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Hypocretinergic system in the medial preoptic area promotes maternal behavior in lactating rats

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ABSTRACT

Hypocretin-1 and 2 (HCRT-1 and HCRT-2, respectively) are neuropeptides synthesized by neurons located in the postero-lateral hypothalamus, whose projections are widely distributed throughout the brain. The hypocretinergic (HCRTergic) system has been associated with the generation and maintenance of wakefulness, as well as with the promotion of motivated behaviors. In lactating rats, intra-cerebroventricular HCRT-1 administration stimulates maternal behavior, whilst lactation *per se* increases the expression of HCRT type 1 receptor (HCRT-R1). Due to the fact that HCRTergic receptors are expressed in the medial preoptic area (mPOA), a region critically involved in maternal behavior, we hypothesize that HCRT-1 promotes maternal behavior acting on this region. In order to evaluate this hypothesis, we assessed the maternal behavior of lactating rats following microinjections of HCRT-1 (10 or 100 μM) and the selective HCRT-R1 antagonist SB-334867 (250 μM) into the mPOA, during the first and second postpartum weeks. While intra-mPOA microinjections of HCRT-1 (100 μM) increased corporal pup licking during the second postpartum week, the blockade of HCRT-R1 significantly decreased active components of maternal behavior, such as retrievals, corporal and ano-genital lickings, and increased the time spent in nursing postures in both postpartum periods. We conclude that HCRTergic system in the mPOA may stimulate maternal behavior, suggesting that endogenous HCRT-1 is necessary for the natural display of this behavior.

1. Introduction

The hypocretinergic (HCRTergic) system has been associated with the promotion of motivated behaviors, including exploratory activity as well as food, sexual and drug seeking behaviors [6,9,19,23,25,42,44,45]. The hypocretins (HCRT) consist of two neuropeptides: HCRT-1 and HCRT-2, also known as orexin A and orexin B respectively. These neuropeptides are synthesized by neurons located in the postero-lateral hypothalamus whose projections are widely distributed throughout the brain [22,32,38,43] HCRT exert their biological functions through two metabotropic receptors: the HCRT type 1 (HCRT-R1) and type 2 receptors (HCRT-R2) that differ in their affinity to HCRT-1 [33].

Several experimental studies indicate that the HCRTergic system plays a role during the lactating period. In this regard, the number of HCRTergic neurons expressing Fos-immunoreactivity is larger in lactating female mice versus virgin ones [14]. Furthermore, prepro-HCRT mRNA and HCRT-R1 mRNA levels in the entire hypothalamus are significantly higher on day 1 of lactation than during late pregnancy and late lactation [48], suggesting that HCRT may be involved in the regulation of certain aspects of maternal behavior in the early stages of lactation. Despite this evidence, only one study has directly examined the effect of HCRT on maternal behavior; D'Anna and Gammie showed that intracerebroventricular (i.c.v.) injections of HCRT-1 increase the number of lickings and groomings of pups, and the number of nursing bouts at intermediate doses in lactating mice [10]. However, the specific areas that mediate this effect remain unknown. Interestingly, HCRT receptors, HCRT-R1 in particular, are expressed in the medial preoptic area (mPOA) [21,46], a critical region involved in the onset and maintenance of maternal behavior [26,28,36].

The mPOA is a key neural site where the hormones of pregnancy and parturition act to synchronize maternal responsiveness [7,27]. In addition, it has been suggested that several neuromodulators, including oxytocin, melanin-concentrating hormone (MCH), amylin and neurotensin mediate the mPOA adjustments,

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underlying the postpartum expression of maternal behavior [3,8,11,18,37,47]. Based on these findings and taking the importance of HCRT on the motivational aspects of behavior into account, as well as its relation with lactation, we hypothesized that HCRT promote maternal behavior acting in the mPOA. In order to evaluate this hypothesis, we assessed the maternal behavior of lactating rats following microinjections of HCRT-1 and the selective HCRT-R1 antagonist SB-334867 into the mPOA during the first and second postpartum weeks.

2. Material and methods

2.1. Animals and housing

Fifty-one primiparous Wistar female rats (240–310 g) and pups were used in this study. The experimental procedures were in strict accordance with the "Guide for the care and use of laboratory animals" (8th edition. National Academy Press, Washington D. C., 2011) and approved by the Institutional Animal Care Committee. All efforts were made in order to minimize the number of animals and their suffering. We used the same experimental protocol as Benedetto et al. [3]. Briefly, animals were housed in a temperature-controlled room under a 12-h light/dark cycle, with *ad libitum* access to food and water. Two days before giving birth, pregnant females were housed individually. On postpartum day 1 (PPD1, birth = day 0), litters were culled to four female and four male pups per mother.

2.2. Stereotaxic surgery

On the morning of PPD3 or PPD11, females were anesthetized with a mixture of ketamine/xylazine/acepromazine maleate (80/2.8/2.0 mg/kg i.p.). Female rats were bilaterally implanted with 22-gauge stainless steel guide cannulae (Plastic One, Roanoke, VA) aimed 2 mm dorsal to the mPOA: AP −0.5 mm (from Bregma); ML ± 0.5 mm (from midline); DV −6.5 mm (from skull) according to Paxinos and Watson [29]. In addition, three stainless steel screws were implanted into the skull as anchors and, together with the guide cannulae, they were secured to the skull with dental cement.

Immediately after surgery, each mother was reunited with her pups in the home cage. All females remained healthy throughout the experiment, exhibiting typical maternal behaviors.

2.3. Experimental design

Animals were randomly assigned to one of the following six independent groups according to drug dosage and the postpartum stage: (1) 10 μM HCRT-1 in the first postpartum week (1stWK) (n = 6), (2) 100 μM HCRT-1 in 1stWK (n = 8), (3) 10 μM HCRT-1 in the second postpartum week (2ndWK) (n = 7), (4) 100 μM HCRT-1 in 2ndWK (n = 8), (5) 250 μM SB-334867 in 1stWK (n = 8) and (6) 250 μM SB-334867 in 2ndWK (n = 8). In every group, each female was microinjected with vehicle and drug on two different days (PPD 6 and 7: 1stWK or PPD 14 and 15: 2ndWK) in a counterbalanced order.

2.4. Drugs

HCRT-1 (Human, mouse, rat; Phoenix Pharmaceuticals Inc., Belmont, CA) was diluted in a sterile saline solution to obtain a final concentration of 10 and 100 μM (the dose was adjusted according to Ref. [49]). Aliquots for these doses were prepared in advance, frozen at −20 °C, and thawed immediately before use. SB-334867, a HCRT-R1 antagonist [35] (provided by Glaxo-Smith-Kline, Essex, UK), was diluted in dimethyl sulfoxide (DMSO) 2% to obtain a final concentration of 250 μM (the dose was adjusted according to Ref. [50]).

2.5. Microinjection procedure

Females were bilaterally microinjected with 0.3 μl of either HCRT-1, SB-334867 or the same volume of vehicle into the mPOA over a period of 3 min, with the injection cannulae (28 gauge; Plastic One, Roanoke, VA) extending 2 mm beyond the tip of the guide cannulae, with a constant-rate infusion pump (Harvard apparatus, USA). The administration cannulae were left in place for an additional minute to allow for the diffusion of the drug. A similar microinjection volume was used in previous studies of the group [3,20].

2.6. Behavioral testing

All behavioral tests were performed during the light phase of the light/dark cycle, between 09:00 and 11:00 AM.

2.6.1. Maternal behavior testing

Following the microinjections procedure, the females were returned to their home cages. 10 min later, the pups were scattered in the mothers' home cages opposite to the nest. Maternal behaviors such as retrievals of the pups to the nest, mouthings, corporal lickings, ano-genital lickings and nest building were counted over a 30 min period. The latencies to retrieve the first pup and to group the entire litter into the nest were also measured. In addition, the latencies and durations of hovering over the pups and nursing postures were recorded. The number of eating, drinking and self-grooming behaviors was also annotated [31].

2.6.2. Locomotor activity

In order to evaluate any non-specific motor disturbance induced by HCRT-1 or SB-334867 administration, the locomotor activity was assessed immediately after the maternal behavioral test. Due to the fact that this test can be conducted only once per animal, it was carried out on independent groups of animals. The following groups were employed: 100 μM HCRT-1-treatment day in the 1stWK (from group 2) and 2ndWK (from group 4), SB-334867-treatment day in the 2ndWK (from group 6) and vehicle-treatment day in the 1stWK (from group 1) and in the 2stWK (from group 3). The number of line-crosses and rearings were measured over a 5-min session in a cage measuring 60 × 40 × 40 cm, that was divided into six equal quadrants (adapted from Ref. [16]).

2.6.3. Elevated plus-maze test

The elevated plus maze (EPM) test was performed after the locomotor activity test in the same animals. The EPM apparatus consists of an elevated (50 cm above the floor) plus-shaped maze with four arms (50 cm long × 10 cm wide), two of which are enclosed by walls 40 cm in height (closed arms), while the other arms lack walls (open arms) [30]. The cumulative time spent in the open arms and the number of open arm entries were recorded over a 5-min session. The data is expressed as the percentage of time spent in the open arms and the number of open arm entries.

2.7. Histological verification of injection sites

At the end of the experiment the animals were euthanized with an overdose of ketamine/xylazine, perfused with 4% paraformaldehyde, and their brains were removed for histological processing. Thereafter, the brains were cut in 100 μm coronal sections with a vibratome. The location of mPOA microinjection sites was verified according to Paxinos and Watson [29].