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Organization of neuropeptide tyrosine-like immunoreactive system in the brain of the Antarctic fish, *Trematomus bernacchii*

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**Abstract** Antarctic notothenioids have developed unique freezing-resistance adaptations, including brain diversification, to survive in the subzero waters of the Southern Ocean surrounding Antarctica. In this study we have investigated the anatomical distribution of neuropeptide tyrosine (NPY)-like immunoreactive elements in the brain of the Antarctic fish *Trematomus bernacchii*, by using an antiserum raised against porcine NPY. Perikarya exhibiting NPY-like immunoreactivity were observed in distinct regions of the brain. The most rostral group of immunoreactive perikarya was found in the telencephalon, within the entopeduncular nucleus. In the diencephalon, three groups of NPY-like immunoreactive perikarya were found in the hypothalamus. Two groups of positive cell bodies were found in distinct populations of the preoptic nucleus, whereas the other group was found in the nucleus of the lateral recess. More caudally, NPY immunoreactivity was detected in large neurons located in the subependymal layers of the dorsal tegmentum of the mesencephalon, medially to the torus semicircularis. NPY-like immunoreactive nerve fibres were more widely distributed throughout the telencephalon to the rhombencephalon. High densities of nerve fibres and terminals were observed in several regions of the telencephalon, olfactory bulbs, hypothalamus, tectum of the mesencephalon and in the ventral tegmentum of the rhombencephalon. The distribution of NPY-like immunoreactive structures suggests that, in *Trematomus*, this peptide may be involved in the control of several brain functions, including olfactory activity, feeding behaviour, and somatosensory and visual information. In comparison with other neuropeptides previously described in the brain of Antarctic fish, NPY is more widely distributed. Our data also indicate the existence of differences in the brain distribution of NPY between *Trematomus* and other teleosts. In contrast with previous results reported in other fish, *Trematomus* contains positive fibres in the olfactory bulbs and immunoreactive perikarya in the nucleus of the lateral recess, whereas NPY-immunopositive cell bodies are absent in the thalamus and rhombencephalon, and no NPY immunoreactivity is present in the pituitary. These differences could be related to the Antarctic ecological diversity of notothenioids living at subzero temperatures.

**Introduction**

Neuropeptide tyrosine (NPY) is a 36-amino-acid C-terminally amidated peptide originally isolated and characterized from the porcine brain (Tatemoto et al. 1982). NPY belongs to a family of structurally related peptides which includes pancreatic polypeptide (PP) and peptide tyrosine-tyrosine (PYY). NPY has been found in the nervous system of all vertebrate groups. The structure of NPY shows remarkable sequence homology from fish to mammals (Larhammar 1996). The concentration of NPY in the brain is higher than that of any other known neuropeptide (McDonald 1988). In mammals, the highest densities of NPY have been found in the cerebral cortex, limbic regions, hypothalamus and brainstem (Vallarino et al. 1995, for review). In the central nervous system of mammals, NPY has a wide range of physiological activities, such as regulation of neuroendocrine secretion (Wahbestedt et al. 1987), stimulation of food intake (Sahu et al. 1988), control of cardiovascular functions (Gibbins and Morris 1988; Martin et al. 1988), inhibition of sexual behavior (Kalra et al. 1987) and regulation of circadian rhythms (Card and Moore 1989). There is a considerable amount of evidence indicating the widespread distribution of NPY also in the brain of
birds, reptiles and amphibians (Vallarino et al. 1995, for review). In contrast, the distribution of NPY-immunoreactive neurons is far more restricted in elasmobranch (Vallarino et al. 1988; Chiba and Homma 1994), teleost fish (Breton et al. 1989; Pontet et al. 1989; Batten et al. 1990; Danger et al. 1991; García-Fernández et al. 1992; Vecino and Ekström 1992; Ceprano and Schreibman 1993) and lungfish (Vallarino et al. 1995).

In teleost fish, the presence of NPY in the brain has been described in detail only in a limited number of species, including Salmo salar (García-Fernández et al. 1992; Vecino and Ekström 1992), Oncorhynchus mykiss (Danger et al. 1991), Gambusia affinis (García-Fernández et al. 1992) and Carassius auratus (Pontet et al. 1989; Pickavance et al. 1992). To the authors’ knowledge, there are no reports on the organization of the NPY system in the brain of fish adapted to subzero waters, as those living in the Antarctic environment. In the ichthyofauna of the Southern Ocean surrounding Antarctica, more than 50% of the species of the suborder of Notothenioidei are present (Gon and Heemstra 1990). In these environmental conditions, notothenioids are protected by various freezing resistance adaptations: for instance, the blood (DeVries 1988) and the cerebrospinal fluid (DeVries and Cheng 1992) contain antifreeze glycoprotein; the serum osmoolality is considerably higher than that reported in the serum of non-Antarctic notothenioids (Eastman 1993). In addition, unique features have been described in the brain of Antarctic notothenioids, for example, in the organization of the subependymal region of the diencephalon (Lannoo and Eastman 1995). Some years ago, we started a project on the chemical neuroanatomy of Antarctic fish, in order to compare the pattern of their neuropeptidergic systems with those occurring in teleosts of temperate waters (Pestarino and Vallarino 1996; Pestarino et al. 1998, 2000). In the present study, we have investigated the distribution of NPY-immunoreactive neurons in the brain of the Antarctic nototheniid, Trematomus bernacchii, in order to determine whether the ecological diversity of this fish is reflected in the organization of the neuropeptidergic system.

Materials and methods

Eight specimens of mature males and females (23–35 cm body length, 85–100 g body weight) of the Antarctic fish, T. bernacchii (Notothenioidei, Nototheniidae), were collected near the Italian Antarctic Station of Terra Nova Bay (Ross Sea), in austral summer 1999/2000. The animals were decapitated, and the brains with the attached pituitaries immediately removed, fixed in Bouin’s fluid for 10 h, dehydrated in ethanol, cleared in xylene and embedded in Paraplast. Serial coronal and sagittal sections were cut (6 μm thick) and mounted in chrome alum/gelatin-coated slides. The sections were rehydrated and treated in a moist dark chamber sequentially with: (1) the normal swine serum (NSS) (Sigma, St. Louis, Mo.) diluted 1:50 in 0.05 M, pH 7.4, phosphate-buffered saline (PBS) at room temperature for 30 min; (2) the NPY primary antiserum, raised in rabbit against porcine NPY (Affinity, Exeter, UK) diluted 1:800 in PBS containing 0.3% Triton X100 and 1% bovine serum albumin at 4°C for 18 h; (3) the secondary fluorescein isothiocyanate-conjugated swine anti-rabbit gammaglobulins (Dukopatts, Denmark) diluted 1:200 in PBS at room temperature for 1 h. Finally, the sections were washed three times in PBS, mounted in glycerol/PBS (1:9) and examined under a Zeiss epifluorescence microscope.

The specificity of the immunoreaction was controlled by: (1) replacement of the primary antiserum with nonimmune rabbit serum diluted 1:40; (2) substitution of the primary antiserum with PBS; (3) preincubation of the primary antiserum with porcine NPY (Affinity) or the related peptides, avian PP or porcine PYY (Peninsula Laboratories, Belmont, Calif.) at a final concentration of 10^{-6} M each.

In order to localize the immunofluorescent cell bodies and fibres, several sections were stained by hematoxylin and eosin at the end of the immunocytochemical procedure. To identify the anatomical regions in the brain of T. bernacchii, we referred to Eastman and Lannoo (1995).

Results

Incubation of brain sections with an antiserum raised against porcine NPY revealed the existence of several populations of NPY-like immunoreactive cell bodies and fibres in the brain of T. bernacchii. The distribution and relative density of NPY-like immunoreactive perikarya and nerve fibres in the brain are shown in Fig. 1. No detectable differences in the distribution of NPY-like immunoreactive elements were observed between males and females.

Diencephalon

The diencephalon contained numerous NPY-like immunoreactive perikarya scattered throughout the entopeduncular nucleus. These elements were round in shape and exhibited a moderate fluorescence (Fig. 2A, B). The other regions of the diencephalon did not show positive cell bodies. By contrast, NPY-like immunoreactive nerve fibres were found in several regions, including the dorsomedial, dorsolateral and ventrolateral subdivision of the diencephalon. In particular, dense accumulations of nerve fibres were present in the dorsolateral diencephalon (Fig. 2C) and within the entopeduncular region (Fig. 2A, B). In contrast, a lower number of immunoreactive nerve fibres was found in the lateral part of the posterior region of the dorsal diencephalon, as well as in the dorsomedial and dorsal subdivisions of the dorsal diencephalon (Fig. 2D). Several beaded immunoreactive nerve fibres were also detected in the ventral part of the rostral diencephalon (Fig. 2E) and within the olfactory bulbs (Fig. 2F).

Diencephalon

In the diencephalon, three groups of NPY-like immunoreactive perikarya were found in the hypothalamus. Two groups of immunoreactive cell bodies were detected in distinct populations of the preoptic nucleus. In particular, one group of positive cells was localized in the rostroventral component of the nucleus, whereas the other group was found in the dorsomedial