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EVIDENCE FOR THE EXPRESSION OF PARATHYROID HORMONE 2 RECEPTOR IN THE HUMAN BRAINSTEM

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Abstract

Background and purpose—The parathyroid hormone 2 receptor (PTH2R) is a G protein coupled receptor. Pharmacological and anatomical evidence suggests that the recently identified tuberoinfundibular peptide of 39 residues is, and parathyroid hormone and parathyroid hormone-related peptide are not, its endogenous ligand. Initial functional studies suggest that the PTH2R is involved in the regulation of viscerosensory information processing. As a first step towards clinical applications, herein we describe the presence of the PTH2R in the human brain-stem.

Material and methods—Total RNA was isolated from postmortem human cortical and brainstem samples for RT-PCR. Good quality RNA, as assessed on formaldehyde gel, was reverse transcribed. The combined cDNA products were used as template in PCR reactions with primer pairs specific for the human PTH2R. In addition, PTH2R immunolabelling was performed on free floating sections of the human medulla oblongata using fluorescent amplification immunochemistry.

Results—Specific bands in the RT-PCR experiments and sequencing of PCR products demonstrated the expression of PTH2R mRNA in the human brainstem. A high density of PTH2R-immunoreactive fibers was found in brain regions of the medulla oblongata including the nucleus of the solitary tract, the spinal trigeminal nucleus, and the dorsal reticular nucleus of the medulla.

Conclusion—Independent demonstration of the presence of PTH2R mRNA and immunoreactivity supports the specific expression of the PTH2R in the human brainstem. The distribution of PTH2R-immunoreactive fibers in viscerosensory brain regions is similar to that reported in mouse and rat suggesting a similar role of the PTH2R in human as in rodents. This finding will have important implications when experimental data obtained on the function of the TIP39-PTH2R neuromodulator system in rodents are to be utilized in human.

Keywords

neuropeptide receptor; neuromodulation; nociceptive information processing; pain; visceral sensory transmission

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The parathyroid hormone 2 receptor (PTH2R) was identified on the basis of its sequence homology to other polypeptide-recognizing seven transmembrane domain receptors[1]. It has about 50% amino acid sequence similarity with the parathyroid hormone 1 receptor (PTH1R). Pharmacological and anatomical evidence suggested that the recently identified tuberoinfundibular peptide of 39 residues (TIP39) is, and parathyroid hormone and parathyroid hormone-related peptide are not, the endogenous ligands of the PTH2R. TIP39 binds to the rat and human receptors with high affinity and is a potent agonist[2, 3]. TIP39 and the PTH2R are abundantly expressed in the rat and mouse brains[1, 4–6] with similar distributions[5, 7] whereas parathyroid hormone has not been detected in the brain[8].

Initial functional studies suggest that the TIP39-PTH2R neuromodulator system is involved in nociceptive information processings. Intrathecal administration of TIP39 increased sensitivity in tail-flick and paw-pressure assays[9]. Furthermore, these pronociceptive effects of TIP39 were antagonized with intrathecal administration of a TIP39 antibody suggesting that some aspects of nociception are potentiated via the PTH2R[9]. Investigation of the physiological functions of TIP39 in human is limited by the lack of information on its expression in the human brain. To alleviate this limitation and to take a step towards clinical applications of future drugs acting on the PTH2R, here we describe the expression of the PTH2R in human brainstem. An RT-PCR experiment was conducted to demonstrate the presence of PTH2R mRNA in the human brainstem. In addition, PTH2R immunoreactivity has also been examined using the sensitive technique of fluorescent amplification immunocytochemistry.

Materials and methods

RT-PCR

Brainstem and control cortical samples were dissected from three *post mortem* human brains to isolate RNA. The degradation of RNA was assessed by running the purified RNAs on denaturing formaldehyde gels. Samples, in which the amount of 28S rRNA was at least equal to that of 18S rRNA were processed further for RT-PCR. RNA was treated with Amplification Grade DNase I (Invitrogen) and cDNA was synthesized with a Superscript II reverse transcriptase kit (Invitrogen) according to the manufacturer's instructions. CDNA of the selected cortical as well as brainstem samples were pooled. The resulting cDNA was used as template in PCR reactions performed with iTaq DNA polymerase (Bio-Rad Laboratories, Hercules, CA). Three different primer pairs were used for PTH2R (GeneBank accession number: NM_005048). A: AATGGAGAGGTTCAGGCAGAAND and TCTCCTTGGCATCCTTCAGT, the calculated lengths of the PCR products is 386 base pair (bp); B: CTGTGGGGCTTCATCTTGA and TGCCTGAACCTCTCCATTG, the PCR product is 441 bp; C: CAATTGCTTGGCTGTAGCTTT and ACAAAATCAATTTGCAGACACAA, the PCR products is 440 bp. The primer pair for GAPDH (GeneBank accession number: NM_002046) was CCACCCAGAAGACTGTGGAT and CCCTGTTGCTGTAGCCAAAT to result in a PCR product of 423 bp. PCR products were run on gel and pictures were taken by a digital camera. The identity of PCR products was verified by sequencing.

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Dissected brain tissue samples were immersion fixed and stored in 4% paraformaldehyde. Then, the tissue blocks were then frozen and 50 μm thick sections were sliced on a freezing microtome. Free-floating sections were pretreated with bovine serum albumin containing Triton X-100. The sections were then placed in anti-PTH2R primary antiserum (1:20000 dilution) for 48 hours, in biotinylated anti-rabbit secondary antibody (1:600 dilution; Vector Laboratories, Burlingame, CA) for 2 hours followed by incubation in avidin-biotin-peroxidase complex (1:300 dilution; Vector Laboratories, Burlingame, CA) for 2 hours. The sections were then treated with FITC-tyramide (1:8000 dilution) and H_2O_2 in Tris hydrochloride buffer (0.1

M, pH 8.0) for 6 minutes, as described previously[10]. The sections were then mounted on positively charged slides, dried, coverslipped, and examined using an Olympus BX60 light microscope.

Results

The intensity of the 28S rRNA band was at least equal to that of the 18S rRNA band in about half of the purified RNA samples run on denaturing formaldehyde gels. These cortical and brainstem RNA samples were separately pooled. PTH2R mRNA was not detected in cortical samples using any of the three primer pairs (Figure 1.). Bands of the expected size were present following amplification of the brainstem sample mixture using primer pairs A and C (Figure 1.). Sequencing of these PCR products verified the presence of PTH2R mRNA in the human brainstem.

Immunocytochemical studies showed a high density of PTH2R-immunoreactive fibers in the medulla oblongata including the nucleus of the solitary tract, the spinal trigeminal nucleus, and the dorsal reticular nucleus of the medulla (Figure 2.). In contrast to dense PTH2R-immunoreactive fiber networks in several brain areas, no PTH2R-immunoreactive cell bodies were detected, which seems to be similar to the PTH2R immunolabeling in rodents[5].

Discussion

Our RT-PCR study provides the first demonstration of the presence of PTH2R mRNA in the human central nervous system. Two different primer pairs resulted in single bands with the expected molecular weights arguing for specific PCR products. Sequencing also confirmed that PTH2R cDNA was specifically amplified. The finding that brainstem, but not cortical samples contained PTH2R mRNA suggests that PTH2R expression is spatially regulated. In addition to the expression of mRNA, the presence of PTH2R immunoreactivity was also demonstrated, supporting the expression of the PTH2R in the human brainstem.

The high density of PTH2R-positive fibers in the spinal trigeminal nucleus and the nucleus of the solitary tract indicates that the PTH2R might have a role in modulating the transmission of autonomic and nociceptive information. The distribution of PTH2R-containing fibers is similar to that previously found in rodents[1, 4, 5] suggesting that a pronociceptive function of the TIP39-PTH2R neuromodulator system demonstrated in rodents[9], is also present in human.

In conclusion, we described the expression of the PTH2R in the human brainstem, which is important for the human application of experimental data obtained on the function of the TIP39-PTH2R neuromodulator system in rodents.

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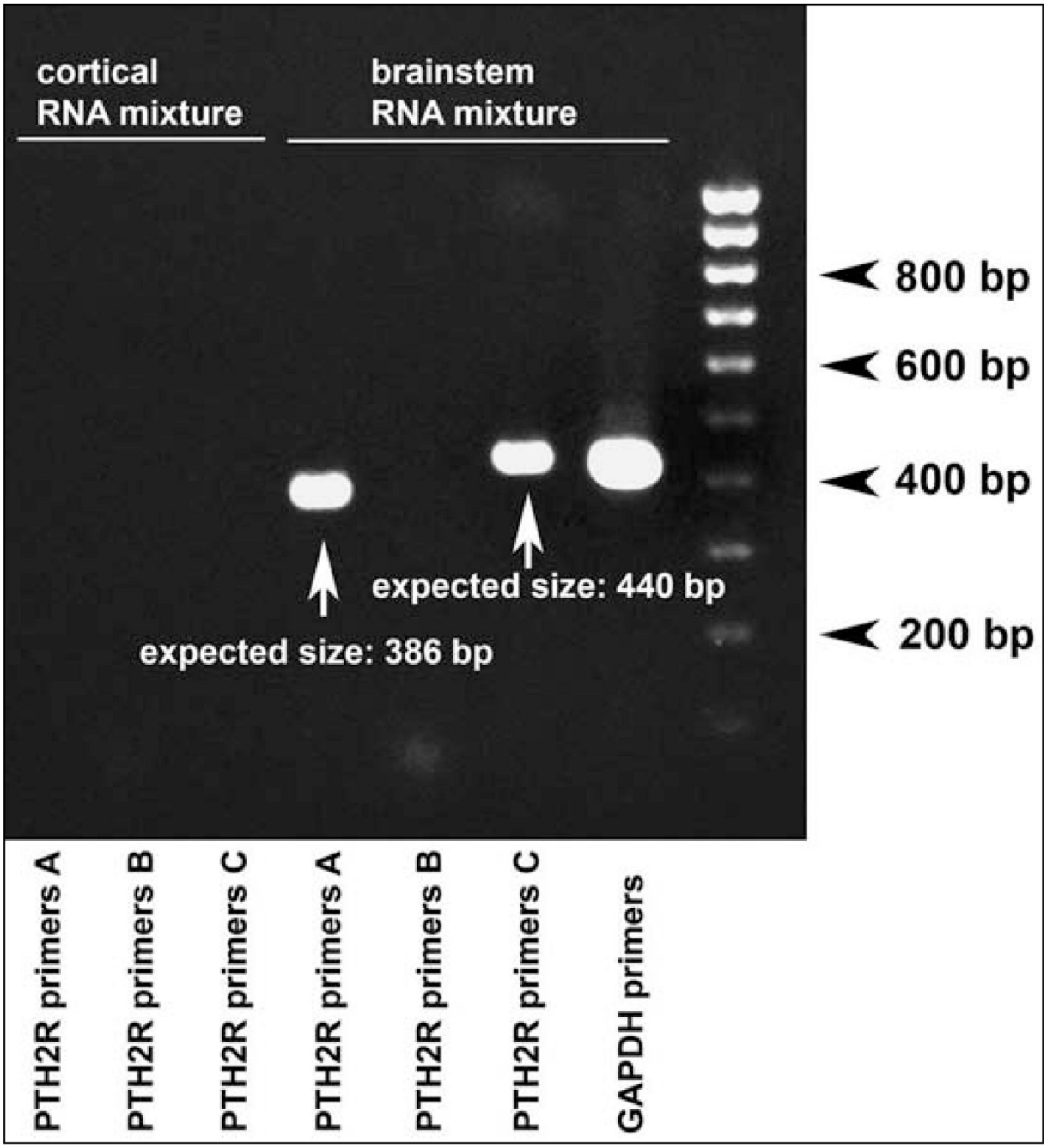

Figure 1.

An RT-PCR experiment demonstrates the expression of PTH2R mRNA in the human brainstem by showing PCR products run on gel. RNA from post mortem brainstem and control cortical samples was isolated and reverse transcribed. Pooled cortical and brainstem cDNA's was used as template in PCR reactions using three different PTH2R-specific primer pairs (PTH2R primers A, B, and C). The image shows PCR products run on gel. PTH2R RNA was not detected in cortical samples using any of the three primer pairs. Arrows indicate bands of the expected size amplified from the brainstem sample mixture using primer pairs A and C. The band of the housekeeping gene GAPDH also appears as positive control

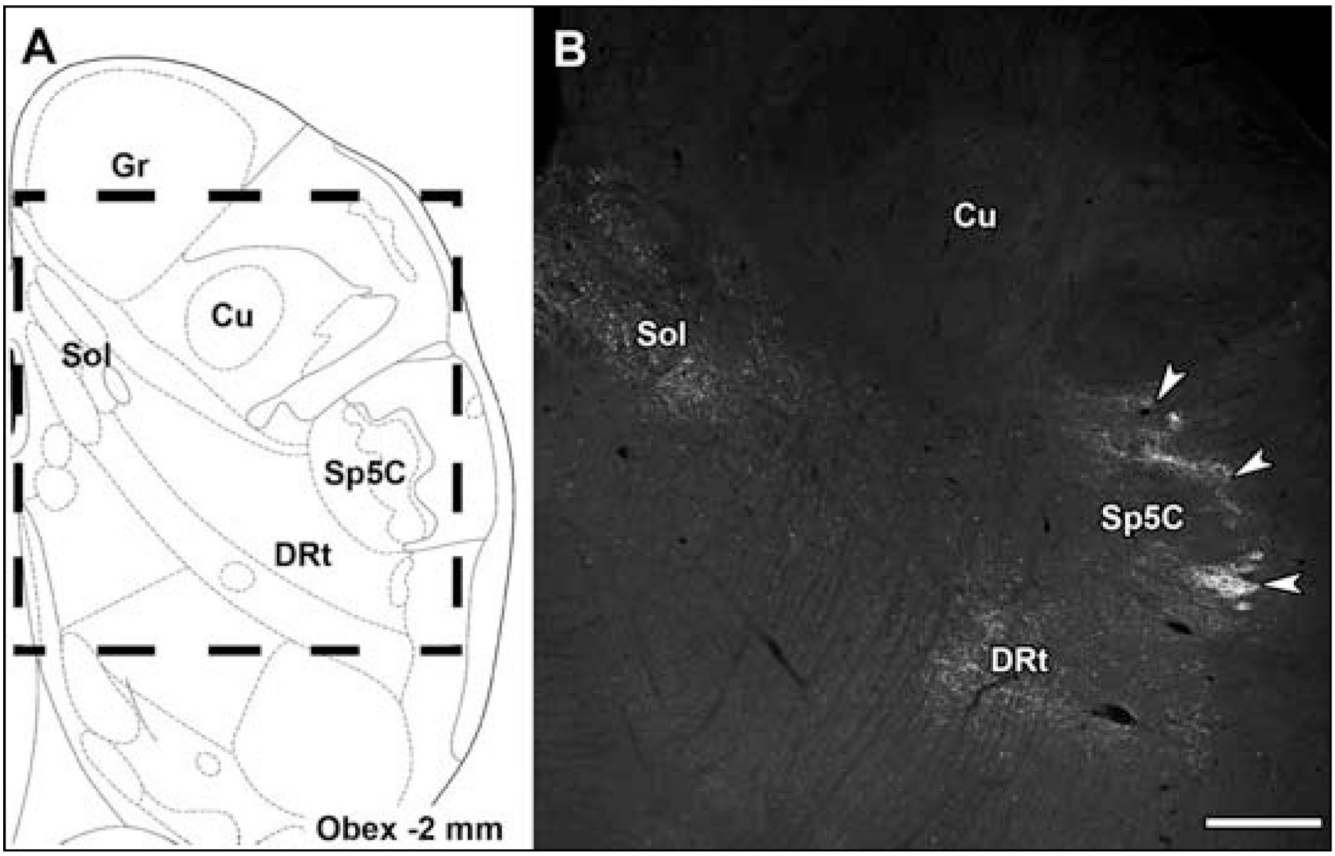

Figure 2.

PTH2R-immunoreactive fiber networks are shown in the human medulla oblongata. **A** A schematic drawing of a coronal section of the brain area 2 mm caudal to the level of the obex based on a human brainstem atlas[11]. **B** A photomicrograph of a section corresponding to the framed area in panel A shows fluorescent immunolabeling of PTH2R in the nucleus of the solitary tract (Sol), the caudal part of the spinal trigeminal nucleus (Sp5C), and the dorsal reticular nucleus (DRt). Arrows point to the superficial laminae of Sp5C where the most intense labeling was found

Cu: cuneate nuclues, Gr: gracile nucleus. Scale bar=1 mm.