European Neuropsychopharmacology (▮▮▮▮) ▮, ▮▮▮–▮▮▮

www.elsevier.com/locate/euroneuro

Inactivation of the melanin concentrating hormone system impairs maternal behavior

Amal Alachkar[a,*], Lamees Alhassen[a], Zhiwei Wang[a], Lien Wang[a], Kara Onouye[a], Nayna Sanathara[a], Olivier Civelli[a,b,c]

[a]*Departments of Pharmacology, School of Medicine, University of California, 369 Med Surge II, Irvine, CA 92697-4625, United States*
[b]*Pharmaceutical Sciences, School of Medicine, University of California, 369 Med Surge II, Irvine, CA 92697-4625, United States*
[c]*Developmental and Cell Biology, School of Medicine, University of California, 369 Med Surge II, Irvine, CA 92697-4625, United States*

Received 20 January 2016; received in revised form 5 August 2016; accepted 24 August 2016

KEYWORDS
Melanin concentrating hormone;
Maternal behavior;
Knockout;
Mice;
Antagonist

Abstract
In order to prepare the mother for the demands of pregnancy and lactation, the maternal brain is subjected to a number of adaptations. Maternal behaviors are regulated by complex neuronal interactions. Here, we show that the melanin concentrating hormone (MCH) system is an important regulator of maternal behaviors.
First, we report that melanin concentrating hormone receptor 1 knockout (MCHR1 KO) mice display a disruption of maternal behavior. Early postpartum MCHR1 KO females exhibit poor nesting, deficits in pup retrieval and maternal aggression. In addition, ablation of MCH receptors results in decreased milk production and prolactin mRNA levels. Then we show that these results are in line with those obtained in wild type mice (WT) treated with the specific MCHR1 antagonist GW803430. Furthermore, following pups retrieval, MCHR1 KO mice display a lower level of Fos expression than WT mice in the ventral tegmental area, and nucleus accumbens. With the progression of the lactation period, however, the MCHR1 KO mice improve maternal care towards their pups. This is manifested by an increase in the pups' survival rate and the decrease in pups' retrieval time beyond the second day after parturition.
In conclusion, we show that the MCH system plays a significant role in the initiation of maternal behavior. In this context, MCH may play a role in integrating information from multiple sources, and connecting brain reward, homeostatic and regulatory systems.

*Correspondence to: Department of Pharmacology University of California, Irvine 355 Med Surge II Irvine CA, 92697-4625, United States.
E-mail address: aalachka@uci.edu (A. Alachkar).

http://dx.doi.org/10.1016/j.euroneuro.2016.08.014
0924-977X/

Please cite this article as: Alachkar, A., et al., Inactivation of the melanin concentrating hormone system impairs maternal behavior. European Neuropsychopharmacology (2016), http://dx.doi.org/10.1016/j.euroneuro.2016.08.014

1. Introduction

There have been separate lines of evidence that support a role of melanin concentrating hormone (MCH) system in various aspects of maternal behavior (Adams et al., 2011; Benedetto et al., 2014).

The anatomical connections and physiological functions of the melanin concentrating hormone (MCH) system are at the basis of its role in regulating maternal behavior. MCH is a hypothalamic neuropeptide that is released by neurons in the lateral hypothalamus and incerto-hypothalamic area (Bittencourt et al., 1992; Sita et al., 2007) and acts through a G protein-coupled receptor, MCHR1 (Chambers et al., 1999; Saito et al., 1999, 2000). The MCHergic neurons project widely throughout the brain. The best-characterized function of MCH system is its regulation of energy homeostasis and food intake (Qu et al., 1996; Rossi et al., 1997). The MCH system, however, regulates other diverse physiological functions such as sleep, stress, anxiety, mood, aggression, and cognition (Blouin and Siegel, 2013; Chung et al., 2011; Fraigne and Peever, 2013; Roy et al., 2006, 2007).

There is a strong anatomical connection between MCH neurons and the maternal neuronal circuit including the medial preoptic area (mPOA) and oxytocin neurons in the paraventricular nucleus (PVN), ventral tegmental area (VTA), nucleus accumbens-shell (NAc-sh), olfactory bulb, lateral septum (LS), and amygdala (Bittencourt et al., 1992; Hervieu et al., 2000; Numan, 2012; Saito et al., 2001) Oxytocin and vasopressin have been shown to excite hypothalamic MCH neurons but not other LH GABAergic neurons (Yao et al., 2012). This suggests that MCH neurons may mediate or modulate some of the oxytocin and vasopressin actions such as maternal behavior. MCHR1 expression in the medial amygdala has been implicated for an important role in maternal aggression towards an unfamiliar male intruder (Niu et al., 2012).

That the MCH system may be involved in maternal behavior is supported by several observations. First, high mortality and cannibalism rate were observed among offspring of MCH KO mothers, an indication of poor mothering (Adams et al., 2011).

Then, the mPOA, which does not express MCH in male or non-lactating female rats, displays mRNA expression and peptide synthesis only during late lactation stage (Knollema et al., 1992; Rondini et al., 2010). The MCH synthesis in mPOA neurons gradually increases during the lactation period, reaching its maximal levels at the end of this period during the weaning stage (Knollema et al., 1992; Rondini et al., 2010).

Finally, Benedetto et al. (2014) have shown that when injected into the medial preoptic area (mPOA) of early postpartum females, MCH inhibits the active components of maternal behavior (Benedetto et al., 2014), suggesting an inhibitory role for the MCH in the mPOA on maternal behavior.

In order to further understand the functional significance of MCH, we study the effects that the genetic deletion of MCHR1 and the pharmacological blockade of MCHR1 have in regulating maternal behavior.

2. Experimental procedures

2.1. Animals and experimental design

Eight-eleven week-old female MCHR1 KO mice (n=48) and background matched wild type B6NTac (n=55) were used.

For the maternal behavior assays, female WT and MCHR1 KO mice were allowed to mate with genotype matched male mice for a period of 3 days. Following this mating period male mice were removed from the cage and the female mice were subsequently monitored daily for signs of pregnancy by visual examination and weight measurements. The date of birth of pups was considered postpartum day 0 (PPD0). We studied the role of the MCH system on nest building, pups' retrieval and maternal aggression in the early period of lactation because this is the time when these behaviors are expressed at their highest levels as described in previous studies (Hennessy et al., 1980; Pedersen et al., 2006; Sato et al., 2010; Thomas and Palmiter, 1997).

Maternal behavior tests (nest building, pups retrieval and maternal aggression) were carried out on the same animals in a sequence: nest building on PPD1, pups retrieval on PPD1–PPD3, and maternal aggression on PPD7. Milk production measurement was carried out on animals that were not tested in maternal behavior tests. Milk production test was carried out on PPD9–PPD11 because it relies on measuring the changes in the pups' daily body weights, which increase with the growth of the pups (Nagai, 1971; Roepke et al., 2009; Sampson and Jansen, 1984).

All experimental procedures were approved by the Institutional Animal Care and Use Committee of University of California, Irvine and were performed in compliance with national and institutional guidelines for the care and use of laboratory animals.

2.2. Drugs

The MCHR1 antagonist GW803430 (gift from Dr. Donald R. Gehlert, Eli Lilly) was dissolved in 2% Tween 80 solution. The effects of GW803430 were studied on two assays: pups' retrieval and milk production. Doses and time of administration of GW803430 were selected based on previous study (Cippitelli et al., 2010). Where GW803430 was used, the control group received the vehicle (2% Tween 80 solution) at a volume of 10 μl/gr of mouse weight.

2.3. Maternal behaviors in post-partum mice

Pup mortality between PPD0–PPD3 was presented as the survival percentage of initial litter size, 149–162 pups of 19–22 dams. Both wild type and knockout mothers were observed for instances of cannibalism.

The following maternal behaviors were assessed by observers who are blinded to the condition of the experimental subjects.

2.3.1. Nest building

The quality of the nest building was scored on PPD1 using a 5-point nest-rating scale (Deacon, 2006). Cages were changed once per week and contained 3 fresh nestlets to ensure proper nest building.

2.3.2. Pup retrieval

The retrieval behavior test measured the mother's latency to retrieve the first pup and the total time required to retrieve 3 pups daily for 3 consecutive days. Pup retrieval test was performed at PPD1, PPD2, and PPD3. All tests were videotaped for a total time of 5 min and analyzed. The mother was removed for one minute from her cage and her pups were removed from the nest. Three pups were placed in each corner of the cage. The female was returned

Please cite this article as: Alachkar, A., et al., Inactivation of the melanin concentrating hormone system impairs maternal behavior. European Neuropsychopharmacology (2016), http://dx.doi.org/10.1016/j.euroneuro.2016.08.014