Neuroanatomy of Tuberoinfundibular Peptide 39 Related to Neuroendocrine and Behavioral Regulations

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Abstract

Tuberoinfundibular peptide of 39 residues (TIP39), also referred to as parathyroid hormone 2 (PTH2), is the endogenous ligand for the parathyroid hormone 2 receptor. TIP39 is synthesized by neurons in three small and distinct brain regions. These neurons project to discrete regions distributed throughout the brain, with highest abundance in the hypothalamus, lateral septum, medial prefrontal cortex, amygdala, periaqueductal gray, nucleus of the solitary tract, locus coeruleus, and spinal cord dorsal horn. Neurons that express the PTH2 receptor are present in each of the regions to which TIP39 neurons project. Experiments have been carried out to evaluate the potential contribution of TIP39-PTH2 receptor signaling to functions thought to be influenced by circuits in regions with high TIP39/PTH2 receptor density. Current evidence supports a role for this peptide/receptor system in multiple homeostatic responses or adaptations, including to nociceptive stimuli, changes in environmental temperature, threat, maternal function, and social awareness.

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Neuropeptide · Hypothalamus · Nociception · Maternal · Lactation · Social · Body temperature

14.1 Introduction of Tuberoinfundibular Peptide of 39 (TIP39)

The identification of TIP39, now also designated parathyroid hormone 2 (PTH2), followed from the discovery and characterization of the parathyroid hormone 2 receptor (PTH2R). The PTH2R was discovered in a screening project aimed at identifying new G-protein coupled receptors (GPCRs). At the time of this project GPCRs selectively activated by secretin, vasoactive intestinal polypeptide (VIP) and parathyroid hormone (PTH) had been identified. Their sequences were homologous and while their predicted 7-transmembrane architecture was like that of other GPCRs their amino acid sequences had little similarity to the majority of known GPCRs. The originally described large GPCR group, which includes the beta-adrenergic receptors and channel rhodopsin as prototypical members, was referred to as the rhodopsin-like receptors and now as GPCR family A. The secretin/VIP related receptors are now designated family B. A fragment of the PTH2R was identified as a novel receptor-like sequence in a collection of PCR products generated using primers designed to recognize common sequences in two regions of the secretin, VIP and PTH receptors with brain-derived cDNA libraries as templates. A full-length receptor cDNA was isolated using the PCR fragment as a probe. The novel receptor cDNA was expressed in tissue culture cells. The cells were exposed to a series of potential receptor ligands selected on the basis of similarity to peptides that specifically activated the secretin, VIP and PTH receptors. PTH, and only PTH, caused a robust increase in cAMP production in cells expressing the novel receptor. Since a receptor for PTH was already characterized the new receptor was designated the PTH2 receptor (Usdin et al. 1995).

Several observations lead to the idea that PTH was not in fact the endogenous physiological ligand for the PTH2 receptor and to a search for that ligand. First, as described in detail below, the PTH2 receptor is abundantly expressed by neurons within the brain (Usdin et al. 1996) while attempts to detect PTH in the brain were unsuccessful. Second, while PTH is a potent activator of the human PTH2 receptor, PTH has very low potency on the rat PTH2 receptor. And third, when compared to an activity in crude hypothalamic extracts that was immunologically distinct from PTH, PTH based peptides were weak partial agonists at the rat PTH2 receptor (Usdin 1997). Using selective stimulation of cAMP production in cells that expressed the cloned PTH2 receptor as an assay a peptide was chromatographically purified from bovine hypothalamus. The sequence of this peptide was determined, and it was chemically synthesized and determined to have a pharmacological profile consistent with that of a physiological ligand for the PTH2 receptor (Usdin et al. 1999), which includes potent activation of the PTH2 receptor from multiple species and little efficacy at the PTH1 or other receptors (Fig. 14.1). The peptide was initially called

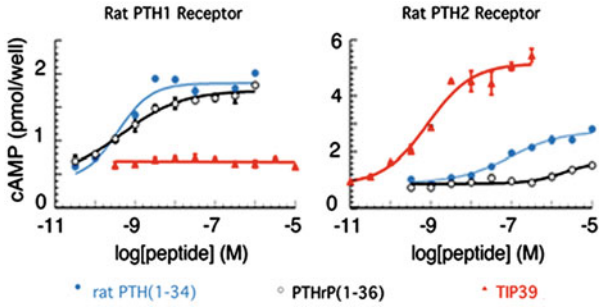

Fig. 14.1 Activation of rat parathyroid hormone 1 (PTH1) and PTH2 receptors. cAMP accumulation is shown in relation to increasing concentrations of PTH, PTH-related peptide, and tuberoinfundibular peptide of 39 residues (TIP39) in COS7 cells expressing the rat PTH1R (A) and the rat PTH2R (B), respectively. The figure was taken from our previous publication (Dobolyi et al. 2012)

Tuberoinfundibular Peptide of 39 Residues (TIP39) and is referred to as this in the original series of publications characterizing its distribution and effects. An international committee on nomenclature subsequently designated it as PTH2 on the basis of its role as a ligand for the PTH2 receptor, which is how it is referred to in databanks (UniGene at http://www.ncbi.nlm.nih.gov/sites/entrez, Mm.207078 for the mouse and Hs.339845 for the human gene) and more recent publications. However, this name is also used for a second form of PTH found in fish that more closely resembles mammalian PTH than does TIP39 (Gensure et al. 2004).

Box 14.1 The Parathyroid Hormone Peptide Family

Mature TIP39 is a secreted peptide of 39 amino acid residues. It is processed from a precursor of approximately 100 residues (depending upon the species). TIP39 is a member of a small peptide family composed of parathyroid hormone (PTH), parathyroid hormone-related peptide (PTHrP), and TIP39 (Usdin et al. 1999). Mature PTH and PTHrP are also polypeptides of about 100 residues. They are products of separate genes but they activate the parathyroid hormone 1 receptor (PTH1 receptor) with equal potency (Gensure and Juppner 2005). Their first 34 or 36 residues are sufficient for high-affinity binding and full efficacy at the PTH1 receptor, and they share twelve of these amino acids (Gillespie and Martin 1994). TIP39 contains only four of the residues that are common to PTH(1–34) and PTHrP(1–36), as well as several additional similar residues (Usdin et al. 1999). However, TIP39 has a backbone structure that can be nearly superimposed on that of PTH (Piserchio et al. 2000). The three peptides of the parathyroid hormone peptide family are members of a larger family that also includes secretin, VIP, calcitonin, gastric

(continued)

Box 14.1 (continued)
inhibitory polypeptide, growth hormone-releasing hormone, pituitary adenylate cyclase-activating polypeptide, and glucagon.

In cells that express the receptors for TIP39 (Goold et al. 2001; Della Penna et al. 2003) and related peptides, both the production of cyclic AMP (cAMP) and an increase in cytoplasmic calcium concentration through a phospholipase C/protein kinase C mechanism via G-protein (Gs and Gq) dependent mechanisms have been demonstrated. The current understanding of TIP39's physiological role is described in the text. PTH and PTHrP act on the same PTH1 receptor through which they are critical regulators of calcium homeostasis and skeletal development and growth, respectively (Rizzoli et al. 1992; Martin et al. 1997).

14.2 Neuroanatomy of TIP39 Cell Groups

14.2.1 TIP39-Expressing Neurons in Adult Males

TIP39 neurons have a highly restricted localization, first described in adult male rats. Developmental stage-dependent and sexually dimorphic expression patterns (Dobolyi et al. 2010 are described in later sections. TIP39 mRNA-expressing neurons and TIP39-immunolabeled neurons had the same distribution pattern: they are present in two brain sites in adult male rats (Dobolyi et al. 2002, 2003b), the medial paralemniscal nucleus (MPL) in the lateral pons and the periventricular gray of the thalamus (PVG) (Fig. 14.2). The latter area may also be referred to as the subparafascicular area because it includes the subparafascicular thalamic nucleus.

Periventricular thalamic TIP39 neurons constitute the largest TIP39 cell group in the brain of young adult rats and mice (Dobolyi et al. 2003b; Faber et al. 2007). TIP39 neurons in the PVG appear rostrally ventral to the central median nucleus of the thalamus, dorsal to the posterior hypothalamic nucleus, and medial to the parvicellular ventral posterior nucleus of the thalamus and mostly medial to the magnocellular subparafascicular nucleus. Additional TIP39 neurons are situated more laterally, ventral to the fasciculus retroflexus. Caudally, TIP39 cells disappear as the PVG becomes the periaqueductal gray of the midbrain at the level of the posterior commissure. In sagittal sections, the distribution of periventricular TIP39 neurons has a sigmoid shape with a rostroventral to posterodorsal orientation, which means that a few TIP39-positive neurons appear in the rostroventral part of the cell group, the density of cells increases postero-dorsally, and finally, only few TIP39 neurons are present in the postero-dorsal extension of the cell group. TIP39 neurons in the PVG are intermingled with tyrosine hydroxylase (TH)-containing neurons corresponding to the A11 dopaminergic cell group. However, no TIP39/tyrosine hydroxylase double-labeled cells were detected in the area (Dobolyi et al. 2010).

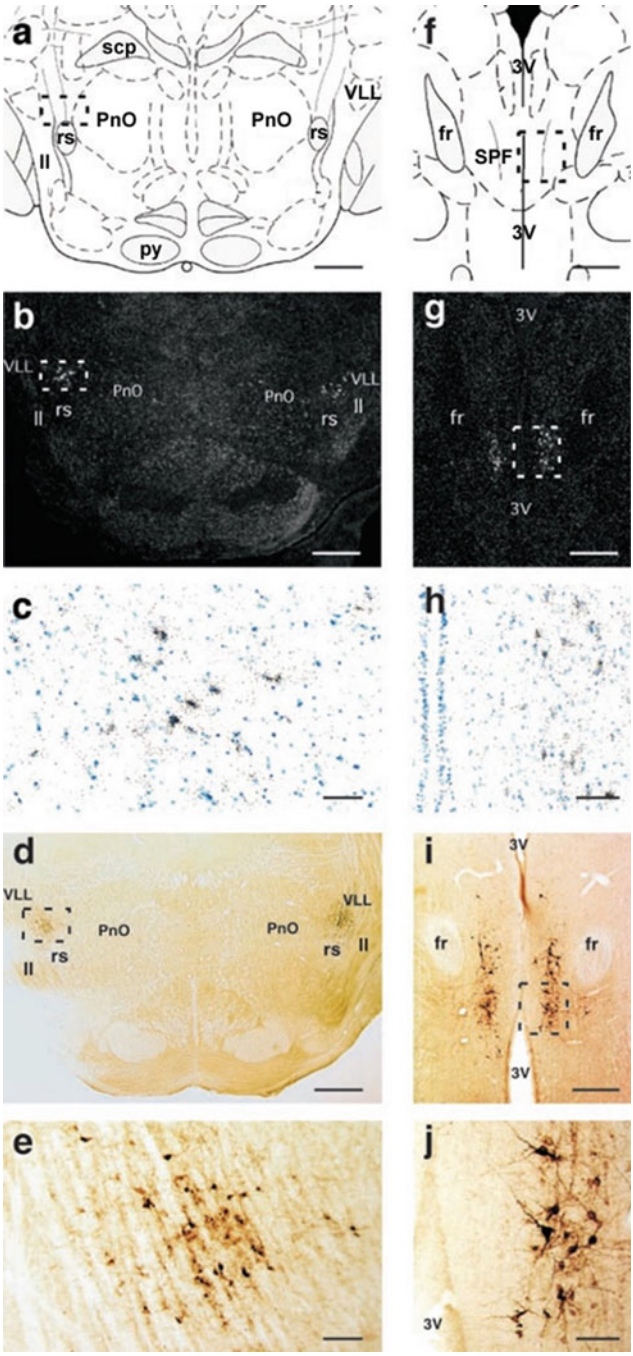

Fig. 14.2 Brain localization of TIP39-containing cells. (**a–e**) medial paralemniscal nucleus at 8.7 mm from the bregma level. (**f–j**) periventricular gray of the thalamus at 4.4 mm from the bregma. Drawings indicate the location of the micrographs (**a**, **f**). The localization of TIP39 mRNA detected by in situ hybridization histochemistry is shown at low magnification in dark-field

Cells of the MPL are distinguished from those in adjacent areas by their organization into dorsolaterally oriented cell columns separated by 20–50-μm wide cell-free zones, probably occupied by fibers of the lateral lemniscus that pass through the region (Varga et al. 2008). Thus, the cone-shaped structure of the MPL can be cytoarchitectonically distinguished from adjacent brain regions in the lateral pontomesencephalic tegmentum. The ventral border of the MPL is the rubrospinal tract. Lateral to the rubrospinal tract, the MPL extends somewhat ventrally, which gives the nucleus a triangular shape with ventral, dorsal, and medial angles. Medially, the MPL borders on the oral part of the pontine reticular formation and the pedunculopontine tegmental nucleus. The MPL narrows dorsally between the caudal part of the pedunculopontine tegmental nucleus and the dorsal nucleus of the lateral lemniscus, giving the nucleus a cone shape. The lateral border of the MPL is the intermediate nucleus of the lateral lemniscus. The caudal borders of the MPL are the region of the A7 noradrenaline cell group medially and the Kölliker-Fuse nucleus laterally. Apart from the cytoarchitectonic differences, a distinct MPL is also supported by its distinct afferent connections. The term "medial paralemniscal nucleus" was introduced by studies on TIP39 in the area (Dobolyi et al. 2003b), and has been adopted by the widely used Paxinos rat brain atlas (Paxinos and Watson 2005).

14.2.2 Identification of a Third Group of TIP39 Neurons, the Posterior Intralaminar Complex of the Thalamus (PIL)

TIP39 expression was investigated during ontogeny, which allowed the identification of a third group of cells in the posterior intralaminar complex of the thalamus (Dobolyi et al. 2006b; Brenner et al. 2008). TIP39 neurons are abundant in this brain region by embryonic day 16.5, and disappear by postnatal day 5 (Fig. 14.3a). This is in sharp contrast to the development of TIP39 neurons in the other thalamic brain region, the periventricular gray of the thalamus, where TIP39 immunoreactivity appears only in the early postnatal period (Fig. 14.3b), even though TIP39 levels also decrease in the PVG and MPL during the period of pubertal development (Dobolyi et al. 2006b). TIP39 neurons in the PIL are located in the posterior intralaminar thalamic nucleus and some adjacent brain areas including the parvicellular subparafascicular nucleus, and the lateral territory of the caudal zona incerta (Cservenak et al. 2010), which together was called as the posterior intralaminar complex of the thalamus (PIL). Since there was another neuropeptide, calcitonin gene-related neuropeptide (CGRP), with similar localization of cell bodies

Fig. 14.2 (continued) micrographs (**b**, **g**) and at greater magnification of the framed areas in bright field (**c**, **h**). The localization of TIP39 protein is demonstrated by peroxidase immunocytochemistry in colchicine-treated animals, shown at low magnification (**d**, **i**) and at greater magnification of the framed areas (**e**, **j**). Scale bars = 1 mm for **a**, **b** and **d**, 100 μm for **c**, **e**, **h**, **j**, and 500 μm for **g**, **h**, and **i**. The figure is taken from our previous publication (Dobolyi et al. 2002)

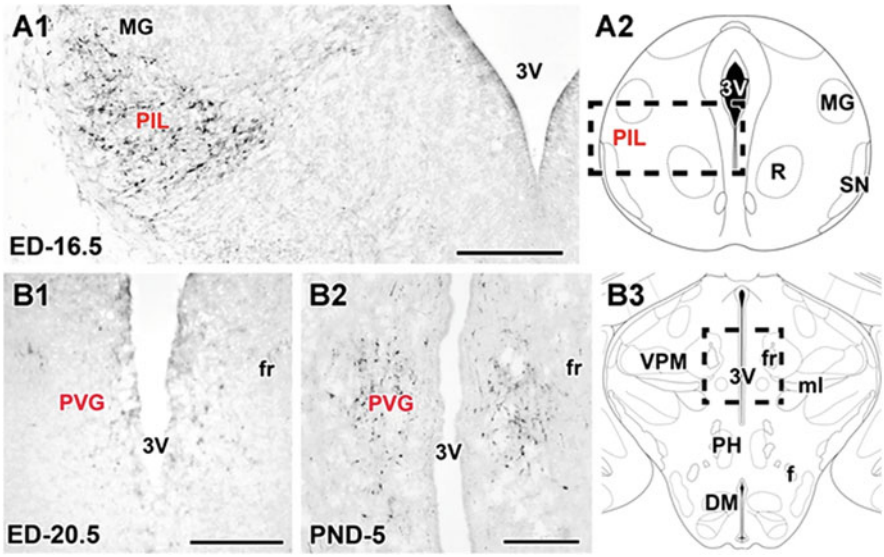

Fig. 14.3 TIP39 neurons in the posterior intralaminar complex of the thalamus at embryonic day (ED)-16.5. A1: TIP39-ir neurons are abundant by ED-16.5 in the posterior intralaminar complex of the thalamus. A2: A drawing of a coronal brain section at ED-16.5 (Paxinos et al. 1991). The framed area corresponds to panels A. B1: Only a few TIP39-ir neurons are faintly labeled in the periventricular gray of thalamus (PVG) between the third ventricle (3 V) and the fasciculus retroflexus (fr) at ED-20.5. B2: TIP39-ir neurons are distinctly labeled in the PVG at PND-5. B3: A drawing of a coronal brain section (Paxinos et al. 1991) indicates the position of the PVG at PND-1. The framed area corresponds to panels B1 and B2. Scale bars = 200 μm for A1, 250 μm for B1, and 300 μm for B2. The panels are selected from different figures of our previous publication (Brenner et al. 2008)

as TIP39, double labeling of the two peptides was performed, which showed no double-labeled cells in the PIL (Dobolyi et al. 2005). Rather, CGRP cells are located immediately lateral to the TIP39 cell group (Brenner et al. 2008).

In seeking to improve demarcation of the posterior intralaminar complex, the distribution of calcium-binding proteins was investigated in and around the PIL area in mother rats (Cservenak et al. 2017b). Parvalbumin-immunoreactive (PV-ir) neurons had low density throughout the PIL, peripeduncular area, and the triangular subdivision of the posterior thalamic nucleus. The distribution of calbindin (CB) immunoreactivity contrasted sharply with that of PV-ir. While the density of CB-ir cell bodies was low in most brain regions adjacent to the PIL, it was high in both the PIL and in the PIL's immediate dorsal neighbor, the triangular subdivision of the posterior thalamic nucleus. TIP- and CB-ir cell bodies were evenly distributed within the PIL. While almost all TIP39-ir neurons contained CB immunoreactivity, the PIL also contained neurons negative for TIP39 that were positive for CB.

Although it is an exciting question, in all three brain areas expressing TIP39, whether the TIP39 neurons contain additional major neurotransmitters, this was experimentally addressed only in the PIL. A combination of TIP39 immunolabeling

with in situ hybridization histochemistry for vesicular glutamate transporter 2 and glutamic acid decarboxylase 67 suggested that the TIP39 neurons contain glutamate but not GABA as their major neurotransmitter (Cservenak et al. 2017a). The excitatory nature of PIL TIP39 neurons was also confirmed by electron microscopy as PIL TIP39 neurons formed asymmetric synapses with their targets and contained glutamate in their terminals (Cservenak et al. 2017b).

14.3 Distribution of TIP39- and PTH2 Receptor-Containing Neuronal Fibers

TIP39 fibers are abundant in a variety of limbic, endocrine, nociceptive, and auditory brain regions including the medial prefrontal cortex, the nucleus accumbens, the lateral septum, the paraventricular thalamic nucleus, the fundus striati, a variety of hypothalamic and amygdaloid areas, the periaqueductal gray, the superior and inferior colliculi, the lateral parabrachial nucleus, the locus coeruleus and subcoeruleus areas, the paraolivary nuclei, and the nucleus of the solitary tract (Dobolyi et al. 2003b). The distribution pattern of TIP39 fibers was found to be very similar to that of the PTH2 receptor (Fig. 14.4).

The distribution of TIP39-ir and PTH2 receptor-ir fibers also shows remarkable similarities within particular brain regions (Dobolyi et al. 2006a; Faber et al. 2007) In the preoptic region, for example, a high density of TIP39- and PTH2 receptor-ir fibers is present in the medial preoptic nucleus whereas a low density of TIP39- and PTH2 receptor-ir fibers is seen in other parts of the medial preoptic area and the lateral preoptic area (Fig. 14.4). In the anterior hypothalamus, a high density of TIP39- and PTH2 receptor-ir fibers is present in the parvocellular subdivisions of the paraventricular nucleus and in the periparaventricular zone. The latter is particularly conspicuous on the side of the magnocellular subdivisions of the paraventricular nucleus, which lack TIP39- and PTH2 receptor-ir fibers. A moderate density of TIP39- and PTH2 receptor-ir fibers is present in the periventricular nucleus and the anterior hypothalamic nucleus whereas only a few immunoreactive fibers can be seen in the lateral hypothalamic area and the supraoptic nucleus, and no immunoreactive fibers are present in the suprachiasmatic nucleus. In the middle part of the hypothalamus, a high density of TIP39- and PTH2 receptor-ir fibers was found in the ventrolateral subdivision of the ventromedial nucleus, and in the dorsomedial and arcuate nuclei (Dobolyi et al. 2003b, 2006a; Faber et al. 2007).

Since PTH2 receptor-containing cell bodies are not, or only faintly, immunolabeled, X-gal histochemistry in mice expressing the beta-galactosidase enzyme driven by the PTH2 receptor promoter was used to describe the distribution of PTH2 receptor-expressing neurons (Faber et al. 2007). This distribution was essentially identical to the distribution of PTH2 receptor mRNA-expressing neurons detected by in situ hybridization histochemistry described in rodents (Dobolyi et al. 2006a; Faber et al. 2007) and also in macaque (Bagó et al. 2009). Most brain regions that contain PTH2 receptor-ir fibers also contained PTH2 receptor-expressing neurons with a very similar distribution (Dobolyi et al. 2006a; Faber et al. 2007).

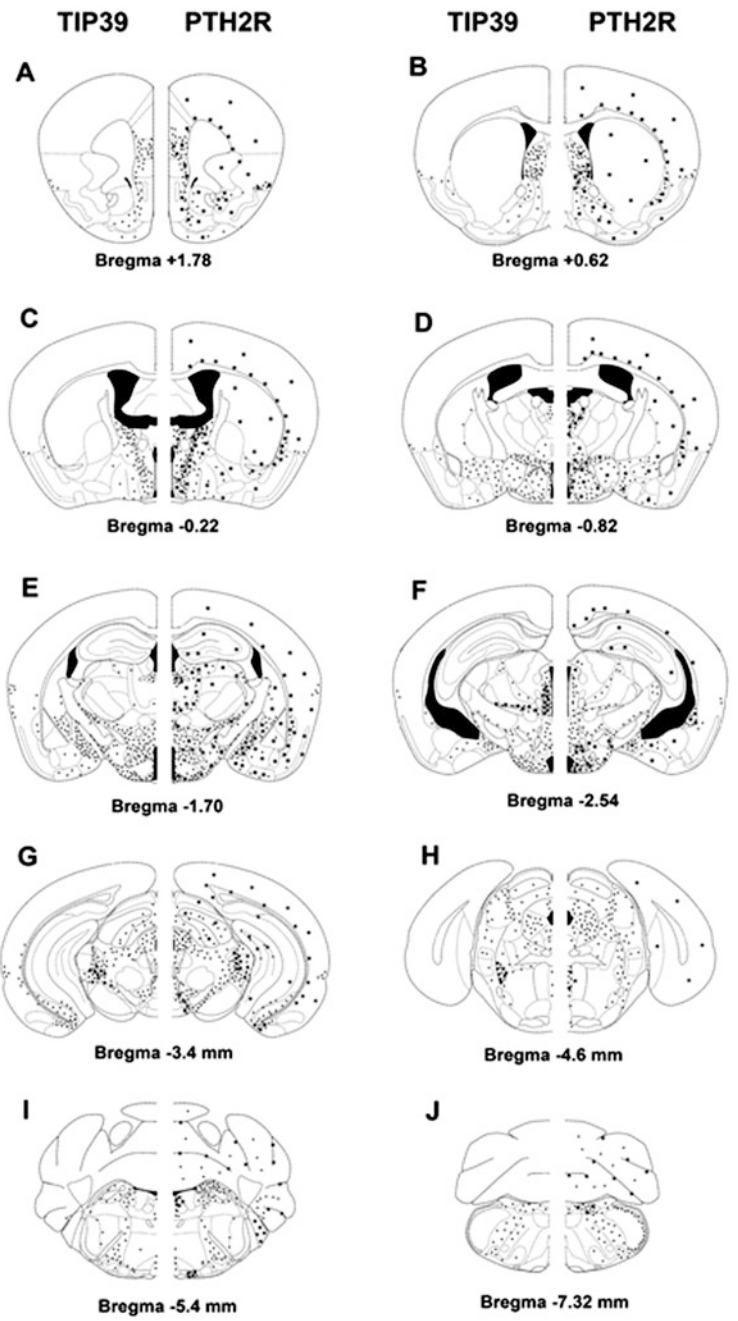

Fig. 14.4 Schematic diagrams demonstrate the distribution of TIP39 (left side) and the PTH2R (right side) in the brain of mice. The diagrams are modifications from a mouse stereotaxic atlas (Franklin and Paxinos 1997). Dots represent fibers and fiber terminals, while squares represent cell bodies. The figure is taken from our previous publication (Dobolyi et al. 2010)

For example, PTH2 receptor-expressing neurons are abundant in many regions of the hypothalamus. In the preoptic region, a high density of PTH2 receptor-expressing neurons is present in the medial preoptic nucleus whereas a low density of PTH2 receptor-expressing neurons is seen in other parts of the medial preoptic area. In the anterior hypothalamic region, a moderate density of PTH2 receptor-expressing neurons is present in the paraventricular and periventricular nuclei whereas the anterior hypothalamic nucleus contains a low density of PTH2 receptor-expressing neurons. In the middle portion of the hypothalamus, a high density of PTH2 receptor-expressing neurons is present in the arcuate nucleus whereas a moderate density was observed in the dorsomedial and perifornical hypothalamic nuclei, and some parts of the lateral hypothalamic area including the so-called far-lateral hypothalamus (Forel's field) immediately next to the internal capsule. In the posterior hypothalamus, a high density of PTH2 receptor-expressing neurons was present in the medial subdivision of the superior mammillary nucleus while its lateral subdivision, and the ventral premammillary, and the tuberomammillary nuclei contained a moderate density of PTH2 receptor-expressing neurons. In contrast, the medial and lateral nuclei of the mammillary body did not contain PTH2 receptor-expressing neurons (Dobolyi et al. 2006a; Faber et al. 2007).

14.4 Comparison of the Distribution of TIP39 to that of the PTH2 Receptor Provides Anatomical Evidence for a TIP39-PTH2 Receptor Neuromodulator System

The localization of cell bodies that express TIP39 and those that express the PTH2 receptor are profoundly different. TIP39 expression is confined to PVG, PIL, and MPL, while considerable PTH2 receptor expression is present in the infralimbic cortex, the innermost layer of other cerebral cortical areas, the basal ganglia, the lateral septum, the posteromedial part of the medial subdivision of the bed nucleus of the stria terminalis, the posterodorsal subdivision of the medial amygdaloid nuclei, the midline thalamic nuclei, the medial geniculate body, the medial preoptic, para- and periventricular, arcuate, dorsomedial, ventral premammillary, tuberomammillary, and supramammillary nuclei of the hypothalamus, and some regions of the lateral hypothalamic area, the lateral subdivisions of the interpeduncular nucleus, the sphenoid nucleus, the nucleus of the trapezoid body, and the nucleus of the solitary tract. In contrast to the profoundly different localization of TIP39- and PTH2 receptor-expressing cell bodies, the distributions of TIP39-ir and PTH2 receptor-ir fibers and cell bodies are markedly similar (Dobolyi et al. 2010). For example, the localization of TIP39 fibers is essentially the same as that of PTH2 receptor immunoreactivity at the light microscopy level in the hypothalamus (Faber et al. 2007). That means the same hypothalamic nuclei and areas contain both TIP39 and PTH2 receptor. Furthermore, their topographical distribution within the nuclei also resembles each other. Such similarities characterize most brain regions that contain TIP39 and PTH2 receptor immunoreactivity, providing anatomical evidence that TIP39 may be the endogenous ligand of the PTH2 receptor as it is

available to activate the receptor upon release from the terminals. This finding supports previous pharmacological data demonstrating that TIP39 can activate the PTH2 receptor. Still, it is worth mentioning that some brain areas such as the caudate nucleus and the cerebral and cerebellar cortices contained some PTH2 receptor immunoreactivity detectable TIP39-ir. Proposed explanations for such a mismatch, which is characteristic of several peptide-receptor systems, include long distance diffusion of the peptide, the existence of another ligand for the receptor, or the lack of sufficient sensitivity of the immunolabeling for the ligand (Herkenham 1987).

14.5 Projections of the Different TIP39 Cell Groups

Bilateral lesions of TIP39 cell groups resulted in the disappearance of TIP39 fibers from their target areas (Dobolyi et al. 2003a). Unilateral lesions also caused a reduction in the density of TIP39 fibers ipsilateral to the lesion. No obvious reduction was found contralateral to the lesion in any brain region as compared to intact animals, suggesting predominantly ipsilateral projections. The residual density was typically somewhat higher for unilateral than bilateral lesions suggesting some contribution of contralateral projection to TIP39 fibers in some brain regions (Dobolyi et al. 2003a; Cservenak et al. 2010). Still, the results of unilateral lesions are shown for demonstration because of the apparently striking difference between the two sides of the brain in the same section.

Lesion studies demonstrated that the forebrain receives most of its TIP39 fibers from the periventricular gray (PVG) and the posterior intralaminar complex of the thalamus (PIL). Lesioning of the third TIP39 cell group, the medial paralemniscal nucleus (MPL), resulted in no visible reduction of the density of TIP39 fibers in the forebrain while lesioning of the PVG and PIL were both effective albeit in different extent in different parts of the forebrain. The accumbens nucleus and the bed nucleus of the stria terminalis may receive more TIP39 input from the PVG, the medial prefrontal cortex and the lateral septum seems to receive similar input from both brain regions (Fig. 14.5). Accordingly, small but visible reductions in the density of TIP39 fibers were observed ipsilateral to the PIL lesion in the infralimbic cortex, the nucleus accumbens, the ventral subdivision of the lateral septum, the bed nucleus of the stria terminalis, and the amygdala (Dobolyi et al. 2003a). In turn, the hypothalamus is generally more abundantly innervated from the PIL than from the PVG as following lesions of the PIL, TIP39 fibers almost completely disappeared from the ipsilateral amygdala and most parts of the ipsilateral hypothalamus (Dobolyi et al. 2003a). Since TIP39 fibers could be followed from the PIL towards the supraoptic decussations (Palkovits et al. 2010) to project in a ventromedial direction, the effect of transaction of this pathway was studied in mother rats. Transections reaching of this tract resulted in the accumulation of TIP39 immunoreactivity immediately caudal to the transection within the fibers of the supraoptic decussations. In the midbrain, only the periaqueductal gray showed a moderate decrease in its density of TIP39 fibers following lesion of the PVG. Other structures of the midbrain, pons,

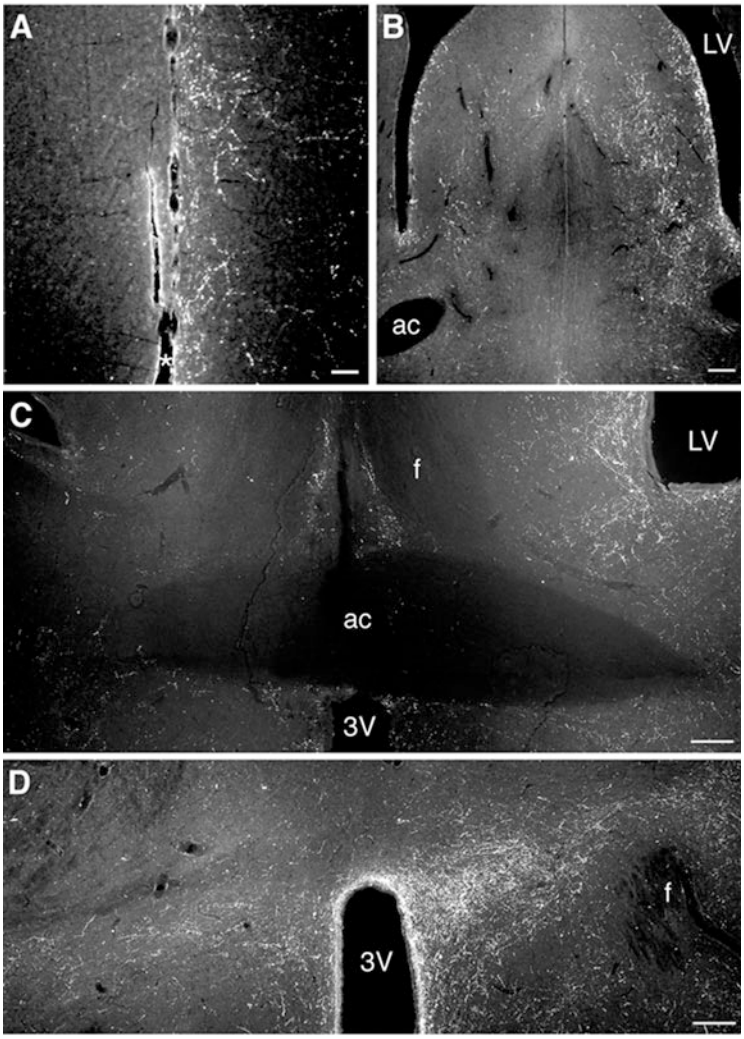

Fig. 14.5 The effect of unilateral (left side) lesions of the PVG on TIP39 fibers. Disappearance of TIP39-immunoreactive fibers ipsilateral to the lesion in the dorsal peduncular and infralimbic cortices (**a**), lateral septum (**b**), bed nucleus of the stria terminalis (**c**), and strong ipsilateral reduction in the hypothalamic paraventricular nucleus (**d**). Scale bars = 200 μm. The figure is from our previous publication (Dobolyi et al. 2003a)

and medulla did not demonstrate visible decrease in their TIP39 content following PVG or PIL lesions.

In contrast, lesions of the medial paralemniscal nucleus (MPL) were effective in reducing the density of TIP39 fibers in the lower brainstem regions. This finding suggests that the nuclei containing TIP39 in this part of the brain, such as relay nuclei of auditory, somato- and viscerosensory information, such as the external cortex of

the inferior colliculus, the spinal trigeminal nucleus, and the nucleus of the solitary tract all receive their TIP39 fibers from the MPL. It also has to be noted that the lesioning technique can detect only robust innervation. Therefore, small contribution to TIP39 fiber density from a non-detected source cannot be fully excluded based on these data.

An alternative method to detect where TIP39 fibers in a given brain regions originate from is the injection of retrograde tracer to their target area coupled with analysis of the location of retrogradely labeled cell bodies in the TIP39 cell groups. Such retrograde studies using cholera toxin beta subunit (CTB) as the retrograde tracer have been performed for the medial preoptic and arcuate nuclei (Cservenak et al. 2013). The position of the injection site was verified by double labeling with TIP39 to demonstrate that TIP39 fibers are indeed dense in the injection sites (Fig. 14.6). Following these injections, TIP39 neurons were retrogradely labeled only in the PIL but not in the PVG and MPL confirming that the hypothalamic regions receive most of their inputs from the PIL. The finding that retrogradely labeled cells were not present around the PIL regions suggests that this projection represents a specific pathway originating only from the PIL and not from other thalamic brain areas. Other retrograde studies also demonstrate the existence of projections from the PIL to the medial preoptic area (Simerly and Swanson 1986), the paraventricular hypothalamic nucleus (Campeau and Watson 2000), the arcuate nucleus (Li et al. 1999a; Szabo et al. 2010), and the amygdaloid nuclei (LeDoux et al. 1990). In these studies, double labeling with TIP39 was not performed, therefore, only the projections of neurons from the PIL can be deduced. However, it is likely that TIP39 neurons were among those (if not exclusively), which projected to the forebrain target areas.

14.6 Afferent Neuronal Connections of TIP39 Neurons

The retrograde tracer CTB was injected into the PIL to identify neurons that project there (Cservenak et al. 2017a). Most projections to the PIL were from the ipsilateral side, with the exception of the gracile and cuneate nuclei, the spinal trigeminal nucleus, and the spinal cord, where there was contralateral dominance. In the spinal cord, CTB-labeled neurons were predominantly located in Rexed laminae IV-VII. Most of the labeled thoracic cells were located in laminae IV-V and the labeled lumbar cells in laminae VI-VII. There was rarely more than one labeled cell in a coronal section. On average, every fourth 50 μm coronal section contained a labeled cell, usually characterized by oval perikarya with multiple dendrites. In the medulla oblongata, the highest density of CTB-labeled cells was in the gracile nucleus, the cuneate nucleus, and the spinal trigeminal nucleus (particularly in the deep layers of its ventral portion). Only a few upper brainstem regions contained CTB-positive neurons. The greatest number was apparent in the external cortex of the inferior colliculus. CTB-labeled neurons were far less numerous in the lateral parabrachial nucleus, periaqueductal gray, and deep layers of the superior colliculus. The infralimbic cortex contains the highest density of labeled cells within the cerebral

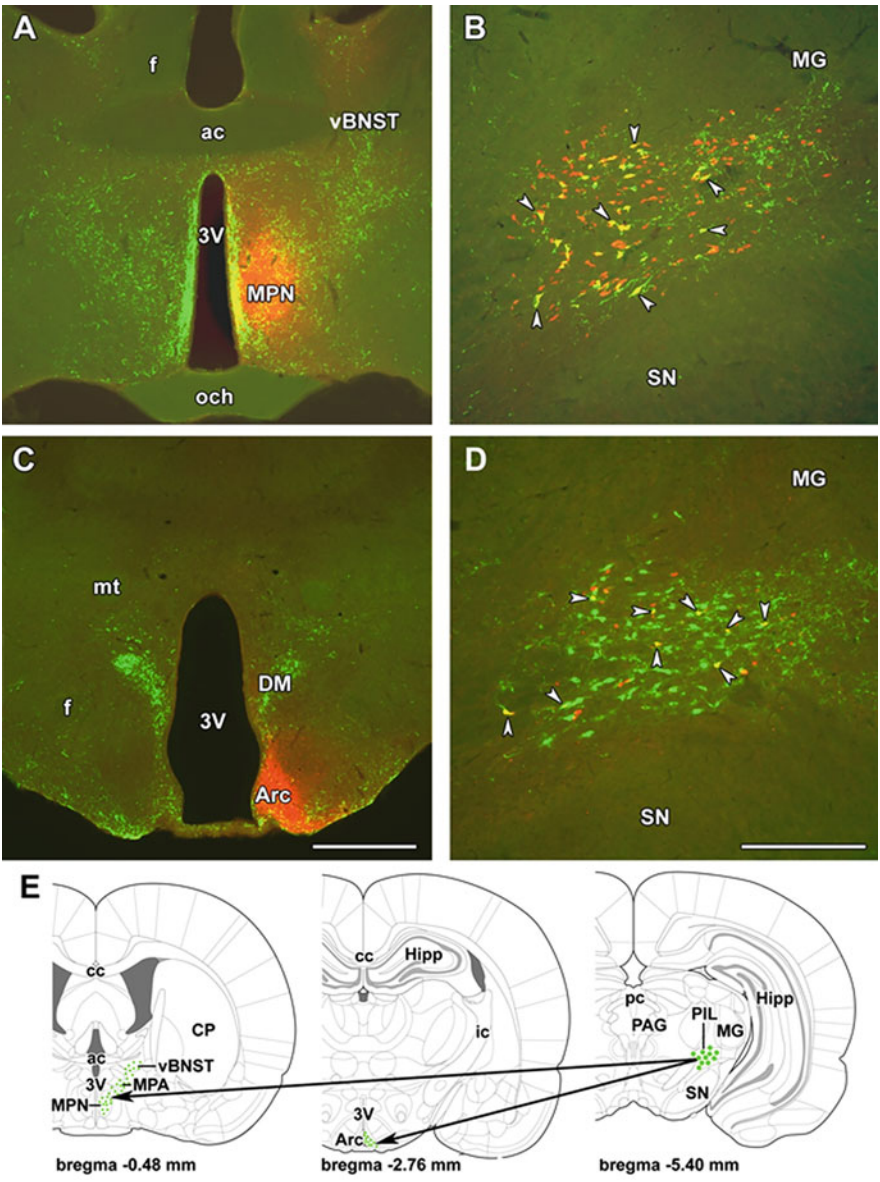

Fig. 14.6 Projections of the PIL into the medial preoptic and arcuate nuclei, demonstrated using the retrograde tracer cholera toxin beta subunit (CTB). CTB is shown in red and TIP39 in green. (**a**): A site of CTB injection into the medial preoptic nucleus (MPN) is shown in relation to TIP39 fibers. (**b**): In the PIL, the majority of TIP39 neurons are labeled with CTB following medial preoptic CTB injection (yellow; white arrowheads). In addition, a number of TIP-negative CTB-labeled neurons are also present. (**c**): A site of CTB injection into the arcuate nucleus (Arc) is shown. (**d**): A portion of the PIL TIP39 neurons are labeled with CTB (shown by white arrowheads) following its injection into the arcuate nucleus. (**e**): A drawing prepared by modifications of panels from a rat brain atlas (Paxinos and Watson 2005) shows the schematics of the PIL-hypothalamic projections. Large green dots in the PIL represent TIP39 cell bodies while small green dots in the preoptic area and the

cortex. There were also a considerable number of retrogradely labeled neurons in auditory areas. In contrast, there were few CTB-positive neurons found in the insular and medial prefrontal cortex. CTB signal was altogether absent from other cortical areas. Retrograde labeling was also largely absent from most other forebrain structures. There was a significant number of labeled neurons only in the central amygdaloid nucleus, the substantia innominata and the anterior portion of the lateral septal nucleus. Within the diencephalon, the largest number of labeled cells was in the ventromedial hypothalamic nucleus, particularly in its ventrolateral subdivision. There were also a considerable number of CTB-containing neurons in the lateral preoptic area and zona incerta.

Another study investigated the neuronal inputs of the MPL using CTB injections into the nucleus (Varga et al. 2008). The injection sites were verified using double labeling with TIP39 to demonstrate that the tracer was injected among TIP39 neurons in the MPL. As a prerequisite in precise tract tracing studies, injections into adjacent brain regions were also performed, which resulted in distinct labeling pattern in this case, too, suggesting that the retrogradely labeled brain areas project specifically into the MPL. Interestingly, a cortical brain region, the secondary auditory cortex and specifically the cortical temporal area 3 (Te3), as defined previously (Roger and Arnault 1989), projected to the MPL. However, adjacent primary (T1) and secondary auditory cortices (T2) also projected to the MPL. These projections were further verified by injecting an anterograde tracer into the primary auditory cortex, which demonstrated that TIP39 neurons in the MPL are indeed closely approached by auditory corticofugal fibers (Varga et al. 2008). In addition to the auditory cortex, another forebrain area, the ventromedial hypothalamic nucleus also projected to the MPL. Interestingly, this is a hypothalamic nucleus which received much less TIP39 innervation than the surrounding hypothalamic areas. In the cerebral cortex, CTB-containing cells were restricted to particular regions (Varga et al. 2008). In addition to these 2 sites, a thalamic auditory region, the medial subdivision of the medial geniculate body also sends descending projections to the MPL. In the midbrain, the external cortex of the inferior colliculus contains the highest density of retrogradely labeled cell bodies. Interestingly, this brain region also contains TIP39 fibers of MPL origin suggesting bidirectional connections between the 2 brain regions. While these inputs were all predominantly ipsilateral to the injection site, the MPL also receives input from the contralateral MPL. Interestingly, the contralaterally projecting neurons are typically negative for TIP39. Finally, the MPL also possesses some lower density inputs, e.g. from the lateral preoptic area, the lateral hypothalamic area, and the zona incerta, as well as periolivary regions of the medulla.

Fig. 14.6 (continued) arcuate nucleus represent TIP39 fiber terminals. The arrows show the projections from the PIL to the medial preoptic area and the arcuate nucleus, respectively. Scale bar = 1 mm for C, and 500 μm for D

14.7 Activation of TIP39 Neurons in Mothers

14.7.1 Assessment of c-Fos Activation in TIP39 Neurons of Lactating Dams

The appearance of c-Fos in response to pup exposure represents the activation of those neurons as c-Fos is the protein product of c-fos, a well-known immediate early gene that appears in activated neurons (Herdegen and Leah 1998). When pups are returned to their mothers after a 20 h separation, the dams begin care for them immediately, and suckling starts within 5 min. Following pup return, c-Fos-positive neurons appeared in a number of regions in the dams' brains including the PIL, MPL, lateral septal nucleus, anteroventral periventricular nucleus, medial preoptic nucleus, medial preoptic area, the ventral subdivision of the bed nucleus of the stria terminalis, and some parts of the periaqueductal gray, but not the periventricular gray of the thalamus. Thus, c-Fos also appears in the nuclei of TIP39 neurons of the PIL and MPL in response to pup exposure, indicating an elevated activity of TIP39 neurons in lactating rat dams in these brain areas. These findings confirmed previously reported expression of c-Fos in the PIL area of lactating rats (Lin et al. 1998) and the area corresponding to the MPL (Li et al. 1999b).

TIP39 neurons represent about half of the neurons demonstrating c-Fos activation in the PIL, as a number of TIP39-negative neurons were also activated in response to suckling (Cservenak et al. 2013). In contrast, within the MPL, c-Fos was located almost exclusively in TIP39 neurons, which is the major neuronal cell group of this nucleus (Varga et al. 2008). Based on the very low number of c-Fos-positive but TIP39-immunonegative neurons, it is likely that other cell types within the MPL are generally not activated in mother rats.

Pup exposure represents a complex stimulus for the mothers. Apart from the suckling reflex, visual, auditory, or olfactory exteroceptive stimuli or hormonal changes associated with the presence of pups could induce prolactin release and maternal behaviors (Terkel et al. 1979; Hashimoto et al. 2001). Theoretically, all these inputs derived from the pups could contribute to the activation of TIP39 neurons in the PIL and MPL by increasing their neuronal activity via specific circuitries. However, the finding that c-Fos appears in TIP39 neurons of the PIL only when physical contact is allowed suggests that TIP39 is induced in the PIL of rat dams by the suckling stimulus and not by other sensory input. These experiments have not been performed in the MPL yet, thus an adequate stimulation other than suckling is conceivable for TIP39 neurons in the MPL. In fact, auditory input could play a major role in the activation of TIP39 neurons in the MPL, because they receive massive input from the auditory cortex, the inferior colliculus, and the periolivary area (Varga et al. 2008). In addition, we have shown that highly intense noise stimulus activates paralemniscal TIP39 neurons (Palkovits et al. 2009). Furthermore, an indirect activation of paralemniscal TIP39 neurons via maternal hormones cannot be excluded either.

It is particularly striking that TIP39 neurons are activated only in the PIL and MPL while TIP39 neurons in the PVG are not activated in lactating dams, which is

consistent with the lack of TIP39 induction in that area as discussed later. Although TIP39 disappears from the PIL earlier than from the PVG and MPL during ontogeny (Brenner et al. 2008), the adult levels are markedly reduced in all three brain regions. Furthermore, brain areas that receive TIP39 axons predominantly from the PVG, including the lateral septal nucleus and the medial prefrontal cortex (Dobolyi et al. 2003b; Wang et al. 2006), also continue to possess a high PTH2 receptor level (Dobolyi et al. 2006b) suggesting that this TIP39 cell group may also be activated in response to some so far unidentified physiological stimuli.

14.7.2 Induction of TIP39 in Mother Rats

Induction of TIP39 mRNA in the PIL and the MPL of lactating mother rats was suggested on the basis of in situ hybridization histochemistry and confirmed by the independent technique of RT-PCR. The temporal pattern of activation of posterior intralaminar and paralemniscal TIP39 neurons was similar (Cservenak et al. 2010). In contrast, TIP39 expression was not changed in the third group of TIP39 neurons, the PVG in mother rats (Cservenak et al. 2010). In the PIL and the MPL, the levels of TIP39 mRNA were elevated specifically in the presence of pups while TIP39 mRNA levels were at their low, basal, non-maternal level in the absence of pups. In a more detailed study, TIP39 expression was found to be markedly upregulated on the first, ninth, and 23rd postpartum days but not on the last day of pregnancy or after weaning, which further supports the idea that elevated activity of these neurons is specific for the period of lactation (Cservenak et al. 2013). Thus, the increase in the level of TIP39 mRNA is a temporary phenomenon during lactation. The induction is likely to take place in all TIP39 neurons within the 2 cell groups as suggested by the increased autoradiography signal in the observed TIP39-expressing neurons following in situ hybridization histochemistry. In turn, the distribution of TIP39 neurons in the PIL and MPL of lactating mother rats was similar to that described previously in young rats (Dobolyi et al. 2003b, 2006b) suggesting that TIP39 reappears in the same neurons, which expressed it during earlier stages of ontogenic development and no additional, TIP39-negative cells are recruited in mothers. Furthermore, an increased TIP39 immunoreactivity was also detected in rat dams suggesting that the increase in TIP39 mRNA level translates into elevated peptide level, which in turn suggests a function of the induced TIP39 in mother rats. A function of the induced TIP39 is also conceivable because the expression level of the receptor of TIP39, parathyroid hormone 2 receptor, does not decrease during postnatal development as TIP39 does (Dobolyi et al. 2006b). Thus, parathyroid hormone 2 receptor is available for maternally induced TIP39 to exert its actions.

14.8 Neuroendocrine Functions of TIP39

14.8.1 The Effect of TIP39 on Prolactin Release

Suckling is known to elevate plasma prolactin levels within minutes of pups return to the mothers deprived of pups for 4 h, and plasma prolactin concentrations peak 30 min after the beginning of suckling (Bodnar et al. 2004; Dobolyi et al. 2020; Phillipps et al. 2020). Experiments to evaluate a potential role of TIP39 took advantage of HYWY-TIP39, a selective antagonist of the PTH2 receptor (Kuo and Usdin 2007). Injection of HYWY-TIP39 into the lateral ventricle dose-dependently blocked the elevation of plasma prolactin levels (Cservenak et al. 2010), suggesting that TIP39 acting on PTH2 receptors contributes to suckling-induced prolactin release.

To further evaluate a potential causal relationship between TIP39 signaling in the arcuate nucleus and prolactin level, cells in the mediobasal hypothalamus were infected near the arcuate nucleus with a virus encoding a secreted form of the PTH2-receptor antagonist peptide (HYWH-TIP39) and enhanced GFP (Fig. 14.7a). At least ten infected cells per injection site were seen in the most densely infected section of the animals, as illustrated in Fig. 14.7b. Mediobasal hypothalamic but not preoptic injection of the HYWH-TIP39 (Fig. 14.7c and d) markedly decreased basal serum prolactin levels and the suckling-induced prolactin release, suggesting that the mediobasal hypothalamus may be the site of action of HYWY-TIP39. PTH2 receptor-expressing neurons are abundant in the periventricular and arcuate nuclei of the hypothalamus (Wang et al. 2000; Faber et al. 2007). PTH2 receptors in these neurons are possible targets mediating the effect of HYWY-TIP39 on prolactin release. Dopaminergic neurons that control prolactin release from the pituitary are also located in the arcuate and periventricular hypothalamic nuclei. However, a direct effect of HYWY-TIP39 on dopaminergic neurons is not likely because the PTH2 receptor was not double-labeled for tyrosine hydroxylase (Usdin et al. 2003; Dobolyi et al. 2006a), and because close appositions between tyrosine hydroxylase neurons and fiber terminals projecting to the mediobasal hypothalamus from the PIL were not detected (Szabo et al. 2010). Therefore, HYWY-TIP39 might influence dopaminergic neurons in the mediobasal hypothalamus via interneurons expressing the PTH2 receptor (Cservenak et al. 2013). Dynorphin-containing neurons in the arcuate nucleus are one of the candidates because they are innervated by axon terminals derived from the PIL (Szabo et al. 2010), innervate tuberoinfundibular dopaminergic neurons (Fitzsimmons et al. 1992), and may be responsible for the effects of opioid peptides on suckling-induced prolactin release by inhibiting tuberoinfundibular dopaminergic neurons (Selmanoff and Gregerson 1986; Arbogast and Voogt 1998; Callahan et al. 2000). It is also a possibility that TIP39 evokes prolactin release by directly or indirectly stimulating prolactin-releasing substance-containing neurons (Freeman et al. 2000; Andrews 2005).

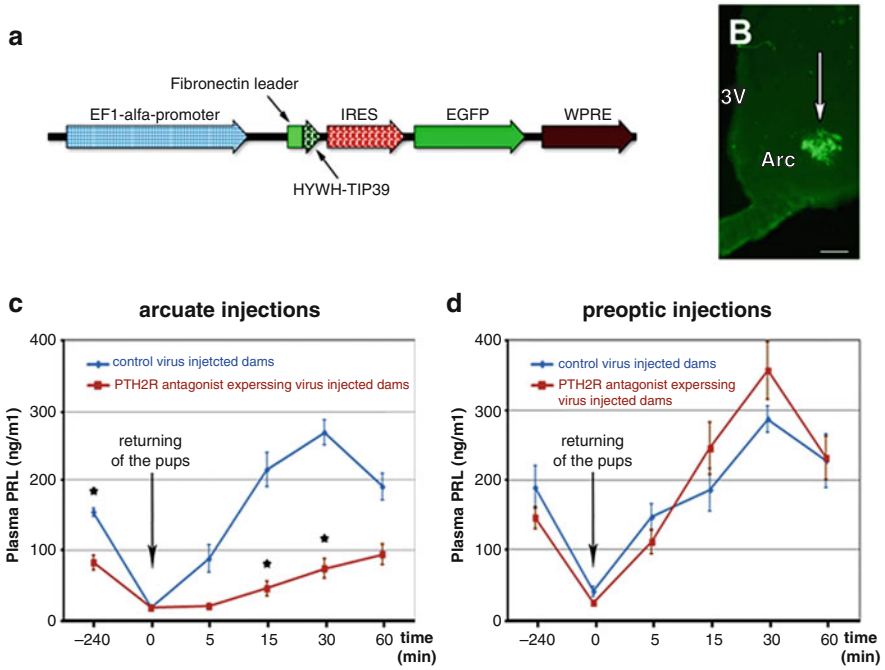

Fig. 14.7 Effect of virus encoding a peptide PTH2 receptor antagonist on prolactin release. (**a**): Structure of the viral construct expressing HYWH-TIP39, an antagonist of the PTH2 receptor. A strong mammalian promoter (EF-1-alfa) drives expression of a fusion protein between the fibronectin leader sequence with signal peptide cleavage site and the HYWH-TIP39 sequence. This is followed by an internal ribosome re-entry site (IRES) and then enhanced green fluorescent protein (EGFP) sequence and a woodchuck hepatitis post-transcriptional regulatory element (WPRE). (**b**): Hypothalamic virus injection site. The white arrow indicates cells infected by the injected virus visualized with EGFP. The injection site is located just lateral to the arcuate nucleus. (**c**): Basal plasma prolactin levels in mother rats injected with the PTH2 receptor antagonist expressing virus were significantly lower than in mothers injected with the control virus, with injections targeted to the arcuate nucleus. After returning their pups, the elevation of serum prolactin level was also blocked in the PTH2 receptor antagonist expressing virus injected mothers (*: $p < 0.01$). (**d**): Prolactin levels did not differ between PTH2 receptor antagonist expressing virus injected and control virus injected mothers with injections targeted to the medial preoptic area. Abbreviations: *Arc* arcuate nucleus, *3V* third ventricle. Scale bar = 100 μm for B (Cservenak et al. 2013)

14.8.2 The Effect of TIP39 on Oxytocin Release

Oxytocin is released in the pituitary and widespread brain areas from terminals of the magnocellular paraventricular and supraoptic neurons during parturition, in response to suckling in mothers and possibly also during adult social interactions. However, neuronal pathways that activate oxytocin neurons are not well established and TIP39-containing PIL neurons are candidates. It was shown, using double labeling in combination with electron microscopy and retrograde tracing, that oxytocin neurons are innervated by TIP39 terminals originating in the PIL (Cservenak et al.

2017a). The excitatory nature of TIP39 neurons was investigated by in situ hybridization histochemistry. Since TIP39 neurons are activated by pup exposure in mother rats as well as in adult females upon social encounter with a familiar conspecific, it was suggested that the PIL-paraventricular projection contributes to the established activation of oxytocin neurons in social contexts (Dobolyi et al. 2018; Tang et al. 2020).

14.8.3 The Effect of TIP39 on the Activity of the Corticotropin-Releasing Hormone (CRH) Neurons

Based on dense networks of PTH2 receptor- and TIP39-containing fibers in the hypothalamic paraventricular nucleus (PVN), Dimitrov and Usdin investigated a potential role of TIP39 in the control of CRH release (Dimitrov and Usdin 2010). There was a large amount of colocalization between the PTH2 receptor and the vesicular glutamate transporter VGlut2 on nerve fibers or terminals that surrounded PVN CRH neurons. TIP39-containing nerve terminals appeared to be very close to these PTH2R/VGlut2-containing processes. A hypothesis derived from these observations is that TIP39 modulates activation of CRH neurons via glutamatergic terminals in the PVN. Several observations are consistent with this idea. TIP39 infusion near the PVN causes an increase in the immediate early gene pCREB within the CRH neuron containing zone of the PVN and this stimulation of pCREB was blocked by a mixture of the glutamate receptor antagonists CNQX and AP-5. This increase in pCREB by TIP39 did not occur in PTH2 receptor knockout mice. Infusion of TIP39 near the PVN also led to an increase in plasma corticosterone that was prevented by co-infusion of glutamate receptor antagonists. Finally, consistent with a physiological role for TIP39 in control of CRH release, the normal diurnal variation in plasma corticosterone was diminished in PTH2 receptor knockout mice.

14.8.4 The Role of TIP39 in Thermoregulation

PTH receptors and TIP39 are present on or in neuronal fibers in several hypothalamic regions, including the preoptic area and one of its subregions, the median preoptic area (MnPO). The MnPO is of interest because it is an important thermoregulatory control region. A series of anatomical tract tracing experiments combined with antibody labeling and in situ hybridization histochemistry established that PTH2 receptors are present on glutamatergic terminals that are presynaptic to projection neurons in the MnPO. These projection neurons are part of thermoregulatory circuitry and provide a multisynaptic input to brown adipose tissue. Pharmacological experiments in which TIP39 was microinjected into the region of the MnPO or the lateral ventricle showed that TIP39 caused a PTH2 receptor dependent increase in body temperature that was mediated by sympathetic output. This effect was not observed when TIP39 was microinjected into the dorsomedial hypothalamic nucleus, a thermoregulatory region that mediates output from the MnPO, which

supports the suggestion that TIP39 effects on thermoregulation are specific to the MnPO. Evidence that TIP39 and the PTH2 receptor have a physiological role in control of body temperature was provided by experiments that evaluated the ability of wild-type or PTH2 receptor knockout mice to defend their body temperature in a cold environment. Wild-type mice placed in a 4° C environment for 1 hour had little change in body temperature. In contrast, the body temperature of PTH2 receptor knockout mice decreased by an average of 3.6° C during the 1-hour exposure to a 4° C environment. A qualitatively similar result was obtained by injecting a PTH2 receptor antagonist into the lateral ventricle (Dimitrov et al. 2011). Furthermore, the peripartum elevation of core body temperature was present but reduced in PTH2R KO mice, even though their locomotor activity increased as core body temperature was reduced (Gellen et al. 2017). Thus, evidence from anatomical, pharmacological, and physiological approaches supports the suggestion that TIP39 signaling plays a significant role in the homeostatic control of body temperature, likely through modulation of glutamatergic signaling in the hypothalamic MnPO. In addition, the PTH2R contributes to the maternally elevated core body temperature as well.

14.8.5 Additional Potential Neuroendocrine Roles of TIP39

There is some evidence available for a role of the TIP39-PTH2 receptor system in the regulation of arginine vasopressin (Sugimura et al. 2003) and growth hormone release (Usdin et al. 2003). Some of these actions could be part of maternal adaptations even though they are not yet proven in mothers (Dobolyi et al. 2012). Another profound neuroendocrine change in mothers is the inhibition of the gonadotropin-releasing hormone (GnRH) neurons, which leads to lactational anestrus. Although experimental data are not available yet, an action of TIP39 on GnRH neurons is plausible given the high density of TIP39 fibers in both the periventricular preoptic nucleus and the arcuate nucleus where kisspeptin neurons regulating GnRH neurons are located.

14.9 Non-neuroendocrine Functions of TIP39

The TIP39-PTH2 receptor system has been implicated in a variety of non-neuroendocrine actions. Acute injection of TIP39 into the lateral ventricle of male rats was observed to have an anxiolytic effect in an elevated plus maze test and an antidepressant-like effect in the forced swim test (LaBuda et al. 2004). Subsequently, anxiety-like behavior in TIP39 knockout (KO) animals was also demonstrated, but only if the animals were previously exposed to mild acute stress (Fegley et al. 2008) and also after fear conditioning (Coutellier and Usdin 2011). In addition, fear incubation, a time-dependent increase in fear responses to trauma-associated cues, is also affected by TIP39 (Tsuda et al. 2015).

PTH2 receptors are expressed in many CNS regions involved in the processing of nociceptive information. These include regions that are within ascending pathways

that convey nociceptive sensory information, as well as within descending pathways to regions involved in modulation of the sensitivity to peripheral stimuli or of responses to nociceptive input. The regions include the spinal cord dorsal horn, PAG, medial and intralaminar thalamic nuclei, several amygdaloid and hypothalamic nuclei, and somatosensory and anterior cingulate cortex (Dobolyi et al. 2002). The potential involvement of TIP39-PTH2 receptor signaling in pain processing was evaluated by comparing performance in several standard tests of acute nociceptive sensitivity between control or wild-type mice and mice in which PTH2 receptor signaling was inhibited either by acute administration of a PTH2 receptor antagonist, by null mutation of the PTH2 receptor or by deletion of TIP39 (Dimitrov et al. 2010). Intracerebroventricular (icv) administration of the PTH2 receptor antagonist HYWH-TIP39 increased latency in acute nociceptive withdrawal assays including the tail-flick and hotplate tests, and in both phases of the formalin test, while administration of TIP39 decreased latency in acute nociceptive sensitivity tests. Observations in the mice with constitutive genetic alterations in PTH2 receptor signaling were generally consistent with these observations. The idea that TIP39-PTH2 receptor signaling contributes to physiological modulation of nociceptive function was also evaluated in animals with more long-lasting perturbations (Dimitrov et al. 2011). Following peripheral nerve injury, both PTH2R- and TIP39-knockout mice developed less tactile and thermal hypersensitivity than controls and returned to baseline sensory thresholds faster. The effects of hind paw inflammatory injury were similarly decreased in knockout mice. Thus the TIP39-PTH2 receptor system appears to have a role in maintaining the normal sensitivity to nociceptive stimuli and modulating responses to injury.

14.10 TIP39 as a Maternal Neuropeptide

Behavioral, endocrine, and psychological changes in mothers represent one of the most profound physiological alterations in the adult central nervous system. Experimental models of maternal behaviors are well established in rodents as control females avoid or even hurt pups while mothers take care of them, retrieving them in the nest, nursing them and performing anogenital licking, etc. (Numan 2020). Additional emotional changes include maternal aggression towards intruders, decreased anxiety in general and reduced responsiveness of the hypothalamo-pituitary-adrenal axis in stress situations (Carter et al. 2001; Neumann 2003).

14.10.1 PIL TIP39 Neurons May Mediate Suckling-Induced Prolactin and Oxytocin Release

Prolactin and oxytocin are major hormones, which evoke many of the maternal adaptations in the brain in addition to their role in lactation. These hormones are released in response to suckling. Lesion and microstimulation studies suggested that the ascending reflex arch conveying sensory information from the nipples for

prolactin and oxytocin release travels through the lateral mesencephalic tegmentum and enters the zona incerta ventromedial to the medial geniculate body (Tindal and Knaggs 1977; Wakerley et al. 1978; Dubois-Dauphin et al. 1985), exactly the position where PIL TIP39 neurons reside (Dobolyi et al. 2018). Excitotoxic lesions of this area blocked the milk-ejection reflex (Hansen and Kohler 1984) and c-fos expression was detected here in lactating mothers (Lin et al. 1998) suggesting relay of the pathway in this position. PIL TIP39 neurons are candidates to be the relay neurons of these hormone-releasing reflex arcs because of a significant portion of c-Fos-positive TIP39 neurons (Cservenak et al. 2017a). Furthermore, injection of the retrograde tracer in a position of the TIP39 neurons in the PIL retrogradely labeled neurons in brainstem sensory relays nuclei as well as in the spinal cord (Cservenak et al. 2017a). In addition, PIL TIP39 neurons project to both the arcuate nucleus and the paraventricular hypothalamic nucleus, which suggests that TIP39 neurons in the PIL convey suckling information to these nuclei where dopaminergic neurons controlling prolactin release and oxytocin neurons reside, respectively. The presence of TIP39 in these neurons suggests the role of this neuropeptide in the regulation of maternal functions. While TIP39 terminals innervate oxytocin neurons, functional evidence is available for the regulation of prolactin release by TIP39. The finding that the body weight of pups is reduced in the absence of a functional TIP39 gene (Coutellier et al. 2011) and that the blockade of PTH2 receptors inhibits suckling-induced prolactin release (Cservenak et al. 2010) suggests that TIP39 plays a physiological role in the regulation of suckling-induced prolactin release. All of these data suggest that the suckling information evoking the release of both hormones in mothers is relayed by PIL TIP39 neurons (Fig. 14.8).

14.10.2 PIL TIP39 Neurons May Represent a Relay Station of Suckling-Induced Non-hormonal Brain Adaptations

Although the behavioral changes are initiated by steroid hormonal alterations in the last days of pregnancy, decreased levels of steroid hormones are detected during lactation leading to anestrous (Siegel 1986; Bridges 2020). Furthermore, maternal motivation remains high following blockade of prolactin and oxytocin actions (Lamming 1994). In addition, maternal behaviors can be induced by prolonged pup exposure even in virgin female rats. These maternally sensitized rats do not lactate and provide a model to separate metabolic regulations from regulations of maternal behaviors (Rosenblatt 1967). Somatosensory inputs derived from the pups play the most important role in maternal sensitization (Stern and Lonstein 2001). The same somatosensory inputs are thought to maintain maternal behaviors in dams after parturition (Febo et al. 2008). Since PIL TIP39 neurons project to the preoptic area, the major forebrain center controlling maternal behaviors, PIL TIP39 neurons may participate not only in the neuroendocrine responses of prolactin and oxytocin release but also in neuronal mechanisms of maternal brain adaptations (Fig. 14.8).

At the preoptic level, the anteroventral periventricular nucleus, the medial preoptic nucleus, the medial preoptic area, and the ventral subdivision of the bed

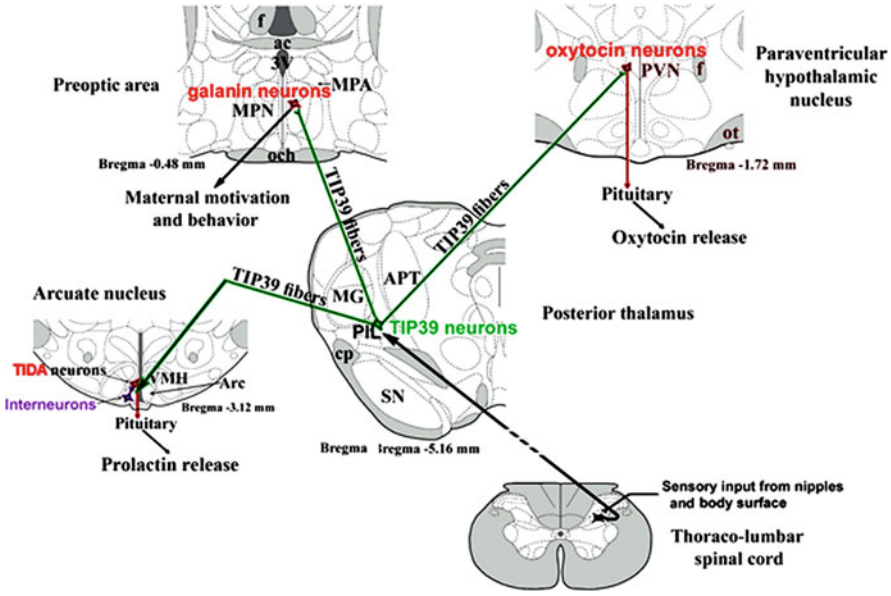

Fig. 14.8 TIP39 neurons in the PIL are proposed relay stations of maternal sensory information towards hypothalamic and limbic centers. During suckling, somatosensory information from the nipples reaches the posterior intralaminar complex of the thalamus (PIL) where TIP39 neurons reside. These neurons project to different hypothalamic sites and other limbic centers (e.g., the medial prefrontal cortex and the lateral septum, not shown in the figure). TIP39 terminals in the paraventricular hypothalamic nucleus lead to oxytocin release, e.g. for milk ejection, TIP39 terminals in the arcuate nucleus evoke prolactin release for the maintenance of lactation, while TIP39 terminals (terminating on galanin neurons) in the medial preoptic area contribute to maternal care

nucleus of the stria terminalis all contain a high density of c-Fos-expressing neurons following suckling. This is a characteristic pattern in the medial preoptic region, often referred to as the medial preoptic area in which c-Fos-expressing neurons have been implicated in pup attachment (Lonstein et al. 1998; Stack and Numan 2000). This c-Fos activation pattern differs from the distribution of prolactin-sensitive neurons in the area, as most of the neuronally activated cells are not prolactin sensitive (Olah et al. 2018). However, the distribution pattern of c-Fos-expressing neurons is very similar to the distribution patterns of TIP39 labeled fibers and terminals observed in the area. TIP39 containing fibers closely appose c-Fos-expressing neurons in all regions of the preoptic area that contain c-Fos-expressing neurons following suckling. Furthermore, galanin neurons in the preoptic area, which are known to be c-Fos activated by pup exposure as a major cell type of the preoptic neuronal network governing maternal behaviors (Wu et al. 2014), were shown to be innervated by TIP39 terminals (Cservenak et al. 2017b).

The position of the MPL immediately next to the nuclei of the lateral lemniscus and its bilateral anatomical connections with auditory brain regions (Dobolyi et al.

2003b; Varga et al. 2008) suggest some auditory functions of paralemniscal TIP39 neurons. Indeed, the paralemniscal TIP39 neurons were specifically activated by high-intensity noise (Palkovits et al. 2009). Rat pups, when isolated, are known to emit high-intensity vocalization in the ultrasonic range (Hofer 1996). Pup ultrasonic vocalizations have been reported to induce maternal behaviors in rats (Terkel et al. 1979; Hashimoto et al. 2001; Febo et al. 2008). Still, there are no data available at present on the anatomical pathway on how ultrasonic vocalization reaches limbic and hypothalamic centers responsible for maternal behavioral and neuroendocrine changes. However, results of application of retrograde tracers suggest that paralemniscal fibers may reach hypothalamic targets, such as the hypothalamic paraventricular nucleus (Palkovits et al. 2004). We hypothesize that paralemniscal TIP39 neurons could mediate pup ultrasonic vocalization towards higher brain centers of their mothers thereby contributing to central maternal adaptations.

14.10.3 Roles of TIP39 in Regulating Maternal Behavior

During the early postpartum period, pup suckling is more rewarding than cocaine (Ferris et al. 2005). A number of different approaches provide evidence that the preoptic area is critically important for maternal motivation (Numan 2020) through its projections to the nucleus accumbens and the ventral tegmental area (Numan et al. 2005). Large electrical and axon-sparing excitotoxic lesions of the MPOA eliminate all maternal behaviors without affecting other behaviors such as feeding and similar effects were found following temporal pharmacological inactivation of MPOA. In contrast to lesions, electrical stimulation of the MPOA increased maternal responsiveness (Morgan et al. 1997). In addition, brain activity is elevated in the MPOA in response to pup exposure based on c-fos (Li et al. 1999b) and fMRI techniques (Febo et al. 2005).

Virally driven constitutive release of HYWH-TIP39, an antagonist of the PTH2 receptor, in the preoptic area resulted from locally infected cells. The behavior of mother rats that received virus injections into the preoptic area was analyzed using a place preference test (Cservenak et al. 2013), which is a sensitive way to assess maternal motivation (Seip and Morrell 2009). The presence of the PTH2 receptor antagonist reduced the number of dams demonstrating preference for the pup-associated cage, and also the amount of time the dams spent in the pup-associated cage, but did not affect the time control females spent in the different cages of the test apparatus (Cservenak et al. 2013). These data provided evidence for the involvement of the TIP39-PTH2 receptor system in maternal motivation. It is also important to note that preoptic injection of the virus expressing the PTH2 receptor antagonist did not affect plasma prolactin levels. Therefore, an indirect mechanism of action on maternal motivation via prolactin can be excluded.

PTH2R KO mothers also showed anxiety-like and depression-like behaviors compared to wild-type mothers (Gellen et al. 2017). These latter data also suggest that the TIP39-PTH2R system could be involved in postpartum depression, the most

frequent psychiatric disorder after childbirth with a prevalence rate of 10% to 15% (Mallikarjun and Oyebode 2005).

14.11 TIP39 in Zebrafish Social Awareness

It was recently observed that there is a dramatic difference in the level of expression of the gene encoding TIP39 (pth2) between zebrafish maintained in social isolation and as a group (Anneser et al. 2020). While there were a number of genes with expression differences between isolated and grouped fish, a difference was only present at multiple developmental states for pth2 and several immediate early genes. The level of pth2 was directly related to the density of fish, and responded to changes in social density within 30 minutes. The signal that controlled pth2 was mediated by the lateral line, a specialized mechanosensory organ, based on the effect of its lesion. The control of pth2 expression was highly selective for agitation of water in the precise pattern created by zebrafish swimming. TIP39/pth2 is expressed in zebrafish by a small group of neurons in a lateral thalamic region and its receptor is widely expressed (estimated to be in 9% of neurons). This pattern is highly reminiscent of the pattern in mammals. It suggests that the observed response to social density may be related to the involvement of signaling by the TIP39/PTH2 receptor system in affective functions that is observed in mammals.

14.12 Perspectives

The TIP39-PTH2 receptor system is pharmacologically and histologically well characterized, which provides an excellent starting point for functional investigations. The functional studies are supported by excellent research tools available, such as new antibodies, transgenic mice lacking the peptide and its receptor, a selective and sensitive peptide antagonist and a lentivirus encoding the peptide antagonist. These research tools led to the implication of TIP39 in different hypothalamic functions, such as thermoregulation, stress response, maternal behavior, and prolactin secretion. In addition, nociceptive and auditory functions of the peptide have been reported. The recent involvement of TIP39 in the social behavior of zebra fish suggests similar functions in rodents. In fact, the established role of TIP39 in the mother–pup relationship represents a special form of social contact (Kinsley and Amory-Meyer 2011), and in addition it is possible that TIP39 may also be involved in adult social interactions in mammals. The effect of TIP39 on oxytocin (Cservenak et al. 2017a; Dobolyi et al. 2018), a well-known social neuropeptide (Neumann 2008) supports this suggestion, which is to be tested in future experiments.

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