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Thalamic neuropeptide mediating the effects of nursing on lactation and maternal motivation

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Summary Nursing has important physiological and psychological consequences on mothers during the postpartum period. Tuberoinfundibular peptide of 39 residues (TIP39) may contribute to its effects on prolactin release and maternal motivation. Since TIP39-containing fibers and the receptor for TIP39, the parathyroid hormone 2 receptor (PTH2 receptor) are abundant in the arcuate nucleus and the medial preoptic area, we antagonized TIP39 action locally to reveal its actions. Mediobasal hypothalamic injection of a virus encoding an antagonist of the PTH2 receptor markedly decreased basal serum prolactin levels and the suckling-induced prolactin release. In contrast, injecting this virus into the preoptic area had no effect on prolactin levels, but did dampen maternal motivation, judged by reduced time in a pup-associated cage during a place preference test. In support of an effect of TIP39 on maternal motivation, we observed that TIP39 containing fibers and terminals had the same distribution within the preoptic area as neurons expressing Fos in response to suckling. Furthermore, TIP39 terminals closely apposed the plasma membrane of 82% of Fos-ir neurons. Retrograde tracer injected into the arcuate nucleus and the medial preoptic area labeled TIP39 neurons in the posterior intralaminar complex of the thalamus (PIL), indicating that these cells but not other groups of TIP39 neurons project to these

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hypothalamic regions. We also found that TIP39 mRNA levels in the PIL markedly increased around parturition and remained elevated throughout the lactation period, demonstrating the availability of the peptide in postpartum mothers. Furthermore, suckling, but not pup exposure without physical contact, increased Fos expression by PIL TIP39 neurons. These results indicate that suckling activates TIP39 neurons in the PIL that affect prolactin release and maternal motivation *via* projections to the arcuate nucleus and the preoptic area, respectively.

1. Introduction

Nursing plays a pivotal role in control of mothers motivation and lactation (Numan et al., 2006). Rat dams that ignore or even hurt pups without maternal sensitization, for instance, vigorously protect them after giving birth (Brunton and Russell, 2008). These abrupt shifts in motivation are also accompanied by metabolic and endocrine adaptations necessary for milk production (Russell et al., 2001; Woodside, 2007). To support lactation, prolactin levels increase enormously in rat dams, and pup suckling is an important stimulus for this (Neville, 2006). While oxytocin (Bosch and Neumann, 2012) and possibly prolactin, too, contribute to maternal motivation (Grattan et al., 2008), suckling can also directly activate specific neuronal pathways to brain centers for maternal behavior (Stern and Lonstein, 2001; Brunton and Russell, 2008). Bilateral lesion of the hypothalamic preoptic area, or the combination of a unilateral lesion with a coronal transection posterior to the preoptic area on the contralateral side of the brain lead to the cessation of maternal care (Olazabal et al., 2002; Numan and Woodside, 2010). Thus, while dopaminergic cells residing in the arcuate nucleus are responsible for suckling-induced prolactin release (Freeman et al., 2000), maternal behaviors are largely regulated by the preoptic area. Although it is known that ascending pathways regulate hypothalamic maternal centers, it has yet to be shown how information about suckling reaches the hypothalamus, and which neurotransmitters are involved in this information transfer.

In earlier studies, we identified a neuropeptide that we named '*tuberoinfundibular peptide of 39 residues*' (TIP39) based on its abundance and that of its receptor, the parathyroid hormone 2 (PTH2) receptor, in the mediobasal hypothalamus (Usdin et al., 1999; Dobolyi et al., 2010). TIP39 neurons are present in three brain regions, the periventricular gray and the '*posterior intralaminar complex*' (PIL) of the thalamus and the medial paralemniscal nucleus in the lateral pons (Dobolyi et al., 2002, 2003). TIP39 levels decrease markedly in all three areas during early postnatal development (Dobolyi et al., 2006b; Brenner et al., 2008). We previously found that in postpartum day 9 dams TIP39 levels are dramatically elevated over that of non-lactating dams in the PIL and the medial paralemniscal nucleus, but not in the periventricular gray of the thalamus (Cservenak et al., 2010; Varga et al., 2012). We also observed that pup exposure induces Fos in TIP39 neurons of the PIL and the medial paralemniscal nucleus (Cservenak et al., 2010; Varga et al., 2012). In addition, the body weight of pups reared by dams lacking TIP39 signaling is reduced during the lactation period (Coutellier et al., 2011). Since TIP39 fibers and the PTH2 receptor are abundant in the preoptic area and arcuate nucleus (Faber et al., 2007), we have now addressed whether the projections of TIP39 neurons to the hypothalamus convey suckling information that regulates maternal motivation and elicits prolactin release. We previously showed that intracerebroventricular injection of a PTH2-R antagonist inhibited suckling stimulated prolactin release (Cservenak et al., 2010). In this study, to learn more about the potential roles of TIP39 during lactation and to clarify its site(s) of action, we antagonized TIP39 actions in the arcuate nucleus or in the preoptic area by means of a virus expressing an antagonist of the PTH2 receptor, and measured maternal motivation, behavior, and the prolactin release. To determine the origin of TIP39 fibers in the arcuate nucleus and the preoptic area, we injected retrograde tracer into these sites and examined the labeling of TIP39 neurons. We also evaluated the time course of TIP39 expression around and during the period of lactation. To test whether suckling itself is the specific signal that activates TIP39 neurons, we compared Fos activation in PIL TIP39 neurons of suckling mothers and mothers with only visual, auditory, and olfactory interaction with their pups.

2. Materials and methods

2.1. Animals

This study was approved by the Semmelweis University, Budapest, Animal Examination Ethical Council of the Animal Protection Advisory Board. Procedures involving rats were carried out in accordance with the Hungarian Ministry of Agriculture's Animal Hygiene and Food Control Department guidelines for experimental protocols and with EU Directive 2010/63/EU for animal experiments.

A total of 106 mother and 10 control female rats (Wistar; Charles Rivers Laboratories, Hungary) were used (12 for retrograde tracer studies, 25 for TIP39 *in situ* hybridization, 15 for Fos activation, 28 for prolactin measurement, and 26 mothers and 10 control females for the behavioral tests). All animals were 90—120 days old when sacrificed. Animals were kept under standard laboratory conditions with 12-h light, 12-h dark periods (lights on at 6.00 AM), and supplied with food and drinking water *ad libitum*. Pregnant and mother rats were housed individually in standard white cages (41 cm × 22 cm × 19 cm) or in blue cages (35 cm × 28 cm × 22 cm) during place preference conditioning. Mother rats delivered their pups on day 22 of pregnancy. Mothers who delivered fewer than 8 pups or whose pups died were excluded from the study. The number of pups was adjusted to 8 within 2 days of delivery. For surgery, perfusions, and dissections, rats were anesthetized with an intramuscular injection of anesthetic mix containing 0.2 ml/300 g body weight ketamine (100 mg/ml) and 0.2 ml/300 g body weight xylazine (20 mg/ml).