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INTERACTION BETWEEN BETA$_1$-ADRENERGIC RECEPTOR AGENTS AND SCOPOLAMINE IN THE BASOLATERAL AMYGDALA ON IMPAIRMENT OF INHIBITORY AVOIDANCE MEMORY PERFORMANCE IN RAT

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Abstract

The current study was designed to examine the involvement of beta$_1$-adrenoceptors in the basolateral amygdala (BLA) in scopolamine-induced memory impairment in adult male Wistar rats. The animals were bilaterally implanted with the cannulas in the BLA and submitted to a step-through type passive avoidance task to measure the memory formation. The results showed that bilateral intra-BLA administration of different doses of scopolamine (0.01, 0.06, 0.1 and 0.6 µg/rat) immediately after the training phase (post-training) impaired memory consolidation. Bilateral microinjection of the beta$_1$-adrenoceptor agonist, xamoterol (0.1, 1 and 2 µg/rat), into the BLA did not have any effect on memory consolidation, but the beta$_1$-adrenoceptor antagonist, atenolol, at the dose of 0.2 µg/rat could significantly impaired memory consolidation. Co-administration of the ineffective dose of xamoterol (1 and 2 µg/rat) with scopolamine (0.6 µg/rat) into the BLA significantly improved scopolamine-induced memory consolidation impairment. On the other hand, co-administration of ineffective dose of atenolol (0.1 µg/rat), with an ineffective dose of scopolamine (0.01 µg/rat), significantly impaired memory consolidation and mimicked the response of a higher dose of scopolamine. In view of the known actions of the drugs used, the present data pointed to the involvement of the BLA beta$_1$-adrenocepors in scopolamine-induced memory consolidation impairment. Furthermore, it seems that a functional interaction between the beta$_1$-adrenergic system and cholinergic muscarinic systems in BLA may be critical for memory formation.

Key words: *Basolateral amygdala, beta$_1$-adrenergic system, passive avoidance task, rat(s), scopolamine.*

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INTRODUCTION:

There is huge body of evidence to support the crucial role of the basolateral amygdala (BLA), as a main part of the amygdaloid complex, in regulating emotional memory (Rezayof et al., 2009). The abundance cholinergic nerves from basal forebrain innervate the BLA which is essential for memory consolidation (Dalmaz et al., 1993). It has been shown that activation of M1 and M2 muscarinic receptors in BLA modulates memory consolidation and synaptic plasticity (Power et al., 2003a). Administration of cholinergic receptor agonists and antagonists improve and impair memory retrieval respectively (Meck et al., 1988; De-Mello et al., 2002). The basolateral nuclei of amygdala shows one of the highest levels of choline acetyltransferase, an essential enzyme in acetylcholine formation, and receives one of the densest cholinergic innervations in the brain (Muller et al. ,2013; Power et al., 2008). Activation of M1 regulates the release of other neurotransmitters in the BLA. Learning tasks such as inhibitory avoidance, food reward magnitude learning, drug-stimulus learning, contextual fear conditioning were impaired following post-training administration of muscarinic cholinergic antagonists into the BLA (Power et al., 2003a; Power et al., 2003b; Muller et al., 2013). These data support the view that BLA muscarinic receptors are needed for memory consolidation.

Various neuromodulators interact with amygdala noradrenergic system to influence memory storage (Valizadegan et al., 2013). Three classes of receptors which are G-protein coupled receptors are considered for noradrenergic system: alpha$_1$-, alpha$_2$-, and beta-receptors (Sirvio et al., 1999). Complex activation of alpha$_1$- and alpha$_2$-adrenoceptors by phenylephrine results in complex effect pattern on memory storage (Ferry et al., 2008). Blocking beta-adrenergic receptors in the amygdala inhibits peripheral stress hormones to modulate memory for inhibitory avoidance training. In addition, post-training intra-amygdala infusions of agonist and antagonist of beta-adrenoceptor shows enhancement and impairment effect on retention of spatial water-maze and inhibitory avoidance training, respectively (Valizadegan et al., 2013). Norepinephrine (NE) has a key role in regulating both consolidation and reconsolidation processes. However, the exact contribution of alpha$_1$- and beta-adrenoceptors in this process is unclear. It has been shown that noradrenaline modulating effects on memory consolidation and reconsolidation has been mainly attributed to the beta-adrenergic receptors activation (Gazarini et al., 2013). Stressful or arousing stimulations as a result of inhibitory avoidance (IA) training release NE in the amygdala, the amount of this release depends on the training experience (Ferry et al., 2008). Studies showed that post-training administration of NE or beta-adrenoceptor agonist increases memory consolidation while the antagonist impairs it (Introini-Collison et al., 1991; Quirarte et al., 1997; LaLumiere et al., 2003). Furthermore, considering that amygdala has high density of beta-adrenoceptor subtypes, the effects of beta-adrenoceptors in the amygdala on memory consolidation could be approved (Bylund et al., 1976). Amygdala post-training infusion of norepinephrine or clenbuterol, beta-adrenoceptor agonist, increases memory storage and enhance retention of inhibitory avoidance and water-maze training (Introini-Collison et al., 1991; Ferry et al., 1999a). Beta-adrenoceptor system in the amygdala effects memory through modulating selectively the BLA (Ferry et al., 1999a).

It has been shown that release of acetylcholine by beta-adrenoceptor activation in the amygdala mediates the memory-modulatory effects. Systemic injection of atropine, antagonist of muscarininc receptors, showed attenuation in the effect of clenbuterol, agonist of beta-adrenoceptor, on the enhancing memory (Roozendaal et al., 2011).

Scopolamine, muscarinic cholinergic receptor antagonist, induced memory impairments has been used as a model for dementia and Alzheimer's disease. The present study aimed to investigate the involvement of BLA beta$_1$-adrenoceptors in the effect of scopolamine, as a non-selective potent antagonist of muscarinic receptors, on memory consolidation. To address this issue, the effect of post-training intra-BLA infusions of scopolamine alone or together with agonist and antagonist of beta$_1$-adrenoceptors were examined.

MATERIALS AND METHODS:

Animals

Adult male Albino-Wistar rats (Pasteur institute, Tehran, Iran) weighting 200-250 at the time of surgery were used. They were housed 8-10 in a big cage with ad libitum access to food and water, and kept at the humidity and temperature- controlled room (22 ± 2°C) under the standard 12-h light/12-h dark cycle. All training and testing were performed during the light phase between 9:00 a.m. and 1:00 p.m. Each animal was used only once. All procedures were performed in accordance with institutional guideline for animal care and use. The Research and Ethics Committee of Tehran University of Medical Sciences, School of Advanced Technologies in Medicine approved the experimental protocol.

Surgery

The animals were given intraperitoneal injection of ketamine–xylazine (50 mg/kg ketamine–5 mg/kg xylazine) mixture for anaesthesia. Two 22-gauge guide steel cannulas were placed 1 mm above the intended sites of injection according to the atlas of Paxinos and Watson (2007). Stereotaxic coordinates for the BLA region were 2.4 mm from bregma, L: ±5 mm from midline and V: 7 mm from the skull surface. The guide cannulae were fixed to the skull using dental cement, then stainless steel stylets (27 gauge) were inserted into the guide cannulae to maintain patency prior to microinfusions and were removed only for the infusion of drugs. After surgery, they were returned to their cage and allowed to recover for 7 days before starting of training process.

For bilateral microinjections of the drugs into the BLA (intra-BLA), each stylet was removed from the guide cannula and replaced by 27-gauge injection needle (1 mm below the tip of the guide cannulae) attached with a polyethylene tube to a 1-µl Hamilton syringe. The BLA was injected with a 0.3 µl per each side (0.6µl/rat) solution over a 60-s period. In order for the drug to completely release in the tissue spaces, the injection needle was kept at the injection site for 60 s before removal.

Drugs and microinfusions procedures

Scopolamine hydrobromide (Sigma-aldrich), muscarinic cholinergic receptor antagonist; Xamoterol (Sigma-aldrich), $beta_1$-adrenergic partial agonist; Atenolol (Sigma-aldrich), $beta_1$-adrenergic antagonist were freshly dissolved in 0.9% sterile saline. The rats were gently restrained by hand and the drugs were infused into BLA via a 27-gauge injection needle which was attached to a 10 µl Hamilton microsyringe through polyethylene (PE-20) tubing. The needles protruded 1mm beyond the tip of cannula. A 0.5 µl of drugs were infused over a period of 5 min via an infusion pump and the injection needle was left within the cannula for an additional 1 min after infusion to maximize diffusion of the drug away from the tip and prevent backflow of it into the cannula. Animals during the injection were awake and after the completion of the infusion were returned into their cage.

Inhibitory avoidance apparatus (Shuttle box)

The inhibitory avoidance apparatus consisted of two equally sized compartments (20 × 20 × 30 cm) with two independent grid floors and a sliding door in the middle could be opened manually. The first compartment consisted of opaque white plastic and was well- illuminated the shock compartment was made of metal plates and was not lighted. The floor of shock compartment consisted of stainless steel bars (2 mm in diameter and 1 cm intervals) that could receive intermittent controllable electric shocks from an isolated stimulator (50 Hz, 3 s, 1 mA intensity).

Behavioral testing

Training

The training was based on our previous study (Zarrindast et al., 2005). All animals were allowed to habituate in the experimental room for at least 30 min before the experiments. Each animal was gently placed in the light compart- ment of the apparatus; after 20 s, the guillotine door was opened, and the animal was allowed to enter the dark compartment. The latency with which the animal crossed into the dark compartment was recorded. Animals that waited more than 100 s to cross to the dark compartment were eliminated from the experiments. Once the animal crossed with all four paws to the next compartment, the guillotine door was closed, and the rat was taken into its home cage. The acquisition trial was repeated after 30 min. In this trial, the guillotine door was opened after 20 s and as soon as the animal crossed to the dark (shock) compartment, the door was closed and a foot shock (50 Hz, 1 mA, and 3 s) was immediately delivered to the grid floor of the dark room. After 20 s, the rat was removed from the apparatus and placed temporarily into its home cage. Two minutes later, the procedure was repeated; if the rat did not enter the dark compartment within 120 s, a successful acquisition of passive avoidance response was recorded. However, if the rat entered the dark compartment before 120 s, the door was closed and the animal received the same shock again. After retesting, if the rat had acquired passive avoidance successfully, it was removed from the apparatus and immediately received injection of the drugs (post-training administration).

Retention test

Twenty-four hours after training, a retention test was performed to determine long-term memory. Each animal was placed in the light compartment for 20 s while the door was open, and the step-through latency was measured for entering into the dark compartment. The maximum cut-off time for step through latency was 300 s. During these sessions, no electric shock was applied.

Drug treatment

In the experiments where the animals received one or two injections, the control groups also received one or two vehicle injections. The intervals of drug administration were based on our previous studies in order to obtain a maximum response.

Experiment 1: Effect of post-training administration of scopolamine on memory consolidation.

In this experiment, five groups of animals were used to examine the effect of scopolamine, a muscarinic antagonist, on memory consolidation. The animals received intra-BLA microinjections of different doses of scopolamine (0.01, 0.06, 0.1 and 0.6 µg/rat) immediately after training (post-training). A control group received post-training injection of saline. On the testing day, step-through latency of each rat was recorded 24 h after the training phase (Fig. 2).

Experiment 2: Effect of post-training intra-BLA microinjection of beta$_1$-adrenoceptor agonist on memory consolidation.

In this experiment, four groups of animals were used to evaluate the effect of post-training intra-BLA microinjection of xamoterol, an agonist of beta$_1$-adrenoceptor on memory consolidation. The animals received intra-BLA microinjections of different doses of xamoterol (0.1, 1 and 2 µg/rat) immediately after training (post-training). A control group received post-training injection of saline. On the testing day, step-through latency of each rat was recorded 24 h after the training phase (Fig. 3).

Experiment 3: Effect of post-training intra-BLA microinjection of beta$_1$-adrenoceptor antagonist on memory consolidation.

In this experiment, four groups of animals were used to evaluate the effect of post-training intra-BLA microinjection of atenolol, an antagonist of beta$_1$-adrenoceptor on memory consolidation. The animals received intra-BLA microinjections of different doses of atenolo (0.01, 0.1 and 0.2 µg/rat) immediately after training (post-training). A control group received post-training injection of saline. On the testing day, step-through latency of each rat was recorded 24 h after the training phase (Fig. 4).

Experiment 4: The effect of post-training intra-BLA microinjection of different doses of scopolamine alone and in combination with xamoterol and atenolol on memory consolidation.

In this experiment, the animals were divided into three groups (Fig. 5). The first group of animals received post-training intra-BLA microinjection of saline or scopolamine (0.01 and 0.6 µg/rat). The second group of animal received post-training intra-BLA microinjection of ineffective dose of scopolamine (0.01 µg/rat) in combination with atenolol (0.01 and 0.1 µg/rat) with 5 min interval. The third group received post-training intra-BLA microinjection of effective dose of scopolamine (0.6 µg/rat) in combination with xamoterol (1 and 2 µg/rat) with 5 min interval (Fig. 5).

Statistics

Retention data are expressed as mean ± SEM and were analyzed with one way ANOVAs. For multiple comparisons, LSD test was used to carry out the Post-hoc comparison of means. A probability level of 0.05 was accepted as statistically significant. The SPSS statistical package was used for calculation.

RESULTS:

Histology

Fig. 1 shows the cannula placements for the injections of saline, vehicle or the drugs into the BLA. The left panel of Fig. 1 shows the representative section taken from the rat brain atlas of Paxinos and Watson, 2007. The right panel of Fig. 1 also shows the representative photomicrograph of the microinjection into the BLA. Shaded and dark areas represent the approximate points in which the cannula was positioned for each animal.

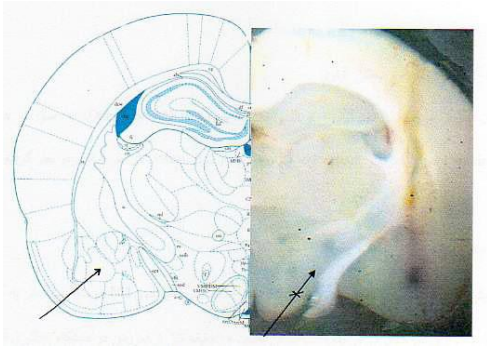

Figure 1. left, Schematic representation of the basolateral nucleus of the amygdala complex which was taken from the atlas of Paxinos and Watson (2007) . Right. The solid line indicates the position of the infusion needle tip in the BLA of the rat.

Figure 2 shows that post-training intra-BLA injections of different doses of scopolamine (0.01, 0.06, 0.1 and 0.6 µg/rat) impaired memory consolidation. One-way ANOVA revealed that scopolamine caused a significant dose-related amnesia [$F(4, 48)=4.16$, $P<0.05$]. The maximum effect was obtained with 0.6 µg/rat of scopolamine.

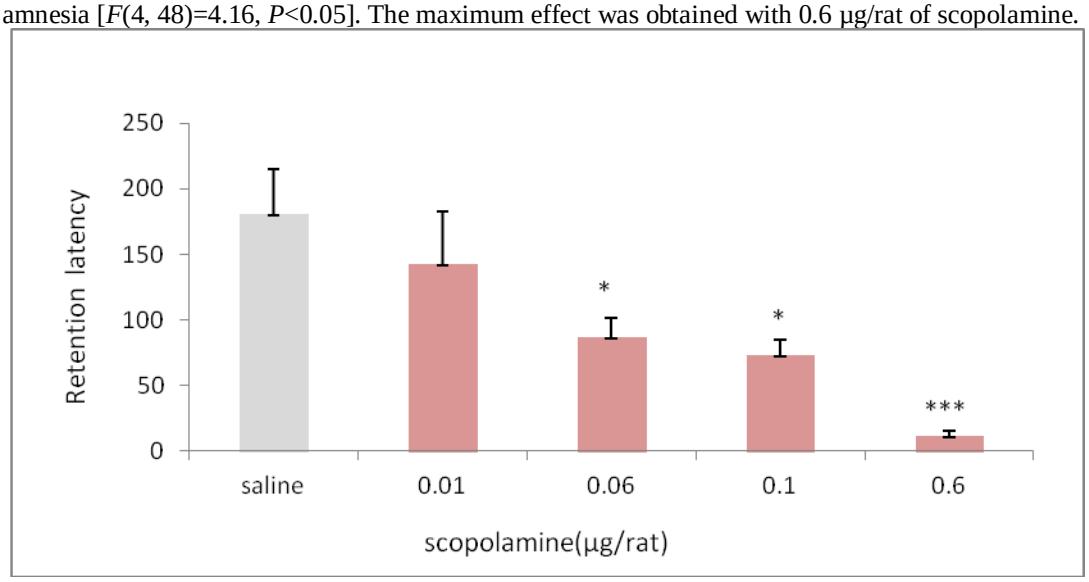

Fig. 2. Effect of post-training intra-BLA administration of scopolamine on memory consolidation. Five groups of animals received post-training administration of scopolamine (0.01, 0.06, 0.1 and 0.6 µg/rat). On the test day, step-trough latencies were measured in all groups. Data are expressed as mean ± SEM. *p < 0.05 and ***p < 0.001 compared to saline control group.

Figure 3 shows the effect of post-training intra-BLA administration of xamoterol on memory consolidation. One-way ANOVA revealed that post-training intra-BLA injections of different doses of xamoterol (0.1, 1 and 2 µg/rat) had no effect on memory consolidation. [$F(3,32)=0.96$, $P>0.05$]

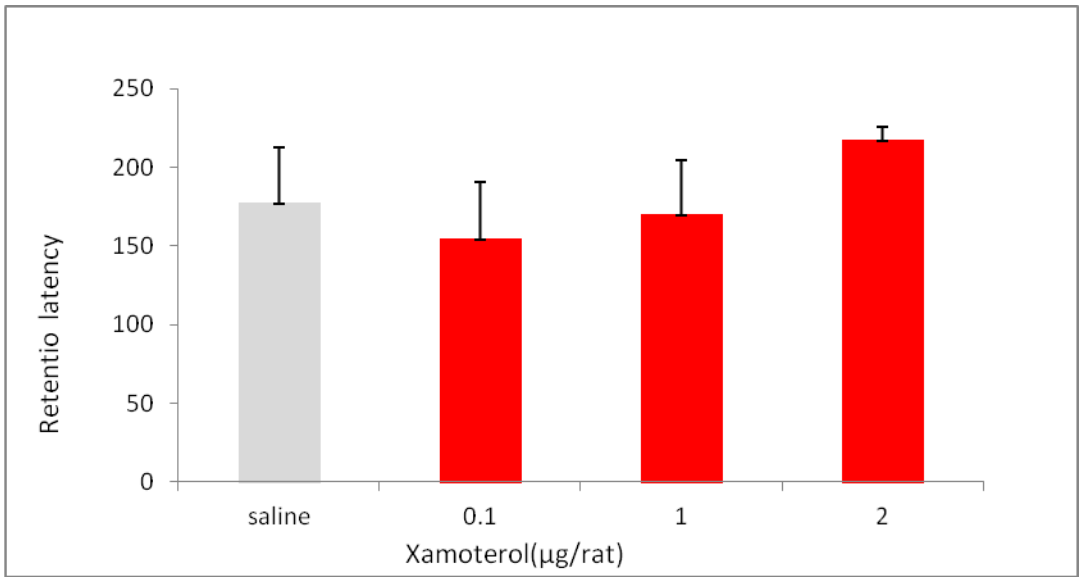

Fig. 3. Effect of post-training intra-BLA administration of xamoterol on memory consolidation. Four groups of animals received post-training administration of xamoterol (0. 1, 1 and 2 µg/rat). On the test day, step-trough latencies were measured in all groups. Data are expressed as mean ± SEM of seven rats per group.

Figure 4 shows the effect of intra-BLA post-training administration of atenolol on memory consolidation. As shown in Fig. 4, post-training intra-BLA injections of atenolol (0.2 µg/rat) impaired memory consolidation. $F(3, 38)=2.06$, $P<0.05$].

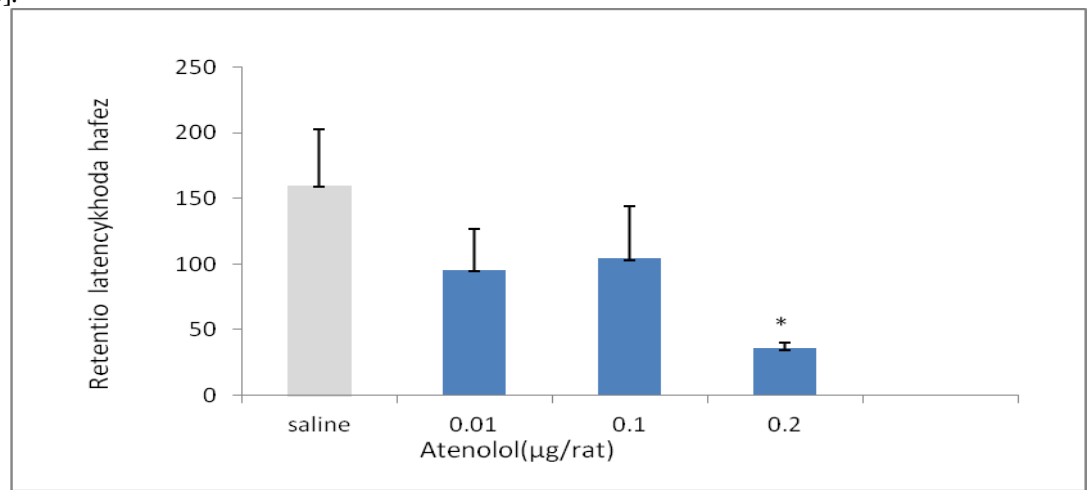

Fig. 4. Effect of post-training intra-BLA administration of atenolol on memory consolidation. Four groups of animals received post-training administration of atenolol (0.01, 0.1 and 0.2 µg/rat). On the test day, step-trough latencies were measured in all groups. Data are expressed as mean ± SEM of seven rats per group. *p < 0.05 compared to saline control group.

Figure 5 shows the retention test latencies of rats given post-training intra-BLA administration of the lower dose of scopolamine (0.01 µg/rat) in combination with atenolol (0.01 and 0.1 µg/rat) and higher dose of scopolamine (0.6 µg/rat) in combination with xamoterol (1 and 2 µg/rat). Significant differences were found among the groups in the escape latencies ($F6,59=3.98$, $P<0.01$). LSD's test showed that higher dose of scopolamine significantly decreased escape latency ($P<0.001$) compared with the control group. When administered together, the ineffective dose of atenolol (0.1 µg/rat) and ineffective dose of scopolamine (0.01 µg/rat) could induce memory impairment (an additive effect) ($P<0.01$). The ineffective doses of xamoterol (1 and 2 µg/rat) could significantly reversed scopolamine induced memory impairment ($P<0.05$ and $P<0.001$ respectively).

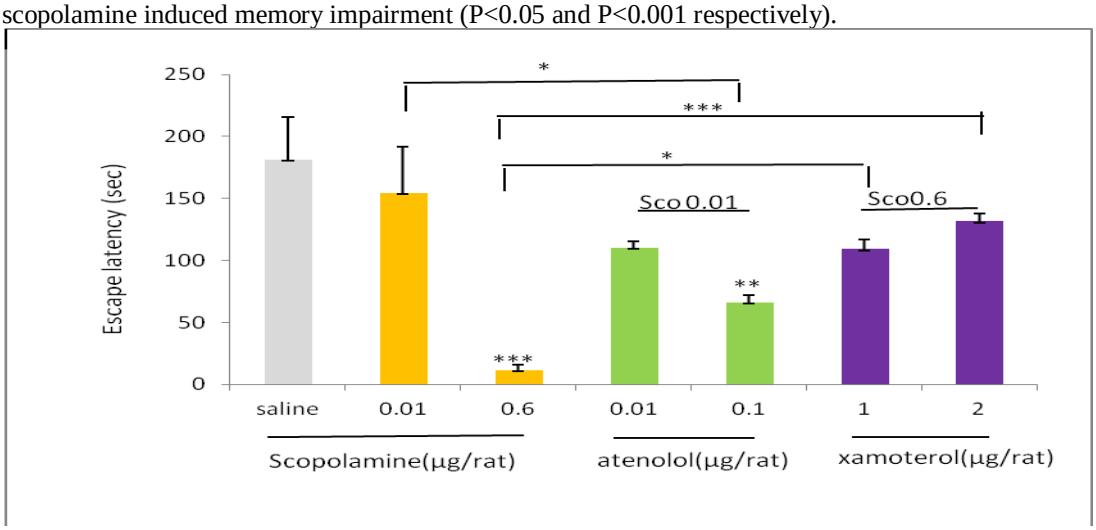

Fig. 5. The effect of post-training intra-BLA microinjection of different doses of scopolamine alone and in combination with atenolol and xamoterol on the step-through latencis. The animals received post-training intra-BLA administration of different doses of scopolamine (0.01 and 0.6 µg/rat) alone and atenolol (0.01 and 0.1 µg/rat) plus lower dose of scopolamine (0.01 µg/rat) or xamoterol (1 and 2 µg/rat) plus higher dose of scopolamine (0.6 µg/rat) and were tested after 24h. Each value represents the mean ± SEM. *p < 0.05 , **P <0.01 and ***P < 0. 001 compared to the saline groups.

DISCUSSION:

The basolateral structures of amygdala (BLA) is highly associated with memory consolidation. Our finding showed that post training intra-BLA administration of various doses of scopolamine (0.01, 0.06, 0.1 and 0.6 µg/rat) decreased retention latencies and induced amnesia in a dose dependence manner. Several studies have shown that activation of muscarinic cholinergic receptor in the BLA enhances memory, while reduction of muscarinic cholinergic receptor activation in the same area during consolidation decreases, memory retention (Power A et al., 2003a). In agreement with our finding, several studies have reported that cholinergic system is essential for learning and memory (Jahanshahi et al., 2012*)*. Scopolamine as an antagonist for muscarinic cholinergic receptor destroys memory in various animal models by directly reducing cholinergic system activity (Bartus et al., 2000). Scopolamine impairs formation of new memories; however it does not affect the retrieval of memories that stored previously (Hasselmo et al., 2004; Atri et al., 2004). Post training administration of scopolamine to rats reduced neuronal proliferation and neurogenesis in the CA1 area of the hippocampus and decreased memory consolidation and induced amnesia (Seifhosseini et al., 2011; Jamali-Raeufy et al., 2011). In the dentate gyrus, scopolamine suppressed expression of phosphorylated cAMP response element binding protein (CREB) which is critical for neural plasticity. This process blocks the survival of newborn neuronal cells without affecting proliferation of neuronal progenitor cells and the differentiation of neuronal cells (Kotani, et al., 2006). Cholinergic system is also involved in regulation of BDNF and NGF mRNAs in the rat hippocampus (Kokaia et al., 1994). Scopolamine induces amnesia is in accordance with scopolamine downregulated-BDNF level is likely associated with M1 receptors. Muscarinic acetylcholine receptors are G-protein-coupled receptors that work through PKC, Ca^{+2}, cAMP and MAP kinase signalling pathways that regulate neuronal long-term potentiation and synaptic plasticity which are crucial in normal neuronal function. BDNF expression is regulated via CREB, Ca^{2+} regulated transcription factor. Likewise, scopolamine affects glial plasticity marker GFAP that could be affected by alternation of downstream cascade of M1 receptors including Ca^{2+}, PKC and MAP kinase pathway observed (Konar et al., 2011). Altogether, here scopolamine inhibiting M1 receptor could affect transcription factors, particularly CREB that regulate BDNF and GFAP level that affects short-term memory and induces amnesia.

Our finding showed that post training intra-BLA administration of different doses of $beta_1$-adrenoceptor agonist, xamoterol (0.1, 1 and 2 µg/rat) had no effect on retention latencies while the $beta_1$-adrenoceptor antagonist, atenolol (0.2 µg/rat) induced amnesia. Noradrenaline affects long-term potentiation which leads to strengthen or persistence memory-related synaptic plasticity (Gazarini et al., 2013). In response to emotion-associated stimuli, central norepinephrine that primarily releases from the locus ceruleus substantially increases noradrenergic activity before peripheral release of epinephrine from the adrenal glands (Abraham et al., 2008). Substantial studies indicated that beta-adrenergic receptors are associated with noradrenergic effects on consolidation and reconsolidation of emotional memories (Gazarini L, 2013). Extensive evidence indicates that the release of peripheral epinephrine on memory consolidation is regulated by bata-adernoreceptor activation in the basolateral nucleus of the amygdala via norepinephrine released in response to stimuli used in inhibitory avoidance training (Ferry et al., 2015). Norepinephrine released in BLA have a direct effect on inhibitory avoindance retention performances (Ferry et al., 2015). Evidence previously demonstrated that $alpha_1$-adrenoceptors modulate the memory storage through the beta-adrenoceptor-mediated cAMP generation (Ferry. et al. 1999b). In the BLA an intense distribution of $beta_1$-adrenoceptor and $beta_2$-adrenoceptor mRNA has been found (Abraham et al., 2008). Immunofluorescence staining, confocal laser scanning and Western-blot analysis revealed that $beta_1$- adrenergic receptors are localized in the membrane and cytoplasm of BLA neurons, while $beta_2$- adrenergic receptors are expressed in the membrane, cytoplasm and nucleus. While both subtypes are essential for auditory fear memory, only synthesis of $beta_1$- adrenergic receptors was observed during the consolidation of auditory fear memory not $beta_2$- adrenergic receptors (Qu et al., 2008). Our results indicated that post-training intra-BLA administration of atenolol as a $beta_1$-adrenergic antagonist, decrease retention latencies and induces amnesia. However, that post-training intra-BLA administration of several doses of xamoterol as a $beta_1$- adrenergic partial agonist was ineffective on retention latencies compared with the control. Studies showed that administration of xamoterol to the amyloid precursor protein (APP) mouse model of AD significantly enhanced levels of nuclear pCREB without affecting their total level of pCREB. Up regulation of CREB results in increasing expression of BDNF which enhances both social and nonsocial short-term memory (Coutellier et al., 2014). Here, we are suggesting that xamoterol by upregulating the levels of nuclear pCREB could affect memory retrieval in long term, but in short

term it would not have enough time for up-regulating CREB expression and protein synthesis in order to affect short-term memory.

In order to support the involvement of the BLA $beta_1$-adrenoceptor in scopolamine-induced memory consolidation, a selective $beta_1$-adrenoceptor antagonist, atenolol, was injected into the target site before post-training administration of scopolamine. The results of this experiment indicated that post-training microinjection of ineffective dose of atenolol (0.1 µg/rat), 5 min before administration of an ineffective dose of scopolamine, could induced memory impairment. Considering that the inhibition of the BLA $beta_1$-adrenoceptors potentiates the response of an ineffective dose of scopolamine on memory consolidation, it can be concluded that the $beta_1$-adrenergic system in the BLA may play a critical role in scopolamine-induced memory impairment. To approve the interaction, the ineffective dose of $beta_1$-adrenoceptor agonist, xamoterol (2 µg/rat) was administered 5 min before the effective dose of scopolamine (0.6 µg/rat). Our result indicated that xamoterol could significantly improve scopolamine-induced memory consolidation impairment. According to our results, it can be suggested that the BLA $beta_1$-adrenergic system may interact with cholinergic system modulation of memory formation. Previous studies have also attempted to explain a correlation between the activation of $beta_1$-adrenoceptors and cholinergic system function (Ohno et al., 1997, Kobayashi et al., 1995). In agreement with our results, it has been shown that intraperitoneal administration of propranolol, the beta-adrenoceptor antagonist, deteriorated the scopolamine-induced working memory impairment (Kobayashi et al., 1995). Another study indicated that ineffective dose of propranolol (10 mg/kg, i. p.) induced a significant increase in working memory errors when administered with intra-hippocampal microinjection of ineffective dose of scopolamine (0.32 µg/side) (Ohno et al., 1997). Taken together, our data indicated that scopolamine-induced memory consolidation impairment may be regulated by the $beta_1$-adrenoceptors signal transduction pathways in the BLA. The evidence suggests that functional cooperation between $beta_1$-adrenoceptors and cholinergic system probably via the BLA mucarinic receptors affects memory formation in the passive avoidance paradigm. Future studies on the current topic are therefore recommended.

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