Modulation of muscimol state-dependent memory by α₂-adrenoceptors of the dorsal hippocampal area

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In the present study, the effects of bilateral intra-dorsal hippocampal (intra-CA1) injections of α₂-adrenoceptor agonist and antagonist, on muscimol state-dependent memory were examined in mice. A single-trial step-down passive avoidance task was used for the assessment of memory retention in adult male NMRI mice. Administration of muscimol (0.1 μg/mouse, intra-CA1) 15 min before training or testing induced impairment of memory retention. Injection of the same dose of the drug 15 min before testing restored memory retention impaired under pre-training muscimol influence. Pre-test intra-CA1 administration of the α₂-adrenoceptor agonist clonidine (0.5 and 1 μg/mouse) impaired memory retention, although the low dose of the drug (0.25 μg/mouse) did not affect memory retention. Pre-test intra-CA1 administration of the α₂-adrenoceptor antagonist yohimbine (1 and 2 μg/mouse) improved memory retention, although the low dose of the drug (0.5 μg/mouse) did not affect memory retention. In other series of experiments, pre-test co-administration of certain doses of clonidine (0.125 and 0.25 μg/mouse, intra-CA1), doses which were ineffective when given alone, and muscimol (0.1 μg/mouse, intra-CA1) significantly inhibited muscimol state-dependent memory. Pre-test intra-CA1 administration of certain doses of yohimbine (0.25 and 0.5 μg/mouse), doses which were ineffective when given alone, improved pre-training muscimol (0.1 μg/mouse)-induced retrieval impairment. Moreover, pre-test co-administration of yohimbine (0.25 and 0.5 μg/mouse, intra-CA1) and muscimol (0.025 μg/mouse, intra-CA1), an ineffective dose, significantly restored the retrieval and induced muscimol state-dependent memory. It may be concluded that the α₂-adrenoceptors of the dorsal hippocampal area play an important role in muscimol state-dependent memory.

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1. Introduction

α₂-adrenoceptors in the central nervous system are involved in the modulation of memory process (Galeotti et al., 2004a, b; Mondaca et al., 2004; Ji et al., 2008). These receptors are widely distributed in the brain, and is localized both pre- and postsynaptically, with high densities in the cortex, on locus coeruleus dendrites and in area CA1 of the hippocampus (Verhage et al., 1992; Nicholas et al., 1996; McDonald et al., 1997; Boehm, 1999; Galeotti et al., 2004a).

Many clinical and experimental studies have shown that systemic administration of α₂-adrenoceptor agonists impairs memory, while their antagonists facilitate memory storage and retrieval in variety of tasks (Haapalinna et al., 1998; Riekkinen et al., 1999; Hall et al., 2001; Chopin et al., 2002; Galeotti et al., 2004a, b; Mondaca et al., 2004).

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α₂-adrenoceptors of the dorsal hippocampal area play an important role in memory processing in the hippocampus (Paulsen and Moser, 1998; Vargas-Caballero et al., 2010).

It is a well known fact that administration of GABA A receptor agonists or antagonists impairs or improves memory storage and retrieval in inhibitory avoidance tasks respectively (Castellano and McGaugh, 1990; Fari et al., 2000; Chapouthier, 2004; Amaral et al., 2007; Jafari-Sabet, 2011).

GABA exerts its action by binding to specific membrane receptors that are divided into two major groups: ionotropic GABA A /GABA C receptors and metabotropic GABA B receptors (Bormann, 2000; Semyanov and Kullmann, 2002; Emson, 2007; Olsen and Sieghart, 2009).

Our previous studies have shown that pre-training intra-dorsal hippocampal (intra-CA1) administration of a GABA A receptor agonist, muscimol induced memory impairment which was restored when the same dose of the drug was administered 24 h later in a pre-test session (Jafari-Sabet and Jannat-Dastjerdi, 2009; Jafari-Sabet, 2011). Thus the retrieval of an event from memory
may require that the organism be in a state that is similar to that in which the event was initially acquired. This phenomenon has been named state-dependent learning (Izquierdo, 1980; Bruins Slot and Colpaert, 1999; Jafari-Sabet et al., 2005; Zarrindast et al., 2006; Jafari-Sabet, 2011).

One of the most well-known and important physiological actions of α2-adrenoceptor activation is modulation of the release of other neurotransmitters in other areas of the brain (Zhang and Ordway, 2003; Alachkar et al., 2006).

Extensive evidence indicates that α2-adrenoceptors activation increases the basal release of GABA in rat hippocampus, cerebral cortex and striatum (Pittaluga and Raiteri, 1987; Maura et al., 1988; Ciranna et al., 2000; Zhang and Ordway, 2003; Alachkar et al., 2006).

Several experimental studies in animals and transgenic approaches suggested that the hippocampal formation plays a crucial role in various types of memory including inhibitory avoidance (Lorenzini et al., 1996; Moser and Moser, 1998; Minichiello et al., 1999; Zola and Squire, 2001; Jafari-Sabet, 2006a; Jafari-Sabet and Jannat-Dastjerdi, 2009). It is well known that the hippocampus receives information from the entorhinal cortex and is connected through it to the amygdala and other cortical areas. Some of these areas have also been involved in memory processing (Izquierdo and McGaugh, 2000; Szapiro et al., 2002; Jafari-Sabet, 2006b; Ferry and McGaugh, 2008).

One-trial step-down inhibitory (‘passive’) avoidance in rodents, has long been a favorite model for biochemical and pharmacological studies of memory (Izquierdo et al., 2006) and induces long-term potentiation (LTP) in CA1 region of the hippocampus (Bliss and Collingridge, 1993; Malenka, 2003; Whitlock and Heynen, 2006).

In this one-trial learning task, animals are placed on a platform and receive a footshock after stepping down from the platform to a grid. When the animals are tested, they are exposed to the training apparatus, but no footshock is administered, which is in fact the method of choice for initiating memory extinction. Animals learn to remain longer on the platform than they do on the training session (Szapiro et al., 2002).

Since the role of CA1 α2-adrenoceptors on muscimol state-dependent memory has not been shown previously, the aim of the present study was to investigate the effects of bilateral intradorsal hippocampal (intra-CA1) injections of α2-adrenoceptor agonist and antagonist on muscimol induced state-dependent memory retrieval in a passive avoidance task in mice.

2. Materials and methods

2.1. Animals

Male albino NMRI mice (Razi Institute, Iran), weighing 25–35 g at the time of the surgery were used. The animals were kept in an animal house with a 12-h light/12-h dark cycle and controlled temperature (22 ± 2°C). Food and water were available ad libitum. Animals were housed in groups of 10 in Plexiglas animal cages. Each animal was used once only. Ten animals were used in each group. Training and testing were done during the light phase of the cycle. All procedures were carried out in accordance with institutional guidelines for animal care and use.

2.2. Surgical and infusion procedures

Mice were anesthetized with intraperitoneal injection of ketamine hydrochloride (50 mg/kg) plus xylazine (5 mg/kg) and placed in a stereotaxic apparatus. The skin was incised and the skull was cleaned. Two 23-gauge guide cannulae were placed (bilaterally) 1 mm above the intended site of injection according to the atlas of Paxinos and Franklin (2001). Stereotaxic coordinates for the CA1 regions of the dorsal hippocampi were AP: −2 mm from bregma, L: ± 1.6 from the sagittal suture and V: −1.5 mm from the skull surface. The cannulae were secured to anchor jewelers’ screws with dental acrylic. Stainless steel stylets (30-gauge) were inserted into the guide cannulae to keep them free of debris. All animals were allowed 1 week to recover from surgery and clear anesthetic.

For drug infusion, the animals were gently restrained by hand; the stylets were removed from the guide cannulae and replaced by 30-gauge injection needles (1 mm below the tip of the guide cannulae). The injector cannula was attached to a polyethylene tube fitted to a 1-μl Hamilton syringe. The injection solutions were administered in a total volume of 1 μl/mouse (0.5 μl in each side, intra-CA1) over a 60 s period. Injection needles were left in place for an additional 60 s to facilitate the diffusion of the drugs.

2.3. Passive avoidance apparatus

Animals were submitted to the behavioral procedure 7 days after surgery. The apparatus was a (30 cm × 30 cm × 40 cm high) wooden box the floor of which consisted of parallel stainless steel bars (0.3 cm diameter spaced 1 cm apart). A wooden platform (4 cm × 4 cm × 4 cm) was placed on the center of the grid floor. In the training session the animals were placed on the platform and their latency to step down on the grid with all four paws was measured. Immediately after stepping down on the grid, animals received electric shocks (1 Hz, 0.5 s, 45 V DC) continuously for 15 s. The shocks were delivered to the grid floor by an isolated (Harvard Stimulator 6002, England) stimulator. If any animal stayed on the platform more than 20 s or stepped up to the platform before the end of 15 s of electric shocks, it was omitted from the experiments. Retention test session was carried out 24 h after training and was procedurally identical to training, except that no shock was delivered to the animals. Step-down latency was used as a measure of memory retention. An upper cut-off time of 300 s was set (Jafari-Sabet et al., 2005; Jafari-Sabet and Jannat-Dastjerdi, 2009; Jafari-Sabet, 2011). The retention test was carried out between 8:00 a.m. and 3:00 p.m.

2.4. Drugs

The drugs used in the present study were muscimol (Tocris Cookson Ltd, UK), clonidine (Tolid Daru Company Tehran, Iran), yohimbine (Nuova-Linnea, Switzerland). All drugs were dissolved in sterile 0.9% saline and were injected into the dorsal hippocampal CA1 regions (intra-CA1) 1 μl/mouse. Control animals received saline.

2.5. Experimental design

Ten animals were used in each experimental group. In experiments where the animals received one or two injections, the control groups also received one or two saline injections.

2.5.1. Experiment 1

This experiment examined muscimol state-dependent memory. In this experiment, four groups of animals were used. Two groups of animals received saline (1 μl/mouse, intra-CA1) 15 min before training and were tested 15 min after pre-test saline (1 μl/mouse, intra-CA1) or muscimol (0.1 μg/mouse, intra-CA1) injection. Two other groups of animals in this experiment were trained 15 min after pre-training muscimol (0.1 μg/mouse, intra-CA1) and were tested 24 h later, 15 min after pre-test saline (1 μl/mouse, intra-CA1) or muscimol (0.1 μg/mouse, intra-CA1) injection (Fig. 2).
Fig. 1. Schematic illustrations of coronal sections of the mouse brain showing the approximate location of dorsal hippocampus sites in the experiments. The numbers indicate AP coordinates relative to bregma. Atlas plates adapted from Paxinos and Franklin (2001).
15 min before testing (Fig. 3).

μ animals received pre-training saline (1 μl/mouse, intra-CA1). Two groups of animals were trained 15 min after muscimol (0.1 μg/mouse, intra-CA1) and were tested 15 min after pre-test saline (1 μl/mouse, intra-CA1) or muscimol (0.1 μg/mouse, intra-CA1) injection. Each value represents the median ± quartiles for 10 animals. **P < 0.001, compared to the pre-training and pre-test saline group.

***P < 0.001, compared to pre-training muscimol (0.1 μg/mouse, intra-CA1) and pre-test saline group.

2.5.2. Experiment 2

In this experiment, eight groups of animals were used. First four groups of animals received saline (1 μl/mouse, intra-CA1) 15 min before training. On the test day, one group of these animals received saline (1 μl/mouse, intra-CA1) and the other three groups received different doses of clonidine (0.25, 0.5 and 1 μg/mouse, intra-CA1) 15 min before testing. The second four groups of animals received saline (1 μl/mouse, intra-CA1) 15 min before training. On the test day, one group of these animals received saline (1 μl/mouse, intra-CA1) and the other three groups received different doses of yohimbine (0.5, 1 and 2 μg/mouse, intra-CA1) 15 min before testing (Fig. 3).

2.5.3. Experiment 3

In this experiment, eight groups of animals were used. The animals received pre-training saline (1 μl/mouse, intra-CA1) or muscimol (0.1 μg/mouse, intra-CA1) 15 min before training. On the testing day, they received different doses of clonidine (0.0625, 0.125 and 0.25 μg/mouse, intra-CA1) 15 min before saline (1 μl/mouse, intra-CA1) or muscimol (0.1 μg/mouse, intra-CA1). All animals were tested 15 min after the last injection (Fig. 4).

2.5.4. Experiment 4

In this experiment, four groups received saline (1 μl/mouse, intra-CA1) 15 min before training and also different doses of yohimbine (0.125, 0.25 and 0.5 μg/mouse, intra-CA1) plus saline (1 μl/mouse) 15 min before testing. Another four groups were trained 15 min after muscimol (0.1 μg/mouse, intra-CA1), and were tested 24 h later, 15 min after pre-test administration of yohimbine (0.125, 0.25 and 0.5 μg/mouse, intra-CA1) plus saline (1 μl/mouse, intra-CA1). Further four groups were trained 15 min after muscimol (0.1 μg/mouse, intra-CA1) and were tested 24 h later, 15 min after pre-test administration of yohimbine (0.125, 0.25 and 0.5 μg/mouse, intra-CA1) plus muscimol (0.025 μg/mouse, intra-CA1) (Fig. 5).

2.6. Verification of cannulae placements

After completion of the experimental sessions, each animal was killed with an overdose of chloroform. Animals received bilateral intra-CA1 injection of ink (0.5 μl/side; 1% aquatic methylene blue solution). The brains were then removed and fixed in a 10% formalin solution for 10 days before sectioning. Sections were examined to determine the location of the cannulae aimed for the CA1 regions. The cannulae placements were verified using the atlas of Paxinos and Franklin (2001). Data from animals with injection sites located outside the CA1 region were not used in the analysis.
2.7. Statistical analysis

Because of wide variations of the data in experimental models of memory study, the retention latencies were expressed as the median and interquartile range. The data were analyzed using the Kruskal–Wallis non-parametric one-way analysis of variance (ANOVA) followed by a two-tailed Mann–Whitney’s U-test, then Holm’s Bonferroni correction for the paired comparisons. In all statistical evaluations, $P < 0.05$ was used as the criterion for statistical significance.

3. Results

3.1. Histology

Fig. 1 shows the approximate point of the drug injections in the CA1 regions of the dorsal hippocampus. The histological results were plotted on representative sections taken from the mouse brain atlas of Paxinos and Franklin (2001).

3.2. Effect of pre-training and pre-test muscimol administration on the memory performance in passive avoidance task

Fig. 1 indicates that there was a significant difference between the responses induced by pre-training and/or pre-test administration of a GABA$_A$ receptor agonist, muscimol (Kruskal–Wallis non-parametric ANOVA, $H(3)=28.76, P<0.001$). Results showed that pre-training and pre-test administration of muscimol (0.1 $\mu$g/mouse, intra-CA1) impaired memory, compared to the pre-training and pre-test saline group. The same dose of the muscimol injected 15 min before testing significantly reversed the impairing effect of pre-training muscimol (0.1 $\mu$g/mouse, intra-CA1) when compared to pre-training muscimol and pre-test saline group.

3.3. Effects of pre-test administration of $\alpha_2$-adrenoceptor agonist and antagonist in mice trained under saline

Fig. 3 indicates that in animals trained after saline treatment and tested following the administration of three different doses of a $\alpha_2$-adrenoceptor agonist, clonidine (0.25, 0.5 and 1 $\mu$g/mouse, intra-CA1) altered the memory retrieval on the test day, compared with saline–saline control group. Lower dose of clonidine (0.25 $\mu$g/mouse) had no significant effect on memory retrieval, while the higher doses of clonidine (0.5 and 1 $\mu$g/mouse) significantly impaired the memory retrieval on the test day (Kruskal–Wallis non-parametric ANOVA, $H(3)=21.81, P<0.001$). The greatest response was obtained with 1 $\mu$g/mouse of drug.

However, in the animals which trained after saline treatment and tested following the administration of three different doses of a $\alpha_2$-adrenoceptor antagonist, yohimbine (0.5, 1 and 2 $\mu$g/mouse, intra-CA1) altered the memory retrieval on the test day, compared with saline–saline control group. Lower doses of yohimbine (0.5 $\mu$g/mouse) had no significant effect on memory retrieval, while the higher doses of yohimbine (1 and 2 $\mu$g/mouse) significantly improved the memory retrieval on the test day (Kruskal–Wallis non-parametric ANOVA, $H(3)=21.81, P<0.001$).

3.4. Effects of pre-test administration of clonidine in mice trained under saline or muscimol (0.1 $\mu$g/mouse)

Fig. 4 indicates that in animals trained after saline treatment and tested following the administration of three lower doses of clonidine (0.0625, 0.125 and 0.25 $\mu$g/mouse, intra-CA1), no significant change was observed in the retention latencies compared to the saline/saline control group [Kruskal–Wallis non-parametric ANOVA, $H(3)=0.72, P>0.05$].

However, in the animals which received pre-training and pre-test administration of muscimol (0.1 $\mu$g/mouse, intra-CA1), pre-test administration of clonidine (0.125 and 0.25 $\mu$g/mouse, intra-CA1) decreased the improvement of memory retrieval by pre-test muscimol (0.1 $\mu$g/mouse, intra-CA1) treatment [Kruskal–Wallis nonparametric ANOVA, $H(3)=19.58, P<0.001$].

3.5. Effects of pre-test administration of yohimbine in mice trained under saline or muscimol (0.1 $\mu$g/mouse)

As shown in Fig. 5, in animals trained after saline treatment and tested following administration of three lower doses of yohimbine (0.125, 0.25 and 0.5 $\mu$g/mouse, intra-CA1), no significant change was observed in the retention latencies as compared with saline/saline control group [Kruskal–Wallis non-parametric ANOVA, $H(3)=6.4, P>0.05$]. In the animals that pre-training administration of muscimol (0.1 $\mu$g/mouse, intra-CA1) impaired memory retrieval, administration of yohimbine (0.25 and 0.5 $\mu$g/mouse, intra-CA1), on the test day, improved the memory retrieval significantly [Kruskal–Wallis, non-parametric ANOVA, $H(3)=29.28, P<0.01$]. Pre-test administration of yohimbine (0.25 and 0.5 $\mu$g/mouse, intra-CA1), in combination with muscimol (0.025 $\mu$g/mouse, intra-CA1) also improved the memory retrieval and mimicked the effects of pre-test muscimol treatment [Kruskal–Wallis non-parametric ANOVA, $H(3)=19.99, P<0.001$].

4. Discussion

Our results show that pre-training and/or pre-test intra-dorsal hippocampal (intra-CA1) administration of certain dose of the GABA$_A$ receptor agonist, muscimol impaired memory retrieval in the step-down passive avoidance task. These results are in agreement with our previous studies (Jafari-Sabet and Jannat-Dastjerdi, 2009; Jafari-Sabet, 2011) and other investigators who found that muscimol impaired memory formation (Jerusalinsky et al., 1994; Castellano et al., 1996; Farr et al., 2000; Chauvetier, 2004; Amaral et al., 2007), indicating the possible existence of an inhibitory influence of the brain GABA$_A$ system on memory.
Furthermore, our results also indicate that pre-training administration of muscimol induced memory impairment which is restored when the same dose of the drug is administered 24 h later in a pre-test session. These results are in agreement with our previous studies (Jafari-Sabet and Jannat-Dastjerdi, 2009; Jafari-Sabet, 2011) and other investigators who found that pre-training administration of muscimol can impair the retrieval of learned tasks in a state-dependent manner, which is reversible by pre-test muscimol administration (Nabeshima et al., 1988; Nakagawa et al., 1993).

Present findings demonstrate that muscimol produces a state of memory in which animals could learn and retrieve a specific response. The present data show that pre-training intra-dorsal hippocampal (intra-CA1) administration of lower doses of a α2-adrenoceptor agonist, clonidine had no significant effect on memory retrieval, while the higher doses of clonidine significantly impaired the memory retrieval in the step-down passive avoidance task. Also, pre-training intra-dorsal hippocampal (intra-CA1) administration of lower doses of a α2-adrenoceptor antagonist, yohimbine had no significant effect on memory retrieval, while the higher doses of yohimbine significantly improved the memory retrieval in the step-down passive avoidance task. These results are in agreement with others who found that α2-adrenoceptor agonists induced a dose-dependent memory impairment, whereas α2-adrenoceptor antagonists induced memory enhancement. (Izquierdo et al., 1992; Haapalinna et al., 1998; Riekkinen et al., 1999; Hall et al., 2001; Chopin et al., 2002; Galeotti et al., 2004a, b; Mondaca et al., 2004; Ferry and McGaugh, 2008).

Also Galeotti et al. (2004a) have shown that clonidine induces amnesia through activation of the G1i, G13, and Go1, but not G12 and G02, protein subtypes in the transduction mechanism. Several studies have shown that α2-adrenoceptor agonists directly or indirectly enhance the release of GABA from the nerve terminals in rat hippocampus, nucleus accumbens, striatum and the cortex (Maura et al., 1988; Murai et al., 1998; Zhang and Ordway, 2003; Alachkar et al., 2006). Conversely, activation of α2-adrenoceptors inhibits the release of norepinephrine, glutamate, acetylcholine, dopamine, and serotonin in various regions of the brain (Pittaluga and Raiteri, 1987; Ciranna et al., 2000; Alachkar et al., 2006; Ferry and McGaugh, 2008).

Thereby suggesting that α2-adrenoceptor agonists and antagonists may modulate memory retrieval by enhancing or decreasing basal GABA release, respectively. In addition, because of the above-mentioned findings it must not be forgotten that, higher doses of clonidine or yohimbine, may be affecting performance by acting at receptors independent of the GABAergic system. Since activation or inhibition of α2-adrenoceptors may impair or improve memory retrieval respectively, the possibility may exist that an α2-adrenoceptors mechanism plays a modulatory role in the muscimol response. Our results also showed that pre-test intra-CA1 administration of lower doses of clonidine do not affect the retrieval of memory by itself, while pre-test intra-CA1 administration of the same doses of the drug with muscimol (0.1 μg/mouse) significantly and dose-dependently inhibited the muscimol-induced memory retrieval improvement, a finding which may show interaction of the α2-adrenoceptor mechanism with the muscimol effect. α2-adrenoceptors can be located both post- and presynaptically on hippocampal neurons (Couill, 1994). Thus, inhibition of the muscimol-induced improvement of memory recall by clonidine may be mediated either through pre- or postsynaptic hippocampal α2-adrenoceptor sites. Therefore, there may be a possibility that activation of α2-adrenoceptors in the hippocampal CA1 regions affects GABA release from the hippocampus, which in turns may modulate memory retrieval in the hippocampus and other target areas. In support of this idea, the involvement of GABA in modulation of memory processes in rat hippocampus, cerebral cortex and striatum has been indicated (Zhang and Ordway, 2003; Ciranna et al., 2004; Alachkar et al., 2006; Vargas-Caballero et al., 2010).

The results of the present experiments show that pre-test intra-CA1 administration of certain doses of yohimbine by itself cannot affect memory retrieval. However, pre-test intra-CA1 administration of the same doses of the yohimbine reversed the memory impairment induced by pre-training administration of muscimol.

This can be supported by the result that the blockade of α2-adrenoceptors by the α2-adrenoceptor antagonist yohimbine may decrease the basal release of GABA in the targets areas and ultimately facilitate memory retrieval. It seems likely that α2-adrenoceptor activation may be coupled to GABA release since GABA release is modulated by α2-adrenoceptors in the brain regions (Pittaluga and Raiteri, 1987) and since GABAergic neurons express α2-adrenoceptors (see Zhang and Ordway, 2003).

In addition, yohimbine when co-administered with the lower dose of muscimol (0.025 μg/mouse) which did not induce state-dependent memory on the test day by itself (Jafari-Sabet and Jannat-Dastjerdi, 2009; Jafari-Sabet, 2011), potentiated pre-test muscimol induced memory improvement. The data are in agreement with previous investigations, indicating an interaction between GABAergic system and α2-adrenoceptors in the laboratory animals, concerning memory consolidation (Pittaluga and Raiteri, 1987; Ciranna et al., 2004). Moreover, it has been reported that yohimbine modulates the release of GABA from the nerve terminals in rat hippocampus (Maura et al., 1988; Murai et al., 1998; Zhang and Ordway, 2003; Alachkar et al., 2006) and consequently improve memory (Chen et al., 1992) Furthermore, Canacho et al. (1996) indicated that α2-adrenoceptors antagonists potentiate the retention enhancement induced by acetylcholinesterase inhibitor in the rat, through blockade of presynaptic α2-adrenoceptors. Thus, by such a mechanism muscimol in combination with yohimbine may improve memory, which in turn elicits a potentiated state-dependent memory.

Recent findings indicating the major molecular steps involved in memory retrieval in selected brain regions of the mammalian brain. Together the findings strongly suggest that memory formation and retrieval may share some molecular mechanisms in the hippocampus and that retrieval initiates extinction requiring activation of several signaling cascades and protein synthesis (Szapiro et al., 2002; Izquierdo et al., 2006).

Furthermore, activity of protein kinases such as protein kinase A (PKA), MAPKs, protein kinase C (PKC), involved in retrieval of one-trial avoidance and are modulated by GABA A receptors, dopamine D1 receptors, β-adrenoceptors, 5HT1A receptors and muscarinic cholinergic receptors in the hippocampus, the entorhinal, parietal and cingulate cortex and the basolateral amygdala (Holt and Maren, 1999; Barros et al., 2001; Szapiro et al., 2002; Rossato et al., 2004; Izquierdo et al., 2006; Jafari-Sabet, 2006a, b; Jafari-Sabet and Jannat-Dastjerdi, 2009; Jafari-Sabet, 2011).

In conclusion, considering the effects of intra-dorsal hippocampal (intra-CA1) injection of clonidine (prevention of memory recall), and the effects of intra-dorsal hippocampal (intra-CA1) injection of yohimbine (enhancement of memory recall) when co-administered with muscimol, it is possible that muscimol-induced memory recall is related to activation of dorsal hippocampal α2-adrenoceptors. In addition, it must not be forgotten that, memory formation involves a complex network of brain systems and serial and parallel molecular events, even for a task as deceptively simple as one-trial avoidance.
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