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Research report

Cannabinoid receptor agonist disrupts behavioral and neuroendocrine responses during lactation

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HIGHLIGHTS

- Cannabinoid reduced maternal care, maternal aggression and maternal anxiolysis.
- Cannabinoid reduced the activity of oxytocinergic neurons in the PVN and SON.
- Cannabinoid receptor agonist disrupts oxytocin secretion in response to suckling.

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ABSTRACT

It has been shown that the endocannabinoid system is involved in the neurohypophyseal hormone secretion produced by exposure to several different stimuli; however, the influence of this system on neuroendocrine responses during lactation is unclear. Therefore, the aim of our study was to investigate the influence of an acute peripheral administration of WIN55,212-2 (cannabinoid receptor agonist) on behavioral and neuroendocrine responses during lactation. On day 6 of lactation, female rats were treated with vehicle or WIN55,212-2 30 min before the start of our experiments. To evaluate maternal behavior, the pups were returned to their home cages to the side of the cage opposite the previous nest, and the resulting behavior of the lactating rats was recorded for the next 30 min. Aggressive behavior was evaluated for 10 min following the placement of an intruder male rat in the home cage. The plasma level of oxytocin and the amount of milk consumption by the pups were evaluated 15 min after the onset of suckling. In addition, double-labelled c-Fos/oxytocin neurons in the medial magnocellular subdivision of the paraventricular nucleus and in the supraoptic nucleus were quantified for each lactating rat. The results show that WIN decreased maternal care, decreased aggressive behaviors, suppressed maternal anxiolysis, decreased plasma oxytocin levels and milk consumption by pups and decreased activation of oxytocinergic neurons in hypothalamic nuclei. Our results indicate that the changes in the behavioral responses of lactating rats treated with WIN maybe can be related to disruption in the neuroendocrine control of oxytocin secretion.

1. Introduction

The behavioral repertoire of some mammals during motherhood differs from that exhibited by females in other periods of their reproductive cycle. Concomitant with the display of maternal care, lactating rats show aggressive behavior and lower levels of anxiety in conflict tests [1]. This behavioral pattern relies, at least partly, on the hormonal changes that characterize late gestation, parturition and lactation [2]. As maternal care parallels the course of these behaviors, it has been suggested that maternal behavior is a condition needed for the expression of aggression and anxiolysis. One potential mediator of these effects on aggression and anxiety-like behavior is the elevation of basal corticosterone; indeed, it has been postulated that the stress hyporesponsiveness of lactating dams mediates the decrease in fear and anxiety associated with the display of maternal aggression [3]. Another potential neural mediator of these behavioral changes is oxytocin [4]. Oxytocin has been suggested to play an important role within the central amygdala to regulate maternal aggression [5]. Using local microdialysis, increased oxytocin release was observed during a maternal defense test in dams. Behavioral changes after birth appear to depend on hormonal changes, such as increased serum levels of oxytocin [6,7]. Importantly, the intracerebroventricular infusion of a selective oxytocin receptor antagonist increased anxiety [8], decreased maternal care [9] and decreased maternal aggression [5].

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Although abundant information is available about the influence of many hormones and neurotransmitters on the maternal behavior of rodents, less is known about the modulation of these signals by endocannabinoids. Studies addressing the effects of *Cannabis* extract on maternal behavior date back to the late 1970s and early 1980s; for example, it was reported that the acute and sub-chronic administration of Δ^9-tetrahydrocannabinol (THC), the primary psychoactive constituent of marihuana, dose-dependently suppressed the retrieval of nesting material in mice [10,11].

In addition, it was demonstrated that THC altered the secretion of pituitary hormones in humans and laboratory animals under a variety of physiological circumstances [12–14]. Moreover, THC decreased suckling-induced oxytocin release by reducing intramammary pressure and pup stretch during suckling [15,16].

Further evidence of the involvement of the cannabinoid system on oxytocin secretion was demonstrated with the administration of a CB1 receptor antagonist, rimonabant, that enhanced the secretion of oxytocin and c-Fos expression in oxytocinergic neurons in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus by inducing blood volume expansion [17]. In addition, several studies have shown that the exogenous administration of cannabinoids inhibits the activity of several hypothalamic-pituitary systems, including the thyroid, gonadal and growth hormone axes [18–20].

Based on the reported involvement of the endocannabinoid system in oxytocin secretion, the aim of our study was to investigate the influence of an acute peripheral injection of WIN55,212-2 (CB1 receptor agonist) on behavioral and neuroendocrine responses during lactation.

2. Material and methods

2.1. Animals

Subjects were adult Wistar nulliparous female rats at approximately 9 weeks of age, which were obtained from the Central Animal Facility of the Federal University of Alfenas and were housed in a temperature-controlled room (22 °C) on a 12 h light–12 h dark cycle (lights on at 7:00 AM) with access to water and food ad libitum. In the experiments, the females were timed-mated by placing them with sexually experienced males. The day on which sperm was observed during vaginal lavage was designated as day 1 of pregnancy. The pregnant females were individually housed in opaque polypropylene cages (42 × 34 × 16 cm). After the rats gave birth (day 0 of lactation), the litters were randomly standardized to eight pups each (four male and four female pups), and the mothers remained with their litters until they were tested for hormonal changes and maternal behavior on day 6 of lactation. To test the aggressive behavior of lactating females, male intruders were used. The male intruders were approximately two months old, were maintained under the same conditions and weighed approximately the same as the resident female. Each male was used only once in the experiment. For testing in the open field, additional female rats in diestrus were used. All of the experiments were conducted in accordance with the declaration of Helsinki on the welfare of experimental animals and with the approval of the Ethics Committee of the Federal University of Alfenas (# 244/2009).

2.2. Drugs

WIN55,212-2 was purchased from Sigma–Aldrich (EUA) and dissolved in a solution containing 0.9% NaCl, tween and dimethyl sulfoxide (DMSO) at a ratio of 8:1:1. For all of the experiments, rats at day 6 of lactation were treated with WIN55,212-2 (1 or 3 mg/kg, i.p.) or vehicle 30 min prior to the start of any experimental procedures. The doses of WIN55,212-2 used in the present study are in agreement with the doses commonly used in other reports that injected these compounds peripherally [21–24]. The injections were followed by the specific procedures and measurements described in the following sections.

2.3. Maternal care

Maternal behavior was assessed between 08:00 and 12:00 h. The initial position of the nest in the home cage was recorded. Next, the litter was removed from the cage and placed in a different cage for 12 h. After 11 h and 30 min of maternal separation, dams were treated with vehicle (1 mL/kg, $n = 8$ animals), WIN55,212-2 (1 mg/kg, $n = 9$ animals) or WIN55,212-2 (3 mg/kg, $n = 10$ animals). After another 30 min, the pups were placed back in their home cages on the side opposite to the location of the previous nest, and the dam's behavior was recorded for the next 30 min. As previously described, we analyzed multiple parameters, including the latency for retrieval of each pup, number of pups brought to the nest, time spent licking the pups, percentage of time spent in the arched-nursing position, percentage of time spent blanket-nursing and percentage of time exhibiting full maternal behavior (the mother staying in the arched-nursing position for 2 min after nursing) [25,26].

2.4. Assessment of maternal aggression

Aggressive behavior was assessed between 08:00 and 12:00 h. Another set of dams were treated with vehicle (1 mL/kg, $n = 9$ animals), WIN55,212-2 (1 mg/kg, $n = 7$ animals) or WIN55,212-2 (3 mg/kg, $n = 9$ animals) and 30 min after injections, an adult male rat (intruder) was placed into the home cage of the female and her litter, and the interaction of the mother and intruder was recorded for 10 min. As previously described, we assessed the latency to first attack (dam lunges quickly at intruder male, usually followed by rolling, biting, and fur pulling directed toward the neck and back regions of the intruder), frontal attack number, lateral attack number, lateral threat number (dam threatens intruder male while approaching laterally) and maternal behavior (i.e., any behavior directed toward caring for the pups) over the 10 min period [25,26].

2.5. Performance in the open field test

Another set of dams were treated with vehicle (1 mL/kg, $n = 7$ animals), WIN55,212-2 (1 mg/kg, $n = 9$ animals) or WIN55,212-2 (3 mg/kg, $n = 9$ animals) and 30 min after the injection, animals were tested in the open field arena to evaluate their anxiolytic and locomotor activity. We also used non-lactating virgin female rats in their diestrus phase that were treated with vehicle ($n = 8$ animals). In this test, each female rat was placed in the center of the novel open field arena. The open field apparatus consists of circular arena with a diameter 60 cm and walls of 45 cm high with a floor is divided into 12 areas. A circle of 30 cm diameter in center divided in four areas was defined as the central areas and the 8 areas along the walls were considered the peripheral area. The number of peripheral (adjacent to the walls) and central (away from the walls) areas that the rat entered with all four paws was recorded for 5 min. Female rat behavior was continuously videotaped by a video camera placed over the structure and was then encoded using a continuous sampling method. The antithigmotactic effect was defined as the proportion of entries into the central part of the arena relative to the total number of entries. The arena was carefully cleaned with a 10% ethanol solution after each test [26,27].