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Immunocytochemical localization of neuropeptide Y (NPY) in the human hypothalamus

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Summary. In order to study the distribution of neuropeptide Y-like immunoreactivity in the human hypothalamus, an immunocytochemical localization of this peptide was performed. Using antibodies developed against synthetic porcine neuropeptide Y (NPY), we have been able to localize immunoreactivity in neuronal cell bodies located exclusively in the infundibular nucleus. Immunostained fibers were found in several regions in the hypothalamus with a high concentration in the periventricular areas. Fibers were also found in the neurovascular zone of the median eminence, the pituitary stalk and the posterior pituitary. These results suggest that immunoreactive material related to porcine NPY is present in the human hypothalamus, with a distribution similar to that observed in the rat.

Key words: Neuropeptide Y – Hypothalamus, human – Immunocytochemistry – Pituitary stalk

Recently, a 36 amino acid residue peptide has been purified from porcine brain and fully characterized (Tatemoto 1982; Tatemoto et al. 1982). Since this peptide has a high degree of homology with pancreatic polypeptide and the newly discovered intestinal peptide YY (PYY), a candidate gut hormone, it has been named neuropeptide Y (NPY). Combining radioimmunoassay and column chromatography, Allen et al. (1983) have shown that immunoreactive material corresponding to NPY was present in high concentrations in several areas of the rat brain. Immunocytochemistry has revealed reactive neuronal cell bodies in the cortex, caudate-putamen nucleus, hypothalamus (arcuate nucleus), hippocampus, anterior olfactory bulb, septum, nucleus accumbens, amygdaloid complex, and periaqueductal grey. High concentrations of fibers and terminals alone were detected in the bed nucleus of the stria terminalis, the peri- and paraventricular regions of the hypothalamus and thalamus, and discrete hypothalamic nuclei, particularly the suprachiasmatic nucleus. At the ultrastructural level, immunostaining was observed in the dense-core vesicles of axon profiles and terminals (Guy et al. 1983; Pelletier et al. submitted). Since the results obtained in the rat suggested that NPY could be a peptide with a transmitter or modulator role, it seemed important to determine whereas NPY-like immunoreactivity (NPY-LI) was also present in the human brain and to identify the morphological structures containing NPY-LI. For this purpose, we conducted an immunohistochemical localization of this peptide in the human hypothalamus and pituitary.

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Materials and methods

Hypothalami and pituitaries from two male and two female patients between 40 to 70 years of age were dissected within 6 h after death and fixed by immersion in Bouin's fluid for 2 days. After dehydration in ethanol, the tissues were embedded in paraffin. Serial coronal sections of the whole hypothalami and pituitaries were cut at 7 μm and mounted for immunohistochemistry.

The immunohistochemical technique used rabbit antiserum to porcine NPY, goat anti-rabbit gamma-globulins and the peroxidase-antiperoxidase complex as described by Sternberger (1972). Antibodies to NPY were raised in rabbits by injecting a mixture of synthetic porcine NPY, methylated bovine serum albumin (Sigma) and complete Freund's adjuvent, as described by Benoit et al. (1982). Each rabbit received at least four boosters. The serum of an immunized rabbit (No. 4603-2) was characterized by radioimmunoassay using iodinated NPY as the tracer. Two peptides with high degree of sequence homology with NPY (Tatemoto 1982), peptide YY and avian pancreatic polypeptide (APP) did not show significant cross-reactivity (less than 0.01%). Several other neuropeptides, such as ACTH, vasoactive intestinal peptide, somatostatin and cholecystokinin did not compete at all with the binding of NPY to antibodies.

For immunohistochemistry, the antiserum was used at a dilution of 1/500 to 1/1000. Control experiments were performed on adjacent sections by substituting normal rabbit serum or NPY antiserum (diluted at 1/500) absorbed with an excess (10^{-7} M) of NPY, PYY, APP, ACTH, β-endorphin, vasoactive intestinal peptide, cholecystokinin, somatostatin and LHRH.

Results

In the four hypothalami, which we have examined, immunostained nerve fibers were observed in a large area extending rostrocaudally from the most anterior portion of the supraoptic nuclei up to the posterior part of the mamillary bodies. Most of these positive fibers were observed in the vicinity of the third ventricle; lesser numbers were found

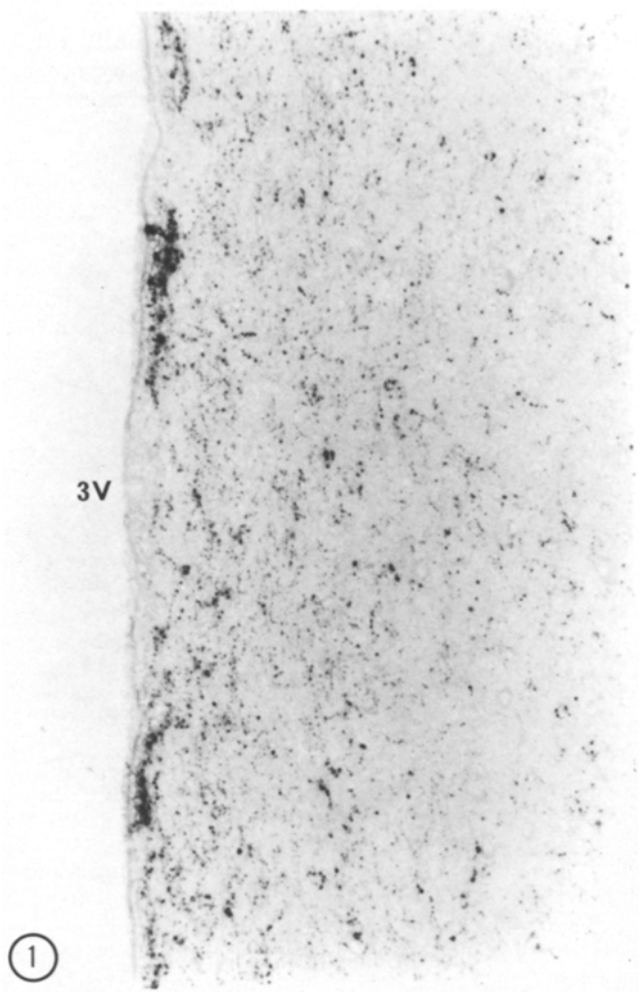

Fig. 1. Localization of NPY-LI in the periventricular nucleus of the human hypothalamus. A large number of positive fibers are located along the third ventricle (*V*). ×120

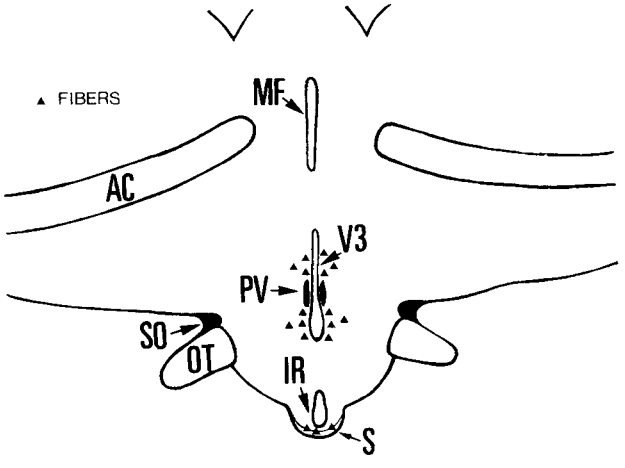

Fig. 2. Diagram modified from Daniel and Prichard (1975) showing the distribution of NPY-containing nerve fibers (△); *MF* midline fissure separating the two hemispheres; *AC* anterior commissure; *V3* third ventricle; *PV* paraventricular nucleus; *SO* supraoptic nucleus; *OT* optic tractus; *IR* infundibular recess of the third ventricle; *S* pituitary stalk

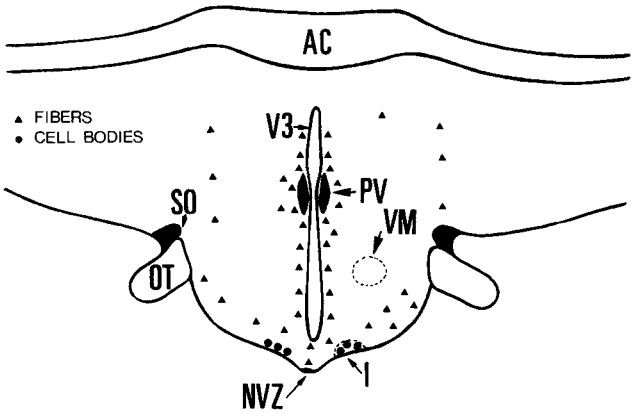

Fig. 3. Diagram modified from Daniel and Prichard (1975) showing the distribution of immunoreactive neuronal cell bodies (○) and fibers (△) in the human hypothalamus. *AC* anterior commissure; *V3* third ventricle; *PV* paraventricular nucleus; *SO* supraoptic nucleus; *VM* ventro-median nucleus, *OT* optic tractus; *NVZ* neurovascular zone of the median eminence; *I* infundibular nucleus

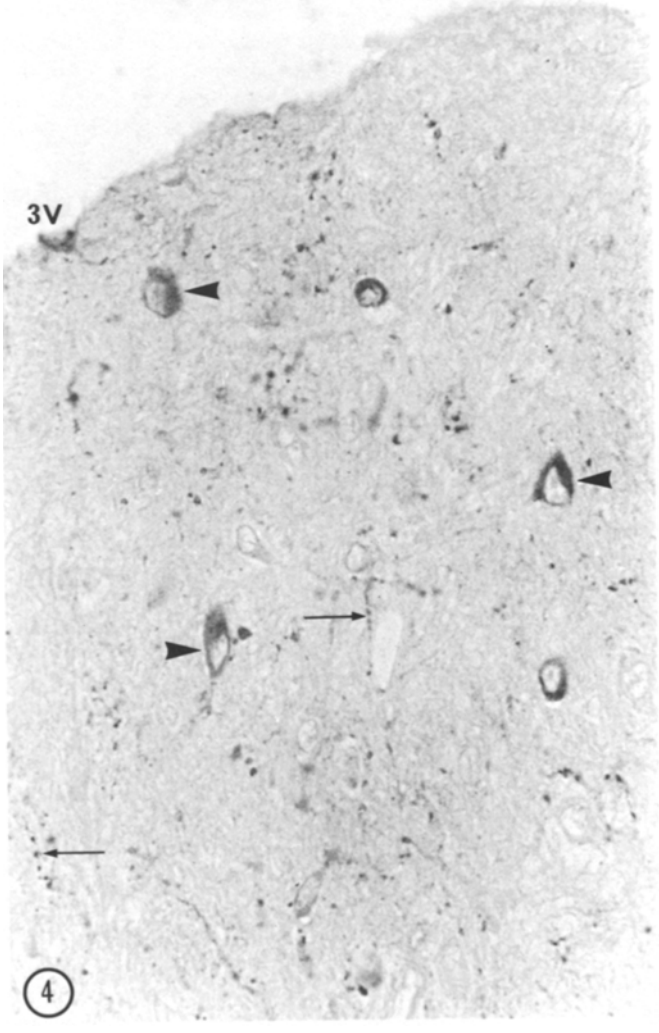

Fig. 4. Localization of NPY-LI in the infundibular nucleus of the human hypothalamus. Immunostained neuronal cell bodies (*arrowheads*) and fibers (*arrows*) can be observed. V: third ventricle. ×320

in the neurovascular zone of the median eminence and the lateral portions of the hypothalamus (Fig. 1). The highest concentration of fibers was observed in the medial portion of the hypothalamus, at the level of the ventro-median nucleus (Figs. 2, 3). Positive fibers were also detected in the pituitary stalk. They were abundant in the proximal portion of the stalk and low in number more distally. Only sparse fibers could be found in the posterior pituitary, whereas absolutely no staining could be detected in the anterior pituitary.

Strongly immunoreactive neuronal cell bodies were only found in a small area of the mediobasal hypothalamus corresponding to the infundibular nucleus (Figs. 3, 4). Immunostained cells extended rostro-caudally from the level of the anterior part of the paraventricular nucleus to the posterior portion of the infundibular nucleus. Occasionally, positive cell bodies were found below in floor of the third ventricle, but not in the pituitary stalk. The NPY-containing cells were characterized by a cytoplasmic staining which appeared granular at high magnification. Their diameter ranged from 18 to 25 μm. Numerous immunostained fibers were generally observed in proximity to the positive cell bodies.

No immunostaining could be detected when normal rabbit serum or anti-NPY absorbed with synthetic NPY was used. Immunoabsorption with PYY, APP, ACTH, VIP, somatostatin, cholecystokinin did not affect the intensity of staining.

Discussion

The present results demonstrate the presence of NPY-LI in the human hypothalamus. Since the antibodies used do not crossreact significantly with PYY and APP and since the staining cannot be abolished by previous immunoabsorption with these two peptides, it is likely that staining is due to the presence of NPY or a very closely related peptide. They concur with recent results indicating that NPY-LI in the rat brain can be detected in hypothalamic neuronal cell bodies mainly located in the arcuate nucleus (Allen et al. 1983; Guy et al. 1983), which is the equivalent of the infundibular nucleus of the human hypothalamus. Very recently, Adrian et al. (1983) have also reported high concentration of NPY-LI in the human hypothalamus. The high concentrations of positive fibers in the periventricular regions also resemble the distribution observed in the rat hypothalamus. Although the origin of the hypothalamic fibers containing NPY-LI could not be traced with certainty, it is likely that most arise from the hypothalamic cell bodies since there is no pathway as yet reported indicating an extra-hypothalamic origin.

The finding of a moderate number of NPY-LI containing fibers in the neurovascular zone and proximal portion of the pituitary stalk suggests that NPY could be released from the nerve endings in these areas eventually to reach the anterior pituitary cells. The rare positive fibers observed in the distal portion of the stalk and posterior pituitary also probably originate from hypothalamic neurons. The wide distribution of the NPY system throughout the hypothalamus suggests that this peptide might have a role in the modulation of hypothalamic function.

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