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Modulation by female sex hormones of the cannabinoid-induced catalepsy and analgesia in ovariectomized mice

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Abstract

Cannabinoids are psychoactive compounds with many pharmacological properties such as analgesia, sedation and catalepsy most of which are mediated by cannabinoid CB_1 receptors. In the present study, we evaluated whether the ovarian sex hormones are involved in the cannabinoid-induced catalepsy and analgesia in ovariectomized female mice. Female NMRI mice (weighing 25–30 g) were divided into 3 main groups: unoperated, sham-operated and ovariectomized. Both the catalepsy and analgesia induced by different doses of the synthetic cannabinoid WIN 55,212-2 (2 and 4 mg/kg, i.p.) were examined in the groups in the presence or absence of the cannabinoid CB_1 antagonist AM251 (0.5 mg/kg). We also evaluated effects of estradiol valerate (10 mg/kg) and progesterone (25 mg/kg) on catalepsy and analgesia induced by WIN 55,212-2 in ovariectomized mice. The antinociceptive effect of WIN 55,212-2 was significantly ($P<0.01$) enhanced in ovariectomized mice, which was prevented by pretreatment with estradiol but not by progesterone. There was no significant difference in the cannabinoid-induced catalepsy between control and ovariectomized mice. However, pretreatment with progesterone but not estradiol potentiated the cataleptic effect of low dose of WIN 55,212-2 (2 mg/kg) in ovariectomized mice ($P<0.01$). The present data demonstrated for the first time that ovarian sex steroids could modulate both cannabinoid-induced catalepsy and analgesia in female ovariectomized mice.

Keywords: Cannabinoids; Catalepsy; Analgesia; Ovarian sex hormones; Estrogen; Progesterone; [Mouse]

1. Introduction

Cannabinoids have important functions in a myriad of physiological and pathophysiological processes which are mainly mediated by at least two types of cannabinoid receptors, denoted as CB_1 and CB_2 receptors (Devane et al., 1988; Matsuda et al., 1990). Cannabinoid CB_1 receptors are the primary mediator of central effects of cannabinoids, including mood, appetite, emesis control, memory, spatial coordination, locomotor activity, catalepsy, and analgesia (Ashton, 1999; Chaperon and Thiébot, 1999; Hao et al., 2000; Goutopoulos and Makriyannis, 2002). The ability of cannabinoids to induce antinociception in virtually every animal model of acute or persistent pain evaluated using different types of noxious stimulation (i.e., thermal, mechanical, and chemical; for review, see Walker and Hohmann, 2005) has encouraged researchers to try to better understand this important nonopioid system of analgesia. However, the exact mechanism by which this system exerts antinociceptive effects has not been fully elucidated. On the other hand, cannabinoids induce profound motor deficits, including immobility and catalepsy (Gough and Olley, 1978; Martin et al., 1991; Prescott et al., 1992; Anderson et al., 1996), which are a confound for behavioral studies that assess motor responses to noxious stimuli. Catalepsy, immobility, ataxia, or the impairment of complex behavioral acts are observed after acute administration of either natural and synthetic cannabinoid

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receptor agonists or the endogenous CB_1 ligand anandamide (Walton et al., 1938; Holtaman et al., 1969; Dewey, 1986; Rodríguez de Fonseca et al., 1998; McLaughlin et al., 2005; McMahon and Koek, 2007). The cannabinoid CB_1 receptor has been found to be present with a very dense intensity in neurons of the cerebellum and the basal ganglia (Herkenham et al., 1990, 1991a,b,c), which explains the ataxia, immobility, and catalepsy observed after acute administration of cannabinoids (Dewey, 1986). Despite these great advances, the functional role of the cannabinoid CB_1 receptor and the underlying mechanism in the regulation of locomotor activity remains to be conclusively established.

Involvement of ovarian sex hormones in the modulation of pain has been reported by previous studies that showed antinociceptive actions of physiological or experimental pregnancy in rats and women (Gintzler, 1980; Cogan and Spinnato, 1986; Dawson-Basoa and Gintzler, 1998). Several studies have evaluated the effect of sex steroids deprivation induced by ovariectomy followed by either estrogen or progesterone supplementation on perception of noxious stimuli in many animal model of pain evaluated (Dawson-Basoa and Gintzler, 1996; Gordon and Soliman, 1996; Walf and Frye, 2003; Kuba et al., 2006; Mannino et al., 2007) and most of the experiments have reported an antinociceptive effect of the ovarian sex steroids. It was also reported that administration of sex steroids in some brain regions, such as amygdala, in ovariectomized rats can produce antinociceptive effects (Walf and Frye, 2003). Besides modulation of nociception, there are several line of evidence showing that sex steroids could influence the locomotor activity and catalepsy induced by a variety of stimuli (Chiodo et al., 1979; Banerjee et al., 1983; Miller, 1983; Nomikos et al., 1987; Palermo-Neto and Dorce, 1990; McCarthy et al., 1993; Rupprecht et al., 1999; Pryce et al., 2003). Although it has been reported that these effects of ovarian hormones could be due to the functional interaction between this system and other related systems such as dopaminergic transmission (Miller, 1983; Palermo-Neto and Dorce, 1990; McCarthy et al., 1993), the underlying mechanisms in the effects of ovarian hormones on catalepsy has not been fully elucidated.

On the other hand, ovarian hormones have been suggested to affect the sensitivity of certain neuronal processes to cannabinoid treatment (Bonnin et al., 1993; Rodríguez de Fonseca et al., 1994; Mani et al., 2001). In one study, Rodríguez de Fonseca et al. (1994) have demonstrated that cannabinoid receptor density and affinity in certain brain areas such as the medial basal hypothalamus, the striatum and the limbic forebrain could vary during different phases of estrous cycle in female rats and ovarian hormone replacement in ovariectomized rats could affect these changes, suggesting the involvement of sex steroids in the alterations in the cannabinoid receptors density in the brain. In another study, Bonnin et al. (1993) reported a possible estrogenic modulation of the effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) on mesolimbic dopaminergic activity. In their experiments, they showed that Δ^9-THC significantly decreased the density of D_1 dopaminergic receptors in the limbic forebrain of ovariectomized rats chronically replaced with estrogens. Considering the possible role of both cannabinoid system and ovarian hormones in the catalepsy and analgesia and also in view of the fact that steroid hormones may mediate certain neurobehavioral effects of cannabinoids, in the present study we evaluated whether behavioral responses such as catalepsy and analgesia induced by acute administration of cannabinoid agonists is modulated by exogenous sex steroids in ovariectomized female mice.

2. Materials and methods

2.1. Animals

Female NMRI mice (Pasteur Institute) weighing 25–35 g were used throughout the study. The animals were housed in a temperature-controlled room (24±1 °C) on a 12-h light/dark cycle with free access to food and water for at least four days before experiments. All experiments were carried out in the same room between 10:00 to 15:00 to minimize diurnal variations. Separate groups of animals were used for each test. All animal experiments were performed according to the institutional guidelines for animal care and use. Animals were divided into 3 main groups: unoperated control, sham-operated and ovariectomized. These main groups were also divided into separate groups for experiments of ovarian hormones and/or cannabinoid agonist and antagonist treatment. Each of these separate experimental groups consisted of 10 animals.

2.2. Ovariectomy

Anesthesia was induced by intraperitoneally injection of 50 mg/kg ketamine (Alfasan, Woerden, Holland). After the onset of anesthesia, the lumbar dorsum was shaved, and the exposed skin prepared for aseptic surgery (a 10% povidene–iodine scrub followed by a sterile saline wipe). Surgery was performed according to the method described by Eddy (1986) with modification (Riazi et al., 2004). In brief, skin was opened with a 1- to 2-cm incision in the midline on the lumbar vertebral line. About 1 cm to each flank, parovarian fatty tissue was identified and pulled out through a small incision. The exposed ovary and associated oviduct were removed. Hemostasis, if needed, was achieved by hemostat pressure for 1 to 2 min. Then the skin incision was sutured (5–0 nonabsorbable). In sham-operated animals, the parovarian fatty tissues and ovaries were just retracted and were replaced.

2.3. Chemicals

The following drugs were used in the study: *R*(+)-[2,3-Dihydro-5-methyl-3[(morpholinyl) methyl] pyrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl) methanone mesylate (WIN 55,212-2) and *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (AM251) were obtained from Tocris (Bristol, UK). Estradiol valerate and progesterone were purchased from Sigma (St. Louis, USA). WIN 55,212-2 and AM251 were dissolved in 10% dimethyl sulphoxide (DMSO) (V/V) in saline. Estradiol

valerate and progesterone were dissolved in olive oil. All drugs were freshly prepared before the test.

2.4. Analgesia

Antinociception was evaluated by the radiant heat tail-flick test (D'Amour and Smith, 1941; Banafshe et al., 2005). Animals were restricted by a restrainer with their tail positioned in apparatus (Type 812, Hugo Sachs Electronics, Germany) for radiant heat stimulation on the dorsal surface of the tail. Tail-flick latency (TFL) was defined as the time interval between the application of standardized beam focused on the tail and the abrupt removal of the tail from nociceptive stimuli. The heat source was set so that baseline latencies were generally between 3 and 4 s. Cut-off time was set at 10 s. Antinociception was quantified as the percentage of the maximal effect (%MPE) using the following formula: %MPE = [(test latency − control latency)/(cut-off time − controllatency)] × 100.

2.5. Catalepsy

The cataleptic response was determined by using a slight modification of the ring test described by Pertwee (1972). Each mouse was placed on a ring (5.5 cm in diameter) which was attached to a stand and raised to a height of 16 cm. Mice were allowed to stay for a period of 5 min. During the test period, the sum of all times during which the mouse was motionless (except for respiratory movements) was measured. This value was divided by 300 s and multiplied by 100 to obtain the catalepsy percentage.

2.6. Treatments

In the first experiments, mice were injected intraperitoneally (i.p.) with either vehicle or WIN 55,212-2 to assess the dose-dependent curves of the analgesic and cataleptic effects. Analgesia was measured 15 min and catalepsy was measured 30 min after WIN 55,212-2 or its vehicle injection. For all animals in these experiments an i.p. injection of DMSO (vehicle instead of AM251) or AM251 (0.5 mg/kg, i.p.) was administered 30 min prior to WIN 55,212-2. Thus, in this stage mice were divided into four groups: vehicle (AM251) + vehicle (WIN 55,212-2), vehicle + WIN 55,212-2, AM251 + vehicle, AM251 + WIN 55,212-2.

In the next series of experiments, the effect of estradiol (10 mg/kg), progesterone (25 mg/kg) or their vehicle (olive oil) was evaluated on the cannabinoid WIN 55,212-2-induced catalepsy or analgesia in ovariectomized mice. Ovariectomized mice were injected with either vehicle (olive oil) or estradiol (10 mg/kg) or progesterone (25 mg/kg) 3.5 h before administration of either AM251 or its vehicle and 4 h before administration of either WIN 55,212-2 or its vehicle.

2.7. Statistical analysis

All data are shown as the mean ± S.E.M. of value for corresponding parameters. Statistical comparison between groups in each experiment was done with one- or two-way analysis of variance (ANOVA) followed by post hoc Student–Newman–Keuls test. A P value less than 0.05 was considered the limit of significance.

3. Results

3.1. Analgesia

Fig. 1 shows that acute administration of WIN 55,212-2 (2 and 4 mg/kg) produced an antinociceptive effect in the tail-flick test and significantly ($P<0.001$) increased %MPE in a dose-dependent manner in three non-operated control, sham-operated and ovariectomized mice. Compared with either control or sham-operated group, the antinociceptive effect of different doses of WIN 55,212-2 was significantly ($P<0.01$) higher in ovariectomized mice. In the absence of WIN 55,212-2 the analgesia measured in the test was not significantly different between the groups. As shown in Fig. 1, the antinociceptive effects of WIN 55,212-2 (4 mg/kg) in all groups was significantly ($P<0.001$) prevented by pretreatment with AM251 (0.5 mg/kg, i.p.).

In the next experiment we probed the role of ovarian sex hormones in the analgesic effects of WIN 55,212-2 in ovariectomized mice. As shown in Fig. 2, in the absence of WIN 55,212-2 neither estradiol (10 mg/kg) nor progesterone (25 mg/kg) had significant effect in the analgesia measured in the tail-flick test. However, pretreatment with estradiol (10 mg/kg) significantly ($P<0.001$) prevented the antinociceptive effect of WIN 55,212-2 (2 and 4 mg/kg) in ovariectomized mice. Progesterone had no significant effect on the antinociceptive effects of WIN 55,212-2 in ovariectomized mice, though the

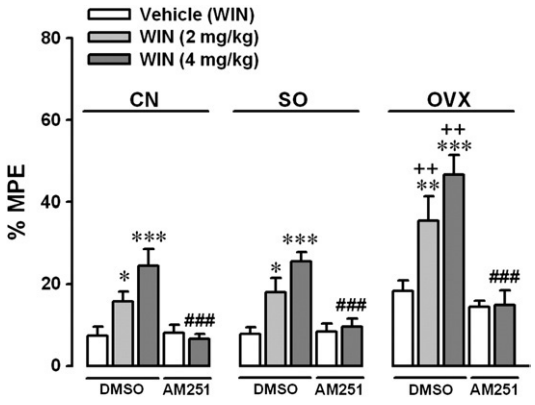

Fig. 1. Antinociceptive effect of the cannabinoid WIN 55,212-2 (WIN, 2 and 4 mg/kg, i.p.) in the tail-flick test in non-operated control (CN), sham-operated (SO) and ovariectomized (OVX) mice. Pretreatment with the selective cannabinoid CB_1 antagonist AM251 (0.5 mg/kg, i.p.) prevented the effects of WIN 55,212-2 on the analgesia measured in the test. Each experimental group consisted of 10 animals. Data are expressed as Mean ± S.E.M. %MPE = [(test latency − control latency)/(cut-off time − control latency)] × 100. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ compared with corresponding DMSO (vehicle for AM251)/vehicle (instead of WIN) group; $^{###}P<0.001$ compared with corresponding DMSO (vehicle for AM251)/WIN (4 mg/kg) group; $^{++}P<0.01$ compared with corresponding DMSO (vehicle for AM251)/WIN group in either CN or SO groups.

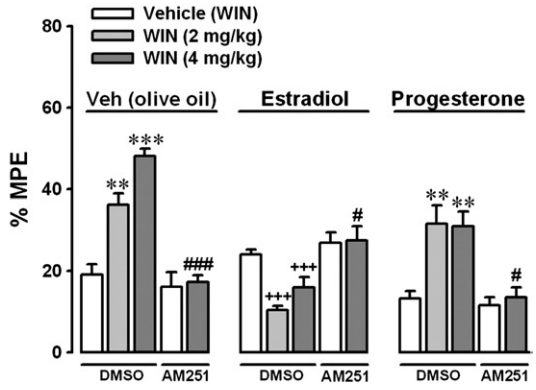

Fig. 2. The effect of pretreatment with estradiol valerate (10 mg/kg) and progesterone (25 mg/kg) on the analgesic effect of WIN 55,212-2 (2 and 4 mg/kg) in the tail-flick test in ovariectomized mice. Each experimental group consisted of 10 animals. Data are expressed as Mean±S.E.M. %MPE=[(test latency−control latency)/(cut-off time−control latency)]×100. $^{**}P<0.01$ and $^{***}P<0.001$ compared with corresponding olive oil or hormone/DMSO (vehicle for AM251)/vehicle (instead of WIN) group; $^{\#}P<0.05$ and $^{\#\#\#}P<0.001$ compared with olive oil or hormone/DMSO (vehicle for AM251)/WIN (4 mg/kg) group; $^{+++}P<0.001$ compared with olive oil (instead of hormone)/DMSO (vehicle for AM251)/corresponding WIN group.

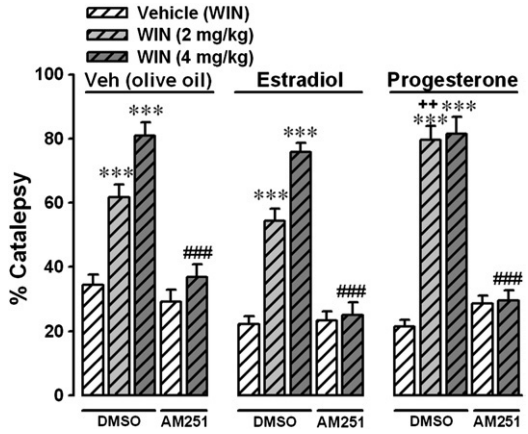

Fig. 4. The effect of pretreatment with estradiol valerate (10 mg/kg) and progesterone (25 mg/kg) on the cataleptic effect of WIN 55,212-2 (2 and 4 mg/kg) in the tail-flick test in ovariectomized mice. Each experimental group consisted of 10 animals. Data are expressed as Mean±S.E.M. $^{***}P<0.001$ compared with corresponding olive oil or hormone/DMSO (vehicle for AM251)/vehicle (instead of WIN) group; $^{\#\#\#}P<0.001$ compared with corresponding olive oil or hormone/DMSO (vehicle for AM251)/WIN (4 mg/kg) group; $^{++}P<0.01$ compared with olive oil (instead of hormone)/DMSO (vehicle for AM251)/ WIN (2 mg/kg) group.

effect of higher dose of WIN 55,212-2 (4 mg/kg) was lower compared with vehicle (olive oil instead of progesterone) group. In addition, the analgesic effect of WIN 55,212-2 (4 mg/kg) in the progesterone-treated ovariectomized mice was significantly ($P<0.05$) prevented by pretreatment with AM251 (0.5 mg/kg, i.p.).

3.2. Catalepsy

Fig. 3 shows that acute administration of WIN 55,212-2 (2 and 4 mg/kg) produced a cataleptic effect and significantly ($P<0.001$) increased the percentage of catalepsy in a dose-dependent manner in three non-operated control, sham-operated and ovariectomized mice. There was no significant difference in the cataleptic effect of WIN 55,212-2 (2 and 4 mg/kg) between control and ovariectomized mice. In the absence of WIN 55,212-2 the catalepsy was not significantly different between the groups. As shown in Fig. 3, the cataleptic effects of WIN 55,212-2 (4 mg/kg) in all groups were significantly ($P<0.001$) prevented by pretreatment with AM251 (0.5 mg/kg, i.p.).

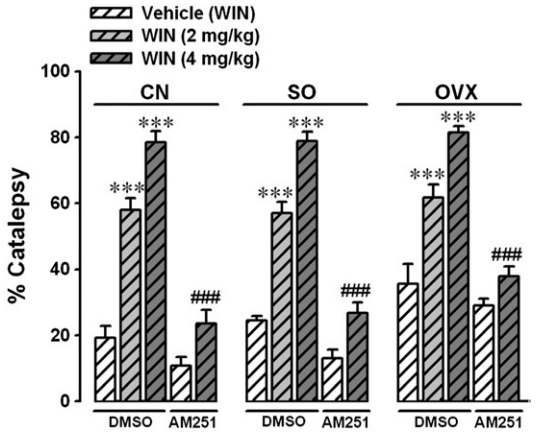

Fig. 3. Cataleptic effect of the cannabinoid WIN 55,212-2 (WIN, 2 and 4 mg/kg, i.p.) in non-operated control (CN), sham-operated (SO) and ovariectomized (OVX) mice. Pretreatment with the selective cannabinoid CB_1 antagonist AM251 (0.5 mg/kg, i.p.) prevented the effects of WIN 55,212-2 on the catalepsy measured in the test. Each experimental group consisted of 10 animals. Data are expressed as Mean±S.E.M. $^{***}P<0.001$ compared with corresponding DMSO (vehicle for AM251)/vehicle (instead of WIN) group; $^{\#\#\#}P<0.001$ compared with corresponding vehicle DMSO (vehicle for AM251)/WIN (4 mg/kg) group.

In the next experiment we probed the role of ovarian sex hormones in the cataleptic effects of WIN 55,212-2 in ovariectomized mice. As shown in Fig. 4, in the absence of WIN 55,212-2 neither estradiol (10 mg/kg) nor progesterone (25 mg/kg) had significant cataleptic effect in ovariectomized mice. Pretreatment with estradiol (10 mg/kg) did not influence the cataleptic effects of WIN 55,212-2 (2 and 4 mg/kg) in ovariectomized mice while progesterone significantly ($P<0.01$) enhanced the cataleptic effect of WIN 55,212-2 (2 mg/kg) in ovariectomized mice. In addition, the cataleptic effect of WIN 55,212-2 (4 mg/kg) in either estradiol- or progesterone-treated ovariectomized mice was significantly ($P<0.001$) prevented by pretreatment with AM251 (0.5 mg/kg, i.p.).

4. Discussion

In the present study, we demonstrated that the cannabinoid WIN 55,212-2 had both antinociceptive and cataleptic effects in either control or ovariectomized mice through involving the cannabinoid CB_1 receptor. The analgesic effect of WIN 55,212-2 was enhanced in ovariectomized mice. Moreover, estradiol but not progesterone significantly prevented the antinociceptive effects of WIN 55,212-2 in the tail-flick test in this group of animals. Although there was no significant alteration in the cannabinoid-induced catalepsy in ovariectomized mice

compared with control or sham-operated ones, progesterone but not estradiol enhanced the cataleptic effect of low dose of WIN 55,212-2 (2 mg/kg) in ovariectomized mice.

According to our data, the cannabinoid WIN 55,212-2 exerted antinociceptive effect in a dose-dependent manner in all experimental groups of animal. This result is in accordance with previous preclinical behavioral studies in which using different types of noxious stimulation (ie, thermal, mechanical, and chemical) demonstrated that cannabinoids effectively induce antinociception (Herkenham et al., 1991c; Tsou et al., 1997; Monhemius et al., 2001; Pertwee, 2001; Hohmann et al., 2005; McLaughlin et al., 2005; Walker and Hohmann, 2005). In 1899, Dixon (1899) reported that delivery of cannabis smoke to dogs produced a failure to respond to pin pricks. Seminal studies on cannabinoid-induced antinociception by Bicher and Mechoulam (1968) and Kosersky et al. (1973) provided a foundation for subsequent work that verified the ability of cannabinoids to profoundly suppress behavioral reactions to acute noxious stimuli and inflammatory and nerve injury-induced pain. The involvement of central cannabinoid system was also derived from the observation that intraventricular administration of cannabinoids WIN 55,212-2, CP 55940, and Δ^9-THC induces antinociception (Martin et al., 1993; Lichtman et al., 1996). Using the tail-flick test, additional studies demonstrated that microinjection of cannabinoids into sites such as the dorsolateral periaqueductal gray (PAG), dorsal raphe nucleus, rostral ventromedial medulla (RVM), nucleus reticularis gigantocellularis pars alpha, amygdala, lateral posterior and submedius regions of the thalamus, superior colliculus, and noradrenergic A_5 in the medulla region produces antinociception (Martin et al., 1995, 1998, 1999; Herkenham et al., 1991a,b,c; Tsou et al., 1997; Manning et al., 2001; Monhemius et al., 2001; Walker and Huang, 2002).

Our data showed that the CB_1-mediated antinociceptive effect of WIN 55,212-2 was significantly higher in ovariectomized mice compared with either control or sham-operated female animals. Since the ovariectomy is a method for removal of the primary source of ovarian hormones, this observation suggested that differences between the antinociception induced by WIN 55,212-2 in ovariectomized mice and sham-operated ones might be due to the ovarian sex hormones. Thus, in our next experiments, we evaluated the effects of exogenous ovarian sex steroids, estradiol and progesterone, on the antinociceptive effects of WIN 55, 212-2 in the tail-flick test in ovariectomized mice. Our data demonstrated that estradiol, but not progesterone, significantly prevented the antinociceptive effects of WIN 55,212-2 in ovariectomized mice. Therefore, it seems that the ovarian steroid estrogen could modulate the cannabinoid-induced analgesia in female mice. Our finding is in line with previous studies which have demonstrated antinociceptive actions of physiological or experimental pregnancy in rats and women (Cogan and Spinnato, 1986; Dawson-Basoa and Gintzler, 1998; Gintzler, 1980). Moreover, several studies have demonstrated the effect of sex steroids deprivation induced by ovariectomy followed by either estrogen or progesterone supplementation on perception of noxious stimuli in many animal model of pain evaluated (Gordon and Soliman, 1996; Dawson-Basoa and Gintzler, 1996; Rhodes and Frye, 2001; Mannino et al., 2007; Kuba et al., 2006; Walf and Frye, 2003). Administration of sex steroids in some brain regions, such as amygdala, in ovariectomized rats has been shown to produce antinociceptive effects (Walf and Frye, 2003). It is noteworthy that although there are scarce data regarding the involvement of sex hormones especially estradiol in the cannabinoid-induced analgesia, the present data showed that ovarian sex steroids could alter the antinociception induced by cannabinoids. These results may suggest that sex differences could affect the cannabinoid-induced analgesia and sex steroids may play a role in this regard. On the other hand, considering the well-known antinociceptive effects of cannabinoids, the present data could suggest that hormone replacement might alter the analgesic effects of cannabinoids. Also, it should be noted that in the present study, neither estradiol nor progesterone at doses used had not individually any antinociceptive effect in the tail-flick test, but estradiol could affect the antinociceptive effect of WIN 55,212-2 in the same test in ovariectomized mice.

Catalepsy is defined as a failure to correct an externally imposed posture. It has been widely used to evaluate motor effects of drugs, particularly those related to extrapyramidal system (Sanberg et al., 1988; Hauber, 1998). Several studies have demonstrated that cannabinoids can induce profound motor deficits, including immobility, catalepsy and ataxia (Walton et al., 1938; Gough and Olley, 1978; Martin et al., 1991; Prescott et al., 1992; Anderson et al., 1996; Rodríguez de Fonseca et al., 1998; McLaughlin et al., 2005; McMahon and Koek, 2007). The cannabinoid CB_1 receptor is present with a very dense intensity in neurons of the cerebellum and the basal ganglia (Herkenham et al., 1990, 1991a,b,c), which explains the ataxia, immobility, and catalepsy observed after acute administration of cannabinoids (Dewey, 1986). Accordingly, our data also showed that the cannabinoid WIN 55,212-2 dose-dependently produced cataleptic effect in mice via involving the cannabinoid CB_1 receptor. Although the cataleptic effects of WIN 55,212-2 were not altered in ovariectomized mice compared with either control ones, the cataleptic effect of low dose of WIN 55,212-2 (2 mg/kg) was significantly increased by pretreatment with progesterone in ovariectomized mice. However, estradiol could not influence the catalepsy induced by WIN 55,212-2. These results suggest that the ovarian sex steroid progesterone could be involved, at least in part, in the cataleptic effect of WIN 55,212-2 in ovariectomized mice.

Consistently, there are several line of evidence showing that sex steroids could influence the locomotor activity and catalepsy induced by a variety of stimuli (Chiodo et al., 1979; Banerjee et al., 1983; Miller, 1983; Nomikos et al., 1987; Palermo-Neto and Dorce, 1990; McCarthy et al., 1993; Rupprecht et al., 1999; Pryce et al., 2003). Moreover, Pryce et al. (2003) demonstrated that progesterone receptors could affect the cannabinoid CB_1-mediated effects of Δ^9-THC on the motility and catalepsy in mice, suggesting a role for ovarian hormones in the cannabinoid-induced catalepsy in mice. Moreover, ovarian sex hormones have been shown to affect the sensitivity of certain neuronal processes to cannabinoid

treatment (Bonnin et al., 1993; Rodríguez de Fonseca et al., 1994; Mani et al., 2001). For instance, Mani et al. (2001) reported that the facilitatory effect of Δ^9-THC on sexual receptivity was inhibited by antagonists to progesterone receptors. They suggested that THC acts on the cannabinoid CB_1 receptor to initiate a signal transduction response that requires intracellular progesterone receptors for effective induction of sexual behavior (Mani et al., 2001). Another study by Rodríguez de Fonseca et al. (1994) also revealed that cannabinoid receptor density and affinity in certain brain areas such as the medial basal hypothalamus, the striatum and the limbic forebrain could vary during the different phases of estrous cycle in female rats and ovarian hormone replacement in ovariectomized rats could affect these changes, suggesting the involvement of sex steroids in the alterations in the cannabinoid receptors density in the brain. Moreover, González et al. (2000) have demonstrated that expression of the cannabinoid CB1 receptor gene in the anterior pituitary gland is regulated by sex steroids in both male and female rats. They also showed that gonadal steroids may affect the response of this gene to chronic cannabinoid administration and observed that anandamide contents in the anterior pituitary gland and the hypothalamus might be controlled by circulating sex steroids (González et al., 2000). Taking our finding together with these reports into consideration, it can be concluded that ovarian sex steroids, especially progesterone, could play a role in the cataleptic effects of cannabinoids in ovariectomized mice. However, more detailed studies are clearly necessary to verify the exact mechanisms underlying this effect.

Nowadays, it is well known that dopaminergic pathway in some particular brain regions such as basal ganglia and mesocorticolimbic plays a crucial role in the control of motor activity and drugs that decrease dopaminergic neurotransmission in such brain regions induce catalepsy in rodents (Koffer et al., 1978; Sanberg et al., 1988). Also, a large body of evidence has indicated that dopaminergic activity could be involved in the catalepsy induced by cannabinoids (for review see De Fonseca et al., 1998). For instance, Anderson et al. (1996) demonstrated that acute stimulation of cannabinoid CB_1 receptors potentiates neuroleptic-induced catalepsy, suggesting a cooperative effect between reduced dopaminergic signaling and activation of CB_1 receptors. The dopaminergic control over cannabinoid-mediated regulation of motor activity was also supported by the observation that chronic neuroleptic treatment induces alterations in the striatal expression of CB_1 receptor mRNA, with a potency that correlates with their affinity for dopamine D_1 receptors (Mailleux and Vanderhaeghen, 1993). On the other hand, it has been shown that ovarian sex hormones could influence some dopamine related behavior in rats such as catalepsy (Palermo-Neto and Dorce, 1990; Speciale et al., 1983; McCarthy et al., 1993; Van Hartesveldt and Joyce, 1986; Miller, 1983). Interestingly, Bonnin et al. (1993) reported a possible modulation by sex steroids of the effects of Δ^9-THC on mesolimbic dopaminergic activity. In their experiments, they showed that Δ^9-THC significantly decreased the density of D_1 dopaminergic receptors in the limbic forebrain of ovariectomized rats chronically replaced with estrogens. Taken together, it seems that dopaminergic transmission may be involved in the effect of ovarian sex hormones on the cataleptic effects of cannabinoids such as WIN 55,212-2 in ovariectomized mice. However, this general assumption has to await more detailed studies.

In summary, our data demonstrated the cannabinoid CB_1-mediated antinociceptive and cataleptic effects of WIN 55,212-2 in either control or ovariectomized mice. The analgesic effect of WIN 55,212-2 was enhanced in ovariectomized mice, which was prevented by pretreatment with estradiol but not by progesterone. There was no significant difference in the cannabinoid-induced catalepsy between control and ovariectomized mice. However, pretreatment with progesterone but not estradiol potentiated the cataleptic effect of low dose of WIN 55,212-2 (2 mg/kg) in ovariectomized mice. The present data suggested the possible involvement of ovarian sex steroids in the analgesia and catalepsy induced by cannabinoids in ovariectomized female mice.

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