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PARATHYROID HORMONE 2 RECEPTOR AND ITS ENDOGENOUS LIGAND TUBEROINFUNDIBULAR PEPTIDE OF 39 RESIDUES ARE CONCENTRATED IN ENDOCRINE, VISCEROSENSORY AND AUDITORY BRAIN REGIONS IN MACAQUE AND HUMAN

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Abstract—Parathyroid hormone receptor 2 (PTH2R) and its ligand, tuberoinfundibular peptide of 39 residues (TIP39) constitute a neuromodulator system implicated in endocrine and nociceptive regulation. We now describe the presence and distribution of the PTH2R and TIP39 in the brain of primates using a range of tissues and ages from macaque and human brain. *In situ* hybridization histochemistry of TIP39 mRNA, studied in young macaque brain, due to its possible decline beyond late postnatal ages, was present only in the thalamic subparafascicular area and the pontine medial paralemniscal nucleus. In contrast, *in situ* hybridization histochemistry in macaque identified high levels of PTH2R expression in the central amygdaloid nucleus, medial preoptic area, hypothalamic paraventricular and periventricular nuclei, medial geniculate, and the pontine tegmentum. PTH2R mRNA was also detected in several human brain areas by RT-PCR. The distribution of PTH2R-immunoreactive fibers in human, determined by immunocytochemistry, was similar to that in rodents, including dense fiber networks in the medial preoptic area, hypothalamic paraventricular, periventricular and infundibular (arcuate) nuclei, lateral hypothalamic area, median eminence, thalamic paraventricular nucleus, periaqueductal gray, lateral parabrachial nucleus, nucleus of the solitary tract, sensory trigeminal nuclei, medullary dorsal reticular nucleus, and dorsal horn of the spinal cord. Colocalization suggested that PTH2R fibers are glutamatergic, and that TIP39 may directly influence hypophysiotropic somatostatin containing and indirectly influence corticotropin releasing-hormone containing neurons. The results demonstrate that TIP39 and the PTH2R are expressed in the brain of primates in locations that suggest involvement in regulation of fear, anxiety, reproductive behaviors, release of pituitary hormones, and nociception. Published by Elsevier Ltd on behalf of IBRO.

Key words: tuberoinfundibular peptide, neuroendocrine modulator, primate hypothalamus, subparafascicular area, medial paralemniscal nucleus, *in situ* hybridization in monkey brain.

Tuberoinfundibular peptide of 39 residues (TIP39) and the parathyroid hormone 2 receptor (PTH2R) constitute a peptide/receptor neuromodulator system. The function of this system is being investigated in rodents. Data suggest the system modulates neuroendocrine, anxiety-related and nociceptive functions. Comparison of the anatomical distribution of the system in primates and rodents is important for extrapolation of experimental results obtained in rodents to humans.

The PTH2R was identified on the basis of its sequence homology to other polypeptide-recognizing receptors (Usdin et al., 1995). It is a seven transmembrane domain receptor, which belongs to the type II (or family B) class of G-protein-coupled receptors (Harmar, 2001; Usdin et al., 2002). The human PTH2R has about 84% amino acid sequence identity with the rodent PTH2R and about 50% with the human parathyroid hormone 1 receptor (PTH1R). TIP39 was purified from bovine hypothalamus on the basis of its selective activation of the PTH2R (Usdin et al., 1999b). Mouse and rat TIP39 sequences, as well as human and bovine TIP39 sequences, are identical while amino acids differ at four positions between rodent and human TIP39 sequences (Dobolyi et al., 2002; John et al., 2002; Usdin et al., 2003). Rodent TIP39 sequences share only six, and four amino acid residues with parathyroid hormone (PTH), and parathyroid hormone–related peptide (PTHrP), respectively, while the corresponding numbers of amino acid similarities for human peptides are eight and five. The PTH1R is activated with subnanomolar potency by parathyroid hormone (PTH) and parathyroid hormone-related peptide (PTHrP), and both peptides are thought to be its physiological ligands. PTH is released from the

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Abbreviations: CRH, corticotropin-releasing hormone; Cy, carbocyanine; DAPI, 4′,6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ir, immunoreactive; PB, phosphate buffer; PTH, parathyroid hormone; PTHrP, parathyroid hormone–related peptide; PTH1R, human parathyroid hormone 1 receptor; PTH2R, parathyroid hormone 2 receptor; TIP39, tuberoinfundibular peptide of 39 residues; VGLUT2, vesicular glutamate transporter 2.

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parathyroid glands and has a critical role in calcium metabolism, whereas PTHrP is produced locally and is involved in the development and remodeling of many tissues, especially the skeleton (Kronenberg et al., 1998). In contrast, TIP39 has no significant effect on the PTH1R (Hoare et al., 2000). The human PTH2R is potently activated by both PTH and TIP39 (Usdin et al., 1999b). TIP39 potency on the rat PTH2R is similar to that on the human receptor, whereas PTH is several thousand-fold less potent and produces only 40% of TIP39's maximal effect (Usdin et al., 1999b). PTHrP does not activate the rat or human PTH2R (Hoare and Usdin, 2001). Thus, there is strong pharmacological evidence that TIP39 is an endogenous ligand for the PTH2R (Usdin et al., 2000).

The PTH2R is most abundant in the brain (Usdin et al., 1995) which contains little if any PTH (Usdin, 1997). The localization of PTH2R-expressing cell bodies as well as nerve fibers and terminals has been described in detail in rat (Wang et al., 2000) and mouse (Faber et al., 2007). Neuronal cell bodies are in general difficult to detect using antibody labeling but their distribution has been demonstrated using *in situ* hybridization histochemistry (Wang et al., 2000; Faber et al., 2007) and labeling of beta-galactosidase in knock-in mice with beta-galactosidase driven by the PTH2R promoter (Faber et al., 2007). The PTH2R is expressed in the cerebral cortex, the caudate nucleus, the lateral septal nucleus, the bed nucleus of the stria terminalis, the central and medial amygdaloid nuclei, the medial preoptic area, the hypothalamic periventricular, paraventricular and arcuate nuclei, midline and intralaminar thalamic nuclei, the medial geniculate body, the pretectal and ventral tegmental areas, the superior colliculus, the pontine tegmentum, the nucleus of the solitary tract, and the cerebellar cortex. In general, these regions contain a matching density of PTH2R-immunoreactive (-ir) fibers, which are also abundant in the median eminence, the periaqueductal gray, the lateral parabrachial nucleus, the sensory trigeminal nuclei, and lamina II of the spinal cord dorsal horn (Wang et al., 2000; Faber et al., 2007). Moreover, in rodents the distribution of TIP39-ir fibers and fiber terminals correlates almost perfectly with the brain areas containing PTH2Rs (Dobolyi et al., 2003b; Faber et al., 2007). Furthermore, even the subregional distribution of TIP39- and PTH2R-ir fibers in these regions showed remarkable similarities in rats (Dobolyi et al., 2006a) as well as in mice (Faber et al., 2007), providing anatomical evidence that TIP39 acts on the PTH2R neurons.

TIP39 neurons restricted to two discrete brain regions give rise to the widely distributed TIP39-ir fibers in both rats and mice (Dobolyi et al., 2003b; Faber et al., 2007). One of them is the subparafascicular area (Wang et al., 2006b), which extends from a medial part in the periventricular gray of the thalamus posterolaterally to the medial geniculate body. The other one is the medial paralemniscal nucleus at the midbrain–pons junction (Varga et al., 2008). It has been established by their disappearance following lesions, as well as by anterograde tracer techniques, that TIP39-ir fibers in limbic and endocrine regions originate in the subparafascicular area while auditory, and nociceptive-viscerosensory regions including the lateral parabrachial nucleus and the spinal cord receive TIP39-ir projections from the medial paralemniscal nucleus (Dobolyi et al., 2003a; Wang et al., 2006c).

Initial functional studies implicate TIP39 in the modulation of some aspects of spinal nociceptive signaling (Dobolyi et al., 2002) and in the modulation of an affective component of nociception (LaBuda and Usdin, 2004). Furthermore, c-Fos activation in brain areas expressing TIP39, suggests that TIP39 neurons may be involved in central regulation of reproduction (Lin et al., 1998; Li et al., 1999; Coolen et al., 2004). Specifically, c-Fos activation has been demonstrated in subparafascicular TIP39 neurons following male sexual behavior (Wang et al., 2006a). An experiment using positron emission tomography to measure increases in regional cerebral blood flow suggests that the subparafascicular area is also activated during human male ejaculation (Holstege et al., 2003). TIP39 has also been suggested to affect the neuroendocrine system. It may regulate the release of pituitary hormones (Ward et al., 2001; Sugimura et al., 2003; Usdin et al., 2003) and is potentially involved in the audiogenic stress response (Palkovits et al., 2004). In addition, intracerebroventricular injection of TIP39 resulted in anxiolytic- and antidepressant-like effects (LaBuda et al., 2004). Furthermore, a very recent study demonstrated increased fear and stress-related anxiety-like behavior in mice lacking TIP39 (Fegley et al., 2008).

There is extremely limited information on TIP39 and the PTH2R in primates. A human PTH2R cDNA has been cloned and both sequences have been identified in the human genome. TIP39 was found in total brain cDNA by RT-PCR and in a preliminary study we reported evidence for the expression of the PTH2R in combined brainstem samples using RT-PCR (Bago et al., 2008). We have now investigated the anatomical expression patterns of TIP39 and the PTH2R in macaque and human brain so that a comparison can be made to the comprehensive mapping that has been performed in rodents. There is a dramatic decline in TIP39 expression during postnatal development in the rat brain (Dobolyi et al., 2006b), so, we used the brain of a 3-day-old macaque to identify the location of TIP39 neurons, by *in situ* hybridization histochemistry. We also mapped the distribution of PTH2R mRNA in a representative set of areas in this brain, because PTH2R expression did not change during postnatal development in the rat (Dobolyi et al., 2006b). In material from an adult macaque we investigated whether PTH2R neurons are glutamatergic by performing double labeling with antibodies to the PTH2R and vesicular glutamate transporter 2 (VGLUT2). We confirmed that humans have a similar distribution of PTH2R synthesis by mapping the expression of the PTH2R in several different brain regions by RT-PCR in adult. We used immunohistochemistry to examine the distribution of PTH2R-ir fibers in material from both young and old humans in a number of brain regions selected to provide comparison with rodent data. Since TIP39 has been suggested to alter plasma growth hormone and corticosterone levels (Ward et al., 2001; Usdin et al., 2003), we also