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Involvement of dorsal hippocampal α1-adrenergic receptors in the effect of WIN55,212-2 on memory retrieval in inhibitory avoidance task

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ABSTRACT

In the present study, the possible role of α1-adrenergic receptors of the dorsal hippocampus on WIN55,212-2-induced amnesia in male Wistar rats has been evaluated. As a model of learning, a step-down passive avoidance task was used. Results indicated that post-training or pre-test intra-CA1 administration of WIN55,212-2 (0.25 and 0.5 μg/rat) reduced the step-down latency, showing an amnestic response. Amnesia produced by post-training WIN55,212-2 (0.5 μg/rat) was reversed by pre-test administration of the same drug dose. Interestingly, pre-test intra-CA1 administration of α1-noradrenergic agonists, phenylephrine alone or with an ineffective dose of WIN55,212-2 (0.25 μg/rat) reversed post-training WIN55,212-2 (0.5 μg/rat)-induced retrieval impairment. On the other hand, pre-test intra-CA1 microinjection of an α1-adrenergic antagonist, prazosin (0.5 μg/rat), 2 min before administration of WIN55,212-2 (0.5 μg/rat) inhibited the pre-test WIN55,212-2 response. It may be concluded that α1-adrenergic receptors of the dorsal hippocampal CA1 regions play an important role in WIN55,212-2-induced amnesia and restoration of memory by pre-test WIN55,212-2 administration.

Cannabinoids cause a wide array of effects in different species. Their effects are mainly produced through cannabinoid receptors; CB1and CB2 subtypes [19]. The CB1 receptors are densely expressed in areas classically involved in learning and memory, such as the hippocampus, cortex, basal ganglia, amygdala and cerebellum [27]. Behavioral studies have suggested direct interactions between cannabinoid receptors and some neurotransmitter systems [8]. It is well known that cannabinoids inhibit the release of different neurotransmitters such as glutamate, acetylcholine and noradrenaline in the hippocampus through the activation of CB1 receptors [24].

There is evidence for the involvement of noradrenaline and adrenergic receptors in learning and memory. For example, noradrenaline when injected into the amygdala [9]; hippocampus and entorhinal cortex [12] enhances memory formation. Furthermore noradrenaline has been implicated in many of the same central processes that are affected by cannabinoids. Previous studies have demonstrated an inhibitory effect of Δ9-tetrahydrocannabinol (THC) and synthetic cannabinoid agonists on noradrenergic functions. Treatment with THC [10] or synthetic cannabinoid agonist [25] decreases noradrenaline in the hippocampus and this decrease correlated to poor performance in the radial arm maze behavioral test. Moreover, pretreatment with 6-hydroxydopamine or lesions of the locus coeruleus (LC) significantly reduce the cataleptogenic effect of THC [14]. On the other hand, brain noradrenergic systems have been implicated in hypothermic [26] and antinociception [15] as induced by THC.

The hippocampal CA1 is a brain region essential for memory [11] and long-term potentiation [1] which receives adrenergic input from the LC and has different types of adrenergic receptors [4]. In the present study, the effects of bilateral microinjections of α1-adrenergic receptor agonists (phenylephrine), and antagonists (prazosin), into the CA1 region of the dorsal hippocampus on WIN55,212-2-induced response in inhibitory avoidance tasks have been investigated.

Adult male Wistar rats (Pasteur Institute, Tehran, Iran) weighing 220–270 g at the time of surgery were used. The animals had free access to food and water, and were kept at 22 ± 2 °C under a 12/12 h

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Table 1
Summary of experimental design.

Figure		Post-training treatment Intra-CA1		Pre-test treatment intra-CA1			
		Vehicle (1 μl/rat)	WIN55,212-2	Vehicle (1 μl/rat)	WIN55,212-2	Phenylephrine	Prazosin
S1	A	Actual histology					
	B	Schematic histology					
1	Left panel		(0–0.5)	1			
	Middle panel	1			(0–0.5)		
	Right panel		0.5		(0–0.5)		
2	Left panel	1		1		(0–0.5)	
	Middle panel		0.5	1		(0–0.5)	
	Right panel		0.5		0.25	(0–0.5)	
3	Left panel	1		1			(0–0.5)
	Right panel		0.5		0.5		(0–0.5)

light:dark cycle (light beginning at 7:00 a.m.). All procedures were performed in accordance with the Institutional Guidelines for Animal Care and Use.

Animals were intraperitoneally anaesthetized with a ketamine/xylazine mixture (100 and 10 mg/kg, respectively) and placed in the flat-skull position within a stereotaxic frame (David Kopf Instruments, USA). Stereotaxic coordinates for the CA1 regions of the dorsal hippocampus were AP: −3 mm from bregma, L: ±2 mm from midline and V: −2.8 mm from the skull surface [18]. The cannulae were anchored to the skull with dental cement.

Drugs used in the present study were WIN55,212-2 mesylate (Tocris, UK), phenylephrine hydrochloride and prazosin hydrochloride (Sigma, UK). WIN55,212-2 was dissolved in a vehicle [dimethylsulphoxide (DMSO; up to 10% (v/v), 0.9% sterile saline and one drop of Tween 80]. Other drugs were dissolved in 0.9% sterile saline.

All drugs were injected bilaterally intra-CA1, with 27-gauge injection needles (1 mm below the tip of the guide cannulae). 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 diffusion of the drugs.

The inhibitory avoidance apparatus was a wooden box (40 cm × 30 cm × 40 cm) whose floor consisted of parallel 3.0-mm stain-less steel bars spaced 1.0 cm apart. A wooden platform (12 cm × 10 cm × 7 cm) was placed on the floor against the left wall. An electric shock (0.4 mA, 5 s) was delivered to the grid floor by an isolated stimulator [22].

A one-trial step-down inhibitory avoidance task was used. Training was based on our previous studies [30]. Each rat was gently placed on the platform. When the rat stepped-down from the platform, a 5 s 0.4-mA shock was applied to the grid. Animals were then immediately withdrawn from the training apparatus. Twenty-four hours after training, the step-down latency was measured 5 min after the last injection. Each rat was again placed on the platform, without any shock. The step-down latency was taken as a measure of retention. An upper cutoff time of 300 s was set. The training and retention test were carried out between 9:00 and 15:00 h.

Eight animals were used in each experimental group. In experiments where animals received either two or three injections; the control groups also received two or three saline or vehicle injections. In order to obtain a maximum response, drug administration intervals were based on previous studies [29]. The experimental design is summarized in Table 1.

Five groups of animals received either saline (1 μl/rat), vehicle (1 μl/rat) or different doses of WIN55,212-2 (0.1, 0.25 and 0.5 μg/rat), immediately after training. On the test day, the animals received saline (1 μl/rat) or vehicle (1 μl/rat) 5 min before the test. The other eight groups of animals received vehicle (1 μl/rat) or WIN55,212-2 (0.5 μg/rat), immediately after training and, after 24 h, they received pre-test injections of different doses of WIN55,212-2 (0, 0.1, 0.25 and 0.5 μg/rat).

All groups received either vehicle (left panel) or 0.5 μg/rat of WIN55,212-2 (middle and right panels), immediately after training and, after 24 h, they were given pre-test injections of different doses of phenylephrine (0, 0.125, 0.25 and 0.5 μg/rat) 2 min before administration of the vehicle (left and middle panel) or 0.25 μg/rat of WIN55,212-2 (left panel).

All groups received post-training administration of the vehicle (1 μl/rat) or WIN55,212-2 (0.5 μg/rat). On the test day, the animals received pre-test intra-CA1 injection of different prazosin doses (0, 0.125, 0.25 and 0.5 μg/rat) alone (Fig. 3, left panel) or in combination with WIN55,212-2 (0.5 μg/rat; Fig. 3, right panel).

After the testing sessions, each rat was deeply anesthetized and 1 μl of a 4% methylene-blue solution was bilaterally infused into the CA1 (0.5 μl/side). Animals were subsequently decapitated, their brains removed and placed in formaldehyde (10%). After several days, the brains were sliced and injection sites were verified according to Paxinos and Watson. Data from animals whose injection sites were located outside the CA1 region were excluded from the experiments (a total number of 15 rats). Such rats were replaced to insure a sample size of eight per group.

Step-down latencies were expressed as the median and interquartile range. Due to large individual variations, data were analyzed with the Kruskal–Wallis nonparametric one-way analysis of variance (ANOVA) followed by a two-tailed Mann–Whitney *U*-test, after which a Bonferoni correction for the paired comparisons. In all statistical evaluations $p < 0.05$ was used as the criterion for statistical significance.

Fig. S1 illustrates the approximate point of drug injections in the animals' CA1 (A). Histological results were plotted on representative sections taken from the rat brain atlas of Paxinos and Watson (B) [18].

Fig. 1 shows the effects of post-training (left panel) or pre-test (middle panel) intra-CA1 administration of WIN55,212-2 on step-down latency. Kruskal–Wallis ANOVA revealed that post-training [$H(4) = 25.63$, $p < 0.001$] or pre-test [$H(3) = 18.74$, $p < 0.001$] WIN55,212-2 (0.25 and 0.5 μg/rat) impaired memory retrieval on the test day. Fig. 1 (right panel) indicates that pre-test WIN55,212-2 (0.5 μg/rat) restored amnesia induced by post-training WIN55,212-2 (0.5 μg/rat) [Kruskal–Wallis nonparametric ANOVA, $H(3) = 16.90$, $p < 0.001$].

Fig. 2 indicates the effects of pre-test intra-CA1 injection of phenylephrine in the presence or absence of WIN55,212-2 on memory retrieval. Kruskal–Wallis ANOVA revealed that pre-test injection of phenylephrine by itself (left panel) had no effect on memory retrieval [Kruskal–Wallis nonparametric ANOVA, $H(3) = 1.89$, $p > 0.05$]. Furthermore, pre-test administration of phenylephrine (0.5 μg/rat) by itself [Kruskal–Wallis, nonparametric ANOVA, $H(3) = 10.58$, $p < 0.05$; middle panel] or phenylephrine (0.25 and 0.5 μg/rat) in combination with a lower dose (0.25 μg/rat) of WIN55,212-2 [Kruskal–Wallis nonparametric ANOVA, $H(3) = 18.10$, $p < 0.001$; left panel] restored