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GLUCOCORTICOID ANTAGONIST ADMINISTRATION INTO THE BASOLATERAL AMYGDALA MODULATES PLACE AVOIDANCE MEMORY

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ABSTRACT

The present study examined the significance of glucocorticoid receptors (GRs) in the basolateral amygdala (BLA) on place avoidance memory in male rats. Rats 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 with the specific GR antagonist RU 38486 (3 ng dissolved in 1 µl 2% ethanol) before acquisition, after training or before retrieval of the place avoidance task. Control rats were injected with vehicle. 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. Acquisition and consolidation were impaired when the drug was injected 10 min before training, immediately after training, or 60 min after training ($P<0.01$), but not 120 min after training. In contrast, injection of the antagonist before the retrieval test was ineffective ($P>0.05$). These findings indicate that glucocorticoids affect memory storage at least in part by binding directly to GR in the BLA and provide further evidences for the opinion that the BLA plays an important role in integrating hormonal and neurotransmitter influences on memory storage.

Key words: Basolateral amygdala, Place avoidance learning, Acquisition, Consolidation, Retrieval, RU38486

INTRODUCTION

There is evidence supporting the view that specific hormonal and brain system are activated by emotional arousal which regulates long-term memory storage (1). Extensive evidence implicates the amygdala in the integration of hormonal and neurotransmitter influences on memory formation (2). Of particular significance are the extensive evidences that memory can be selectively enhanced by post-training administration of drugs and hormones. Whether memory is enhanced or impaired by post-training treatment depends on the specific experimental conditions as well as the post-training treatment (1,3). It is well established that glucocorticoids readily enter the brain and activate adrenal steroid receptors. There is also evidence that post-training systemic injections of adrenal glucocorticoid modulate memory reten-tion (4). With both types of adrenal stress hormones (adrenal catecholamines and glucocorticoids) administered after training, the effects on memory are dose and time-dependent (5). Enhancement is found with moderate doses and maximal enhancement is obtained with injections administered immediately after training. There is also evidence that these adrenal stress-related hormones interact in modulating memory storage (4, 5, 6). Recent reports indicate that systemic post-training administration of the synthetic glucocorticoid, dexamethasone enhanced memory for inhibitory avoidance training and that the enhancement was blocked in animals with excitotoxic lesions of the basolateral nucleus of the amygdala (BLA) (7). Furthermore BLA lesions also selectively blocked deficits in water-maze escape training induced by adrenalectomy

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and by intracerebroventricular infusions of a specific glucocorticoid receptor (GR or Type-II) antagonist (8). These finding together with evidence that the BLA contains a moderate density of GR (9), suggest that glucocorticoid effects on memory modulation may be mediated at least in part by activation of GR in the BLA. In order to evaluate this implication further the present study examined the effect of post-training infusions of a specific glucocorticoid receptor (GR or Type-II) antagonist into the BLA on memory for place avoidance learning.

MATERIAL AND METHODS

Subjects: Adult male rats of Long-Evans strain, weighting between 300-350 grams, were obtained from the Institute breeding colony of academy of sciences in Prague. 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 after the conclusion of the procedures to keep at 90% of their free feeding weight. A total of 80 rats (40 treatment and 40 vehicle) in ten groups (n=8) were used in experiment.

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. The room could be completely dark when the lights were switched off. An electromotor under the control of a PC could rotate the arena about its center at 1 rpm. A feeder mounted 2 m above the arena dropped 20 mg pasta pellets to random places in the arena at 10 sec intervals. Over 3 days, the rats were trained in a daily 20 min session to forage for scattered food.

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 analyzed the television signal. Position was recorded with a spatial resolution of 0.4 cm and a temporal resolution of 100 ms. The system allowed interactive control of the experiment, for example when various behavioral criteria were met like entering and staying within a region, foot shock could be activated. A second LED was attached to the periphery of the arena so that when the arena rotated the rat's position relative to both room cues, the position of any landmarks on the floor of the arena could be determined.

Surgical Procedure: For surgery, rats were pre-medicated with intraperitoneal injection of atropine sulfate (0.5 mg/kg) and 10 min later anaesthetized with thiopental (50 mg/kg i.p). 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, uninsulated silver wire 200 um in diameter was implanted subcutaneously at the back of neck and was attached to connector cemented to the rat's skull. Permanent stainless steel guide cannulae (22 gauge, 12mm) aimed at either the basolateral amygdala (anterior-posterior AP= 3 mm from bregma, medial-lateral ML= ± 4.9 mm from midline, dorsal-ventral DV= 6.4 mm from surface of skull, (10) nose bar of 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. Immediately after surgery, each rat received penicillin G (15000-30000 unit i.m) and was placed in a temperature-controlled chamber until recovery from anesthesia.

Behavioral Training: Avoidance began after a week of recovery. A counter-balanced cable was attached to the connector to power the shock and an infrared light-emitting diode was attached between the rat's shoulders by a latex harness. A custom made computer-based system tracked the light-emitting diode position in the reference frame of the room every 100 msec by using an overhead television camera. The room frame position of a second LED on the outside of the arena also was tracked and was used to calculate the rat's position in the reference frame of the arena. A prohibited sector was defined in both reference frames as a 60° partial sector centered in one of the four quadrants. Whenever the rat entered the prohibited sector for >0.5 sec, 50 Hz

current (<0.6 mA) was delivered for 0.5 sec between the implanted wire and the high impedance contact between the rats feet and the grounded arena floor. The shock was repeated after 3 sec if the animal did not leave the prohibited area. The shock condition was intended to be unpleasant, and, once trained, the rats continued to forage over the unpunished surface of the arena without signs of fear. Retention test of the place avoidance training was assessed 24 h later by a 30 min extinction trial in which the time to first enter and the number of entrances to shocked area was measured the avoidance memory.

Drug and Injection Procedure: The specific GR antagonist RU 38486 (3 ng/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. RU 38486 (Russell UCLA Co. France) was dissolved initially in 100% ethanol and diluted to a final concentration of 2% ethanol in 0.9% saline. A 2% ethanol solution in saline was used for vehicle control injection. All solutions were freshly prepared before each experiment. Doses of drugs were selected as used previously (2,7,8).

Histology: After completion of the behavioral tests, the rats were anaesthetized with an overdose of thiopental sodium (100 mg/kg i.p). 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. Cannula location was determined using a light microscope and standardized atlas plates (10) by an observer blind to the behavioral results. If the cannula tip was not located in BLA, the rat was not included into the statistical analysis.

Statistics: Retention was analyzed using analysis of variance and t-test. An alpha level of 0.05 was used as a criterion of statistical significance.

RESULTS

Figures 2,3 illustrate typical results obtained in the retention tests. The time to the first entrance and the numbers of entrances during extinction were used to measure the avoidance memory. The data indicated that acquisition and consolidation was impaired when the injections were applied immediately after training according to the both retention measures. In comparison with the retention performance of control rats receiving intracerebral injection of the vehicle, rats given infusions of the specific GR antagonist RU 38486 (3 ng/μl) into the basolateral amygdala had significantly shorter latency of first entrance and higher number of entrances ($P<0.01$). The effects of delayed infusions of specific GR antagonist RU 38486 (3 ng/μl) were examined in rats with cannulae aimed at the basolateral amygdala. The results indicated that the memory-impairing effects of post-training infusion of specific GR antagonist RU 38486 (3 ng/μl) into the basolateral amygdala were time-dependent. Rats given infusions of specific GR antagonist RU 38486 (3 ng/μl) into the basolateral amygdala 60 min after training, had significantly shorter latency of first entrance and higher number of entrances in comparison with control group ($P<0.01$). However, post-training infusions of specific GR antagonist RU 38486 (3 ng/μl) into the basolateral amygdala administrated 120 min after training did not affect retention performances ($P>0.05$). Retrieval was also not impaired under injections ($P>0.05$).

DISCUSSION

The major finding of the present experiment is that infusion of the drugs into the BLA affecting GR modulates memory storage and impairs retention of the place avoidance task assessed during a 30 min extinction trial 24h later. The BLA injection of GR antagonist impaired acquisition and consolidation when applied 10 min before and immediately or 60 min after training but had no effects 120 minutes after training. Acquisition and retrieval were also not impaired under BLA inactivation. This treatment produces deficits in retention performance as assessed by two behavioral measures: the time to the first entrance and the number of entrances during extinction. Therefore, post-training administration of the GR antagonist impaired memory

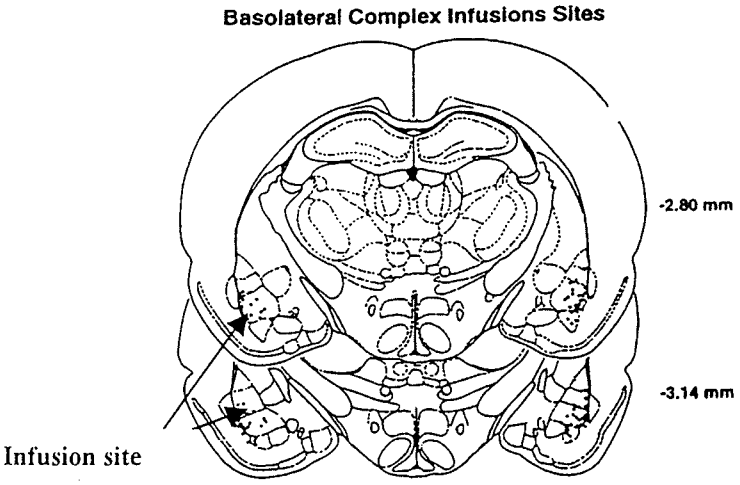

Fig 1. Illustration of approximate location of injection needle tips observed for animals in the BLA (10).

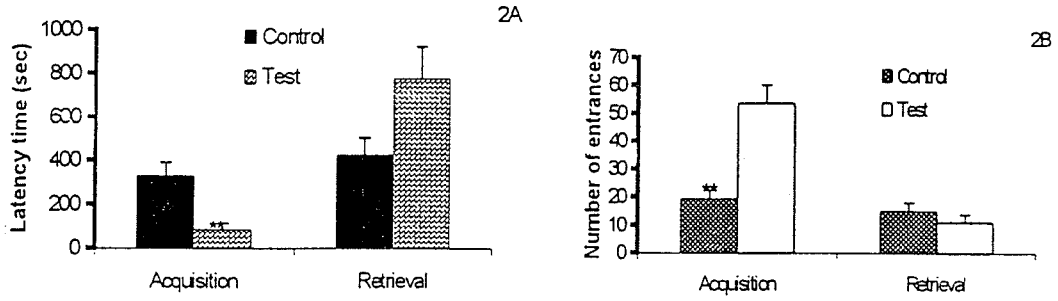

Fig 2. The effects of 10 min before training or extinction injections of GR antagonist (RU 38486) into the BLA on acquisition and retrieval of place avoidance learning. (A) Mean±SEM latency time (the time of the first entry to the zone shock) and (B) Mean±SEM of numbers of entrances during extinction on the 30 min. **P <0.01 compared with control group.

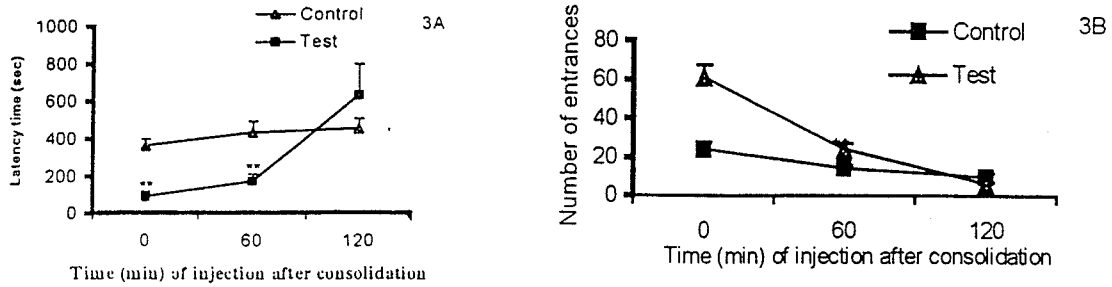

Fig 3. The effects of immediate and delayed (60,120 min) injections of GR antagonist (RU 38486) after training into the BLA on consolidation of place avoidance learning. (A) Mean ± SEM latency time (the time of the first entry to the zone shock) and (B) Mean ± SEM of numbers of entrances during extinction on the 30 min. **P < 0.01 compared with control group.

for place avoidance training. The present experimental results support the hypothesis that binding of glucocorticoid to GR in the BLA affects memory storage. However, GR are widely distributed in the brain (4), and binding of glucocorticoid to GR in other brain regions such as the hippocampus also profoundly affects learning and memory (11). Previous finding indicate that lesions of the BLA block the effects on memory storage. The present finding is supported by a report (7) that effects of other drugs and hormones on memory storage are mediated by amygdala influences. Extensive evidence indicates that activation of adrenal steroid receptors in the hippocampus plays an important role in mediating the effects of glucocorticoid on memory storage (9,13). However, recent findings indicate that glucocorticoids also affect memory storage through influences involving the amygdala. Moreover, lesions of the amygdala restricted selectively to the basolateral nucleus also block the memory-enhancing effects of post-training systemic injections of dexamethasone (5). It is important to note that the basolateral lesions alone do not impair retention of inhibitory avoidance. Post-training infusions of a specific glucocorticoid receptor agonist (RU 28362) enhances retention when administered into the basolateral nucleus but are ineffective when administered into the central nucleus (5). The results of this experiment strongly suggests that the central nucleus is not involved in mediating the memory-modulating effects of glucocorticoid. Additionally, as was found in studies of the effects of epinephrine, the memory-modulating effects of glucocorticoid involve noradrenergic activation within the amygdala. Propranolol infused into the basolateral nucleus blocks the memory-enhancing effects of post-training systemic injections of dexamethasone (14).

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