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The parathyroid hormone 2 receptor participates in physiological and behavioral alterations of mother mice

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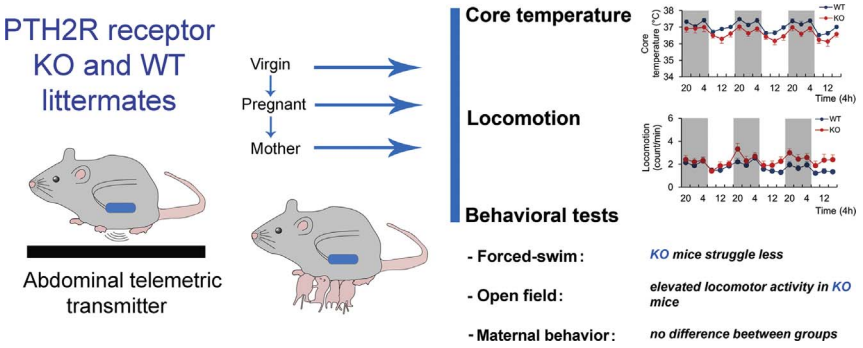
GRAPHICAL ABSTRACT



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ABSTRACT

Parathyroid hormone 2 receptor (PTH2R) and its ligand, tuberoinfundibular peptide of 39 residues (TIP39) have been implicated in the maintenance of homeostasis including body temperature in males. The system was recently shown to be activated in mothers. Therefore, we addressed some of its functions during pregnancy and lactation, comparing PTH2R-knockout (PTH2R-KO) mice with their wild type (WT) littermates. Core body temperature (T_c) was recorded via a telemetric device, anxiety was assessed in an open field, depression-like behavior in the forced-swim test while spontaneous and pup retrieval-induced maternal behavior was also observed. Nulliparous virgin KO and WT mice showed a circadian pattern of T_c and locomotion with no differences between groups. WT mothers had an increased T_c during pregnancy and lactation as compared to virgins. This peripartum elevation of T_c was present but reduced in KO mice, even though their locomotor activity was increased. The greater locomotor activity of the KO mice was confirmed in the open field test but no other behavior differences were detected in the test. The maternal behavior was similar in the 2 genotypes except that the KO mice spent more time in the nest. In turn, a marked difference was found in the forced-swim test as the KO mice spent significantly less time struggling and more time floating compared to the WT littermates. The data suggest that the PTH2R contributes to the elevated T_c and reduced depression-like behavior of mother mice.

Abbreviations: BAT, brown adipose tissue; FST, forced-swim test; KO, knockout; min, minute; OF, open field; PIL, posterior intralaminar complex of the thalamus; PND, postnatal day; POA, preoptic area; PTH2R, parathyroid hormone receptor type 2; T_c, core body temperature; TIP39, tuberoinfundibular peptide of 39 residues; WT, wild type

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These findings are consistent with specific maternal functions of the activated TIP39-PTH2R neuromodulator system in mothers.

1. Introduction

Parathyroid hormone 2 receptor (PTH2R) is a G-protein coupled receptor originally identified based on its sequence homology to the parathyroid hormone 1 receptor (PTH1R) [1]. It turned out, however, that PTH2R is most abundant in the brain and its endogenous ligand was determined to be the newly purified neuropeptide, tuberoinfundibular peptide of 39 residues (TIP39) rather than parathyroid hormone or parathyroid hormone-related peptide, ligands of the PTH1R involved in calcium homeostasis. TIP39 selectively activates the PTH2R [2], and TIP39 fibers have a distribution within the brain similar to that of the PTH2R positive neurons. Both are abundant in limbic brain regions, such as the infralimbic cortex, the lateral septum, several parts of the preoptic area (POA), the peri- paraventricular, and arcuate hypothalamic nuclei [3].

The TIP39-PTH2R system has been implicated in the regulation of body temperature. Local injection of TIP39 into the POA increased the core body temperature (T_c) of male mice by about 2 °C [4]. In turn, mice lacking the PTH2R or wild type mice injected with a PTH2R antagonist via lateral ventricle cannulae, showed a significant decrease in T_c compared to control mice in a cold environment [4]. It was concluded that sympathetic brown adipose tissue (BAT) activation was not appropriately regulated in the absence of PTH2R activation.

Apart from the above described homeostatic regulation, activation of the PTH2R by intracerebroventricular TIP39 injection in male rats resulted in anxiolytic-like, and antidepressant-like effects in the elevated plus maze and forced-swim test (FST), respectively [5]. Furthermore, the lack of the PTH2R in male mice increased anxiety-related behaviors following stress-inducing environmental perturbation [6].

Other lines of evidence suggested specific functions of the TIP39-PTH2R system in mothers [7]. TIP39 mRNA and peptide levels were markedly elevated in the posterior intralaminar complex of the thalamus (PIL) in lactating mother rats as compared to nulliparous control females [8]. In addition, TIP39 neurons in the PIL showed c-fos activation in response to suckling in rats and were shown to project to the medial preoptic area [9].

In mice and rats, maternal T_c is elevated throughout lactation [10–12]. The lactational hyperthermia occurs despite the animals' activity level being reduced, and is unrelated to maternal food intake and litter mass or size [11,12]. The elevation in T_c was suggested to result from milk production and reduced ability to dissipate heat because of contact with pups [13]. However, the latter possibility was questioned, as the maximum T_c of the mother was not the highest during nesting bouts. Rather, the T_c was greatest during exploration or nest building, suggesting that heat dissipatory issues were not the cause of lactational hyperthermia [14]. It was suggested instead that interaction with developing pups may elevate T_c by stimulating hormone secretion, as prolactin, progesterone and corticosterone have all been implicated in the heat production and retention of mothers [15,16]. The thermoregulatory pathways consist of the spinal dorsal horn, the lateral parabrachial nucleus, the preoptic area (with warm-sensitive and inhibitory output neurons), the dorsomedial hypothalamic nucleus, the premotor neurons in the rostral ventromedial medulla, and the thermal effectors. Thus, all of these brain regions represent potential targets of both hormonally and axonally mediated influences of pup-derived inputs.

Besides the physiological changes seen in rodent mothers, there are vast changes in their behavior during the period of lactation. Behavioral changes include licking, nursing, retrieving the pups to the nest, aggression toward intruders and decreased anxiety [17]. Coutellier and coworkers showed that the pups of PTH2R-knockout (PTH2R-KO) mouse dams were smaller than those of WT, and that this was due to postpartum and not pup genotype effects [18]. No differences in maternal behaviors from those of WT mice were detected. Only the anxiety-like behavior was observed to be affected by the absence of PTH2R as PTH2R-KO mothers spent less time in the open arms in the elevated zero maze test [19].

Based on the previous findings described above, we addressed the role of the PTH2R in pregnant and mother mice in thermoregulation and in their locomotor pattern by monitoring core body temperature and movement with implanted telemetric transmitters in WT and PTH2R-KO mice continuously for 2 months throughout pregnancy and lactation, as thermal physiology in these periods presents unique challenges [20]. In addition, anxiety and depression-like as well as maternal behavior of the mice was measured by open field (OF), FST and pup retrieval tests.

2. Methods

2.1. Animals

The procedures were carried out in accordance with the Hungarian Act of Animal Care and Experimentation (1998, XXVIII) and within the guidelines of the European Communities Council Directive, 2010/63/EU. All procedures were performed with attention to minimize animals' pain and discomfort. A combined 20 female (10 WT and 10 KO) C57BL/6J mice were used in the experiments. Development and genotyping of KO animals were previously described [18]. Animals were 3–6 months old when mated with males with the opposite genotype (WT mothers with KO males and KO mothers with WT males so that all pups are heterozygous) for 2 weeks, and kept under standard laboratory conditions with 12 h:12 h light:dark periods (lights on at 6.00 AM), at 21 ± 1 °C, in 50–60% humidity, supplied with food and drink ad libitum. After mating animals were separated, and the pregnant females were housed individually until the end of the experiment in standard (41 × 22 × 19 cm) cages in a separate room dedicated to the experiment. The weight of the females was monitored weekly. The litter size was adjusted to six within two days following parturition,

2.2. Measurement of core body temperature

E-mitters (STARR Life Sci Corp, Oakmont, PA) were implanted into the abdominal cavity under ketamine-xylazine anesthesia (0.1 ml/30 g body weight ketamine (20 mg/ml) and 0.1 ml/30 g body weight xylazine (4 mg/ml), Le Vet B·V, Oudewater, the Netherlands). After the implantation, the animals were put on a heat-pad, and administered antibiotics (subcutaneous Gentamicin 5 mg/kg, Sandoz GmbH, Kundl, Austria) for 3 days to avoid infectious complications. A week after the operation, female mice were mated with males. We monitored the animals' core body temperature (T_c) and locomotion with the implanted transmitters continuously for 2 months throughout pregnancy and lactation.

2.3. Analysis of core body temperature

The core body temperatures (T_c) were calculated for 3 days in nulliparous virgin, prepartum (days 15–18 of pregnancy), early lactational (postpartum days 3–6) and late lactational (postpartum days 13–16) periods as averages of four hours. We made comparisons between dark and light phases within genotypes, and also between KO