

## Original Article

# Modulation of WIN55,212-2 State-Dependent Memory by $\alpha$ 2-Adrenergic Receptors of the Dorsal Hippocampus

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## Abstract

**Background:** An overlapping distribution of  $\alpha$ 2-adrenergic receptors with cannabinoid receptors has been reported in certain brain structures such as the dorsal hippocampus. Thus, functional interactions between cannabinoid and  $\alpha$ 2-adrenergic systems in cognitive control seem possible. In the present study, we examine the possible role of  $\alpha$ 2-adrenergic receptors of the dorsal hippocampus on WIN55,212-2 state-dependent learning.

**Methods:** Adult male Wistar rats were bilaterally implanted with chronic cannulae in the CA1 regions of their dorsal hippocampi trained in a step-down type inhibitory avoidance task and tested 24 hr after training, to measure step-down latency.

**Results:** Post-training or pre-test intra-CA1 administration of the cannabinoid receptor agonist, WIN 55,212-2 (0.25 and 0.5  $\mu$ g/rat) induced amnesia. Amnesia produced by post-training WIN55,212-2 (0.5  $\mu$ g/rat) was reversed by pre-test administration of WIN55,212-2, that was due to a state-dependent effect. Pre-test intra-CA1 microinjections of clonidine (0.25, 0.5 and 1  $\mu$ g/rat) or yohimbine (0.5, 0.75, and 1  $\mu$ g/rat) did not affect memory retrieval per se. Pre-test intra-CA1 administration of clonidine (0.5 and 1  $\mu$ g/rat) or clonidine (0.25, 0.5, and 1  $\mu$ g/rat) with an ineffective dose of WIN 55,212-2 (0.25  $\mu$ g/rat) reversed post-training WIN55,212-2 (0.5  $\mu$ g/rat, intra-CA1) induced memory impairment. Pre-test intra-CA1 microinjection of yohimbine (1  $\mu$ g/rat) before administration of WIN55,212-2 (0.5  $\mu$ g/rat, intra-CA1), however, dose-dependently inhibited WIN55,212-2 state-dependent memory.

**Conclusion:** Modulation of  $\alpha$ 2-adrenergic receptors in the dorsal hippocampal CA1 regions can influence WIN55,212-2 induced amnesia and WIN55,212-2 state-dependent learning of an inhibitory avoidance task by pre- or post-synaptic mechanism(s).

**Keywords:** clonidine, rat, state-dependent memory, yohimbine, WIN55,212-2

## Introduction

Several lines of evidence indicate that cannabinoids modify learning and memory processes.<sup>1,2</sup> Cannabinoids exert an amnesic effect in different models of memory assessment such as the radial maze, spatial alternation in a T-maze and a delayed matching/non-matching to position task with lever presentation.<sup>3-5</sup> Our previous experiments have demonstrated that post-training administration of WIN55,212-2 (a cannabinoid receptor agonist) dose-dependently impairs memory consolidation in step-down or step-through passive avoidance learning.<sup>6,7</sup> It is important to note that pre-test administration of WIN55,212-2 facilitates memory retrieval in amnesia induced by post-training administration of WIN55,212-2.<sup>6,7</sup> This is known as state-dependent memory, in which the newly acquired information in one drug state cannot be recalled or used unless the retrieval is tested in the same drug state.<sup>8,9</sup> Since recall of learned information is possible only if the subject is in the same state as during the encoding phase, thus this type of learning is known as state-dependent learning.<sup>9,10</sup> Two different CB1 and CB2 receptor sites mediate the effects of cannabi-

noids in the central nervous system and immune cells.<sup>11</sup> CB1 receptors are densely expressed in areas classically involved in learning and memory, such as the hippocampus, cortex, basal ganglia, amygdala, and cerebellum.<sup>3,12</sup>

Pharmacological studies have suggested a direct interaction between cannabinoids and some neurotransmitter systems.<sup>13-15</sup> There is evidence indicating that cannabinoids can inhibit the release of several neurotransmitters such as glutamate,<sup>16</sup> acetylcholine,<sup>17</sup> and noradrenaline<sup>12,18</sup> throughout the brain via activation of CB1 receptors.<sup>18</sup> Evidence exists for the involvement of noradrenaline and adrenergic receptors in learning and memory. For example, noradrenaline when injected into the amygdala,<sup>19,20</sup> hippocampus, and entorhinal cortex<sup>21</sup> enhances memory formation. Noradrenaline signals are mediated by two major classes of receptors,  $\alpha$  and  $\beta$ , both coupled with G-proteins.<sup>22</sup>  $\alpha$ -adrenergic receptors are distinguished into two subtypes ( $\alpha$ 1 and  $\alpha$ 2), by differences in ligand specificity, kinetics, and effects. These receptors are expressed widely in the central nervous system.<sup>22,23</sup>  $\alpha$ 2-adrenoceptors are localized both pre-synaptically and post-synaptically.<sup>24</sup> Previous studies indicate that  $\alpha$ 2-adrenoceptors play an important role in spatial working memory,<sup>25-28</sup> but have little effect on behavioral tasks dependent on the medial temporal lobe or the parietal cortex.<sup>29,30</sup> It has been suggested that improving the effect of  $\alpha$ 2-adrenoceptor stimulation is task-dependent. In contrast to these reports, some studies indicate that  $\alpha$ 2-adrenergic receptors of the medial temporal lobe influence inhibitory avoidance memory.<sup>31,32</sup> Our recent studies have shown that activation of  $\alpha$ 2-adrenergic receptors in the dorsal hippocampal CA1 regions restore amnesia induced by scopolamine (a muscarinic cholinergic receptor

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antagonist), while an  $\alpha$ 2-adrenergic antagonist inhibited scopolamine state-dependent memory in an inhibitory avoidance task.<sup>33</sup> Clinical studies indicated that clonidine improved performance of memory recall in patients with Korsakoff's amnesia<sup>34</sup> and those with schizophrenia.<sup>35</sup> Recent studies have shown that clonidine improves spatial working memory in patients with Parkinson's<sup>36</sup> or Alzheimer disease.<sup>37</sup>

The CA1 region of the dorsal hippocampus is essential for learning, memory<sup>38</sup> and long-term potentiation.<sup>39</sup> It receives adrenergic input from the locus coeruleus and contains different types of adrenergic receptors.<sup>19</sup> Noradrenaline has been implicated in many of the same central processes that are affected by cannabinoids. For example, brain noradrenergic systems have been implicated in the hypothermia<sup>40</sup> and antinociception<sup>41</sup> induced by  $\Delta$ 9-tetrahydrocannabinol (THC). Prenatal exposure to THC increases the expression of the catecholamine synthesizing enzyme tyrosine hydroxylase in neurons during early fetal brain development.<sup>42</sup> Furthermore, THC<sup>43</sup> and synthetic cannabinoid agonists such as CP-55,940 and WIN 55,212-2<sup>44</sup> decrease noradrenaline release in the hippocampus. This decrease correlates to poor performance in the radial arm maze behavioral test. Thus, functional interactions between cannabinoids and noradrenergic systems in inhibitory avoidance learning seem possible.  $\alpha$ 2-adrenergic receptors are involved in state-dependent memory.<sup>33</sup>

Since the role of the CA1  $\alpha$ 2-adrenergic receptor on WIN55,212-2 state-dependent memory has not been shown, the aim of the present study was to investigate the effects of bilateral microinjections of  $\alpha$ 2-adrenergic receptor agonists and antagonists into the CA1 region of the dorsal hippocampus on WIN55,212-2 state-dependent memory. In the first step, we investigated the effect of WIN55,212-2 on memory consolidation and retrieval by using the step-down passive avoidance task. Secondly, the effects of microinjections of an  $\alpha$ 2-adrenergic receptor agonist, clonidine, and an  $\alpha$ 2-adrenergic receptor antagonist, yohimbine, into the CA1 region of the hippocampus on WIN55,212-2 response were evaluated.

## Materials and Methods

### Animals

Adult male Wistar rats 4 months old (Pasteur Institute, Tehran, Iran) and weighing 220 – 270 g at the time of surgery were used. Animals had free access to food and water, and were housed four to a cage at 22±2°C under a 12/12 hr light:dark cycle (lights on at 7:00 am). All experiments were carried out during the light phase between 9:00 and 15:00. Experimental groups consisted of eight animals and each animal was tested once. All procedures were performed in accordance with the Institutional Guidelines for Animal Care and Use.

### Surgery

Animals were intraperitoneally anesthetized with a mixture of ketamine (100 mg/kg) plus xylazine (10 mg/kg) and placed in the flat-skull position within a stereotaxic frame (David Kopf Instruments, USA). A midline incision was made and the skin and underlying periosteum retracted. 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.<sup>45</sup> 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. All animals were allowed one week to recover from surgery and clear the anesthetic.

### Drugs

Drugs used in the present study were: WIN55,212-2 mesylate (Tocris, UK), clonidine hydrochloride and yohimbine (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. Control animals received either saline or vehicle. All drugs were injected bilaterally intra-CA1.

### Intra-CA1 injections

For bilateral drug infusion, the animals were gently restrained by hand. Stylets were removed from the guide cannulae and replaced 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  $\mu$ L/rat (0.5  $\mu$ L 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.

### Inhibitory avoidance apparatus

The inhibitory avoidance apparatus was a wooden box (40×30×40 cm) whose floor consisted of parallel 3.0 mm stainless steel bars spaced 1.0 cm apart. A wooden platform (12×10×7 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.<sup>46-48</sup>

### Behavioral procedures

#### Training

A one-trial step-down inhibitory avoidance task was used. Training was based on our previous studies.<sup>49</sup> Each rat was gently placed on the platform. When the rat stepped down from the platform and placed all four paws on the grid floor, a five second 0.4-mA shock was applied to the grid after which animals were immediately withdrawn from the training apparatus. This training procedure was carried out

#### Retention test

Twenty-four hours after training, 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 retention test was also carried out between 9:00 and 15:00.

#### Experimental procedure

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. The drug administration intervals were based on previous studies.<sup>33,50</sup>

#### Experiment 1: effect of WIN55,212-2 on inhibitory avoidance memory

The effect of pre-training and pre-test administration of WIN55,212-2 on an inhibitory avoidance task was examined using thirteen groups (n=8/group). Five groups of animals received saline (1  $\mu$ L/rat) or different doses of WIN55,212-2 (0, 0.1, 0.25, and 0.5  $\mu$ g/rat) immediately after training. On the test day, animals received saline (1  $\mu$ L/rat) or vehicle (1  $\mu$ L/rat) 5 min before the test (Figure 1A). The other eight groups of animals received vehicle (1  $\mu$ L/rat; Figure 1B) or an effective dose of WIN55,212-2 (0.5  $\mu$ g/rat; Figure 1C) immediately after training. On the test day, the animals received different doses of WIN55,212-2 (0, 0.1, 0.25, and 0.5  $\mu$ g/rat) 5 min before testing.

### Experiment 2: effects of pre-test administration of clonidine on memory retrieval in the presence or absence of WIN55,212-2

The effect of pre-test intra-CA1 microinjection of an  $\alpha_2$ -adrenoceptor agonist, clonidine, alone, or in combination with WIN55,212-2 on memory retrieval was examined using twelve groups ( $n=8$ /group). Four groups of animals received post-training vehicle (1  $\mu$ L/rat) and on the test day they received microinjections of clonidine (0, 0.25, 0.5, and 1  $\mu$ g/rat). Two min later, animals received vehicle (1  $\mu$ L/rat; Figure 2A). Step-down latency was measured 5 min after vehicle injection.

Eight groups of animals received a post-training effective dose of WIN55,212-2 (0.5  $\mu$ g/rat). On the test day, four groups of animals received clonidine (0, 0.25, 0.5, and 1  $\mu$ g/rat) and after 2 min, were injected with vehicle (1  $\mu$ L/rat; Figure 2B). The other four groups of animals received clonidine (0, 0.25, 0.5, and 1  $\mu$ g/rat) and after 2 min, were injected with WIN55,212-2 (0.25  $\mu$ g/rat; Figure 2C). Step-down latency was measured 5 min after vehicle (B) or WIN55,212-2 (C) injections.

### Experiment 3: effects of pre-test administration of yohimbine on memory retrieval in the presence or absence of WIN55,212-2

On the training day, all groups received post-training administration of vehicle (1  $\mu$ L/rat) or WIN55,212-2 (0.5  $\mu$ g/rat). On the test day, four groups of animals received yohimbine (0, 0.5, 0.75, and 1  $\mu$ g/rat) and after 2 min, were injected with vehicle (1  $\mu$ L/rat; Figure 3A) Step-down latency was measured 5 min after vehicle injection. The other four groups of animals received yohimbine (0, 0.5, 0.75, and 1  $\mu$ g/rat) and after 2 min, were injected with WIN55,212-2 (0.5  $\mu$ g/rat; Figure 3B). Step-down latency was measured 5 min after WIN55,212-2 injection.

### Histology

After the testing sessions, each rat was deeply anesthetized and 1  $\mu$ L of a 4% methylene-blue solution was bilaterally infused into the CA1 (0.5  $\mu$ L/side), as described in the drug section. 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.<sup>45</sup> Data from animals whose injection sites were located outside the CA1 (less than 5%) were excluded from the experiments. Those rats were replaced to insure a sample size of eight per group.

### Data analysis

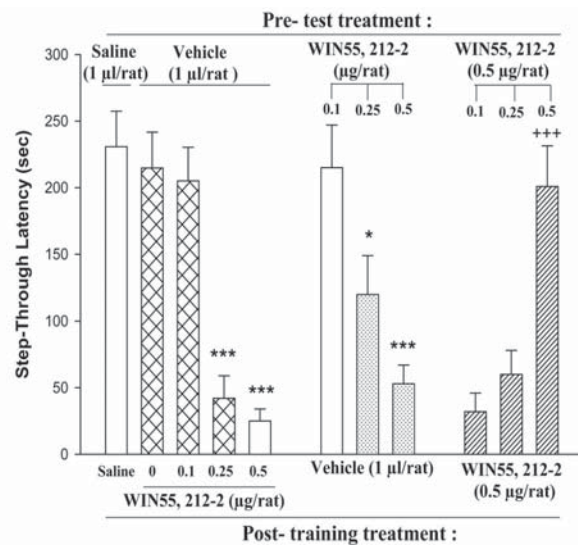
Step-down latencies were expressed as the median and interquartile range. Because of large individual variations, data were analyzed with the Kruskal-Wallis nonparametric one-way analysis of variance (ANOVA) followed by a two-tailed Mann-Whitney U-test, after which a Bonferoni correction for the paired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

## Results

### Effect of WIN55,212-2 on inhibitory avoidance memory

Figure 1 shows the effects of post-training (Figure 1A) or pre-test (Figure 1B) intra-CA1 administration of WIN55,212-2 on step-down latency. Kruskal-Wallis ANOVA revealed that post-training [ $H(4)=24.72$ ,  $P<0.001$ ] or pre-test [ $H(3)=19.38$ ,  $P<0.001$ ] WIN55,212-2 (0.25 and 0.5  $\mu$ g/rat, intra-CA1) impaired inhibitory avoidance memory on the test day when compared with saline-

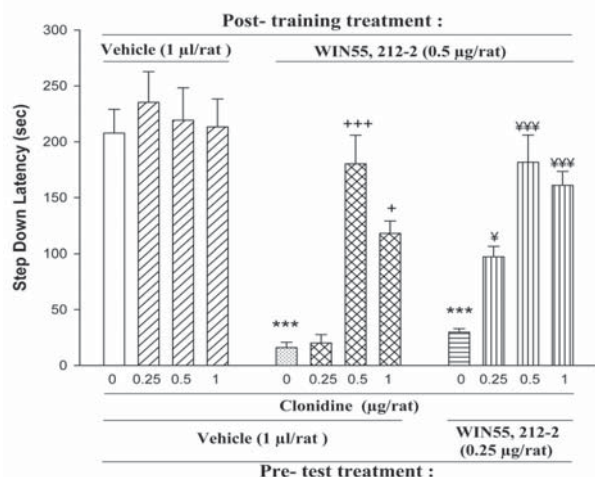
treated animals. Figure 1A indicates that in the animals in which memory consolidation was impaired due to post-training administration of WIN55,212-2 (0.5  $\mu$ g/rat, WIN55,212-2-induced amnesia), pre-test WIN55,212-2 (0.5  $\mu$ g/rat) restored retrieval to the control level (WIN55,212-2 memory state) [Kruskal-Wallis nonparametric ANOVA,  $H(3)=17.05$ ,  $P<0.001$ ].



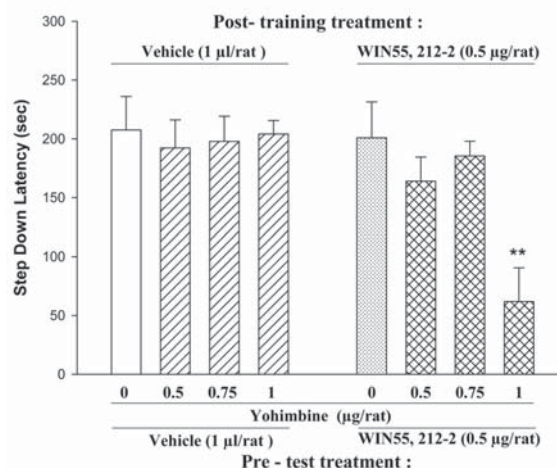
**Figure 1.** The effects of WIN55,212-2 on inhibitory avoidance memory. Thirteen groups of animals were used. Five groups of animals (Panel A) received post-training saline (1  $\mu$ L/rat, intra-CA1), or different doses of WIN55,212-2 (0, 0.1, 0.25, and 0.5  $\mu$ g/rat, intra-CA1). On the test day, the animals received saline (1  $\mu$ L/rat, intra-CA1) or vehicle (1  $\mu$ L/rat, intra-CA1) 5 min before testing. Four groups of animals (Panel B) received post-training injections of vehicle (1  $\mu$ L/rat) and pre-test injections of different doses of WIN55,212-2 (0, 0.1, 0.25, and 0.5  $\mu$ g/rat, intra-CA1). The remaining four groups of animals (Panel C) received post-training injections of a high dose of WIN55,212-2 (0.5  $\mu$ g/rat, intra-CA1) and pre-test injections of different doses of WIN55,212-2 (0, 0.1, 0.25, and 0.5  $\mu$ g/rat, intra-CA1). Test session step-down latencies were expressed as median and quartile for eight animals. \* $P<0.05$ , \*\*\* $P<0.001$ , different from post-training saline/pre-test saline group. +++ $P<0.001$ , different from post-training WIN55,212-2 (0.5  $\mu$ g/rat)/pre-test vehicle group.

### Effect of pre-test administration of clonidine on memory retrieval in the presence or absence of WIN55,212-2

Figure 2 shows the effect of pre-test intra-CA1 injection of clonidine in the presence or absence of WIN55,212-2 on memory retrieval. In animals that received vehicle (1  $\mu$ L/rat) after training who were tested following intra-CA1 administration of clonidine (Figure 2A), no significant change was observed in the step-down latencies as compared with the vehicle/vehicle control group [Kruskal-Wallis nonparametric ANOVA,  $H(3)=1.90$ ,  $P>0.05$ ]. In the animals that post-training administration of WIN55,212-2 (0.5  $\mu$ g/rat) impaired memory consolidation, pre-test administration of clonidine (0.5 and 1  $\mu$ g/rat) significantly reversed memory impairment [Kruskal-Wallis, nonparametric ANOVA,  $H(3)=20.48$ ,  $P<0.001$ ] (Figure 2B). In addition, the lower dose of pre-test WIN55,212-2 (0.25  $\mu$ g/rat) *per se*, did not induce significant WIN55,212-2 state-dependent learning. However, pre-test administration of different doses of clonidine (0.25, 0.5, and 1  $\mu$ g/rat) with a lower dose of WIN55,212-2 (0.25  $\mu$ g/rat) significantly improved memory retrieval and mimicked the effects of pre-test administration of a higher dose of WIN55,212-2 [Kruskal-Wallis nonparametric ANOVA,  $H(3)=16.60$ ,  $P<0.001$ ] (Figure 2C).



**Figure 2.** The effects of pre-test administration of clonidine with or without WIN55,212-2 on step-down latencies. Twelve groups of animals were used. In one series (Panel A), all animals received post-training administration of vehicle (1 µl/rat, intra-CA1) and pre-testing administration of clonidine (0, 0.25, 0.5, and 1 µg/rat, intra-CA1) before vehicle (1 µl/rat, intra-CA1). The other eight groups of animals received post-training administration of WIN55,212-2 (0.5 µg/rat, intra-CA1). On the test day, four groups of animals received clonidine (0, 0.25, 0.5, and 1 µg/rat, intra-CA1) and after 2 min, animals were injected with vehicle (1 µl/rat, intra-CA1) 5 min before testing (Panel B). The other four groups of animals received clonidine (0, 0.25, 0.5, and 1 µg/rat, intra-CA1) and after 2 min, were injected with WIN55,212-2 (0.25 µg/rat, intra-CA1) 5 min before testing (Panel C). Test session step-down latencies were expressed as median and quartile for eight animals. \*\*\* $P < 0.001$  different from post-training vehicle/pre-test vehicle group. +  $P < 0.05$ , +++  $P < 0.001$  different from post-training WIN55,212-2/pre-test vehicle group. ¥  $P < 0.05$ , ¥¥¥  $P < 0.001$  different from post-training WIN55,212-2/pre-test WIN55,212-2 group.



**Figure 3.** The effects of pre-test administration of yohimbine with or without WIN55,212-2 on step-down latencies. Eight groups of animals were used. All animals received post-training administration of vehicle (1 µl/rat, intra-CA1) or WIN55,212-2 (0.5 µg/rat, intra-CA1). On the test day, four groups of animals received yohimbine (0, 0.5, 0.75 and 1 µg/rat, intra-CA1) and after 2 min, were injected with vehicle (1 µl/rat, intra-CA1) 5 min before testing (Panel A). Another four groups of animals received yohimbine (0, 0.5, 0.75, and 1 µg/rat, intra-CA1) and after 2 min, were injected with WIN55,212-2 (0.5 µg/rat, intra-CA1) 5 min before testing (Panel B). Test session step-down latencies were expressed as median and quartile for eight animals. \*\* $P < 0.01$  different from post-training WIN55,212-2/pre-test WIN55,212-2 group.

Effects of pre-test administration of yohimbine on memory retrieval in the presence or absence of WIN55,212-2

Figure 3 shows the effect of pre-test intra-CA1 administration of yohimbine in the presence or absence of WIN55,212-2 on memory retrieval. In the animals trained before vehicle treatment and tested following intra-CA1 administration of three different doses of yohimbine (0.5, 0.75, and 1 µg/rat), no significant change was observed in the step-down latencies as compared with vehicle/vehicle control group [Kruskal-Wallis nonparametric ANOVA,  $H(3)=0.47$ ,  $P > 0.05$ ]. Furthermore, in the animals which received post-training and pre-test administration of WIN55,212-2 (0.5 µg/rat), pre-test intra-CA1 administration of yohimbine (1 µg/rat) decreased the improvement of memory retrieval by pre-test WIN55,212-2 (0.5 µg/rat) treatment [Kruskal-Wallis nonparametric ANOVA,  $H(3)=11.13$ ,  $P < 0.05$ ].

## Discussion

The step-down passive avoidance method has been used to study learning and memory in rodents.<sup>51</sup> In accordance with previous studies,<sup>5,12,43,52</sup> our present data showed that post-training or pre-test intra-CA1 administration of a cannabinoid receptor agonist, WIN55,212-2 dose-dependently decreased rats' performances in the acquisition and retrieval of an inhibitory avoidance task. In hippocampal preparations, cannabinoids that acted via CB1 receptors have been shown to inhibit the release of different neurotransmit-

ters such as glutamate,<sup>16</sup> acetylcholine,<sup>17</sup> and noradrenaline.<sup>12,18</sup> Thus, WIN55,212-2 can impair memory retrieval by decreasing the release of glutamate, acetylcholine and/or noradrenaline in the hippocampus. The present data show that amnesia induced by post-training WIN55,212-2 (0.5 µg/rat) was completely inhibited by a pretest injection of the same dose of WIN55,212-2 in male rats. Similar results have been obtained by intra-CA1 microinjections of WIN55,212-2 in mice.<sup>7</sup> The data agree with our recent study, which indicate that pre-test intracerebroventricular injections of WIN55,212-2 can restore memory impairment induced by pre-training intracerebroventricular administration of WIN55,212-2 in mice.<sup>53</sup> A similar response has been shown for morphine,<sup>54,55</sup> lithium,<sup>56</sup> and histamine,<sup>57,58</sup> which has been considered to be state-dependent memory. State-dependent memory denotes the fact that information that has been learned while the animal is under the influence of a certain drug (state) can only be recalled and used to solve a task when the animal is in the same state in which the information was learned, but not in a different, i.e., undrugged state.<sup>9,10,59</sup>

Cannabinoids and morphine show similarities in their effects.<sup>60</sup> The receptors of both drugs belong to the G-protein that couples to the Gi/Go GTP-binding proteins<sup>61</sup> and activation of both receptors may inhibit the release of several neurotransmitters.<sup>18</sup> We have shown previously that an  $\alpha$ 2-adrenergic receptor modulated morphine state-dependent learning. Clonidine reversed amnesia induced by morphine, whereas yohimbine inhibited morphine state-dependent learning.<sup>62</sup> It can be proposed that  $\alpha$ 2-adrenoceptor

drugs may mediate the effect of WIN55,212-2 on memory on the test day. Thus, in this study the effects of pre-test administration of  $\alpha$ 2-adrenoceptor agonists or antagonists on inhibitory avoidance memory impaired by post-training administration of WIN55,212-2 have been investigated.

The present data indicated that pre-test intra-CA1 microinjections of different doses of an  $\alpha$ 2-adrenergic agonist, clonidine, restored memory impairment induced by post-training administration of WIN55,212-2. Pre-test co-administration of non-effective doses of clonidine with a lower dose of WIN55,212-2 (0.25  $\mu$ g/rat), which by itself did not induce state-dependent memory, have shown reversal of memory impairment. Our results may be in agreement with previous investigations showing that clonidine effectively ameliorated memory deficits produced by phencyclidine or MK-801, a noncompetitive NMDA receptor antagonist typically used for blocking NMDA receptors. The effect of  $\alpha$ 2-adrenergic receptor agonists on norepinephrine transmission and signaling in the brain is complex. Several investigations have indicated that administration of  $\alpha$ 2-adrenergic receptor agonists<sup>63</sup> and epinephrine<sup>64</sup> can improve memory. The mechanisms underlying clonidine's reversal/restoration of the memory deficits produced by WIN55,212-2 are unclear and need further investigations. However, the ability of clonidine to modulate the efficacy of glutamate synaptic transmission via activation of G-protein-coupled adrenergic receptors seems likely.<sup>65</sup>

In addition, the present study investigated the effects of pre-test microinjections of the  $\alpha$ 2-adrenergic receptor antagonist, yohimbine, with or without WIN55,212-2 on memory retrieval. Memory retrieval was not affected in animals trained before saline treatment and tested following intra-CA1 administration of yohimbine. The doses of yohimbine used in the present study were selected based on our previous study where the drugs, alone, were ineffective on the step-down latencies of inhibitory avoidance memory (Zarrindast et al., unpublished observations).

In the animals, which received post-training and pre-test administration of WIN55,212-2 (0.5  $\mu$ g/rat), pre-test intra-CA1 administration of yohimbine decreased improvement of memory retrieval by pre-test WIN55,212-2 (0.5  $\mu$ g/rat) treatment. Yohimbine significantly inhibited WIN55,212-2 state-dependent memory, which may indicate that the WIN55,212-2 response is mediated through the CA1  $\alpha$ -adrenergic receptor system. There are contradictory results about the role of  $\alpha$ 2-adrenergic receptors during acquisition and storage of information. It has been reported that  $\alpha$ 2-adrenoceptor antagonists potentiate the retention enhancement induced by acetylcholinesterase inhibitors in rats, through blockade of pre-synaptic  $\alpha$ 2-adrenoceptors.<sup>31</sup> There are reports that the  $\alpha$ 2-adrenoceptor antagonist, yohimbine, increased memory retention.<sup>66</sup> However, some studies have demonstrated that post-training administration of yohimbine did not affect memory consolidation,<sup>67</sup> whereas other studies have indicated that yohimbine could impair spatial working memory in monkeys.<sup>25-27</sup> The cause of these discrepancies is not clear; however, the effect may be dependent upon the animals, testing methods and doses used.  $\alpha$ 2-adrenoceptors are located both post- and pre-synaptically on hippocampal neurons.<sup>24</sup> Inhibition of pre-synaptic  $\alpha$ 2-adrenoceptors receptors increases the release of norepinephrine, acetylcholine, and glutamate.<sup>68-72</sup> However, inhibition of post-synaptic  $\alpha$ 2-adrenoceptors inhibits the effect of norepinephrine.<sup>73</sup> Thus, inhibition of these pre- or post-synaptic  $\alpha$ 2-adrenoceptors may have different effects on memory and learning.

These results suggest that CA1 plays an important role in WIN55,212-2 state-dependent learning. The present findings also suggest that modulation of  $\alpha$ 2-adrenergic receptors in CA1 can influence WIN55,212-2 induced amnesia and WIN55,212-2 state-dependent learning in an inhibitory avoidance task.  $\alpha$ 2-adrenergic receptor agonists can mimic WIN55,212-2 response and potentiate WIN55,212-2 state-dependent learning, while  $\alpha$ 2-adrenergic receptor antagonists inhibit WIN55,212-2 state-dependent learning. It should be considered that  $\alpha$ 2-adrenoceptors located both pre- and post-synaptically on hippocampal neurons have contradictory responses. More experiments may be necessary to determine the involvement of pre- or post-synaptic  $\alpha$ 2-adrenoceptor mechanism(s) in WIN55,212-2 state-dependent learning.

## References

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Loonak waterfall, near Siahkal-Gilan Province (Photo by M.H Azizi MD)