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Melanin-concentrating hormone in the medial preoptic area reduces active components of maternal behavior in rats

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ABSTRACT

Melanin-concentrating hormone (MCH) is an inhibitory neuropeptide mainly synthesized in neurons of the lateral hypothalamus and incerto-hypothalamic area of mammals that has been implicated in behavioral functions related to motivation. During lactation, this neuropeptide is also expressed in the medial preoptic area (mPOA), a key region of the maternal behavior circuitry. Notably, whereas MCH expression in the mPOA progressively increases during lactation, maternal behavior naturally declines, suggesting that elevated MCHergic activity in the mPOA inhibit maternal behavior in the late postpartum period. To explore this idea, we assessed the maternal behavior of early postpartum females following bilateral microinfusions of either MCH (50 and 100 ng/0.2 μl/side) or the same volume of vehicle into the mPOA. As expected, females receiving 100 ng MCH into the mPOA exhibited significant deficits in the active components of maternal behavior, including retrieving and nest building. In contrast, nursing, as well as other behaviors, including locomotor activity, exploration, and anxiety-like behavior, were not affected by intra-mPOA MCH infusion. The present results, together with previous findings showing elevated expression of this neuropeptide toward the end of the postpartum period, suggest that modulation of mPOA function by MCH may contribute to the weaning of maternal responsiveness characteristic of the late postpartum period.

Introduction

Melanin-concentrating hormone (MCH) system has been recently demonstrated to be involved in various aspects of motivated behaviors [5,13,14,24,52]. MCH is an inhibitory neuropeptide [11] synthesized mainly by neurons in the lateral hypothalamus and incerto-hypothalamic area of mammals [4,23,53] that acts through one type of receptor (MCHR1) in rats [42,50]. These MCHergic neurons project widely throughout the brain, and send a relatively dense projection to the medial preoptic area (mPOA) [4], an area known to be critically involved in maternal behavior [25,26,29,49].

Intriguingly, MCH mRNA expression and peptide synthesis are transiently induced only during lactation in neurons of the mPOA, a region that does not otherwise express MCH in males or non-lactating female rats [17,40]. The expression of MCH in mPOA neurons, but not in lateral hypothalamus and incerto-hypothalamic area, progressively increases throughout the postpartum period, reaching its maximal expression at the end of lactation, during weaning. On the basis of these findings, we hypothesized that the MCH system in mPOA may negatively modulate the expression of maternal behavior toward the end of the postpartum period. As a first step to evaluate this hypothesis, we examined the effect of intra-mPOA injection of MCH on maternal behavior during the early postpartum period, when endogenous MCH levels are naturally low and the caregiving behavior is at its highest expression.

Methods

Animals and housing

A total of twenty-one primiparous Wistar female rats (275–315 g) and pups were used for this study. All animal use and 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

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to minimize the number of animals used and their suffering. Animals were housed in a temperature-controlled room (22 ± 1 °C) under a 12-h light/dark cycle (lights-on at 05.30 am), with ad libitum access to food and water. Two days before giving birth, pregnant females were housed individually in transparent cages (40 cm × 30 cm × 20 cm) containing shredded paper towels as nest-building material. On postpartum day 1 (PPD1, birth = day 0), litters were culled to four female and four male pups per mother (litter weight average on PPD1 was 46.1 ± 0.4 g).

Stereotaxic surgery

On the morning of PPD2, females were anesthetized with a mixture of ketamine/xylazine/acepromazine maleate (80/2.6/2.0 mg/kg, i.p.). To obtain a flat-skull position in the stereotaxic device, the incisor bar was adjusted for each animal until lambda and bregma were at the same dorsoventral level [33]. 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 surface) [33]. In addition, three stainless steel screws were implanted into the skull as anchors, and the guide cannulae were secure to the skull with dental cement. Immediately after surgery, a dummy cannula was inserted into each guide cannula to maintain patency.

During surgery, pups were maintained in their home cage under a heat lamp. Immediately after surgery, each mother was reunited with her pups in the home cage. All females remained healthy throughout the experiment, fully exhibiting typical maternal behaviors, and their pups gained weight and developed normally.

Experimental design

Animals were randomly assigned to one of the following two independent groups: (1) 50 ng MCH (n = 8), or (2) 100 ng MCH (n = 10). Within each group, each female received two microinjections in a counterbalanced order, such that half of the females were first microinjected with MCH (on PPD5) and the next day (PPD6) with saline, whereas the other half of the females received the opposite treatment order.

Drug

MCH (Human, mouse, rat; Phoenix Pharmaceuticals Inc., Belmont, CA, #070-47) was diluted in sterile saline to obtain a final concentration of 0.25 and 0.5 μg/μl. Aliquots for these doses were prepared in advance, frozen at −20 °C, and thawed immediately before use.

Microinjection procedure

Females were bilaterally microinjected with either MCH (50 or 100 ng in 0.2 μl of saline) or the same volume of saline into the mPOA over a period of 2 min, with the injection cannulae (28 gauge; Plastic One, Roanoke, VA) extending 2 mm beyond the tip of guide cannulae. The administration cannulae were left in place for an additional minute to allow for the diffusion of the drug. The doses and volume of MCH were chosen on the basis of previously published studies [3,7,13,15,18,19,38,41].

Behavioral testing

All testing was conducted on PPD 5 and 6, three days after surgery, during the light phase of the light/dark cycle [3,6,8].

Maternal behavior testing

Following the microinjections of either MCH or saline, females were returned to their home cage. Ten minutes after, the pups were scattered in the mother' home cage opposite to the nest. The number of the following maternal behaviors was recorded during 30 min: retrievals of the pups to the nest, mouthings (oral re-arrangement of a pup within the nest), lickings and nest building (each retrieval or re-arrangement of nest material). Also, the latencies to retrieve the first pup and to group the entire litter into the nest were measured. In addition, the latencies (first episode ≥ 2 min in duration) and durations of hovering over the pups and nursing, in both low and high kyphotic postures, as well as the total time in contact with the pups (hover over plus nursing) were recorded. A latency of 1800 s was given to any behavior that was not initiated or completed within the 30-min test period. The number of eating, drinking and self-grooming behaviors was also annotated [36,37].

Locomotor activity

In order to evaluate any nonspecific motor disturbance induced by MCH administration, the locomotor activity of vehicle-treated females from group 1 (50 ng of MCH) and MCH-treated females from group 2 (100 ng of MCH) was evaluated immediately after the maternal behavioral test. The number of line crosses, rearings and self-groomings were measured over a 5-min session, in a cage measuring 60 cm × 40 cm × 40 cm, divided in six equal quadrants (adapted from [9]).

Elevated Plus-Maze

Following completion of the locomotor activity test, females underwent testing for anxiety-like behavior using the elevated plus maze (EPM). 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 lacked walls (open arms) [34]. Each mother rat was placed on the center of the platform (10 cm × 10 cm) of the EPM, facing a closed arm and allowed to explore the maze for 5 min. The cumulative time spent in the open arms, and the number of open and closed arm entries were recorded during a 5-min session. Entry into an arm was defined as the animal placing all four paws into it. Data are expressed as the percentage of time spent in the open arms (100 × time spent in open arms/total time), the percentage of open arm entries (100 × number of entries in open arms/[total number of entries in open + closed arms]) and the percentage of closed arm entries (100 × number of entries in closed arms/[total number of entries in open + closed arms]). The maze was cleaned with a 5% ethanol/water solution and dried thoroughly between test sessions.

Histological verification of injection sites

At the end of the experiment, animals were euthanized with sodium pentobarbital (60 mg/kg, i.p.), 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 were verified according to the neuroanatomical Atlas of Paxinos and Watson [33].

Statistics

All behavioral data are presented as median (semi-interquartile ranges [SIQR]). As data did not follow a normal distribution (Kolmogorov–Smirnov test, $p > 0.05$), non-parametric tests were utilized [45]. Mann–Whitney U tests were used for comparisons of independent groups, and Wilcoxon matched-pair signed-ranks tests were used for intragroup comparisons. A $p < 0.05$ was used in all analyses to discard the null hypothesis.

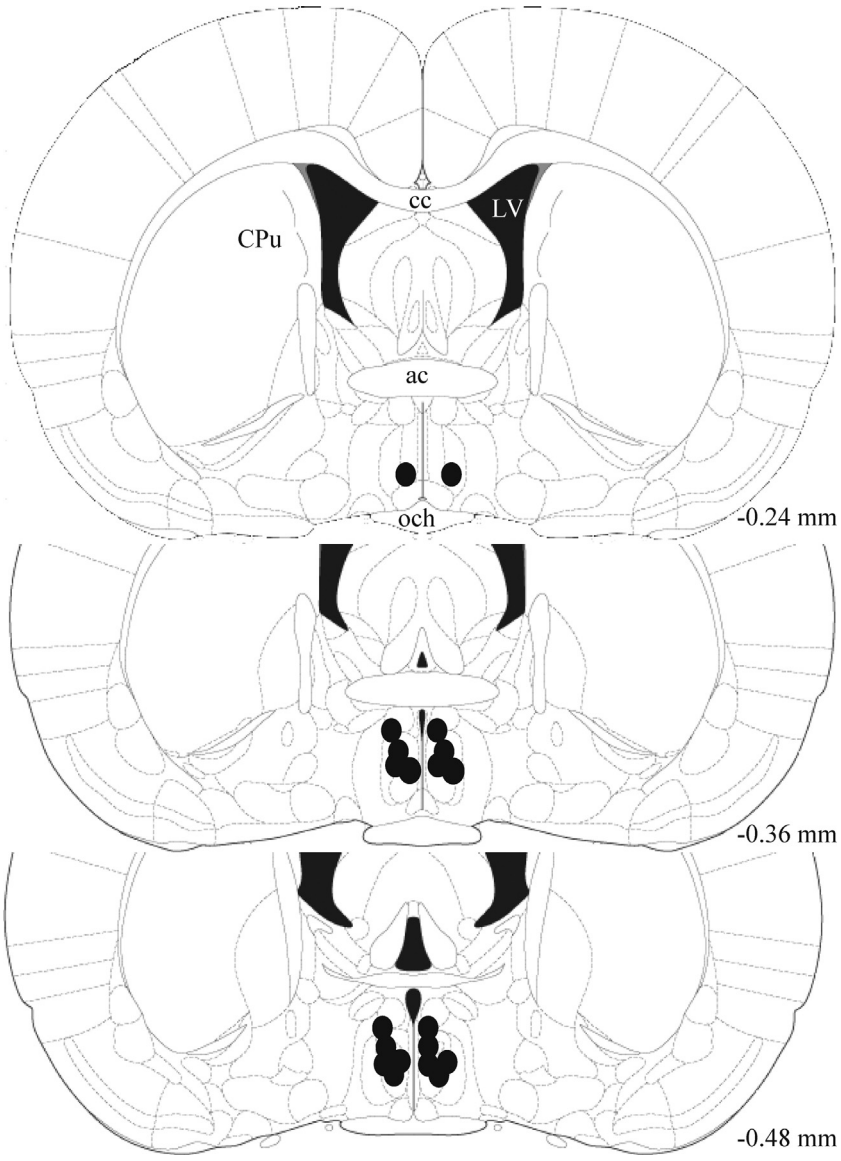

Fig. 1. Microinjections sites. Schematic representations of coronal sections at the level of mPOA. Black full circles indicate the microinjection sites of the MCH 100 ng group ($n = 10$); bottom numbers indicate distance from bregma. Plates were taken from the atlas of Paxinos and Watson [33]. ac, anterior commisure; cc, corpus callosum; CPu, caudate putamen; LV, lateral ventricle; och, optic chiasm.

Results

Sites of injection

All microinjections were located within the mPOA between −0.24 and −0.48 mm from bregma, based on examination of cannula tracks in histological sections [33]. Three animals had misplaced cannulas and were excluded from subsequent data analysis, resulting in a total of eighteen animals being included in the study. Fig. 1 shows the microinjection sites of the MCH 100 ng group ($n = 10$). The location of the microinjection sites had similar distribution in the MCH 50 ng group ($n = 8$).

Effect of MCH on maternal behavior

Infusion of the low dose of MCH (50 ng) into the mPOA significantly increased the latency to group the entire litter into the nest ($T_8 = 3.0$, $p = 0.035$, Table 1). All other measures of maternal behavior were not affected by this dose of MCH (Table 1 and Fig. 2).

Microinjections of 100 ng MCH into the mPOA decreased the active components of maternal behavior, including the number of retrievals (Saline = 8.5 ± 1.6; $MCH_{100} = 5.5 \pm 2.3$; $T_{10} = 7.0$, $p = 0.036$), mouthings (Saline = 5.5 ± 3.5; $MCH_{100} = 3.0 \pm 1.2$; $T_{10} = 6.0$, $p = 0.050$), and nest building (Saline = 23.0 ± 6.3; $MCH_{100} = 3.5 \pm 1.3$; $T_{10} = 1.5$, $p = 0.008$), as compared to saline microinjections (Fig. 2). Also, the latency to group the entire litter into the nest following administration of the high dose of MCH tended to be higher than that following vehicle but the difference did not reach significance ($T_{10} = 10.0$, $p = 0.074$). In contrast, huddling/nursing behaviors were not affected by MCH 100 ng microinjections (p = ns, Table 1).

Non-maternal behaviors, including self-grooming, drinking and eating were not affected by any of the MCH doses used (data not shown).

Locomotor activity and anxiety-like behavior

No significant differences were found in any locomotor parameter examined between MCH_{100} and vehicle-treated groups

Table 1
Latencies and durations of maternal behaviors of postpartum female rats following either MCH (50 and 100 ng/side) or saline treatment.

	Saline	MCH 50 ng	Saline	MCH 100 ng
Active behaviors				
Latency (*s*)				
First retrieval	13.5 ± 3.8	23.5 ± 56.1	22.5 ± 55.6	14.0 ± 10.0
Reunion litter	246 ± 172.2	1800.0 ± 834.9*	181.0 ± 135.3	1800.0 ± 843.7
Huddling and nursing behaviors				
Latency (*s*)				
Hover over	189.0 ± 46.0	238.5 ± 320.0	243.0 ± 227.5	321.5 ± 264.0
Nursing postures	938.5 ± 178.5	1199.0 ± 178.5	1343.0 ± 371.5	900.5 ± 561.6
Duration (*s*)				
Hover over	998.5 ± 208.2	713.0 ± 150.4	934.0 ± 186.6	570.5 ± 302.4
Nursing postures	688.5 ± 95.0	1195 ± 223.2	538.0 ± 209.0	377.0 ± 396.8
Time with pups	1605.5 ± 22.6	1766.5 ± 198.2	1470.0 ± 173.2	1358.0 ± 136.2

Data are expressed as median ± SIQR. Within-group comparisons were analyzed by Wilcoxon match pairs signed-ranks test; significant differences in responding relative to the vehicle infusion are indicated by asterisks;
* $p < 0.05$.

(Table 2). There was no effect of intra-mPOA MCH administration on anxiety-like behavior in the EPM; MCH- and vehicle-treated mothers spent a similar amount of time in the open arms and made a similar number of entries into the open arms of the EPM (all ps = ns; Table 2).

Discussion

The present study provides the first evidence suggesting an inhibitory role for MCH in the mPOA in the expression of maternal behavior. Specifically, results show that intra-mPOA microinjections of MCH selectively decreased the expression of active maternal behaviors in early postpartum female rats, to levels characteristic of the late postpartum period [37], when endogenous levels of MCH in the mPOA are naturally high [40]. Importantly, the effects of the intra-mPOA administration of MCH were specific to maternal behavior, as other behaviors, including locomotor activity, exploration, and anxiety-like behavior, were not affected by this neuropeptide. Viewed collectively, the evidence favors the idea that modulation of mPOA function by MCH may contribute to the waning of maternal responsiveness, characteristic of the late postpartum period.

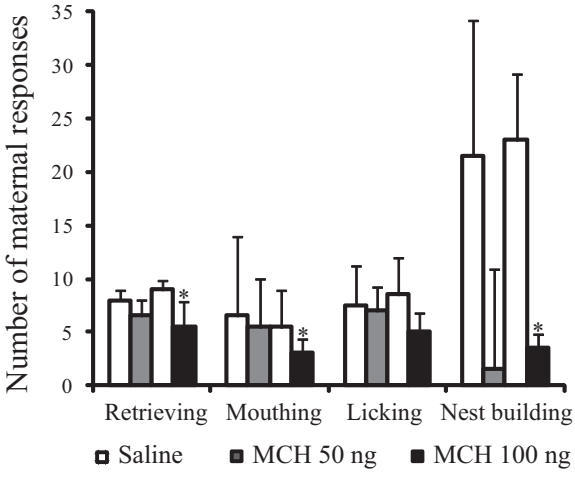

Fig. 2. Effect of MCH microinjections into the mPOA on active components of maternal behavior. The chart shows the number of different active maternal responses of postpartum female rats following bilateral administration of either saline or MCH (50 and 100 ng/side) over a 30 min maternal test. Within-group comparisons were analyzed with Wilcoxon matched-pair signed-ranks test; significant differences are indicated by asterisks (* $p < 0.05$).

One previous research demonstrated that postpartum MCH knockout mice exhibit increased cannibalism of their pups over the first 3 postpartum days [1]. This increased pup mortality was concluded to be secondary to the olfactory defects caused by a lack of MCH. Olfaction is essential for maternal behavior in mice, and interference with the function of the olfactory bulb, following bulbectomy or chemical lesions, has been shown to similarly impact maternal behavior in female mice, leading to high levels of cannibalism [10,54]. Beyond the third postpartum day, however, survival and weights of MCH knockout and wild type pups over the next 14 days were not different. Notably, this report did not include direct observations of maternal behavior, and therefore it is not known whether deletion of the MCH gene may have impacted expression of maternal behavior.

Administration of MCH into the mPOA, a region critically involved in maternal behavior [26], selectively and severely disrupted many active components of maternal behavior, while leaving huddling and nursing behaviors relatively intact. The amount of time MCH-treated mothers remained with their pups was normal, indicating that they remained interested in approaching the pups and maintaining physical contact with them. Moreover, MCH-treated mothers showed similar levels of locomotor activity, exploration and anxiety-like behavior relative to controls. These results are consistent with a substantial body of evidence showing a selective involvement of the mPOA in motivational aspects of maternal behavior [2,16,22,51], and suggest that the primary effect of MCH was specific to maternal behavior via its direct action on mPOA function, and not caused by nonspecific sensory, motor and/or affective alterations elicited by this neuropeptide.

MCH expression is transiently up-regulated at the mRNA and peptide level in the mPOA of postpartum females, but not in the

Table 2
Locomotor activity and elevated plus maze. Effect of bilateral microinjections of MCH (100 ng/side) into the mPOA on locomotor activity and elevated plus maze behavior.

Parameters	Saline	MCH
Locomotor activity (*counts/5 min*)		
Crossings	47.0 ± 11.5	56.5 ± 10.0
Rearings	22.0 ± 4.5	24.0 ± 6.0
Self grooming	3.0 ± 0.5	2.5 ± 2.1
Elevated plus maze		
% Entries in open arms	45.0 ± 12.3	50.0 ± 7.9
% Time spent in open arms	18.5 ± 15.0	14.3 ± 12.9
% Entries in closed arms	55.0 ± 12.3	50.0 ± 7.9

All data are expressed as median ± SIQR. Group comparisons were analyzed with the Mann–Whitney *U* test.

lateral hypothalamus and incerto-hypothalamic area. Given this region-specific increase in MCH gene expression [17], coupled with the numerous cells labeled for ppMCH mRNA and MCH immunoreactivity in the postpartum mPOA [40], it is likely that MCH is produced locally in mPOA neurons (and not transported to the mPOA for release). Importantly, the majority of these neurons expressing ppMCH mRNA in the mPOA also co-express glutamate decarboxylase (GAD)-67 mRNA, the enzyme that synthesizes GABA [40]. Several studies indicate that GABAergic transmission in the mPOA mediates aspects of maternal behavior through local inhibition (interneurons) and/or providing inhibitory input to various neural sites [2,20]. For instance, more than half of all neurons in the mPOA that are active during performance of maternal behavior are GABAergic [20]. In addition, activation of GABA-A receptors in the mPOA by local administration of muscimol induces significant deficits in active components of maternal behavior [2], in a manner similar to functional inactivation of the mPOA [16,25,28,37], and both outcomes are similar to those seen in the present study after intra-mPOA MCH administration. Although the present study does not provide information on how MCH affected mPOA function to produce the observed deficits in early postpartum maternal behavior, it is reasonable to speculate that one possible mechanism of elevated MCH in the mPOA toward the end of lactation might be to inhibit maternal behavior by depressing the output of the mPOA that is important for maternal behavior, including activity of GABAergic efferent projection neurons.

Several studies indicate that the mPOA mediates motivational aspects of maternal behavior through its interaction with components of the mesolimbic dopamine system, including the ventral tegmental area (VTA) and the nucleus accumbens (NA) [31,47]. Specifically, there is a significant amount of evidence indicating mPOA inhibition of dopamine function participates in activating maternal behavior [12,22,43,48]. MCH expression progressively increases in the postpartum mPOA, reaching its maximal expression at the end of lactation, when maternal responsiveness has waned [40]. Since MCH exerts pre- and postsynaptic inhibitory effects on cell firing [11], it is possible that MCH inhibits maternal behavior by depressing the mPOA GABAergic projections to NA [2,20], and/or mPOA excitatory projections to VTA, which contains dopamine neurons projecting to the NA [30,44]. This latter possibility would result in attenuation of the magnitude of the increase in NA dopamine release during late postpartum mother-pup interactions, which is highly consistent with the decreased NA dopamine activity observed in late postpartum females relative to early postpartum ones [35]. An alternative, although not exclusive, possibility is that MCH exerts its effects at presynaptic terminals by blunting the release of a number of neurotransmitters, thus fine-tuning the final output of the mPOA [39]. The mPOA is a chemically heterogeneous structure, and many neurotransmitters and modulators are released in the postpartum mPOA to regulate expression of maternal behaviors. For example, various aspects of maternal behavior are disrupted by interference with GABAergic, dopaminergic, serotonergic or noradrenergic activity in the mPOA [2,21,22,27,32,46].

Recent research has revealed that the mPOA is differentially engaged throughout the postpartum period to orchestrate maternal responses to the changing needs of the developing pups, from a necessary facilitatory role during early postpartum period to an inhibitory role during late postpartum period [37]. Viewed together, we propose that elevated MCH in the postpartum mPOA toward the end of lactation is one mechanism contributing to the changing role of the mPOA in maternal responsiveness across the postpartum period. Future studies, including those that use MCH antagonists, are necessary to test this model and further refine our understanding of the contribution of MCH to maternal motivation.

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