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# Distribution and characterization of neuropeptide Y-like immunoreactivity in the brain and pituitary of the goldfish

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**Summary.** The distribution of neuropeptide Y (NPY) immunoreactivity has been studied by means of immunocytochemistry and radioimmunoassay in the brain of the goldfish. It was found that NPY had a widespread distribution in the entire brain in particular in the telencephalon, diencephalon, optic tectum and rhombencephalon. In the pituitary gland, positive type-B fibers were observed in the various lobes frequently in direct contact with secretory cells, in particular the gonadotrophs, somatotrophs and MSH (melanocyte-stimulating hormone) secreting cells. When measured by radioimmunoassay, the highest NPY concentrations were found in the pituitary and telencephalon, confirming the results of immunocytochemistry. The displacement curves obtained with serial dilutions of brain extracts were parallel to that of synthetic porcine NPY. Following high performance liquid chromatography, the NPY-like material extracted from goldfish brain co-eluted as a single peak with synthetic porcine NPY. These data demonstrate the presence of an NPY-like substance widely distributed in the goldfish brain. The observation of NPY-immunoreactive fibers in the pituitary gland suggests that, among its other functions, NPY may play a role in the neuroendocrine regulation of pituitary function.

**Key words:** Neuropeptide – Pituitary – Brain – Immunocytochemistry – HPLC – *Carassius auratus* (Teleostei)

Neuropeptide Y (NPY) is a member of the pancreatic polypeptide family that has been isolated from porcine brain extracts (Tatemoto et al. 1982) and that has a widespread distribution in the peripheral (Lundberg et al. 1982; Uddman et al. 1985) and central nervous system of mammals (Allen et al. 1983; Everitt et al. 1984; Chronwall et al. 1985) including man (Adrian et al. 1983; Pelletier et al. 1984). Among other putative functions (for review, see Gray and Morley 1986), considerable interest has been focused on the possible role of NPY on the neuroendocrine modulation of various pituitary hormone secretions, in particular the control of gonadotrophin, growth hormone (Kalra and Crowley 1984; Kerkerian et al. 1985; McDonald et al. 1985) and melanotropin release (Danger et al. 1985). However, there is very little information from other groups of vertebrates. In amphibians, a peptide closely related to NPY has been identified and localized in various brain areas (Danger et al. 1985; Cailliez et al. 1987), but to date little data are available in fish, with the exception of the description of immunoreactive amacrine cells in the goldfish retina (Osborne et al. 1985). Recently, a peptide having 83% homology with porcine NPY has been isolated from coho salmon endocrine pancreas (Kimmel et al. 1986).

Considering the quantitative and qualitative importance of this peptide in other vertebrates and in particular its putative neuroendocrine functions, we initiated investigations first to demonstrate the presence of an NPY-like substance in the brain of teleosts and then to study its possible involvement in the regulation of pituitary functions.

We report here our results on the localization of NPY immunoreactivity by means of immunohistochemistry and radioimmunoassay in the brain and pituitary gland of the goldfish.

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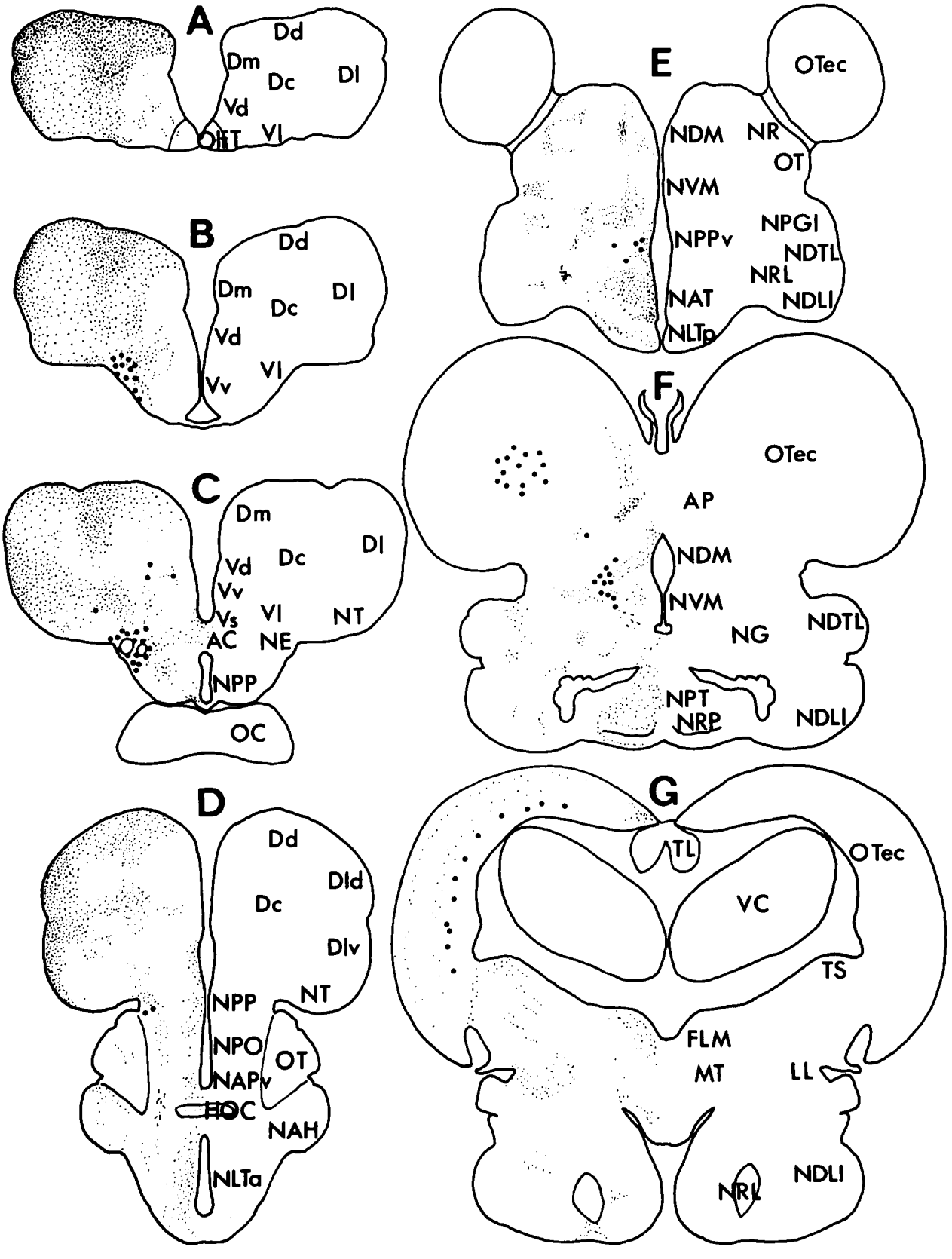
## Materials and methods

Adult male and female goldfish (*Carassius auratus*) (25–35 g) were collected from a natural pond located near Bordeaux and kept in running tap water under a 16L:8D photoregime.

*Immunohistochemistry:* The animals (N = 11) were perfused under anesthesia (MS 222, Sandoz) through the aortic bulb with 50 ml of 4% formaldehyde in phosphate buffer (PB, 0.1 M, pH 7.4). After dissection, the brain and pituitary were postfixed for 12 h in the same fixative at 6° C and cut using a Vibratome. After washing, the sections (50 µm) were pretreated for 30 min with 0.5% hydrogen peroxide to inactivate endogenous peroxidases, and for 30 min with swine serum (2% in PB). The slices were then incubated overnight at room temperature with antibodies against NPY (Pelletier et al. 1984) diluted 1:3000 in PB containing 0.1% Triton X100. After washing, they were exposed to peroxidase-labelled Fab fragments diluted 1:2000 for 2 h at room temperature, and the peroxidase activity was revealed using 0.025% diaminobenzidine and 0.006% hydrogen peroxide.

The nomenclature of forebrain nuclei used in this study is that of Peter and Gill (1975).

*Immunoelectron microscopy:* Brains and pituitaries ($N = 5$) were immersed in the above fixative to which 0.1% glutaral-


A
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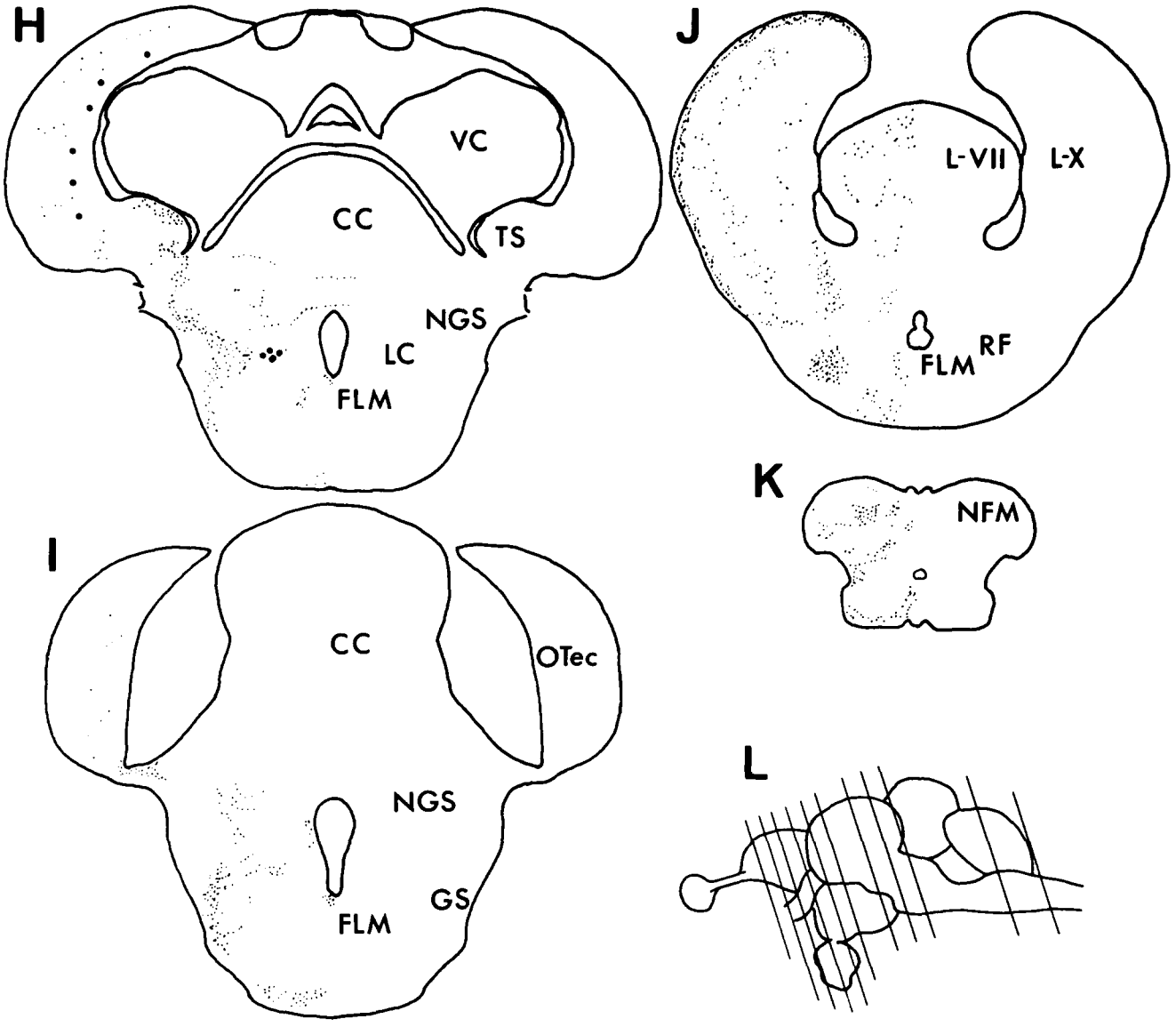



**Fig. 1 A–K.** Selected cross sections (levels given on Fig. 1 L) providing a semi-schematic representation of the distribution of NPY-immunoreactive perikarya (*full circles*) and fibers (*dots*) in the brain of the goldfish. *AC* anterior commissure; *AP* area praetectalis; *CC* corpus of the cerebellum; *Dc* area dorsalis telencephali, pars centralis; *Dd* area dorsalis telencephali, pars dorsalis; *Dl* area dorsalis telencephali, pars lateralis; *Dld* area dorsalis telencephali pars lateralis dorsalis; *Dlv* area dorsalis telencephali, pars lateralis ventralis, *Dm* area dorsalis telencephali pars medialis; *FLM* fasciculus longitudinalis medialis; *GS* tractus gustus secundus; *HOC* horizontal commissure; *LC* locus coeruleus; *LL* fasciculus longitudinalis lateralis; *L–VII* lobus facialis; *L–X* lobus vagi; *MT* midbrain tegmentum; *NAH* nucleus anterior hypothalami; *NAPv* nucleus anterior periventricularis; *NAT* nucleus anterior tuberis; *NDLI* nucleus diffusus lobi inferioris; *NDM* nucleus dorsomedialis thalami; *NDTL* nucleus diffusus tori lateralis; *NE* nucleus entopeduncularis; *NFM* nucleus funicularis medialis; *NG* nucleus glomerulosus; *NGS* nucleus gustus secundus; *NLTa* nucleus lateralis tuberis, pars anterior; *NLTp* nucleus lateralis tuberis, pars posterior; *NPG1* nucleus praeglomerulosus, pars lateralis; *NPO* nucleus praeopticus; *NPP* nucleus praeopticus periventricularis; *NPPv* nucleus posterior periventricularis; *NPT* nucleus posterior tuberis; *NR* nucleus rotundus; *NRL* nucleus recessus lateralis; *NRP* nucleus recessus posterioris; *NT* nucleus teniae; *NVM* nucleus ventromedialis thalami; *OC* optic chiasma; *OlfB* olfactory bulb; *OlfT* olfactory tract; *OT* optic tract; *OTec* optic tectum; *RF* reticular formation; *TL* torus longitudinalis; *TS* torus semicircularis; *VC* valvula cerebelli; *Vd* area ventralis telencephali, pars dorsalis; *Vl* area ventralis telencephali, pars lateralis; *Vs* area ventralis telencephali, pars supracommissuralis; *Vv* area ventralis telencephali, pars ventralis

dehyde was added, and cut at 50 µm. Some of the sections were processed for immunocytochemistry as described above, postfixed in 1% osmium tetroxide and flat embedded in Epon.

For post-embedding immunocytochemistry, Vibratome sections were embedded without osmium fixation. White ultrathin sections were collected on nickel grids, etched in hydrogen peroxide (10%), washed, exposed to bovine serum albumin (3%) and then to the specific antiserum diluted 1:1000 for 90 min. After washing, they were incubated with colloidal-gold-(10 nm)-labelled anti-rabbit immunoglobulins (Janssen) diluted 1:100 and counterstained with uranyl acetate.

*Tissue extractions:* For the detection and measurement of NPY, 30 brains were rapidly dissected and 7 main regions (olfactory bulbs, telencephalon, optic tectum and thalamus, inferior lobe, cerebellum, vagal lobe and pituitary) were separated. The tissues were immersed in boiling 2 N acetic acid for 10 min and sonicated. Because of the small quantity of tissue, the same regions of 5 individuals were pooled. After centrifugation (15 min; 15000 *g*), the supernatants were collected, freeze-dried and kept in a dry atmosphere until assay. The pellets were used for the measurement of protein content according to Lowry et al. (1951).

*Radioimmunoassay (RIA):* All radioimmunoassays were carried out in duplicate as described by Danger et al. (1985). Serial dilutions ($r = {}^1/_2$) of tissue homogenates from various brain regions were assayed for NPY in order to study parallels between the inhibition curves and the standard curve. All HPLC (high performance liquid chromatography) fractions were resuspended in 500 µl veronal buffer (20 mM veronal; 0.4% bovine serum albumin, 2 ml/l mercaptoethanol, pH 8.6) and the NPY concentration was measured in duplicate on 100 µl aliquot of each fraction.

*Chromatographic analysis.* Dry extracts from whole goldfish brains (N = 10) were resuspended in distilled water and centrifuged at 10000 g for 15 min. The supernatant was loaded onto a C-18 Sep-pak reverse phase chromatography cartridge (Alltech). The column was then washed with 20% acetonitrile – 0.1% trifluoroacetic acid and the NPY-containing fraction was eluted with 40% acetonitrile. After lyophilization, the extracts were resuspended in distilled water and subjected to reverse phase HPLC analysis on a Beckman liquid chromatograph (Model 114 M) equipped with a gradient controller (Model 421 A) and an Interchim/C-18 column (250 × 4.1 mm). The samples were eluted at 22° C using a gradient of acetonitrile – 0.1% trifluoroacetic acid with a flow rate of 1 ml/min. The gradient used is shown in Fig. 16. One-ml fractions were collected and air dried in a Speed Vac Concentrator (Savant, USA). The HPLC standard consisted of 1 µg synthetic NPY.

## Results

Immunohistochemistry: The distribution of NPY-immunoreactive (ir) structures, i.e. cell bodies and fibers, is presented on representative transverse sections from rostral to caudal (Fig. 1).

A few isolated stained perikarya were detected in the external layer of the olfactory bulbs, whereas ir fibers were observed in various parts of the bulbs and within the medial olfactory tracts (Fig. 2).

The telencephalon contained the highest density of both cell bodies and terminals (Fig. 1A to 1D). The cell bodies were located in the lateral parts of the caudal ventral telencephalon, in particular at the level of the nucleus entopeduncularis in which the ir cells surrounded the lateral forebrain bundle (NE; Fig. 4). This important cell cluster consisted of small round perikarya and, in its caudal part, it extended towards the dorsal part of the ventral telencephalon (Vd; Fig. 5). In terms of ir fibers, the dorsal telencephalon received a more massive innervation compared with the ventral part. In particular, there was a striking difference in the density of terminals observed between the central part of the dorsal telencephalon (Dc) and the dorsal part of the ventral telencephalon (Vd; Fig. 3). A few ir fibers were also detected at the level of the anterior commissure (AC) and in the area supracomissuralis of the ventral telencephalon (Vs; Fig. 1C).

Positive fibers were observed in most periventricular nuclei of the preoptic region (Fig. 1C to 1F), in particular the nucleus praeopticus periventricularis (NPP), the nucleus praeopticus (NPO), the nucleus anterior periventricularis (NAPv), and all levels of the nucleus lateralis tuberis (Fig. 6). These fibers were located in a subependymal position and never contacted the ventricle. Positive fibers entered the pituitary stalk and innervated the different lobes of the pituitary gland. More caudally, a few weakly ir cell bodies were detected in the nucleus posterior periventricularis (NPPv) and lateral to it (Fig. 1E). In the posterior hypothalamus, the nucleus anterior tuberis (NAT) received numerous ir fibers. Positive profiles were also detected around the nucleus recessus posterioris (NRP) and within the nucleus posterior tuberis (NPT). Laterally, a few scattered ir fibers were observed in the nucleus anterior hypothalami (NAH) and in the nucleus diffusus lobi inferioris (NDLI).

In the thalamic region, the most prominent feature was the presence of a dual population of ir cell bodies (Fig. 8), one consisting of small round cells, whereas the other was represented by large perikarya. These perikarya were located lateral to the nucleus dorsomedialis and ventromedialis thalami (NDM and NVM). In the surrounding area,

**Fig. 2.** Ir cell body (*arrow*) and fiber (*arrowhead*) at the periphery of the olfactory bulb (*Olf B*). Bar: 200 µm, × 70

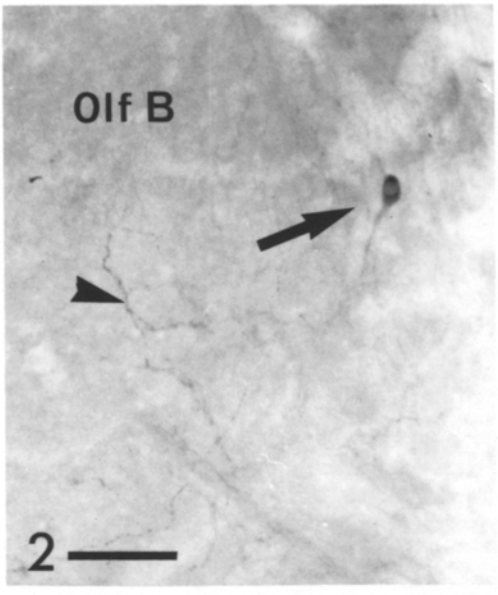
**Fig. 3.** Transverse section through the anterior telencephalon showing the striking difference between the NPY innervation of the dorsal part of the ventral telencephalon (*Vd*) and the central portion of the dorsal telencephalon (*Dc*). Bar: 200 µm, × 70

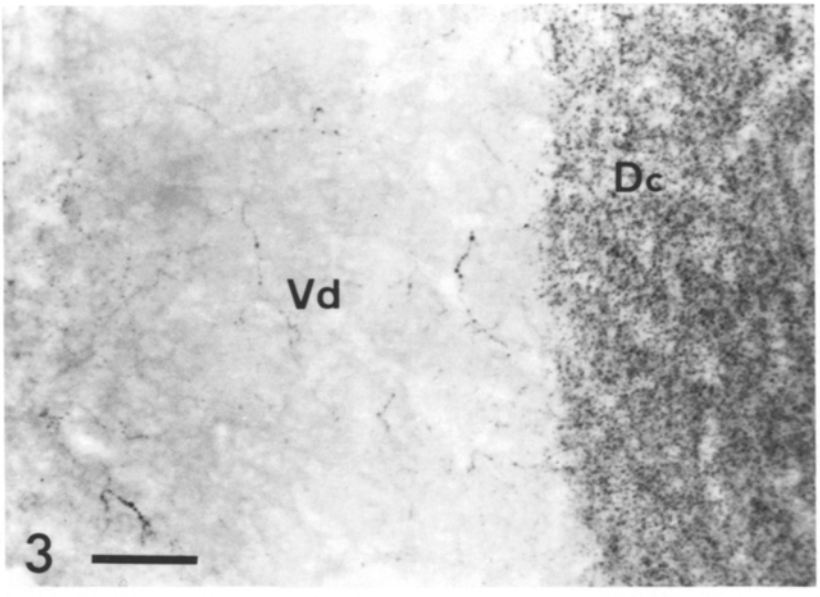
**Fig. 4.** The ir cell bodies located at the level of the nucleus entopeduncularis (*NE*). Bar: 200 µm, × 40

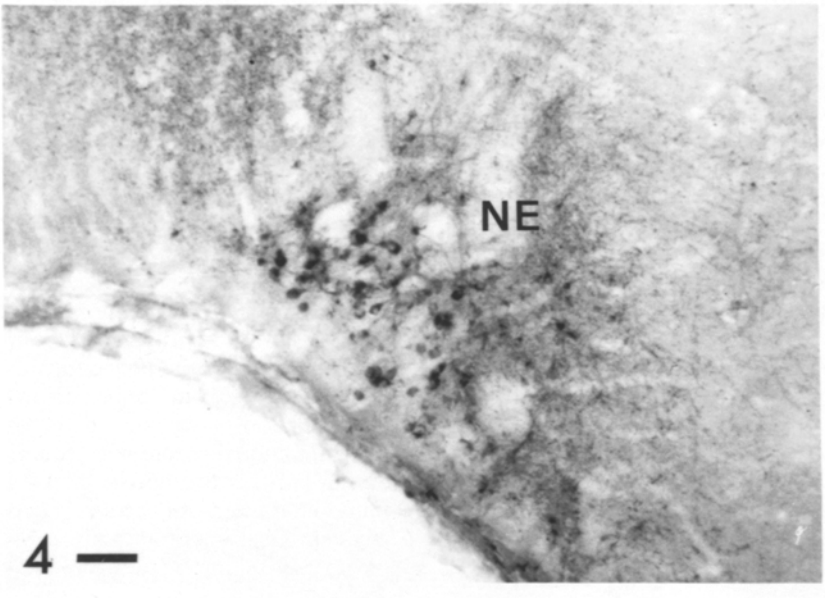
**Fig. 5.** A few scattered ir perikarya and fibers in the dorsal extent of the ventral telencephalon (*Vd*). Bar: 200 µm, × 75

**Fig. 6.** Transverse section of the hypothalamus at the level of the pituitary stalk showing the dense periventricular plexus of ir fibers. *NLTa* nucleus lateralis tuberis, pars anterior. Bar: 200 µm, × 32

**Fig. 7.** Transverse section of the anterior part of the pretectal area (*AP*). Bar: 200 µm, × 70


Olf B
2


Dc
Vd
3


NE
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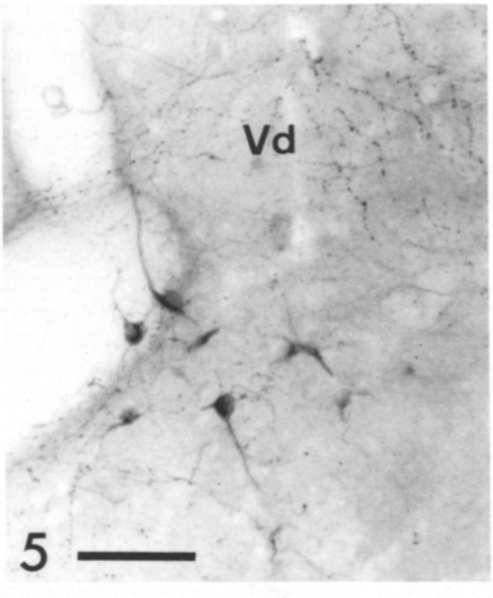
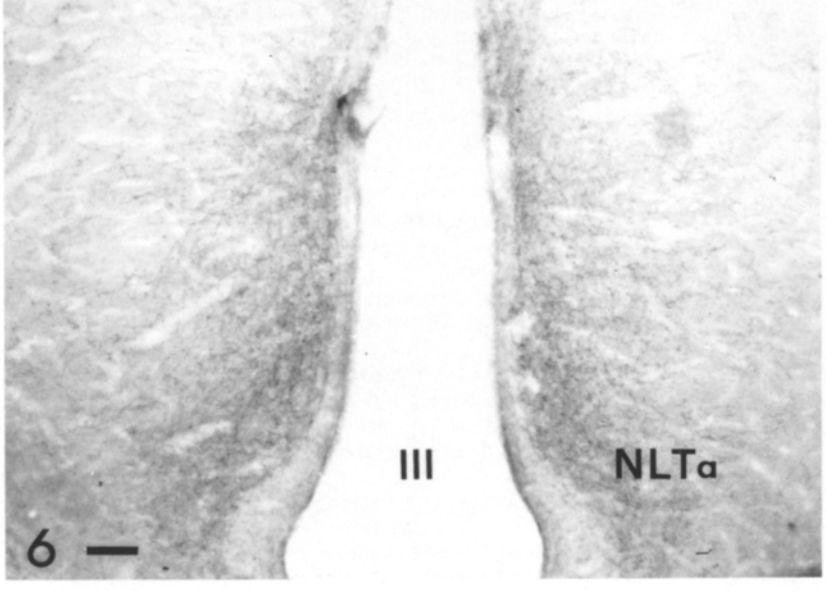

Vd
5


III
NLTa
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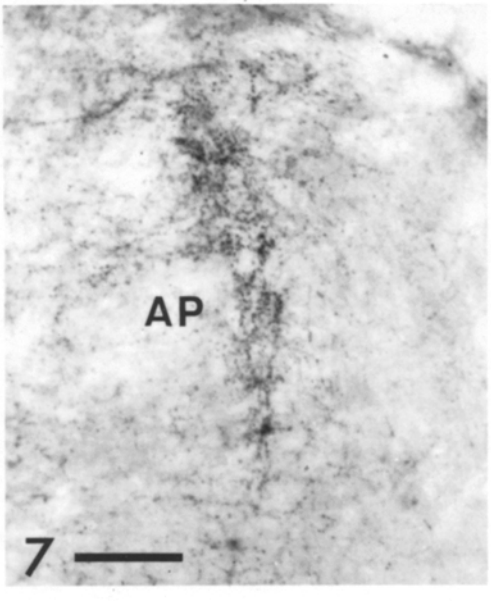

AP
7

numerous ir fibers and dendrites were also noted. Another remarkable feature was the heavy innervation of the area praetectalis (AP; Fig. 7). In the ventrolateral extent of this latter structure a few isolated ir cells could be detected (Fig. 1F).

From the pretectal region, fibers seemed to ascend towards the optic tectum (Fig. 1F to 1H), which, in addition to positive fibers, also contained numerous small ir cell bodies in the periventricular layer.

In the midbrain (MT), ir perikarya were found only at the level of the locus coeruleus (LC; Fig. 1G, 1H). These cells formed a well-defined nucleus that sent projections ventrolaterally (Fig. 10). Part of this tract appeared to ascend towards the torus semicircularis, in which the density of fibers was particularly high (Fig. 9). Positive fibers were observed along the tractus longitudinalis medialis (FLM) and lateralis (LL). No immunoreactivity could be detected in the cerebellum, the corpus or the valvula (Fig. 1G to 1I).

The vagal (L–X), glossopharyngeal (L–IX) and facial (L–VII) lobes all contained ir fibers, in particular the third layer of the vagal lobe (Fig. 11). Fibers appeared to run ventro- and dorso-laterally in the medulla oblongata and extended to the spinal cord (Fig. 1H to 1K). Neuronal processes were found in the nucleus funicularis medialis (NFM) and the dorsal and ventral horn of the spinal cord.

Electron-microscope examination following pre- or post-embedding immunocytochemistry at the level of the telencephalon and hypophysis enabled the positive processes to be characterized as type-B fibers; these fibers contained small (under 100 nm) neurosecretory vesicles on which immunoreactivity was located. In the adenohypophysis, positive endings were observed in direct contact with most cell types namely gonadotrophs, growth hormone (Fig. 12) cells and melanocyte-stimulating hormone (MSH) secreting cells (Fig. 13). Positive fibers were also detected in the rostral neurohypophysis.

Radioimmunoassay: The displacement curves obtained with serial dilutions of goldfish brain extracts dissected as shown on Fig. 14 were parallel to that of synthetic porcine NPY (Fig. 15). The concentrations of NPY-like material in the various brain regions dissected as shown are reported in Table I. When reported to the protein content, the highest concentrations of NPY were found in the pituitary and telencephalon.

HPLC: The elution profile of goldfish brain extracts is shown in Fig. 16. The NPY-like material co-eluted as a single peak with synthetic porcine NPY (retention time 48 min).

## Discussion

The present study demonstrates that a peptide immunologically related to porcine NPY is present in large amounts in the brain of the goldfish. Preliminary immunohistochemical studies indicate that such a peptide with a similar distribution is also present in the brain of the rainbow trout (*Salmo gairdneri,* unpublished observations) and probably in that of other teleosts. Thus, an NPY-like factor appears to be present in the brain of vertebrates and also invertebrates, since a substance immunologically related to NPY has recently been found in specific neurons of crustaceans (Charmentier-Daurès et al. 1987; Shoofs et al. 1988).

Our results demonstrate that, with the exception of the cerebellum, this NPY-like peptide has a ubiquitous distribution in the goldfish brain. Such a widespread distribution of NPY has already been documented in mammals (Allen et al. 1983; Pelletier et al. 1984; Chronwall et al. 1985) and amphibians (Danger et al. 1985). Interestingly, in both groups, NPY is not detected in the cerebellum – results in agreement with those obtained in goldfish. The NPY measured in the cerebellum-anterior medulla region probably comes from the anterior medulla oblongata, since none is detected in the cerebellum by immunohistochemistry. Although it is difficult to make direct comparisons, the overall distribution of NPY in the goldfish brain resembles that reported in other classes of veretebrates. However, it must be pointed out that positive perikarya were found in only 6 brain nuclei of the goldfish, whereas ir cells were detected in 35 nuclei in the rat brain following intraventricular injection of colchicine (Chronwall et al. 1985). Whether this difference is caused by the lack of colchicine pretreatment in our study or by a less differentiated system in teleosts is not known.

As in the rat (Chronwall et al. 1985), a few ir cell bodies are found in the olfactory bulbs of the goldfish. In the telencephalon, ir cells are only found in the latero-posterior region of the ventral telencephalon (Vl) and the entopeduncular nucleus. According to Northcutt (1981), these areas correspond to a migrated subpallial nucleus. Similarly NPY-ir perikarya have been reported in different pallial (dorsal and medial) nuclei and in the entopeduncular nucleus of the frog brain (Danger et al. 1985). It is interesting that, on the basis of topographical, connectional and histochemical data, Northcutt and Bradford (1980) propose that Vl is homologous to the olfactory tubercles of higher vertebrates, a region, that, in the rat brain, contains numerous NPY-positive perikarya (Chronwall et al. 1985). A few ir cells are also found in the ventral part of the area ventralis telencephali (Vv), an area presumably homologous to the medial septum in which NPY cell bodies were also detected in the rat (Chronwall et al. 1985). In the goldfish telencephalon, the most intriguing feature concerns the striking difference between the highly innervated dorsal region and the ventral area, which only exhibits a few ir-axons.

**Fig. 8.** The two populations, small (*arrowheads*) and large (*arrow*), of ir cells located lateral to the nucleus ventromedialis thalami (*NVM*). Bar: 200 μm, × 75

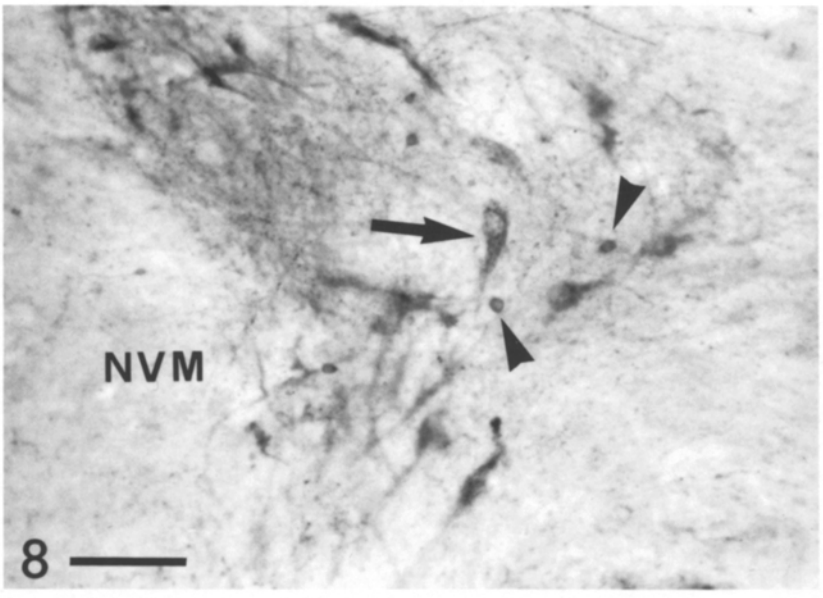
**Fig. 9.** Transverse section of the torus semicircularis (*TS*) showing the high density of ir fibers. Bar: 200 μm, × 75

**Fig. 10.** Transverse section through the locus coeruleus (*LC*). Note the ir perikarya and their ventrolateral projections. Bar: 200 μm, × 70

**Fig. 11.** The dense plexus of ir fibers (*arrows*) in the third layer of the vagal lobe (*L–X*). Bar: 200 μm, × 75

**Fig. 12.** Pre-embedding immunocytochemistry of NPY in the anterior lobe of the pituitary gland. An ir type-B fiber (*arrow*) is located in the vicinity of a growth hormone (*GH*) cell and a negative nerve profile (*arrowhead*). Bar: 500 nm, × 26300

**Fig. 13.** Post-embedding immunocytochemistry of NPY in the intermediate lobe of the pituitary gland. r (*arrow*) and a negative (*arrowhead*) type-B fiber in the vicinity of a melanocyte-stimulating hormone (*MSH*)-secreting cell. Bar: 500 nm, × 36200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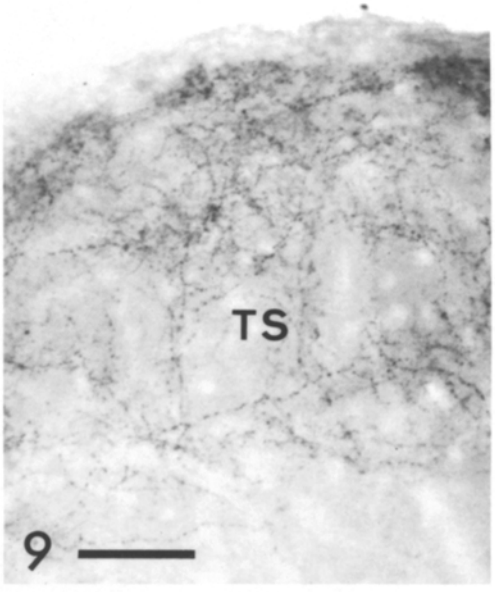
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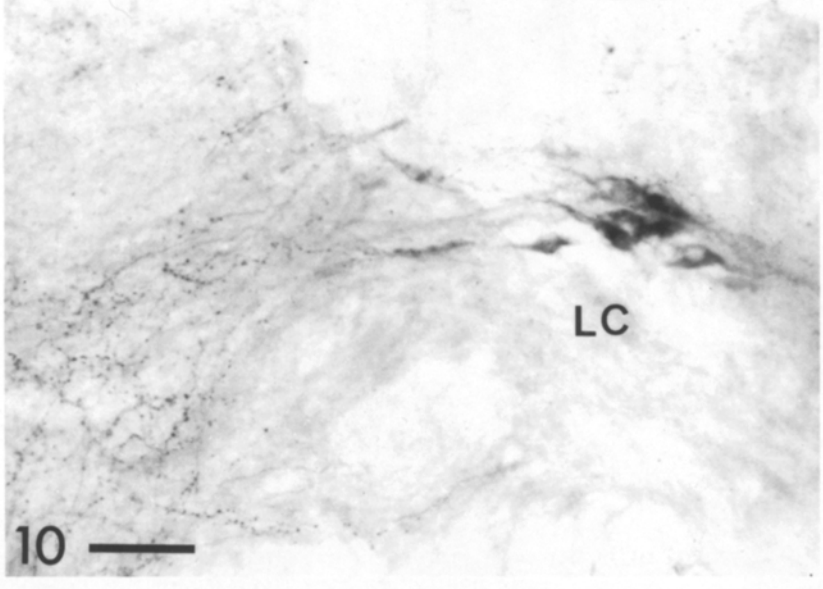
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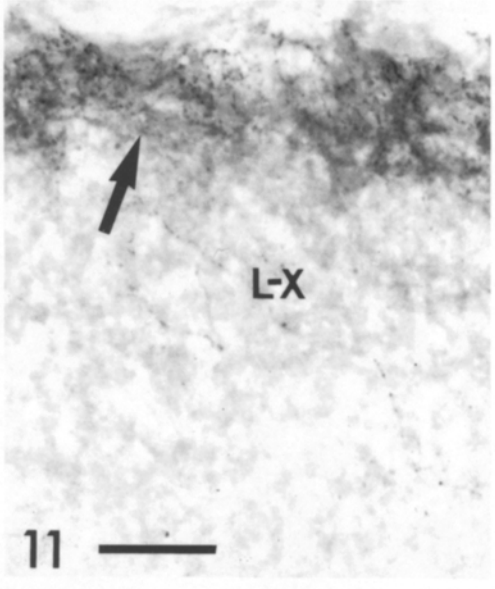
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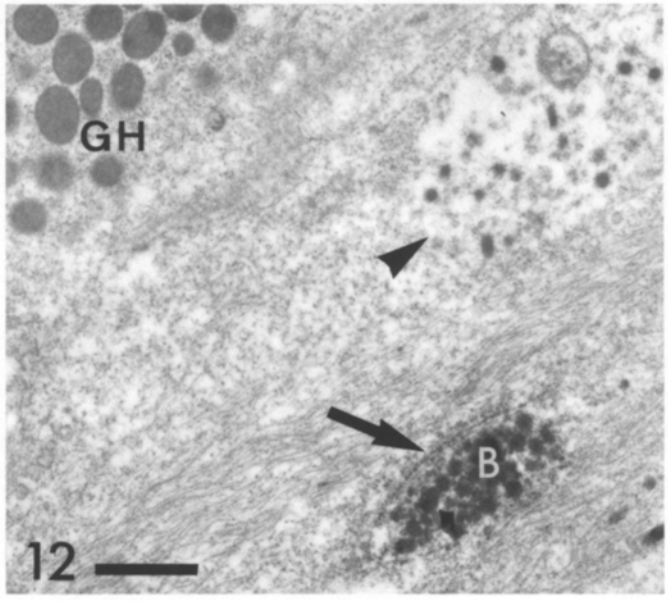
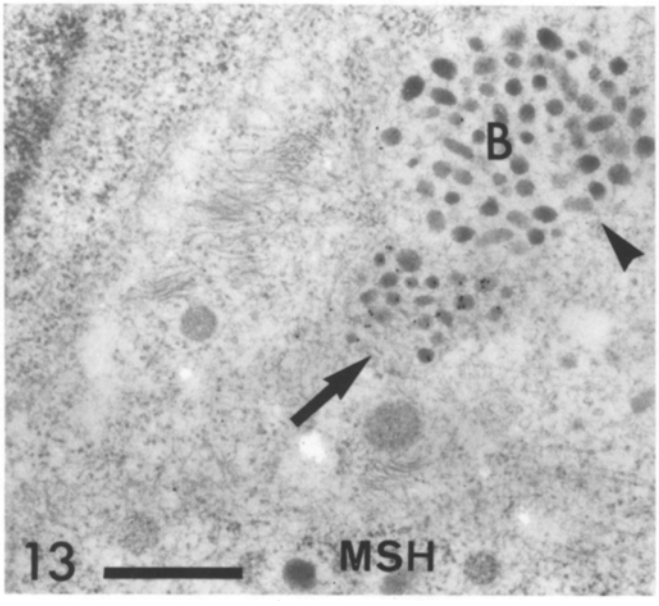

GH
B
12


B
MSH
13

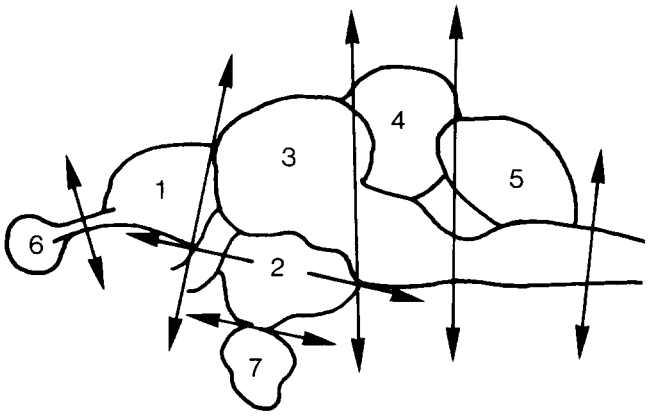



**Fig. 14.** Dissection of the brain regions used for radioimmunoassay. Numbers refer to Table 1

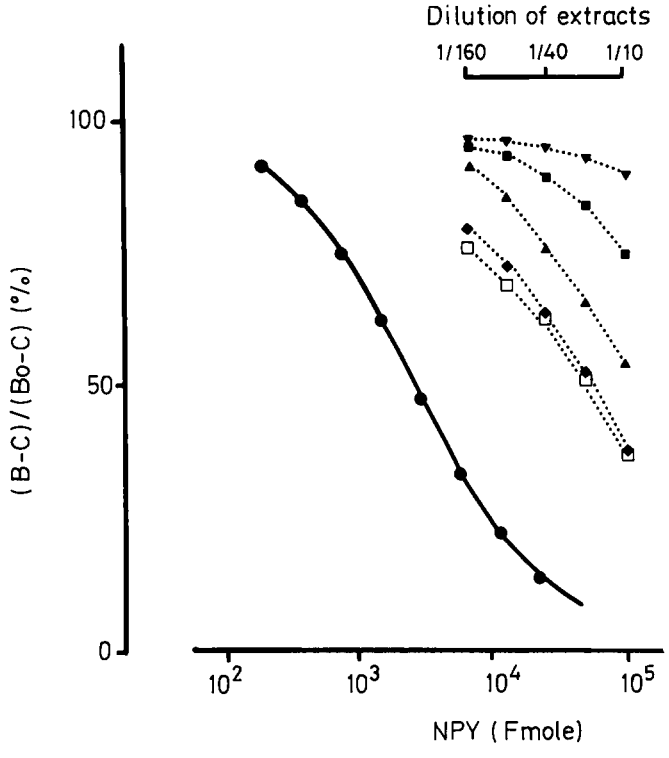



**Fig. 15.** Semi-logarithmic curves comparing competitive inhibition of antibody-bound $^{125}$I-labeled NPY by synthetic NPY (●) and various brain extracts. Five dilution curves from independent tissue extracts were set up (□ Telencephalon; ■ Inferior lobe; ◆ Optic tectum and thalamus; ▲ Vagal lobe; ▼ Pituitary)

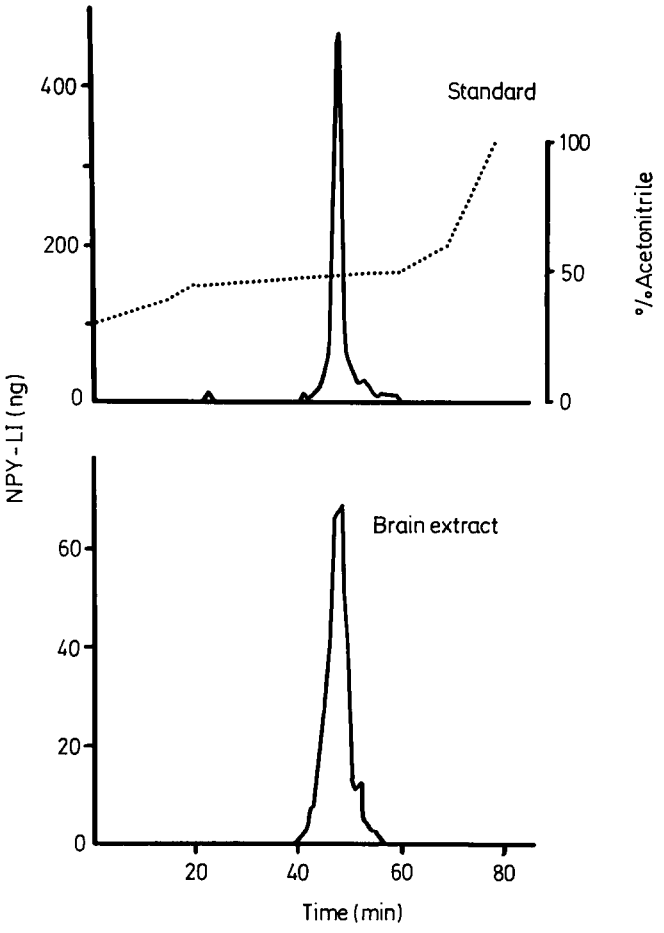



**Fig. 16.** Reverse-phase HPLC analysis of synthetic porcine NPY and fish brain extracts

In both amphibians and mammals, ir neurons are found in the periventricular regions of the hypothalamus, mainly in the preoptic and infundibular regions (Allen et al. 1983; Pelletier et al. 1984; Danger et al. 1985; Chronwall et al. 1985; Gray and Morley 1986; Ciofi et al. 1987). Although numerous fibers are seen in the periventricular region of the goldfish hypothalamus, a few ir cell bodies are found only at the level of the nucleus posterior periventricularis, a nucleus known for its high catecholaminergic content (Geffard et al. 1984). However, we cannot rule out the possibility that more ir neurons may be demonstrated following colchicine injection in this area.

In the present study, an important group of ir cell bodies was observed in the posterior thalamic region, in a position similar to that reported in the frog (Danger et al. 1985). In the goldfish this cell grouping was composed of two cell types: small round ir perikarya and larger cell bodies, which could be negative, but because they are surrounded by numerous ir terminals, they appeared as positive perikarya. At the light-microscope level, it was not possible to ascertain whether these cells were NPY-containing perikarya or targets for NPY terminals.

As in the frog brain (Danger et al. 1985), numerous small ir cells are located in the optic tectum. However, in the goldfish, these cells are strictly located in the periventricular layer, whereas in amphibians, they are detected not only in this position but also in more superficial layers (Danger et al. 1985), which in goldfish receive an abundant innervation.

One of the most interesting results of the present investigation is the finding of ir perikarya in the locus coeruleus (LC). This nucleus is known to contain catecholamine (Parent et al. 1978; Kah 1983) and has recently been found to react to antibodies against noradrenaline (NA; A Pontet and O Kah, unpublished observations). In mammals, it is now well-documented that NPY coexists with NA. In particular, some of the noradrenergic perikarya of the LC also contain NPY (Everitt et al. 1984). Coexistence of NA and NPY within the same neuron is also well-documented in the peripheral nervous system (Lundberg et al. 1982). The ir cells of the goldfish locus coeruleus probably send projec-

**Table 1.** Immunoreactive NPY contents and concentrations in goldfish brain extracts. Values are given as mean ± SEM

| Brain region | NPY content (ng/pool) | NPY concentration (ng/mg proteins) |
|---|---|---|
| 1. Telencephalon | 11.6 ± 0.8 | 3.97 ± 0.82 |
| 2. Inferior lobe | 1.9 ± 0.2 | 0.91 ± 0.31 |
| 3. Optic tectum and thalamus | 16.9 ± 1.6 | 0.80 ± 0.17 |
| 4. Cerebellum and ant. medulla | 4.9 ± 1.4 | 0.45 ± 0.31 |
| 5. Vagal lobe and post. medulla | 6.4 ± 1.4 | 0.40 ± 0.09 |
| 6. Olfactory bulbs | not detectable | not detectable |
| 7. Pituitary | 3.2 ± 0.7 | 8.75 ± 4.95 |

tions to the torus semicircularis, and the medulla oblongata and spinal cord.

Freshwater cyprinids, such as the goldfish, are well-known for their hypertrophied visceral areas, i.e. facial, glossopharyngeal and vagal lobes, which all exhibit an important NPY innervation, in particular the third layer (Morita et al. 1980) of the vagal lobe, which according to Herrick (1905) contains second order relay neurons for the ascending projections of the vagal taste system. These data suggest that, in fish, NPY could be involved in the control of feeding, as has already been documented for mammals (Clark et al. 1984).

Unlike the frog (Danger et al. 1985) and mammals (Gibson et al. 1984), the goldfish spinal cord does not exhibit any ir perikarya, but contains a high density of fibers, in particular at the level of the dorsal horn. These fibers may originate either from more rostral cell bodies or from a local circuit since, according to Gibson et al. (1984), the NPY content of the spinal cord is not modified following spinal transection.

The results of our RIA investigations are in good agreement with our immunohistochemical observations and show that the highest concentrations of NPY are found in the pituitary and telencephalon. The fact that the dilution curves obtained with goldfish extracts parallel that of synthetic NPY indicate that goldfish brain contains a peptide closely related to porcine NPY. However, concentration-dependent displacement of labeled NPY by tissue extracts does not provide information concerning the chemical structure of the immunoreactive peptide detected. HPLC analysis further demonstrates that the goldfish immunoreactive peptide co-elutes with porcine NPY. These results, in agreement with those obtained by Kimmel et al. (1986) suggest that the goldfish peptide may be considered as authentic NPY.

The widespread distribution of NPY in various territories of the goldfish brain suggests that this neuropeptide may be involved in various functions within the central nervous system, as already documented in mammals (for review see Gray and Morley 1986). Furthermore, the fact that the distribution of NPY appears similar in mammals and fish would suggest that this factor serves similar functions in both groups. Of particular interest is the observation of a dense innervation in the mediobasal hypothalamus and the presence of positive endings within the different lobes of the pituitary. Such fibers may represent the neuroanatomical substrate underlying a neuroendocrine regulation of some pituitary functions similar to those reported in mammals and amphibians. Indeed, NPY has been shown to be a potent inhibitor of melanotropin release in frogs (Danger et al. 1987) and to be involved in the regulation of luteinizing hormone (Kerkerian et al. 1985; McDonald et al. 1985; Rodriguez-Sierra et al. 1987) and growth hormone release (McDonald et al. 1985) in rodents. The detection and characterization of endings contacting melanotrophs, gonadotrophs and somatotrophs in the goldfish pituitary suggest that NPY may play similar functions in teleosts. Indeed, in vitro experiments have demonstrated that NPY modulates the release of gonadotrophin in goldfish (Kah et al. 1988) and rainbow trout (Breton et al. 1988).

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