).

In conclusion, our findings suggest that modulation of adrenergic system in the BLA can influence place avoidance memory. The results of this study indicate that memory for place avoidance task, a hippocampus-dependent task, can be enhanced or impaired by treatments that increase NE or block β-adrenergic receptors in the BLA and supports further evidence for the opinion that the BLA plays an important role in integrating hormonal and neurotransmitter influences on memory storage.

ACKNOWLEDGEMENTS

The authors would like to thank the Prof. June Bures and all employee of department of neurobiology of learning and memory, institute of physiology of Prague in Czech Republic for their useful comments and technical assistance during the study. The Iranian Ministry of Health and Medical Education (Under-secretary of Student Affairs) supported this work.

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