Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Involvement of basolateral amygdala $GABA_A$ receptors in the effect of dexamethasone on memory in rats*

Lotfollah KHAJEHPOUR†, Acieh ALIZADEH-MAKVANDI,
Mahnaz KESMATI, Hooman ESHAGH-HAROONI
(*Department of Biology, College of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran*)
†E-mail: khajehpour@scu.ac.ir

Received Nov. 5, 2010; Revision accepted Mar. 8, 2011; Crosschecked Sept. 30, 2011

Abstract: In this study we investigated whether $GABA_A$ receptors of the basolateral amygdala (BLA) interact with the effect of dexamethasone on the retrieval stage of memory. Adult male Wistar rats were bilaterally cannulated in the BLA by stereotaxic surgery. The animals were trained in step-through apparatus by induction of electric shock (1.5 mA, 3 s) and were tested for memory retrieval after 1 d. The time of latency for entering the dark compartment of the instrument and the time spent by rats in this chamber were recorded for evaluation of the animals' retrieval in passive avoidance memory. Administration of dexamethasone (0.3 and 0.9 mg/kg, subcutaneously (s.c.)), immediately after training, enhanced memory retrieval. This effect was reduced by intra-BLA microinjection of muscimol (0.125, 0.250 and 0.500 μg/rat), when administered before 0.9 mg/kg of dexamethasone. Microinjection of bicuculline (0.75 μg/rat, intra-BLA) with an ineffective dose of dexamethasone (0.1 mg/kg, s.c.) increased memory retrieval. However, the same doses of muscimol and bicuculline without dexamethasone did not affect memory processes. Our data support reports that dexamethasone enhances memory retrieval. It seems that $GABA_A$ receptors of the BLA mediate the effect of dexamethasone on memory retrieval in rats.

Key words: Basolateral amygdala, Dexamethasone, Muscimol, Bicuculline, Passive avoidance task
doi:10.1631/jzus.B1000340 **Document code:** A **CLC number:** Q42

1 Introduction

Several studies show that glucocorticoids, the main stress hormones, modulate processes of memory by influencing the limbic system. The activation of glucocorticoid-sensitive pathways by the administration of agonists of these hormones after training, enhances memory consolidation (Power *et al*., 2000; Paré, 2003; Roozendaal *et al*., 2006). Glucocorticoids act via intracellular receptors (genomic pathway) and membrane receptors (Makara and Haller, 2001). Therefore, the effects of glucocorticoids on memory can be mediated via these two receptor mechanisms by interaction with several neurotransmitter systems in the brain including noradrenergic, cholinergic and opioidergic systems (Rashidy-Pour *et al*., 2004; Roozendaal *et al*., 2004; Khaksari *et al*., 2007).

The amygdala, which is located in the medial temporal lobe of the brain, has an important role in cognitive behaviors such as learning and memory (Baxter and Murray, 2002; Sah *et al*., 2003). The basolateral nuclei of the amygdala mediate the effects of some hormones and neurotransmitters on memory formation (Power *et al*., 2000; McGaugh and Roozendaal, 2008) and connect the amygdala to other regions of the brain, such as the hippocampus and the prefrontal cortex, that are involved in the processes of memory (Roozendaal *et al*., 2009).

There is a high accumulation of glucocorticoid receptors in the basolateral amygdala (BLA) region

* Project supported by the Shahid Chamran University of Ahvaz, Iran

(Cintra *et al*., 1994). Post-training intra-BLA microinjection of glucocorticoid agonists in contextual fear conditioning or passive avoidance facilitates memory retention, whereas infusion of their antagonists impairs memory retrieval (Roozendaal and McGaugh, 1997; Donley *et al*., 2005). In addition, the memory enhancing effect of post-training systemic injection of dexamethasone, a glucocorticoid agonist, in passive avoidance tasks, is blocked by lesion of the BLA (Roozendaal and McGaugh, 1996; McGaugh and Roozendaal, 2008; Roozendaal *et al*., 2009). BLA mediates cognitive functions via several neurotransmitter and neuropeptide systems including opioidergic, noradrenergic, cholinergic, dopaminergic, and GABAergic systems (Quirarte *et al*., 1997; Wilensky *et al*., 2000; Stevenson and Gratton, 2003; Stevenson *et al*., 2003; McGaugh and Roozendaal, 2008).

γ-Aminobutyric acid (GABA) is one of the neurotransmitters of the peripheral and central nervous system, and it has an inhibiting action (Bormann, 2000; Watanabe *et al*., 2002). This neurotransmitter acts through three types of receptors: ionotropic $GABA_A$ and $GABA_C$ receptors and metabotropic $GABA_B$ receptor (Macdonald and Olsen, 1994; Bormann, 2000; Ong and Kerr, 2005; Emson, 2007; Olsen and Sieghart, 2009). Neuropharmacological findings obtained from studies of certain types of memory, such as reward, Y-maze, and passive avoidance, have demonstrated that memory processes are affected by GABAergic agents. For example, post-training peripheral or central (intra-amygdala) injections of antagonists of GABA receptors increase retrieval in these memory tasks, whereas their agonists impair memory formation (Stackman and Walsh, 1995; Ferry *et al*., 1999; Hatfield *et al*., 1999; Myhrer, 2003).

Several studies have shown that $GABA_A$ receptors are involved in modulation of memory processes (Izquierdo *et al*., 1990; Myhrer, 2003; Zarrindast *et al*., 2004). There is a high density of $GABA_A$ receptors in most regions of the brain including the amygdaloid complex (Salinas and McGaugh, 1996) and the hippocampus (Mao and Robinson, 1998). Therefore, memory formation is enhanced by intra-amygdala infusion of picrotoxin or bicuculline, antagonists of $GABA_A$ receptors, and impaired by infusion of muscimol, an agonist of $GABA_A$ receptors (Hatfield *et al*., 1999; Roozendaal *et al*., 2009). Since the BLA has an expansive distribution of GABAergic neurons and receptors, it also has a high concentration of receptors of glucocorticoid hormones. In this study we investigated whether $GABA_A$ receptors of the BLA interact with the effect of dexamethasone on memory retrieval in rats.

2 Materials and methods

2.1 Animals

Adult male Wistar rats (from 200–270 g in weight, from the Pasture Institute, Iran) were used in this study. The animals were placed, four per cage, in an animal house with a constant temperature [(24± 2) °C] and a 12-h light cycle (07:00 to 19:00) with water and food available ad libitum. To allow adaptation of the animals to the conditions in the laboratory, they were transferred to the laboratory room at least 7 d prior to surgical practice. They were also handled before the training day (3 min/d). The experiments were carried out between 09:00 and 14:00. Procedures used in this research were performed in compliance with the National Academy's guide for the care and use of animals in research.

2.2 Drugs

Dexamethasone (Synopharm, Italy) was dissolved in a vehicle (saline containing 2% ethanol) and injected subcutaneously (s.c.). Muscimol and bicuculline (Sigma, St. Louis, CA, USA) were dissolved in sterile 9 g/L saline and microinjected as an intra-BLA treatment. The doses of these drugs were derived from our pilot studies and other related reports (Power *et al*., 2000; Zarrindast *et al*., 2004).

2.3 Surgery and microinjections

One week before the beginning of the behavioral experiments, the animals were anesthetized by administration of ketamine hydrochloride (50 mg/kg) and xylazine (4 mg/kg) intraperitoneally, and then secured in the stereotaxic instrument (Stoelting, USA). Two sterile guide cannulas (22-gauge and 13 mm in length made from stainless steel) were placed bilaterally 1 mm above the region of injection based on the stereotaxic characteristics of the BLA, in accordance with the rat brain atlas of Paxinos and Watson (1997): anterior to bregma (AP) −2.8 mm,

lateral to the sagittal suture (L) ±5 mm, and ventral from the surface of the skull (V) 6.6 mm. The cannulas were affixed at the surface of the skull with dental cement. A Hamilton syringe (2 μl) and a needle (27-gauge and 14 mm in length), joined together by a polyethylene tube, were used for the microinjection of the drugs. The BLAs (in the right and the left sides of the brain) were infused with a 0.5-μl solution per side (1 μl/rat) within a period of 120 s (for microinjection and diffusion of drugs in the BLA).

2.4 Behavioral procedure

2.4.1 Instrument

The step-through instrument, used for training and testing of the animals, consisted of two compartments (each 20 cm×20 cm×30 cm): one light or white compartment and another dark or black, which were connected via a small door (7 cm×9 cm). Rods (2.5 mm in diameter and made from stainless steel) were placed at 1 cm intervals in the floor of the dark chamber. A stimulator delivered intermittent electric shocks (50 Hz, 1.5 mA for 3 s duration) to the floor of the dark chamber via these rods.

2.4.2 Training

One hour before the training, the animals were transferred to the experimental room. Each animal was placed in the light chamber of the apparatus and after 10 s the door to the dark compartment was opened. Immediately after the animal entered the dark compartment, the door was closed and the rat was transferred to the home cage. The time of latency for entering the dark compartment was recorded. Animals with a latency of more than 100 s were omitted from this research.

The acquisition trial was carried out 30 min later. Each animal was placed in the light compartment and after 10 s the door was opened. As soon as the animal entered the dark compartment and all four paws had been placed on the grid floor, the door was closed and an electric shock (50 Hz, 1.5 mA for 3 s) was immediately delivered to this chamber. The rat was temporarily transferred from the apparatus to its home cage about 20 s after receiving the shock. After 2 min, the rat was again placed in the apparatus (as in the prior trials); if the latency (to enter the dark chamber) was more than 120 s, the acquisition stage of passive avoidance memory was successfully completed. This procedure was repeated for animals that had not completed it successfully (each rat received the foot shock for a maximum of three times). In this research, all drugs were administered immediately after training (post-training).

2.4.3 Testing

Retention tests were performed to assess long-term memory 24 h after training. The animals were placed in the light chamber and after 10 s the door was opened. Without using the electrical shock during this phase, the latency of entering the dark compartment (step-through latency) and the time spent in the dark chamber, for assessment of memory retrieval, were recorded for 300 s.

2.5 Experiments

Post-training, each rat received either saline, muscimol, or bicuculline via an intra-BLA microinjection, and either dexamethasone or the vehicle s.c. 5 min later.

2.5.1 Effect of dexamethasone on memory retrieval

This experiment examined the effect of dexamethasone on memory retrieval. Four groups (n=8 each group) of rats were used. One group (control) received saline (1 μl/rat, intra-BLA) plus the vehicle (1 ml/kg, s.c.). The other groups of animals first received microinjections of saline (1 μl/rat, intra-BLA) and then different doses of dexamethasone (0.1, 0.3 and 0.9 mg/kg, s.c.). The animals were tested after one day and the step-through latency and the time spent in the dark chamber for each rat were recorded.

2.5.2 Effect of intra-BLA microinjection of muscimol on the effect of dexamethasone on memory retrieval

Eight groups (n=8 each group) of animals were used. Four groups received microinjection of muscimol (0, 0.125, 0.250 and 0.500 μg/rat, intra-BLA), an agonist of $GABA_A$ receptors, plus the vehicle (1 ml/kg, s.c.). The other four groups of animals received the muscimol (0, 0.125, 0.250 and 0.500 μg/rat, intra-BLA) plus dexamethasone (0.9 mg/kg, s.c.). The rats were tested one day later and the step-through latency and the time spent in the dark chamber for each rat were recorded.

2.5.3 Effect of intra-BLA microinjection of bicuculline on the effect of dexamethasone on memory retrieval

Eight groups (n=8 each group) of rats were used in this experiment. The first four groups received microinjection of bicuculline (0, 0.25, 0.50 and 0.75 μg/rat, intra-BLA) plus the vehicle (1 ml/kg, s.c.). The other four groups of animals received bicuculline (0, 0.25, 0.50 and 0.75 μg/rat, intra-BLA) plus dexamethasone (0.1 mg/kg, s.c.). The rats were tested one day later and the step-through latency and the time spent in the dark chamber for each rat were recorded.

2.6 Confirmation of injection sites

After the behavioral test, the rats were killed with ether and received a microinjection of 1% methylene blue (0.5 μl/side, intra-BLA). They were then decapitated and their brains were placed in a 10% formalin solution for 10 d. For the determination of the cannula locations and sites of injections, brain sections were examined for the BLA by reference to the rat brain atlas of Paxinos and Watson (1997). Data from rats that received drugs outside of the BLA were omitted.

2.7 Analysis of data

The data were analyzed by one or two-way analysis of variance (ANOVA) and Tukey's test using SPSS statistical software and the results expressed as mean±standard error of the mean (SEM). The statistical significance level was set at P<0.05.

3 Results

3.1 Effect of dexamethasone on memory retrieval

Fig. 1 shows the effects of dexamethasone (administered post-training) on step-through latency (Fig. 1a) and total time spent in the dark chamber (Fig. 1b). Statistical analysis by one-way ANOVA showed that different doses of dexamethasone (0.1, 0.3 and 0.9 mg/kg, s.c.) increased the step-through latency in a dose-dependent manner [F(3, 28)=11.517, P<0.001] and decreased the time spent in the dark compartment [F(3, 28)=7.209, P<0.01] in the passive avoidance task compared to the control group, indicating an enhancement of memory retrieval.

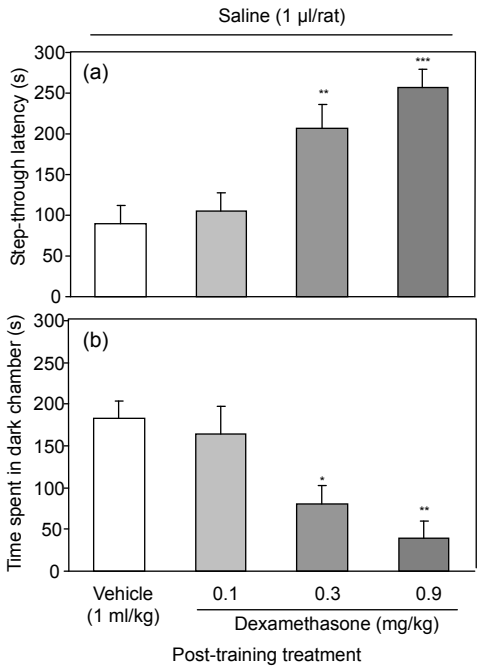

Fig. 1 Effect of dexamethasone on memory retrieval
(a) Step-through latencies; (b) Time spent in the dark chamber. Post-training, the animals received the vehicle (1 ml/kg, s.c.) or dexamethasone (0.1, 0.3, or 0.9 mg/kg, s.c.) plus saline (1 μl/rat) and were tested one day later. The columns show the mean±SEM (n=8). * P<0.05, ** P<0.01, *** P<0.001 compared to the control group (vehicle/saline)

3.2 Effect of intra-BLA microinjection of muscimol on the effect of dexamethasone on memory retrieval

Fig. 2a indicates the effect of muscimol (injected post-training) on the dexamethasone effect on step-through latencies. Statistical analysis by two-way ANOVA showed that there was an interaction effect on memory retrieval between the groups that received the muscimol (0, 0.125, 0.250 and 0.500 μg/rat, intra-BLA) plus the vehicle (1 ml/kg, s.c.) and the groups that received the muscimol (0, 0.125, 0.250 and 0.500 μg/rat, intra-BLA) plus dexamethasone (0.9 mg/kg, s.c.) [within group comparison: treatment effect, F(1, 56)=9.13, P<0.01; dose effect, F(3, 56)=5.22, P<0.01; treatment×dose interaction, F(3, 56)=4.26, P<0.01]. Furthermore, one-way ANOVA indicated that there was no significant change among the groups that received different doses of muscimol (0, 0.125, 0.250 and 0.500 μg/rat, intra-BLA) plus the vehicle [F(3, 28)=0.145, P>0.05]. The analysis revealed that the enhancement effect of 0.9 mg/kg of dexamethasone on memory retrieval was significantly

reduced by these doses of muscimol [$F(3, 28)=9.102$ $P<0.001$]. Tukey's test showed that the maximum response was obtained with 0.500 μg/rat of muscimol ($P<0.01$).

Fig. 2b shows the effect of muscimol on the dexamethasone effect on the time spent in the dark chamber. Two-way ANOVA showed that there was an interaction effect on memory retrieval between the groups that received muscimol (0, 0.125, 0.250 and 0.500 μg/rat, intra-BLA) plus the vehicle (1 ml/kg, s.c.) and those that received muscimol (0, 0.125, 0.250 and 0.500 μg/rat, intra-BLA) plus dexamethasone (0.9 mg/kg, s.c.) [within group comparison: treatment effect, $F(1, 56)=0.75$, $P>0.05$; dose effect, $F(3, 56)=2.44$, $P>0.05$; treatment×dose interaction, $F(3, 56)=5.05$, $P<0.01$]. One-way ANOVA indicated that there was no significant change in the time spent in the dark compartment among the groups which received muscimol (0, 0.125, 0.250 and 0.500 μg/rat, intra-BLA) plus the vehicle (1 ml/kg, s.c.) [$F(3, 28)=0.7283$, $P>0.05$]. However, one-way ANOVA indicated that there was a significant difference in the time spent in the dark compartment between the animals that received muscimol (0.125, 0.250 and 0.500 μg/rat, intra-BLA) plus dexamethasone (0.9 mg/kg, s.c.) and the control group (saline/dexamethasone) [$F(3, 28)=8.819$, $P<0.001$]. This experiment indicated that the enhancement effect of dexamethasone on memory retrieval was significantly reduced by muscimol.

3.3 Effect of intra-BLA microinjection of bicuculline on the effect of dexamethasone on memory retrieval

Fig. 3a indicates the effects of bicuculline with or without dexamethasone on step-through latencies. Two-way ANOVA showed that there was an interaction effect on memory retrieval between the groups that received bicuculline (0, 0.25, 0.50 and 0.75 μg/rat, intra-BLA) plus the vehicle (1 ml/kg, s.c.), and the groups that received bicuculline (0, 0.25, 0.50 and 0.75 μg/rat, intra-BLA) plus dexamethasone (0.1 mg/kg) [within group comparison: treatment effect, $F(1, 56)=20.08$, $P<0.0001$; dose effect, $F(3, 56)=2.83$, $P<0.05$; treatment×dose interaction, $F(3, 56)=2.84$, $P<0.05$].

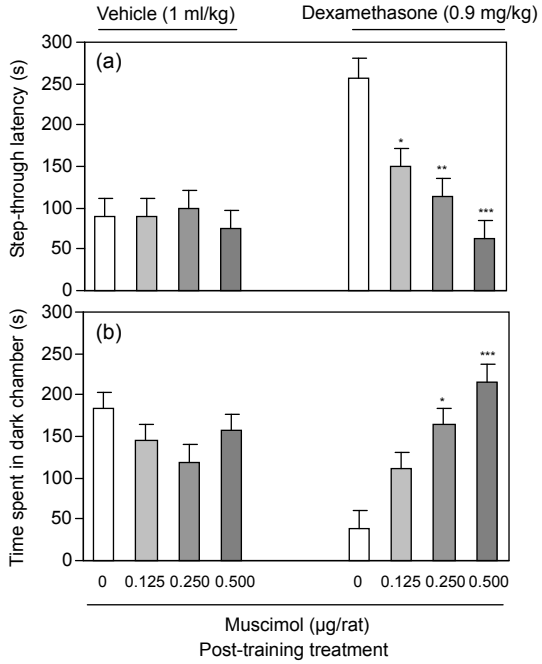

Fig. 2 Effect of muscimol on dexamethasone effect on memory retrieval

(a) Step-through latencies; (b) Time spent in the dark chamber. The rats received saline (1 μl/rat, intra-BLA) or muscimol (0.125, 0.250 and 0.500 μg/rat, intra-BLA) plus the vehicle (1 ml/kg, s.c.) or dexamethasone (0.9 mg/kg, s.c.), as post-training injections. Each column shows the mean±SEM (n=8). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to the group treated with saline/dexamethasone

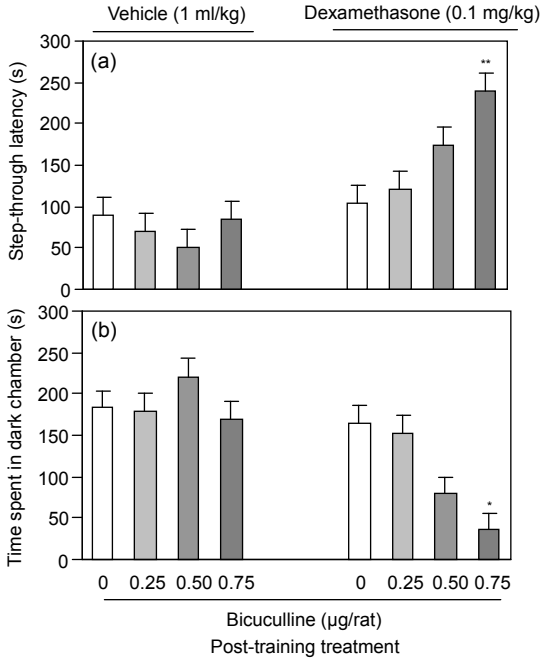

Fig. 3 Effect of bicuculline on dexamethasone effect on memory retrieval

(a) Step-through latencies; (b) Time spent in the dark chamber. The rats received saline (1 μl/rat, intra-BLA) or bicuculline (0.25, 0.50 and 0.75 μg/rat, intra-BLA) plus the vehicle (1 ml/kg, s.c.) or dexamethasone (0.1 mg/kg, s.c.), as post-training injections. Each column shows the mean± SEM (n=8). $^{*}P<0.05$, $^{**}P<0.01$ compared to the group treated with saline/dexamethasone

Furthermore, one-way ANOVA indicated that there was no significant change in the latencies among the groups that received bicuculline (0, 0.25, 0.50 and 0.75 μg/rat, intra-BLA) plus the vehicle (1 ml/kg, s.c.) [$F(3, 28)=0.5630$, $P>0.05$]. Analysis showed that memory retrieval was significantly potentiated by co-administration of an ineffective dose of dexamethasone (0.1 mg/kg, s.c.) and bicuculline (0.75 μg/rat, intra-BLA) [$F(3, 28)=5.104$, $P<0.01$].

Fig. 3b shows the effect of bicuculline on the effect of dexamethasone on time spent in the dark chamber. Statistical analysis, on the basis of two-way ANOVA, showed that there was an interaction effect on memory retrieval between the groups that received bicuculline (0, 0.25, 0.50 and 0.75 μg/rat, intra-BLA) plus the vehicle (1 ml/kg, s.c.), and the groups that received bicuculline (0, 0.25, 0.50 and 0.75 μg/rat, intra-BLA) plus dexamethasone (0.1 mg/kg, s.c.) [within group comparison: treatment effect, $F(1, 56)=17.81$, $P<0.0001$; dose effect, $F(3, 56)=2.80$, $P<0.05$; treatment×dose interaction, $F(3, 56)=3.05$, $P<0.05$]. Furthermore, one-way ANOVA indicated that there was no significant change in the time spent in the dark chamber among the groups which received bicuculline (0, 0.25, 0.50 and 0.75 μg/rat, intra-BLA) plus the vehicle (1 ml/kg, s.c.) [$F(3, 28)=0.74$, $P>0.05$]. One-way ANOVA indicated that there was a significant change in the time spent in the dark chamber in the group that received bicuculline (0.75 μg/rat, intra-BLA) plus dexamethasone (0.1 mg/kg, s.c.) compared to the saline (1 μl/rat)/dexamethasone (0.1 mg/kg, s.c.) group [$F(3, 28)=4.922$, $P<0.01$]. Therefore, the effect on memory retrieval of an ineffective dose of dexamethasone (0.1 mg/kg) was significantly increased by ineffective doses of bicuculline.

4 Discussion

This study aimed to investigate the involvement of $GABA_A$ receptors of the BLA in the effect of dexamethasone, an agonist of glucocorticoid receptors, on memory retrieval using intra-BLA microinjections of muscimol and bicuculline, an agonist and an antagonist, respectively, of $GABA_A$ receptors. In accordance with other investigations, the findings of our experiments showed that systemic subcutaneous injection of moderate doses of dexamethasone after training increased memory retrieval in the passive avoidance memory in a dose-dependent manner.

Studies have shown that administration of glucocorticoid hormones modulates memory formation in many different types of retrieval assessment, including inhibitory avoidance, water-maze, and contextual fear conditioning, in experimental animals (Power *et al*., 2000; McGaugh, 2002; Paré, 2003; Venturella *et al*., 2005; Roozendaal *et al*., 2006). The effects of dexamethasone are mediated by genomic pathways, via intracellular glucocorticoid receptors (GRs), and nongenomic mechanisms, via membrane-associated receptors (Venturella *et al*., 2005; Khaksari *et al*., 2007). Post-training central administration of antagonists of these receptors impairs memory formation (Roozendaal and McGaugh, 1997). Several studies have shown that certain neurotransmitters and neuropeptide systems, including cholinergic, noradrenergic, and opioidergic systems, are involved in these effects of glucocorticoids (Rashidy-Pour *et al*., 2004; Khaksari *et al*., 2007; Roozendaal *et al*., 2009). In this study, we determined the involvement of BLA $GABA_A$ receptor mechanisms in the effect of dexamethasone on memory retrieval.

The role of the BLA $GABA_A$ receptors in the effect of dexamethasone on retrieval was evaluated. In this research, the effect of dexamethasone on memory retrieval was increased by intra-BLA injection of muscimol, an agonist of $GABA_A$ receptors, when administered after training and before injection of dexamethasone. Several studies have suggested that muscimol affects memory retention (Michelot and Melendez-Howell, 2003; DiSorbo *et al*., 2009). For example, Castellano and McGaugh (1990) reported that muscimol, administered systemically after training, impaired retrieval in a passive avoidance memory model. There are reports that the injection of muscimol into certain regions of the brain induces memory impairment or amnesia. For instance working memory was impaired by intra-septal (Yamamoto *et al*., 2007) and intra-hippocampal (Ohno *et al*., 1992) injections of this drug. It was also shown that intra-BLA injection of muscimol reduced the acquisition of conditioned place preference (CPP) induced by morphine (Zarrindast *et al*., 2004).

However, our research indicated that intra-BLA microinjection of different doses of bicuculline used in our experiments failed to affect memory retrieval

by itself, but potentiated the effect of systemic injection of dexamethasone (ineffective dose; 0.1 mg/kg, s.c.) on the retrieval stage of memory. Several studies have shown that central and peripheral administration of bicuculline improves memory processes (Castellano and McGaugh, 1990; Ohno *et al*., 1992; Michelot and Melendez-Howell, 2003; Yamamoto *et al*., 2007). Other studies have shown that the activation or inactivation of $GABA_A$ receptors of BLA by their agonists and antagonists, disrupts or improves memory performance, respectively (McGaugh and Roozendaal, 2008). In this study, the doses of muscimol and bicuculline were selected on the basis of a pilot study in which they did not affect memory retrieval.

Our results showed that a $GABA_A$ receptor of BLA can interfere with the effect of dexamethasone on this kind of memory. These findings agree with those of other investigations. Duvarci and Paré (2007) reported that corticosterone, another glucocorticoid agonist, reduces the activation of GABAergic neurons in BLA. Rodriguez Manzanares *et al*. (2005), using in vitro field potential recordings, have shown that the facilitating effect of stress on retrieval is accompanied by the inhibition of the GABAergic system in the BLA. Therefore, it may be that $GABA_A$ receptors of BLA interact with glucocorticoid hormones indirectly via other neurotransmitter systems.

Several lines of evidence indicate that noradrenergic activity in the BLA has an important role in mediating the effects of other neurotransmitter systems and hormones on memory retrieval. For example, intra-BLA administration of antagonists of the β-noradrenergic receptors inhibits the effect of the glucocorticoid on memory retrieval (Roozendaal *et al*., 2002). Furthermore, it is reported that stimulation of β-adrenoreceptors enhances the long-term potentiation (LTP) of cortical projections to lateral amygdala (Faber *et al*., 2005). Therefore, it seems that the activation of BLA β-adrenoreceptor mechanisms is required for the effect of glucocorticoids on retrieval. However, intra-amygdala infusion of a β-adrenoreceptor antagonist inhibits the enhancement effect of bicuculline on memory (McGaugh and Roozendaal, 2008) and conversely, administration of muscimol reduces levels of amygdala norepinephrine (Hatfield *et al*., 1999). Therefore, it may be that the activity of the BLA noradrenergic system is also required for modulation of the interaction of the BLA $GABA_A$ receptor mechanisms and dexamethasone effect on memory retrieval.

The cholinergic system also has an important role in the hippocampus and amygdala for modulating memory processes (Khajehpour *et al*., 2008; Rezayof *et al*., 2009). BLA cholinergic activity modulates memory consolidation (Roozendaal *et al*., 2009) and is also important for the memory enhancement effect of glucocorticoids (Dalmaz *et al*., 1993). The infusion of atropine (intra-BLA), an antagonist of muscarinic cholinergic receptors, impairs the effect of dexamethasone on memory formation, when these drugs are co-administered after training (Power *et al*., 2000). However, the muscarinic cholinergic and noradrenergic systems in the amygdala interact in their effects on memory storage and retrieval (Dalmaz *et al*., 1993). Finally, opioidergic (Roozendaal *et al*., 2009) and glutamatergic (Yamada *et al*., 1999) systems also may be mediating the involvement of $GABA_A$ receptors in the effect of dexamethasone on memory retrieval.

5 Conclusions

In this research, moderate doses of dexamethasone, a glucocorticoid hormone agonist, increased retrieval in the passive avoidance type of memory. It can be inferred that the mechanisms of $GABA_A$ receptors of the BLA may directly or indirectly (through other neurotransmitter systems such as noradrenergic, cholinergic and opioidergic systems) be involved in the effect of dexamethasone on memory retrieval.

Acknowledgements

We would like to thank Dr. Abbas EMAM (Faculty member of the English Department of Shahid Chamran University of Ahvaz, Iran) for his careful editing of the English manuscript of this research.

References

Baxter, M.G., Murray, E.A., 2002. The amygdala and reward. *Nat. Rev. Neurosci.*, **3**(7):563-573. [doi:10.1038/nrn875]

Bormann, J., 2000. The 'ABC' of GABA receptors. *Trends Pharmacol. Sci.*, **21**(1):16-19. [doi:10.1016/S0165-6147(99)01413-3]

Castellano, C., McGaugh, J.L., 1990. Effects of post-training bicuculline and muscimol on retention: lack of state dependency. *Behav. Neural Biol.*, **54**(2):156-164. [doi:10.1016/0163-1047(90)91352-C]

Cintra, A., Zoli, M., Rosén, L., Agnati, L.F., Okret, S., Wikström, A.C., Gustafsson, J.A., Fuxe, K., 1994. Mapping and computer assisted morphometry and microdensitometry of glucocorticoid receptor immunoreactive neurons in rat central nervous system. *Neuroscience*, **62**(3):843-897. [doi:10.1016/0306-4522(94)90481-2]

Dalmaz, C., Introini-Collison, I.B., McGaugh, J.L., 1993. Noradrenergic and cholinergic interactions in the amygdala and the modulation of memory storage. *Behav. Brain Res.*, **58**(1-2):167-174. [doi:10.1016/0166-4328(93)90101-U]

DiSorbo, A., Wilson, G.N., Bacika, S., Hoxhaa, Z., Biadaa, J.M., Mickleya, G.A., 2009. Time-dependent retrograde amnesic effects of muscimol on conditioned taste aversion extinction. *Pharmacol. Biochem. Behav.*, **92**(2):319-326. [doi:10.1016/j.pbb.2008.12.020]

Donley, M.P., Schulkin, J., Rosen, J.B., 2005. Glucocorticoid receptor antagonism in the basolateral amygdala and ventral hippocampus interferes with long-term memory of contextual fear. *Behav. Brain Res.*, **164**(2):197-205. [doi:10.1016/j.bbr.2005.06.020]

Duvarci, S., Paré, D., 2007. Glucocorticoids enhance the excitability of principal basolateral amygdala neurons. *J. Neurosci.*, **27**(16):4482-4491. [doi:10.1523/JNEUROSCI.0680-07.2007]

Emson, P.C., 2007. $GABA_B$ receptors: structure and function. *Prog. Brain Res.*, **160**:43-57. [doi:10.1016/S0079-6123(06)60004-6]

Faber, E.S., Delaney, A.J., Sah, P., 2005. SK channels regulate excitatory synaptic transmission and plasticity in the lateral amygdala. *Nat. Neurosci.*, **8**(5):635-641. [doi:10.1038/nn1450]

Ferry, B., Roozendaal, B., McGaugh, J.L., 1999. Basolateral amygdale noradrenergic influences on memory storage are mediated by an interaction between β and α_1-adrenoceptors. *J. Neurosci.*, **19**(12):5119-5123.

Hatfield, T., Spanis, C., McGaugh, J.L., 1999. Response of amygdalar norepinephrine to footshock and GABAergic drugs using in vivo microdialysis and HPLC. *Brain Res.*, **835**(2):340-345. [doi:10.1016/S0006-8993(99)01566-8]

Izquierdo, I., Cunha, C., Medina, J.H., 1990. Endogenous benzodiazepine modulation of memory processes. *Neurosci. Biobehav. Rev.*, **14**(4):419-424. [doi:10.1016/S0149-7634(05)80064-8]

Khajehpour, L., Rezayof, A., Zarrindast, M.R., 2008. Involvement of dorsal hippocampal nicotinic receptors in the effect of morphine on memory retrieval in passive avoidance task. *Eur. J. Pharmacol.*, **584**(2-3):343-351. [doi:10.1016/j.ejphar.2008.02.030]

Khaksari, M., Rashidy-Pour, A., Vafaei, A.A., 2007. Central mineralocorticoid receptors are indispensable for corticosterone-induced impairment of memory retrieval in rats. *Neuroscience*, **149**(4):729-738. [doi:10.1016/j.neuroscience.2007.08.016]

Macdonald, R.L., Olsen, R.W., 1994. $GABA_A$ receptor channels. *Annu. Rev. Neurosci.*, **17**:569-602. [doi:10.1146/annurev.ne.17.030194.003033]

Makara, G.B., Haller, J., 2001. Non-genomic effects of glucocorticoids in the neural system: evidence, mechanisms and implication. *Prog. Neurobiol.*, **65**(4):367-390. [doi:10.1016/S0301-0082(01)00012-0]

Mao, J.B., Robinson, J.K., 1998. Microinjection of GABA-A agonist muscimol into the dorsal but not the ventral hippocampus impairs non-mnemonic measures of delayed non-matching-to-position performance in rats. *Brain Res.*, **784**(1-2):139-147. [doi:10.1016/S0006-8993(97)01324-3]

McGaugh, J.L., 2002. Memory consolidation and the amygdala. *Trends Neurosci.*, **25**(9):456-461. [doi:10.1016/S0166-2236(02)02211-7]

McGaugh, J.L., Roozendaal, B., 2008. Memory Modulation. *In*: Learning and Memory: A Comprehensive Reference. Elsevier Ltd., Vol. 3, p.521-553. [doi:10.1016/B978-012370509-9.00113-3]

Michelot, D., Melendez-Howell, L.M., 2003. *Amanita muscaria*: chemistry, biology, toxicology, and ethnomycology. *Mycol. Res.*, **107**(pt2):131-146. [doi:10.1017/S0953756203007305]

Myhrer, T., 2003. Neurotransmitter systems involved in learning and memory in the rat: a meta-analysis based on studies of four behavioral tasks. *Brain Res. Rev.*, **41**(2-3):268-287. [doi:10.1016/S0165-0173(02)00268-0]

Ohno, M., Yamamoto, T., Watanabe, S., 1992. Intrahippocampal injections of benzodiazepine and muscimol impair working memory but not reference memory of rats in the three-panel runway task. *Eur. J. Pharmacl.*, **219**(2):245-251. [doi:10.1016/0014-2999(92)90302-K]

Olsen, R.W., Sieghart, W., 2009. $GABA_A$ receptors: subtypes provide diversity of function and pharmacology. *Neuropharmacology*, **56**(1):141-148. [doi:10.1016/j.neuropharm.2008.07.045]

Ong, J., Kerr, D.I., 2005. Clinical potential of $GABA_B$ receptor modulators. *CNS Drug Rev.*, **11**(3):317-334. [doi:10.1111/j.1527-3458.2005.tb00049.x]

Paré, D., 2003. Role of the basolateral amygdala in memory consolidation. *Prog. Neurobiol.*, **70**(5):409-420. [doi:10.1016/S0301-0082(03)00104-7]

Paxinos, G., Watson, C., 1997. The Rat Brain in Stereotaxic Coordinates, 3 Ed. Academic Press, San Diego, CA, p.15-31.

Power, A.E., Roozendaal, B., McGaugh, J.L., 2000. Glucocorticoid enhancement of memory consolidation in the rat is blocked by muscarinic receptor antagonist in the basolateral amygdala. *Eur. J. Neurosci.*, **12**(10):3481-3487. [doi:10.1046/j.1460-9568.2000.00224.x]

Quirarte, G.L., Roozendaal, B., McGaugh, J.L., 1997. Glucocorticoid enhancement of memory storage involves noradrenergic activation in the basolateral amygdale. *PNAS*, **94**(25):14048-14053. [doi:10.1073/pnas.94.25.14048]

Rashidy-Pour, A., Sadeghi, H., Taherain, A.A., Vafaei, A.A., Fathollahi, Y., 2004. The effects of acute restraint stress and dexamethasone on retrieval of long-term memory in rats: an interaction with opiate system. *Behav. Brain Res.*, **154**(1):193-198. [doi:10.1016/j.bbr.2004.02.007]

Rezayof, A., Khajehpour, L., Zarrindast, M.R., 2009. The amygdala modulates morphine-induced state-dependent memory retrieval via muscarinic acetylcholine receptors. *Neuroscience*, **160**(2):255-263. [doi:10.1016/j.neuroscience.2009.02.069]

Rodriguez Manzanares, P.A., Isoardi, N.A., Carrer, H.F., Molina, V.A., 2005. Previous stress facilitates fear memory, attenuates GABAergic inhibition, and increases synaptic plasticity in the rat basolateral amygdala. *J. Neurosci.*, **25**(38):8725-8734. [doi:10.1523/JNEUROSCI.2260-05.2005]

Roozendaal, B., McGaugh, J.L., 1996. Amygdaloid nuclei lesions differentially affect glucocorticoid-induced memory enhancement in an inhibitory avoidance task. *Neurobiol. Learn. Mem.*, **65**(1):1-8. [doi:10.1006/nlme.1996.0001]

Roozendaal, B., McGaugh, J.L., 1997. Glucocorticoid receptor agonist and antagonist administration into the basolateral but not central amygdala modulates memory storage. *Neurobiol. Learn. Mem.*, **67**(2):176-179. [doi:10.1006/nlme.1996.3765]

Roozendaal, B., Quirarte, G.L., McGaugh, J.L., 2002. Glucocorticoids interact with the basolateral amygdala β-adrenoceptor–cAMP/cAMP/PKA system in influencing memory consolidation. *Eur. J. Neurosci.*, **15**(3):553-560. [doi:10.1046/j.0953-816x.2001.01876.x]

Roozendaal, B., Hahn, E.L., Nathan, S.V., de Quervain, D.J.F., McGaugh, J.L., 2004. Glucocorticoid effects on memory retrieval require concurrent noradrenergic activity in the hippocampus and basolateral amygdala. *J. Neurosci.*, **24**(37):8161-8169. [doi:10.1523/JNEUROSCI.2574-04.2004]

Roozendaal, B., Okuda, S., Quatrain, D.J.F., McGaugh, J.L., 2006. Glucocorticoids interact with emotion-induced noradrenergic activation in influencing different memory functions. *Neuroscience*, **138**(3):901-910. [doi:10.1016/j.neuroscience.2005.07.049]

Roozendaal, B., McEwen, B.S., Chattarji, S., 2009. Stress, memory and the amygdala. *Nat. Rev. Neurosci.*, **10**(6):423-433. [doi:10.1038/nrn2651]

Sah, P., Faber, E.S.L., Lopez de Armentia, M., Power, J., 2003. The amygdaloid complex: anatomy and physiology. *Physiol. Rev.*, **83**(3):803-834. [doi:10.1152/physrev.00002.2003]

Salinas, J.A., McGaugh, J.L., 1996. The amygdala modulates memory for changes in reward magnitude: involvement of the amygdaloid GABAergic system. *Behav. Brain Res.*, **80**(1-2):87-98. [doi:10.1016/0166-4328(96)00023-X]

Stackman, R.W., Walsh, T.J., 1995. Anatomical specificity and time dependence of chlordiazepoxide-induced spatial memory impairments. *Behav. Neurosci.*, **109**(3):436-445. [doi:10.1037/0735-7044.109.3.436]

Stevenson, C.W., Gratton, A., 2003. Basolateral amygdala modulation of the nucleus accumbens dopaminergic response to stress: role of the medial prefrontal cortex. *Eur. J. Neurosci.*, **17**(6):1287-1295. [doi:10.1046/j.1460-9568.2003.02560.x]

Stevenson, C.W., Sullivan, R.M., Gratton, A., 2003. Effects of basolateral amygdala dopamine depletion on the nucleus accumbens and medial prefrontal cortical dopamine responses to stress. *Neuroscience*, **116**(1):285-293. [doi:10.1016/S0306-4522(02)00553-5]

Venturella, R., Lessa, D., Luft, T., Roozendaal, B., Schwartsmann, G., Roesler, R., 2005. Dexamethasone reverses the memory impairment induced by antagonism of hippocampal gastrin-releasing peptide receptors. *Peptides*, **26**(5):821-825. [doi:10.1016/j.peptides.2004.12.010]

Watanabe, M., Maemura, K., Kanbara, K., Tamayama, T., Hayasaki, H., 2002. GABA and GABA receptors in the central nervous system and other organs. *Int. Rev. Cytol.*, **213**:1-47. [doi:10.1016/S0074-7696(02)13011-7]

Wilensky, A.E., Schafe, G.E., LeDoux, J.E., 2000. The amygdala modulates memory consolidation of fear-motivated inhibitory avoidance learning but not classical fear conditioning. *J. Neurosci.*, **20**(18):7059-7066.

Yamada, J., Saitow, F., Satake, S., Kiyohara, T., Konishi, S., 1999. $GABA_B$ receptor-mediated presynaptic inhibition of glutamatergic and GABAergic transmission in the basolateral amygdala. *Neuropharmacology*, **38**(11):1743-1753. [doi:10.1016/S0028-3908(99)00126-4]

Yamamoto, Y., Mikami, A., Fujii, Y., Kamei, C., 2007. Effect of histamine on muscimol-induced working memory deficits in radial maze performance. *J. Pharmacol. Sci.*, **104**(3):252-257. [doi:10.1254/jphs.FP0061472]

Zarrindast, M.R., Ahmadi, S., Haeri-Rohani, A., Rezayof, A., Jafari, M.R., Jafari-Sabet, M., 2004. $GABA_A$ receptors in the basolateral amygdala are involved in mediating morphine reward. *Brain Res.*, **1006**(1):49-58. [doi:10.1016/j.brainres.2003.12.048]