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Effects of cannabinoid and glutamate receptor antagonists and their interactions on learning and memory in male rats

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ABSTRACT

Introduction: Despite previous findings on the effects of cannabinoid and glutamatergic systems on learning and memory, the effects of the combined stimulation or the simultaneous inactivation of these two systems on learning and memory have not been studied. In addition, it is not clear whether the effects of the cannabinoid system on learning and memory occur through the modulation of glutamatergic synaptic transmission. Hence, in this study, we examined the effects of the simultaneous inactivation of the cannabinoid and glutamatergic systems on learning and memory using a passive avoidance (PA) test in rats.
Materials and methods: On the test day, AM251, which is a CB1 cannabinoid receptor antagonist; MK-801, which is a glutamate receptor antagonist; or both substances were injected intraperitoneally into male Wistar rats 30 min before placing the animal in a shuttle box. A learning test (acquisition) was then performed, and a retrieval test was performed the following day.
Results: Learning and memory in the PA test were significantly different among the groups. The CB1 receptor antagonist improved the scores on the PA acquisition and retention tests. However, the glutamatergic receptor antagonist decreased the acquisition and retrieval scores on the PA task. The CB1 receptor antagonist partly decreased the glutamatergic receptor antagonist effects on PA learning and memory.
Conclusions: These results indicated that the acute administration of a CB1 antagonist improved cognitive performance on a PA task in normal rats and that a glutamate-related mechanism may underlie the antagonism of cannabinoid by AM251 in learning and memory.

1. Introduction

Many chemical factors, including neurotransmitters, influence learning and memory through actions in different brain regions (Phale and Korgaonkar, 2009). Decreased or increased levels of neurotransmitters or the activation or blockade of their receptors may alter learning and memory (Levin, 2006). Glutamate (Glu), which is the major excitatory neurotransmitter in the brain, has a prominent role in learning and memory processes (Phale and Korgaonkar, 2009). The endocannabinoid system is one of the main neuromodulators of the mammalian central nervous system (López-Moreno et al., 2008). In addition, cognitive effects have been described after cannabinoid use in humans (Riedel and Davies, 2005; Schoedel et al., 2012) and, more recently, in animals (Swartzwelder et al., 2012; Stumm et al., 2013; Renard et al., 2013).

Glu neurotransmitter, which is stored in presynaptic vesicles, has been estimated to be released in up to half of the synapses in the brain (Olney, 1990). Glu receptors have been identified as important interfaces in learning and memory paradigms as well as in mechanisms of synaptic plasticity (Phale and Korgaonkar, 2009), such as long-term potentiation (LTP) and long-term depression, which are believed to be the underlying cellular basis of at least some forms of learning (Riedel and Reymann, 1996; Riedel et al., 2003; Phale and Korgaonkar, 2009). The N-methyl-D-aspartate (NMDA) receptor (NMDAR) subtype of Glu receptors plays a substantial role in neural physiology, synaptic plasticity, and behavioral learning and memory (Shapiro, 2001).

A large body of evidence from animal models and human studies has indicated that *Cannabis sativa* preparations, such as marijuana, induce numerous and complex effects on cognitive functions, including attention, learning, emotional reactivity, enhancement of the perceptions of the senses, and impairments in short-term memory (Pattij et al., 2008). These preparations act through two types of receptors—CB1 and CB2 (Wise et al., 2009).

Despite numerous studies on the effects of the cannabinoid system in learning and memory, there have been conflicting results (Terranova et al., 1995; Puighermanal et al., 2009). In addition, the effects of the cannabinoid system on synaptic plasticity and LTP remain controversial

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(Collins et al., 1994; Terranova et al., 1995; Misner and Sullivan, 1999; Carlson et al., 2002; de Oliveira Alvares et al., 2006; Lin et al., 2011). According to one of these studies, cannabinoid receptor agonists impair memory formation, while antagonists reverse these deficits or act as memory enhancers, and they have revealed reductions in neural plasticity following cannabinoid treatment and increased plasticity following antagonist exposure (Riedel and Davies, 2005).

A series of biochemical, molecular, and pharmacological studies have demonstrated functional interactions between the CB1 receptor and the Glu NMDAR (Rodríguez-Muñoz et al., 2012; Sánchez-Blázquez et al., 2013, 2014). However, an understanding of the exact mechanism underlying the neurochemical interactions and/or signaling pathways between CB1 and NMDA receptors requires further studies (Ferraro et al., 2009).

Unlike the available data on the effects of the cannabinoid system on learning and memory and the role of the glutamatergic system in learning and memory, the simultaneous stimulation or inactivation effects of these two systems on learning and memory have not been studied. Therefore, in this study, we test the hypothesis that the effects of the cannabinoid system on learning and memory are the result of its effects on glutamatergic synaptic transmission. The interactions of these two systems in the modulation of learning and memory could have important therapeutic implications in clinical settings.

2. Material and methods

2.1. Animals

We used male Wistar rats of a laboratory strain weighing 200–240 g, obtained from the Pasteur Institute, Tehran, Iran. The animals were fed a standard diet and housed in plastic cages, five animals per cage, in an air-conditioned and temperature-controlled (22 ± 2 °C) room under a 12-h light/dark cycle (lights on at 8:00). Food and water were freely available. All of the experiments were conducted in a quiet, diffusely lit room between 9:00 and 13:00. Each experimental group consisted of 10 naïve animals. All research and animal care procedures were approved by the Veterinary Ethics Committee of this University and were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Treatments

Saline was administered intraperitoneally (i.p.) in the first (control) group 30 min before the tests. AM251 (1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-1-piperidinyl-1H-pyrazole-3-carboxamide), which is a selective cannabinoid CB1 receptor antagonist (AM251; Sigma-Aldrich Co. LLC, St. Louis, MO, USA) and MK-801 (Tocris Bioscience, Bristol, UK) were administered i.p. 30 min before the tests at doses of 1 and 0.1 mg/kg, respectively. In the final group, AM251 + MK-801 were administered i.p. 30 min before the tests.

2.3. Apparatus and procedures

Passive avoidance behavior was studied in a one-trial-learning, step-through-type passive avoidance task that utilized the natural preference of rats for a dark environment (Gold, 1986). The apparatus consisted of two compartments that had a steel-rod grid floor (3 mm in diameter, 10 mm apart). One of the compartments (30 × 20 × 20 cm) was equipped with a 20 W lamp that was located centrally at a height of 50 cm, and the other was a dark compartment of the same size. The compartments were connected with a guillotine door (20 × 15 cm). A dark room was used during the experimental session. In the training trial, the guillotine door between the light and dark compartment was closed. When each rat was placed in the light compartment with its back to the guillotine door, the door was opened, and, at the same time, the time (the step-through latency) was measured with a stopwatch until the rat entered the dark compartment. After the rat entered the dark compartment, the door was closed (Shahidi et al., 2008; Lashgari et al., 2009; Rasuli et al., 2011; Sarihi et al., 2011).

2.4. Training procedure

The test lasted 2 days. On the first day, all of the rats in the experimental groups became habituated to the apparatus. The rat was placed in the illuminated compartment, and, 5 s later, the guillotine door was raised. Upon entering the dark compartment, the door was closed, and the rat was taken from the dark compartment into the home cage. The habituation trial was repeated after 30 min. It was followed after the same interval by the acquisition trial, during which the guillotine door was closed, and a 50-Hz 1-mA constant current shock was applied for 1.5 s immediately after the animal had entered the dark compartment (Shahidi et al., 2008; Lashgari et al., 2009; Rasuli et al., 2011; Sarihi et al., 2011). In the experiment, the rat was retained in the apparatus, and it received a foot shock each time it reentered the dark compartment. Training was terminated when the rat remained in the light compartment for 120 consecutive seconds. The number of trials to acquisition (entries into the dark chamber) was recorded (Sarihi et al., 2011).

2.5. Retention test

Twenty-four hours after the passive avoidance training, the rat was placed in the illuminated chamber, and, 15 s later, the guillotine door was raised. The latency time for entering the dark compartment (step-through latency) and the time spent there over 10 min was recorded (Sarihi et al., 2011).

2.6. Statistical analysis

The statistical significance of the results was computed by analysis of variance (ANOVA), which was followed by a post hoc Tukey test. In all of the comparisons between particular groups, a probability of 0.05 or less was considered significant.

3. Results

3.1. Effects of AM251, MK-801, and AM251 + MK-801 on acquisition in the passive avoidance test

The animals in all of the experimental groups and the control group learned the passive avoidance task (number of trials to acquisition). For the number of trials to acquisition, no significant differences were found between the MK-801, AM251, and MK-801 + AM251 groups compared to the control group ($P > 0.05$ for all). The difference between the MK-801 and AM251 groups was significant ($P < 0.05$). The effects of these substances on acquisition are summarized in Fig. 1.

3.2. Effects of AM251, MK-801, and AM251 + MK-801 on passive avoidance retrieval

3.2.1. Step-through latency

One-way ANOVA indicated that there were significant differences in the step-through latency (STL) among the groups in the retrieval test. A Tukey's multiple comparison test revealed that the STL of the AM251 group ($P < 0.05$) was significantly higher and the MK-801 ($P < 0.01$) and AM251 + MK-801 ($P < 0.05$) groups were significantly lower than that of the control group. The STL of the AM251 + MK-801 group was significantly higher ($P < 0.05$) than that of the MK-801 group and less ($P < 0.05$) than that of the AM251 group. The difference between the MK-801 and AM251 groups was significant ($P < 0.01$). The effects of these substances on step-through latency are shown in Fig. 2.