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Involvement of dorsal hippocampal α-adrenergic receptors in the effect of scopolamine on memory retrieval in inhibitory avoidance task

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ABSTRACT

The present study evaluated the possible role of α-adrenergic receptors of the dorsal hippocampus on scopolamine-induced amnesia and scopolamine state-dependent memory in adult male Wistar rats. The animals were bilaterally implanted with chronic cannulae in the CA1 regions of the dorsal hippocampus, trained in a step-through type inhibitory avoidance task, and tested 24 h after training to measure step-through latency. Results indicate that post-training or pre-test intra-CA1 administration of scopolamine (1 and 2 μg/rat) dose-dependently reduced the step-through latency, showing an amnestic response. Amnesia produced by post-training scopolamine (2 μg/rat) was reversed by pre-test administration of the scopolamine that is due to a state-dependent effect. Interestingly, pre-test intra-CA1 microinjection of α1-adrenergic agonist, phenylephrine (1 and 2 μg/rat) or α2-adrenergic agonist, clonidine improved post-training scopolamine (2 μg/rat)-induced retrieval impairment. Furthermore, pre-test intra-CA1 microinjection of phenylephrine (0.25, 0.5 and 1 μg/rat) or clonidine (0.25, 0.5 and 1 μg/rat) with an ineffective dose of scopolamine (0.25 μg/rat), synergistically improved memory performance impaired by post-training scopolamine. On the other hand, pre-test injection of α1-receptors antagonist prazosin (1 and 2 μg/rat) or α2-receptors antagonist yohimbine (1 and 2 μg/rat) prevented the restoration of memory by pre-test scopolamine. It is important to note that pre-test intra-CA1 administration of the same doses of prazosin or yohimbine, alone did not affect memory retrieval. These results suggest that α1- and α2-adrenergic receptors of the dorsal hippocampal CA1 regions may play an important role in scopolamine-induced amnesia and scopolamine state-dependent memory.

1. Introduction

The importance of cholinergic systems in learning and memory has been shown previously (Blokland, 1995). Evidence suggested that acetylcholinesterase inhibitors, which enhance the availability of acetylcholine in the synaptic cleft improve performance in several cognitive models in both rodents and humans, whereas anticholinergic drugs impair learning and memory in a variety of tasks (Fibiger, Damsma, & Day, 1991; Gallagher & Colombo, 1995; Power, Vazdarjanova, & McGaugh, 2003; Zarrindast, Bakhsha, Rostami, & Shafaghi, 2002). There is also evidence that learning functionally modifies cholinergic neurons, which become progressively more active. For instance, release of Ach in the hippocampus increases during performance of a learned spatial memory task (Stancampiano, Cocco, Cugusi, Sarais, & Fadda, 1999). The increase in Ach is positively correlated with performance improvement during learning (Fadda, Robinson, Fratta, Pertwee, & Riedel, 2006). Scopolamine, a muscarinic cholinergic receptor antagonist, impairs memory performance that has been proposed as an animal model of dementia (Collerton, 1986; Jensen, Stephens, Sarter, & Petersen, 1987; Quartermain & Leo, 1988). Similarities in the memory deficits between Alzheimer patients and scopolamine treated animals have been reported. Thus, it has been proposed that scopolamine, could serve as a useful pharmacological tool to produce a partial model of the disorder (Bartus, 2000). Furthermore, along with cholinergic atrophy, monoamines are reduced in Alzheimer's disease and the possibility may exist that enhancement of monoaminergic functions may elicit beneficial

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effects on behavior and cortical activity (Dringenberg, 2000; Hertz, 1989).

The various areas of the brain, including the neocortex and the hippocampus are innervated by the ascending noradrenergic system that originates in the locus coeruleus. Noradrenaline acts through two classes of receptors (α and β), both coupled with G-proteins (Sirvio & MacDonald, 1999). α-adrenergic receptors are divided into two subtypes, differing in ligand specificity, kinetics, and effects. These receptors are expressed widely in the central nervous system (Berridge & Waterhouse, 2003; Sirvio & MacDonald, 1999). α1-adrenoceptors were found to be mainly post-synaptic, whilst α2-adrenoceptors are localized both pre-synaptically and post-synaptically (Stuchlik, Petrasek, & Vales, 2008). Adrenergic receptors are involved in learning and memory. For example, noradrenaline enhances memory formation when administered into various brain regions including the hippocampus and entorhinal cortex (Izquierdo et al., 2000); and amygdale (Clayton & Williams, 2000; Hatfield & McGaugh, 1999). Brain noradrenaline concentrations after training have been correlated with retention performance (Gold & van Buskirk, 1978).The mechanisms underlying the function of this neurotransmitter in memory processing are unknown. However, the ability of noradrenaline to modulate the efficacy of glutamate synaptic transmission via activation of G-protein-coupled adrenergic receptors seems likely (Scheiderer, Dobrunz, & McMahon, 2004).

Based on these findings the aim of the present study was to investigate the effects of bilateral microinjections of α1- or α2-adrenergic receptor agents into the CA1 region of the dorsal hippocampus on scopolamine-induced amnesia and scopolamine state-dependent memory, by using an inhibitory avoidance task has been investigated.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (Pasteur institute, Tehran, Iran) weighing 220–270 g at time of surgery were used. They had free access to food and water, were housed four in a cage, and kept at (22 ± 2) °C under a 12/12 h light–dark cycle (light beginning at 7:00 a.m.). All experiments were carried out during the light phase between 8:00 and 14:00. Experimental groups consisted of eight animals and each animal was tested once. All procedures were performed in accordance with institutional guidelines for animal care and use.

2.2. Surgery

Animals were anaesthetized intraperitoneally with a ketamine/xylazine mixture (100 and 10 mg/kg, respectively) and placed in a stereotaxic frame (David Kopf Instruments, USA) with flat-skull position. A midline incision was made and the skin and underlying periosteum retracted. Stereotaxic coordinates for the CA1 regions of dorsal hippocampi were AP: −3 mm from bregma, L: ±2 mm from midline and V: −2.8 mm from the skull surface (Paxinos & Watson, 2007). The cannulae were anchored to the skull with dental cement, and then stainless steel stylets (27 gauge) were inserted into the guide cannulae to maintain patency prior to microinfusions.

2.3. Drugs and microinfusions

The drugs included scopolamine hydrobromide, yohimbine (Tocris, UK) phenylephrine hydrochloride, prazosin hydrochloride and clonidine hydrochloride (Sigma, UK). All drugs were dissolved in sterile saline and were injected into CA1 of dorsal hippocampus.

2.4. Intra-CA1 injections

For bilateral drug infusion, the animals were gently restrained by hand; the stylets were removed from the guide cannulae and replaced by 27-gauge injection needles (1 mm below the tip of the guide cannula). The injection solutions were administered in a total volume of 1 μl/rat (0.5 μl in each side) over a 60 s period. Injection needles were left in place for an additional 60 s to facilitate the diffusion of the drugs.

2.5. Inhibitory avoidance apparatus

A step-through inhibitory avoidance apparatus consisted of two compartments of the same size ($20 \times 20 \times 30$ cm^3). In the middle of a dividing wall, a guillotine door (7.9 cm^2) could be lifted manually. The walls and floor of one compartment consisted of white opaque resin, the walls of the other compartment were dark. Stainless steel bars (3 mm in diameter and 1 cm intervals) constituted the floor of the dark compartment. Intermittent electric shocks (50 Hz, 3 s, 1 mA intensity) were delivered to the grid floor of the dark compartment by an isolated stimulator.

2.6. Behavioral procedures

Training was based on our previous studies (Zarrindast, Eidi, Eidi, & Oryan, 2002; Zarrindast, Farajzadeh, Rostami, Rezayof, & Nourjah, 2005a). All animals were allowed to habituate in the experimental room (with light and sound attenuated) for at least 30 min prior to the experiments. Then, each animal was gently placed in the brightly lit compartment of the apparatus; after 5 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 immediately withdrawn from the compartment. The trial was repeated after 30 min as in the acquisition trial where after 5 s the guillotine door was opened 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 animal was retested in the same way as in the previous trials; if the rat did not enter the dark compartment during 120 s a successful acquisition of IA response was recorded. Otherwise, when the rat entered the dark compartment (before 120 s) a second time, the door was closed and the animal received the shock again. After retesting, if the rat learned inhibitory avoidance response successfully, it was removed from the apparatus and received post-training injection of saline or scopolamine (intra-CA1) immediately. On the test day, intra-CA1 infusions were performed 5 min prior to the test. For the study of memory 24 h after training, each animal was gently placed in the light compartment and after 5 s (sec) the door was opened, and Step-through latency (sec) was measured in absence of electric foot shocks, as indicators of inhibitory avoidance behavior. An upper cut-off of 300 s was set. The retention test was also carried out between 8:00 a.m. and 2:00 p.m.

2.7. Data analysis

The data are expressed as mean ± SEM. The statistical analysis was performed using one- and two-way analysis of variance (ANOVA). Post-hoc comparison of means was carried out with the Tukey test for multiple comparisons, when appropriate. The level of