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Neuropeptide Y: localization in the central nervous system and neuroendocrine functions

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Summary – Neuropeptide Y (NPY) is a 36–amino acid peptide first isolated and characterized from porcine brain extracts. A number of immunocytochemical investigations have been conducted to determine the localization of NPY-containing neurons in various animal species including both vertebrates and invertebrates. These studies have established the widespread distribution of NPY in the brain and in sympathetic neurons. In the rat brain, a high density of immunoreactive cell bodies and fibers is observed in the cortex, caudate putamen and hippocampus. In the diencephalon, NPY-containing perikarya are mainly located in the arcuate nucleus of the hypothalamus; numerous fibers innervate the paraventricular and suprachiasmatic nuclei of the hypothalamus, as well as the paraventricular nucleus of the thalamus and the periaqueductal gray. At the electron microscope level, using the pre- and post-embedding immunoperoxidase techniques, NPY-like immunoreactivity has been observed in neuronal cell body dendrites and axonal processes. In nerve terminals of the hypothalamus, the product of the immunoreaction is associated with large dense core vesicles. In lower vertebrates, including amphibians and fish, neurons originating from the diencephalic (or telencephalic) region innervate the intermediate lobe of the pituitary where a dense network of immunoreactive fibers has been detected. At the ultrastructural level, positive endings have been observed in direct contact with pituitary melanotrophs of frog and dogfish. These anatomical data suggest that NPY can act both as a neurotransmitter (or neuromodulator) and as a hypophysiotropic neurohormone.

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In the rat a few NPY-containing fibers are found in the internal zone of the median eminence and high concentrations of NPY-like immunoreactivity are detected in the hypothalamo–hypophyseal portal blood, suggesting that NPY may affect anterior pituitary hormone secretion. Intrajugular injection of NPY causes a marked inhibition of LH release but does not significantly affect other pituitary hormones. Passive immunoneutralization of endogenous NPY by specific NPY antibodies induces stimulation of LH release in female rats, suggesting that NPY could affect LH secretion at the pituitary level. However, NPY has no effect on LH release from cultured pituitary cells or hemipituitaries. In addition, autoradiographic studies show that sites for ^{125}I-labeled Bolton–Hunter NPY or ^{125}I-labeled PYY (2 specific ligands of NPY receptors) are not present in the adenohypophysis, while moderate concentrations of these binding sites are found in the neural lobe of the pituitary. It thus appears that the inhibitory effect of NPY on LH secretion must be mediated at the hypothalamic level. This hypothesis is supported by the following observations: i), intracerebroventricular injection of NPY causes a reduction in plasma LH; ii), NPY fibers have been observed in contact with LHRH neurons in the preoptic region; iii), the organum vasculosum of the lamina terminalis, where LHRH neurons are located, lacks a blood–brain barrier (NPY injected peripherally can thus reach LHRH neurons in this area). The fact that intracerebroventricular injection of NPY in 5,7-dihydroxytryptamine treated rats causes stimulation of LH release (instead of an inhibition in control animals) also suggests that the effect of NPY may in part be mediated *via* serotoninergic neurons.

In amphibians, the existence of a NPY-neuronal system originating from the hypothalamus and terminating in the pars intermedia suggest that NPY may play a role in the control of pituitary melanotrophs. *In vitro* data show that NPY induces a marked inhibition of α-melanotropin (α-MSH) release in frog and toad. The biologically active determinant of NPY is located in the C-terminal region of the molecule. Experiments conducted with acutely dispersed pituitary cells and electrophysiological data using the patch-clamp technique indicate that NPY exerts a direct effect on pituitary melanotrophs. Administration of NPY in black background-adapted toads causes aggregation of dermal melanophores. Taken together, these data indicated that in amphibians NPY can be considered as a melanotropin-release inhibiting factor (MIF).

From these studies, it is concluded that NPY was: i), a neurotransmitter which mediates a number of processes in the brain and in sympathetic nerves; and ii), a hypophysiotropic neurohormone which regulates the secretion of various pituitary hormones such as LH in mammals and α-MSH in amphibians.

neuropeptide Y / immunocytochemistry / neuroendocrinology / gonadotropin-releasing hormone (LHRH) / melanocyte-stimulating hormone (α-MSH) / luteinizing hormone / pituitary / anterior lobe / intermediate lobe

Introduction

Besides classical neurotransmitters, several regulatory neuropeptides act as chemical messengers of cell communication. These peptides are usually synthesized as precursor proteins which are further cleaved by proteolytic enzymes. Processing of pro-peptides also includes various post-translational modifications such as glycosylation, phosphorylation, amidation and acetylation (Jenks *et al*, 1986). Following their release, regulatory peptides interact with specific receptors which are generally located on the membrane of the target tissue. This interaction triggers a series of intracellular events which induce the physiological response. Owing to

their variety, their broad distribution and their various physiological activities, regulatory peptides are now considered as key molecules of neuronal communication. During the past 2 decades, intensive research has been conducted in order to identify and localize biologically active peptides in the brain and to determine the precise functions of these neuropeptides.

Among the neuropeptides discovered recently, neuropeptide tyrosine (NPY) has raised considerable interest. NPY belongs to a family of peptides including peptide tyrosine–tyrosine (PYY) and pancreatic peptides (PP) which exhibit close structural homologies with NPY. Avian pancreatic polypeptide (APP) was the first PP-related peptide to be discovered (Kimmel *et al*, 1975). APP is a 36-amino acid peptide which was initially isolated from chicken pancreatic extracts as a side-product of insulin purification (Kimmel *et al*, 1975). Homologous peptides have been subsequently characterized in the islets of Langerhans of several mammalian species (Lin and Chance, 1972; 1974; Schwartz and Tager, 1981; Boel *et al*, 1984; Schwartz and Hansen, 1984; Yamamoto *et al*, 1986). All these peptides are composed of 36-animo acid residues and their primary structure has been highly preserved during evolution.

A number of gut or pancreatic peptides have also been detected in the brain. For instance, vasoactive intestinal peptide (VIP), cholecystokinin (CCK) and neurotensin were initially isolated in the digestive tract and have been subsequently detected in specific neurons of the central nervous system (Vanderhaeghen *et al*, 1975; Hökfelt *et al*, 1982). As expected, immunohistochemical techniques using APP-directed antibodies have revealed an extensive PP-like neuronal system in rat and human brain. It has thus been established that the brain of mammals contain a PP-related peptide (Loren *et al*, 1979; Lundberg *et al*, 1980; Hunt *et al*, 1981; Card and Moore, 1982; Card *et al*, 1983). However the attempts to characterize the immunoreactive peptide by means of radioimmunoassays for APP have led to inconsistent results. Despite the intensive staining observed by immunocytochemistry, only traces of immunoreactive material could be detected by radioimmunological techniques (Lundberg *et al*, 1984).

Since many gut peptides appear to possess a carboxyterminal tyrosine-amide residue, Tatemoto and Mutt have developed an original chemical method for the detection of such Tyr-NH_2-terminated peptides (Tatemoto and Mutt, 1982). Using this approach, these authors have isolated from the porcine gut a new peptide exhibiting high homology with the PP family. This peptide with tyrosine residues at both amino- and carboxyterminal ends was named peptide YY (PYY). Thus, PYY became a good candidate for the PP-like immunoreactivity previously reported in the brain. However, the same year, Tatemoto *et al* (1982) isolated and characterized a new peptide of the PP/PPY family in porcine brain extracts. This brain peptide was named neuropeptide tyrosine or NPY.

As with PPY, NPY is 36-amino acid peptide and has a C-terminal tyrosine amide residue. The NPY molecule exhibits 5 tyrosines in positions 1, 20, 21, 27, 36. The primary structure has been determined in 5 mammalian species, *ie* hog,

rat (Allen *et al*, 1987; Larhammar *et al*, 1987), man (Corder *et al*, 1984; Minth *et al*, 1986), guinea pig and rabbit (O'Hare *et al*, 1988). The sequence of NPY in rabbit, guinea pig and man is identical to that of rat NPY and only differs from the porcine sequence by the substitution of a leucine residue by a methionine in position 17. Computer-assisted analysis has revealed that the spatial structure of NPY consists of 2 main domains: the N-terminal portion has a β-turn-like structure and the C-terminal region is organized as a β-helix (Allen *et al*, 1987). Owing to the structural homologies with PP-related peptides, NPY has been classified within the PP-family. NPY exhibits a high degree of sequence homology with porcine PYY and PP (61 and 47%, respectively). Phaeochromocytomal tissues are generally rich in NPY material (Emson *et al*, 1984). As a matter of fact, Corder *et al*, (1984) have succeeded in isolating and characterizing human NPY from adrenal medullary tumors. These adrenal medulla tumors have also been used as a source of messenger RNA encoding NPY to synthesize and clone the corresponding complementary DNA (Minth *et al*, 1984). Translation of the encoding region gives rise to a pro-peptide of 97-amino acid residues with a molecular weight of 10.9 kDa. The genes encoding man (Minth *et al*, 1986) and rat (Allen *et al*, 1987; Larhammar *et al*, 1987) NPY have subsequently been isolated, cloned and sequenced. Comparison of the human and rat pro-hormones reveals the presence of 4 substitutions (2 of which are located in the signal peptide and the others in the flanking peptide). An additional methionyl residue is also present at the N-terminal end of the human pre-pro-molecule.

The various steps of the translational processing of NPY are now well known. The signal peptide is first removed co-translationally. Then, the pro-peptide is cleaved at the level of a pair of basic amino-acids (Lys_{66}-Arg_{67}) to generate an intermediate product (NPY-Gly_{37}-Lys_{38}-Arg_{36}-COOH) and a 30-amino acid peptide named C-terminal flanking peptide of neuropeptide tyrosine (C-PON). The Arg- and Lys-residues of the intermediate peptide product are cleaved by a carboxypeptidase and NPY is amidated by a peptidyl glycine alpha-amidating monooxygenase, the Gly_{37} residue as an amine donor.

In rat and in man, the gene encoding NPY consists of 4 exons and 3 introns. The first exon encodes for the 5'-unstranslated region and for the methionine codon necessary to initiate translation. The second exon encodes for the remainder of the signal peptide and for most of the peptidic sequence of NPY. The third exon encodes for the C-terminal tyrosine and its site of amidation and in addition, for the 23 first amino acids of the C-PON molecule. Finally, the 3rd exon encodes for the 7 last amino-acids of C-PON and for the 3'-untranslated region.

The gene encoding NPY is located on chromosone 7 (Takeuchi *et al*, 1986). In Sprague–Dawley rats, there is only one gene coding for NPY; however, there is evidence of polymorphism in allelic genes. In fact when the allelic genes are compared, 8 modifications are noted, but none is found in the exons (Allen *et al*, 1987).

Localization of NPY in the central nervous system

In mammalian brain, the concentrations measured by means of radioimmunoassay are in the range of one picomol per mg wet tissue (YS Allen *et al*, 1983; Dawbarn *et al*, 1984; DeQuidt and Emson, 1986). The highest concentrations are found in the paraventricular and arcuate nucleus of the hypothalamus. High amounts of NPY-like material have also been measured in the paraventricular nucleus of the thalamus, the nucleus accumbens, the septum and medial amygdala (YS Allen *et al*, 1983; Dawbarn *et al*, 1984). Concurrently, very low amounts of NPY have been detected in the cerebellum (Lundberg *et al*, 1984). In the medulla, the concentrations of NPY are higher in the sacral region than in lumbar, thoracic or cervical segments (Gibson *et al*, 1984).

The localization of NPY-containing neurons in the brain of mammals has been intensively studied. In the rhinencephalon, NPY perikarya are mainly located in the anterior and medial olfactory nuclei (Chronwall *et al*, 1985; Gall *et al*, 1986). Most of these neurons are multipolar with short processes. They are generally located in the deep granular cell layer or in the gray matter, in the vicinity of the ependymal zone (Scott *et al*, 1987).

In the telencephalon, all subdivisions of the cerebral cortex contain NPY fibers (figure 1). The distribution of NPY immunoreactivity in the visual cortex has been

Fig 1. Section through the rat cerebral cortex. Several cell bodies and beaded fibers are labelled by the NPY antiserum (×200).

thoroughly studied in various species including rat (Chronwall *et al*, 1984), cat (Wahle *et al*, 1986), monkey (Hendry *et al*, 1984a, b) and man (Van Reeth *et al*, 1987). Immunopositive perikarya are numerous in cortical layers II, III, V and VI. NPY-containing fibers are also present in all cortical layers, but the density of nerve processes is greater in neocortical layers I and IV. In layer I, the fibers are often seen in the vicinity of cerebral arteries, suggesting that NPY may play a role in the regulation of cerebral blood flow. Immunoreactive cell bodies are also located in other telencephalic areas such as the hippocampus and striatum. The distribution of NPY immunoreactive neurons has been determined in the hippocampus of rat (Köhler *et al*, 1987), monkey (Köhler *et al*, 1986a) and man (Chan-Palay, 1987). A relatively high density of immunoreactive cell bodies is located in the internal regions of Ammon's horn. The subiculum also contains numerous NPY-neurons. These cell bodies are primarily located in the pyramidal cell layer.

In the subiculum and Ammon's horn, NPY-nerve processes are mainly found in the molecular layer; immunopositive fibers are particularly dense in the entorhinal area where some large multipolar and bipolar cells are present in the deep layers (IV–VI). Immunohistochemical techniques have also revealed the presence of NPY perikarya in the caudate and putamen nuclei, the claustrum, the endopiriform and accumbens nuclei (Massari *et al*, 1984; Kerkérian *et al*, 1986) and in the dorso-lateral septum (Gaspar *et al*, 1987). In the amygdala, NPY cells are located in the basolateral and medial nuclei. The whole amygdala contains NPY-nerve processes. The density of fibers is, however, higher in the central nucleus of the amygdala (Gustafson *et al*, 1986).

In the diencephalon of all species studied, such as hamster (Sabatino *et al*, 1987), dogfish (Vallarino *et al*, 1988), goldfish (Pontet *et al*, 1989), trout (Danger *et al*, submitted), frog (Danger *et al*, 1985; Caillez *et al*, 1987a, b), newt (Perroteau *et al*, 1988), rat (Chronwall *et al*, 1984; Everitt *et al*, 1984; Pelletier *et al*, 1984b), cat (Wahle *et al*, 1986; Léger *et al*, 1987; Hu *et al*, 1987), monkey (Smith *et al*, 1985; Bons *et al*, submitted) and man (Pelletier *et al*, 1984a; Chan-Palay and Yasargil, 1986), the arcuate nucleus of the hypothalamus contains the highest density of NPY-neuronal systems (figs 2–4). In the rat brain, cell bodies have also been visualized in the dorsal and dorsolateral preoptic area. However, in the cat the preoptic hypothalamus does not contain any NPY-immunoreactive perikarya (Léger *et al*, 1987). The paraventricular, periventricular and arcuate nuclei, as well as the preoptic portion of the bed nucleus of the stria terminalis contain particularly dense plexus of NPY-fibers. In the cat brain, only a few immunoreactive fibers are present in the suprachiasmatic area (Léger *et al*, 1987) whereas in other mammalian species, numerous nerve processes innervate this nucleus. NPY-nerve fibers are also present in the neurovascular part of the median eminence and scarce positive fibers are observed in the posterior lobe of the pituitary (Pelletier *et al*, 1984b). In the thalamus, a relatively high density of NPY-perikarya and nerve fibers is found in the geniculate nucleus. The cell bodies are mainly located

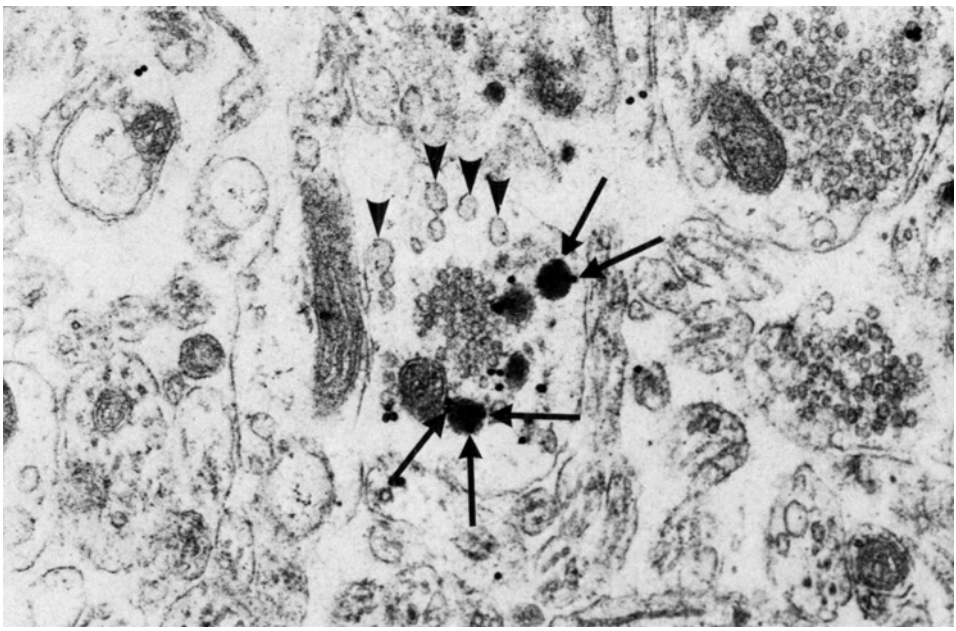


Fig 2. Immunoelectron microscopic localization of NPY in the frog brain using the peroxidase–antiperoxidase method. One positive terminal of the infundibulum is immunostained. The gold particles are located in dense core vesicles (arrows). Note that clear vesicles are unstained (arrowheads); (×55,000). (From Danger JM *et al, Peptides* 6, 1225–1236, 1985; With permission).

in the areas known to receive contralateral retinal afferences such as the antero-external part of the ventro-lateral geniculate body. In addition, NPY-positive cell bodies and nerve fibers are present in the intergeniculate leaflet (Moore *et al*, 1984; Ueda *et al*, 1986; Sabatino *et al*, 1987). NPY containing neurons are also located in other thalamic nuclei such as the pretectal oliva and the habenula (Smith *et al*, 1985).

In the mesencephalon of rats, NPY-positive perikarya are located in the ventral tegmentum, the pars compacta of the substantia nigra, the laterocentral gray matter, the reticular formation, and in dorsal and lateral inferior colliculi (Everitt *et al*, 1984; Chronwall *et al*, 1985). Immunoreactive fibers are mainly located in the central gray matter (Chronwall *et al*, 1985; see Gray and Morley, 1986 for review). In the monkey brain, numerous NPY-terminals have been visualized in the interpeduncular nucleus (Smith *et al*, 1985). With the exception of the locus coeruleus, the lateral parabrachial white matter, most regions of the mesencephalon are poorly innervated (Adrian *et al*, 1983; Chronwall *et al*, 1985; Smith *et al*, 1985). In rats, a relatively high number of NPY cell bodies are located in the dorsal part of the locus coeruleus. However, in monkey, no positive cell bodies can be observed in this region (Smith and Parent, 1986). The presence of a small

cluster of NPY-perikarya surrounding the dorsal nucleus of the tegmentum should also be mentioned. In the medulla oblongata, NPY-cell bodies are located in the whole rostro-caudal region of the nucleus of the solitary tract (Harfstrand *et al*, 1987a, b) and in the ventro-lateral medulla (Carter *et al*, 1985). The nucleus of the tractus solitarius, the dorsal vagal nucleus, the raphe and the giganto-cellular nucleus are moderately innervated by NPY-nerve fibers.

NPY-immunoreactive fibers are located in the entire medulla (Gibson *et al*, 1984). A comparative study conducted on 5 different species has shown that immunoreactivity is particularly dense in the lumbosacral segment of the medulla, around the central canal, and in the dorsal horn (Gibson *et al*, 1984). In the cat medulla, NPY-perikarya have been detected in the dorsal horn (Gibson *et al*, 1984; Krukoff, 1987), suggesting that at least part of the innervation of the medulla may have an intrinsic origin. Similar results have also been reported in non-mammalian vertebrates (Danger *et al*, 1985). Taken together, these results suggest that NPY is synthesized, and stored in numerous brain areas. The presence of messenger RNA in the rat hypothalamus has been demonstrated using *in vitro* translation (Ivell *et al*, 1984). Recently, *in situ* hybridization studies have established that NPY is actually produced in brain neurons (Terenghi *et al*, 1987; Chan-Palay, 1988).

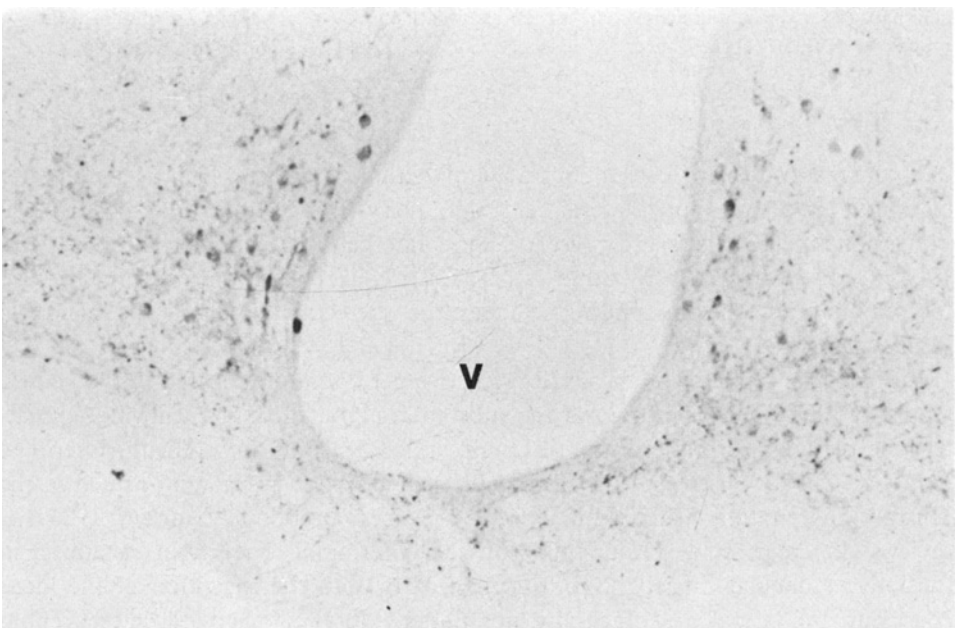


Fig 3. Coronal section through the medial basal hypothalamus of the rat. Numerous cell bodies are labelled by the NPY antiserum, using the peroxidase–antiperoxidase technique in the arcuate nucleus. A dense network of immunopositive fibers can also be observed in the internal zone of the median eminence. V, ventricule; (×200).

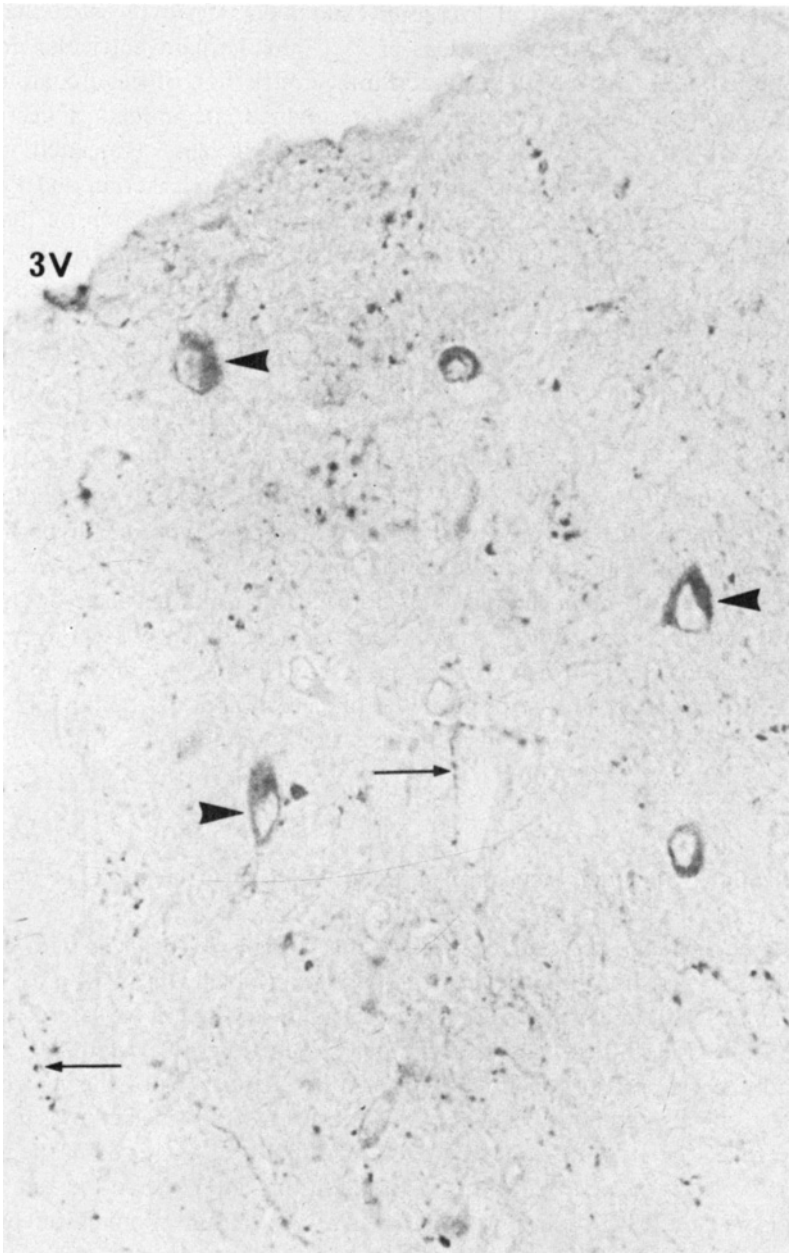


Fig 4. Section through the human basal hypothalamus. Several cell bodies are labelled by the NPY antiserum, in the infundibular nucleus (arrowheads). Immunoreactive beaded fibers are also immunostained (arrows). 3V, third ventricle; (×450).

The widespread distribution of NPY neuronal systems makes it difficult to determine precisely the organization of the NPY neuronal pathways in the brain. However, a few studies have revealed that NPY may send projections over a long distance in the brain. Electrical lesioning experiments have revealed that NPY perikarya located in the arcuate nucleus project into the paraventricular and dorsal hypothalamus (Bai *et al*, 1985). In addition, destruction of the arcuate nucleus using post-natal treatment with monosodium-L-glutamate induces a decrease in the density of NPY fibers in the paraventricular nucleus, associated with an increase of the number of NPY perikarya in this nucleus (Kerkérian and Pelletier, 1986). Thus the arcuate nucleus may exert a tonic inhibitory action on the NPY-containing neurons located in the paraventricular nucleus. Electrical lesioning experiments have also shown that the NPY-nerve fibers located in the suprachiasmatic nucleus originate in part from the lateral geniculate nucleus (Harrington *et al*, 1985). Depletion of NPY-fibers of the suprachiasmatic nucleus has also been observed following transection of the stria terminalis (Allen *et al*, 1984a). NPY-neurons located in the amygdala complex may be the origin of the fibers located in the suprachiasmatic area. Retrograde labelling experiments indicate that the NPY-innervation of the dorsal vagal complex originates partly from the NPY-perikarya located in the parvo-cellular region of the periventricular hypothalamus (Gray *et al*, 1986). Finally, retrograde tracing techniques have shown that the innervation of the rat hippocampus is of both intrinsic and extrinsic origins. The intrinsic system is constituted of cortical neurons located in the deep layers of the perirhinal area of the piriform cortex. The extrinsic innervation originates from cortical neurons located in the deep of the perirhinal area of the piriform cortex from the rostro-caudal endopiriform nucleus, the lateral amygdala and the locus coeruleus (Köhler *et al*, 1986b).

Co-localization with other neurotransmitters and/or neuropeptides in the brain

Immunohistochemical studies have revealed that NPY is co-localized with biogenic amines in various areas of the central nervous system. In rats, most of the noradernergic cells and some adrenergic neurons of the ventro-lateral medulla oblongata are also stained using an NPY-antiserum (Everitt *et al*, 1984). Numerous noradrenergic perikarya and a few dopaminergic neurons of the ventromedial locus coeruleus also contain NPY-like immunoreactivity (Sawchenko *et al*, 1985). In man, Hökfelt *et al*, (1983a) have shown that NPY is co-localized in the medulla oblongata with tyrosine hydroxylase. In addition, NPY is co-located with γ aminobutyric acid (GABA) in pyramidal cells of the cat and monkey cerebral cortex (Hendry *et al*, 1984b).

Co-localization of NPY with other neuropeptides has also been investigated by means of immunohistochemistry (table I). NPY is present in somatostatinergic neurons of the cortex (Vincent *et al*, 1982; Chronwall *et al*, 1984), hippocampus,

where the co-localization rate is particularly high (Köhler *et al*, 1987a; Chan-Palay, 1987), the olfactory bulb (Beal *et al*, 1986), pericentral nucleus of the colliculus and the periaqueductal gray layer (DeQuidt and Emson, 1986). NPY has also been detected in perikarya containing the molluscan cardio-excitatory peptide (FMRF-amide) in the medulla oblongata, the hypothalamus and in the medulla (Hökfelt *et al*, 1983b; see Chronwall, 1985 for a review; Sasek and Elde, 1985). NPY is co-localized with galanin in the locus coeruleus (Holets *et al*, 1985), with VIP in neurons of the cranial parasympathetic ganglia (Leblanc *et al*, 1987) and with CCK in perikarya of the nucleus of the solitary tract (Harfstrand *et al*, 1987a). Recently, Ciofi *et al*, (1988) have shown that in rats NPY is present in GRF-containing neurons of the hypothalamus.

The structural relationship between NPY and other neuronal systems has been investigated using various approaches. In rats, unilateral and selective lesion of the nigrostriatal dopaminergic neurons induces an increase in the number and staining intensity of the ipsilateral striatum (Kerkérian *et al*, 1986). These results suggest that the NPY-neuronal system of the rat striatum is under the inhibitory control of dopaminergic afferents of the nigrostriatal pathway. Ultrastructural studies combining immunohistochemistry and autoradiography have revealed axo-axonic contacts between NPY-ergic and serotoninergic fibers (Guy *et al*, 1988b). In the rat accumbens nucleus, synaptic contacts have been observed between NPY-ergic dendrites and glutamic acid decarboxylase (GAD) immunoreactive terminals (Massari *et al*, 1984). Axo-somatic synapses between NPY-fibers and

Table I. Overview of co-localization of NPY with other putative neurotransmitters in the brain.

Substance	*Localization*	*References*
Catecholamines	Noradrenergic and adrenergic cell groups of the medulla oblongata, noradrenergic cells of the locus coeruleus and medulla oblongata	Everitt *et al*, 1984 Hökfelt *et al*, 1983a Sawchenko *et al*, 1985
Somatostatin	Cortex, striatum, hippocampus, olfactory bulb, pericentral nucleus of the inferior colliculus, periaqueductal gray	Chronwall *et al*, 1984 Beal *et al*, 1986 Vincent *et al*, 1982 Köhler *et al*, 1987 Chan-Palay, 1987
GABA	Pyramidal cells of the cerebral cortex	Hendry *et al*, 1984b
Galanin	Locus coeruleus	Holets *et al*, 1985
GRF	Arcuate nucleus	Ciofi *et al*, 1988
FMRF-amide	Hypothalamus, medulla oblongata, spinal cord	Hökfelt *et al*, 1983b Chronwall, 1985 Sasek and Elde, 1985
VIP	Parasympathetic cranial ganglion	Leblanc *et al*, 1987

corticotropin-releasing factor (CRF) have recently been demonstrated in the parvocellular region of the periventricular nucleus of the rat hypothalamus (Liposits *et al*, 1988).

Ontogeny of NPY in the brain

A few studies have been conducted in order to determine the ontogeny of NPY in the brain of mammals. In rats NPY is present as early as day 14 of intra-uterine life (E14) in the diencephalon, but appears only at E19 in the cerebral cortex. NPY-concentrations increase rapidly following birth (Allen *et al*, 1984b). The embryonic development of NPY-neuronal systems is homologous to that of catecholaminergic neurons (Allen *et al*, 1984b). In fact, NPY and tyrosine hydroxylase are co-localized in neurons of the medulla oblongata from E17 (Foster *et al*, 1984). According to these authors, NPY-immunoreactivity appears at E13 but development of NPY neurons is independent from that of tyrosine hydroxylase-immunopositive neurons. The results of this study indicate that NPY and tyrosine hydroxylase are not systematically associated and that the factors which regulate the synthesis of these products are not identical.

Receptors

The presence of receptors to NPY in the rat brain was initially reported by Unden *et al*, (1984). The highest concentration of NPY binding sites has been detected in membrane preparations from rat cerebral cortex, hypothalamus, hippocampus (Unden *et al*, 1984), and in the vasomotor region of the medulla oblongata (Nakajima *et al*, 1986). NPY binding on membrane receptors depends on the presence of calcium (Goldstein *et al*, 1986) and is inhibited by magnesium (Unden and Bartfai, 1984). Using the C-terminal fragment NPY^{13-36} (Sheikh *et al*, 1989) have recently identified two subtypes of neuropeptide Y (named Y_1 and Y_2), which differ in affinity and specificity. The Y_1 subtype binds NPY with a dissociation constant in the nanomolar range, but does not bind NPY^{13-56}. The Y_2 subtype exhibits a high affinity for both NPY and NPY^{13-36}. The NPY receptor is sensitive to proteolytic enzyme treatment and to reducing agents (Unden and Bartfai, 1984). Table II gives the relative quantities of ^{125}I-labeled peptide YY (^{125}I-labeled PYY) and ^{125}I-labeled Bolton-Hunter neuropeptide Y (^{125}I-labeled BH-NPY) binding sites in the hypothalamus and pituitary gland of rat as determined by *in vitro* receptor autoradiography (Martel *et al*, 1989). The labeling of various hypothalamic nuclei and the pituitary gland is generally stronger when using ^{125}I-labeled PYY. The 2 ligands used here most likely interact with a similar population of receptor sites in the CNS (Walker and Miller, 1988; Martel *et al*, 1989). However, PYY differentiates better than NPY 2 of the affinity states (high and

Table II. Comparative distribution of [^{125}I]peptide YY and ^{125}I-labeled Bolton–Hunter neuropeptide Y receptor sites in hypothalamic nuclei and pituitary gland in the rat. Relative densities in lateral septum frontal cortex and hippocampus are given for comparison. + + + : high density; + + : moderate density; + : low density; − : very low density.

Area	*[^{125}I]PYY Relative density*	*[^{125}I]BH-NPY Relative density*
Hypothalamus		
Paraventricular nucleus	+ +	+
Preoptic area		
(medial and lateral)	+ +	+
Medial preoptic nucleus	+	+
Lateral hypothalamic area	+ +	+
Septohypothalamic nucleus	+ +	+
Suprachiasmatic nucleus	+	+ +
Lateroanterior nucleus	+ +	+
Ventromedial nucleus	+ +	+
Supraoptic nucleus	+ +	+
Pituitary gland		
Anterior lobe	−	−
Intermediate lobe	−	−
Neural lobe	+ +	−
Other areas		
Lateral septum	+ + +	+ + +
Frontal cortex (layers I–II)	+ +	+ +
Hippocampus (stratum radiatum)	+ + +	+ + +

moderate) of the receptors (Walker and Miller, 1988). Accordingly, with the concentration of radioligands used in the present study (35 pM), ^{125}I-labeled PYY should label a greater proportion of the high affinity states of the receptors. It is also important to point out that PYY-like immunoreactivity has been detected in the hypothalamus, brainstem and spinal cord of the rat (Ekman *et al*, 1986). It is therefore possible that specific ^{125}I-labeled PYY receptors demonstrating low affinity for ^{125}I-labeled BH-NPY may exist in these brain areas. It should be noted, however, that neither Y_1 nor Y_2 subtype of binding site distinguish between NPY and PYY (Sheikh *et al*, 1989).

In comparison to other regions such as the hippocampus and the cortex, the amounts of ^{125}I-labeled PYY binding sites in the hypothalamus and pituitary gland vary from low to moderate (table II). On the other hand, comparatively low levels of ^{125}I-labeled BH-NPY are seen in most hypothalamic nuclei and the pituitary gland (table II). The moderate levels of ^{125}I-labeled PYY binding observed in the paraventricular hypothalamic nucleus is of particular interest in regard to the stimulatory effect of discrete injection in this nucleus of NPY or PYY on food intake (Stanley *et al*, 1985b). It has also been shown that PYY is more potent than NPY in eliciting this behavior, supporting our finding of higher-levels of ^{125}I-labeled PYY than ^{125}I-labeled BH-NPY sites in the paraventricular

nucleus. The existence of moderate concentrations of ^{125}I-labeled PYY sites in the preoptic area is consonant with the view that NPY plays a pivotal role in the regulation of LH secretion (see below).

The moderate quantities of ^{125}I-labeled PYY binding observed in the neural lobe of the pituitary gland may suggest a role for NPY and/or PYY on the release of neurohypophysial hormones such as vasopressin and/or oxytocin. It has already been shown that injection of NPY in the supraoptic nucleus, which contains a moderate density of ^{125}I-labeled PYY sites, induced the secretion of vasopressin (Willoughby and Blessing, 1987). The presence of ^{125}I-labeled PYY sites in the neural lobe of the pituitary gland may also suggest possible effect of this peptide family directly at the level of vasopressin nerve terminals. Finally, the absence of significant labeling with either ^{125}I-labeled PYY or ^{125}I-labeled BH-NPY in the anterior pituitary of male rats is in agreement with the lack of direct effect of these peptides on the release of pituitary hormones (see below). However, a sex difference in the expression of these receptors cannot be excluded, since a direct effect of NPY on the release of anterior pituitary hormones has been reported in female rats (McDonald *et al*, 1985a). The absence of ^{125}I-labeled BH-NPY and/or ^{125}I-labeled PYY binding sites in certain areas may also reflect species differences in the distribution of these sites. Such a situation may occur in the intermediate lobe of the pituitary, since NPY inhibits MSH release in the frog (Danger *et al*, 1986) while it does not affect MSH secretion in the rat (Kraicer *et al*, 1988). It should also be pointed out that some areas may contain ^{125}I-labeled BH-NPY and/or ^{125}I-labeled PYY receptor sites which cannot be detected with the relatively low concentration of radioligands used in this study. Finally, the presence of ^{125}I-labeled PYY receptor sites in other hypothalamic nuclei such as the septo-hypothalamic, latero-anterior and ventromedial nuclei deserves further investigation.

Behavioral actions of NPY

The widespread distribution of NPY in the brain suggests that the peptide exerts various behavioral actions. Clark *et al*, (1984) initially showed that injection of NPY in the third ventricle increased feeding behavior in rats. This powerful orexigenic action of NPY was subsequently confirmed by Levine and Morley (1984). Stanley and Leibowitz (1984) and Clark *et al*, (1985). In addition, these groups have observed that stimulation of food intake also occurs after direct injection of NPY in the paraventricular nucleus of the hypothalamus. NPY induces feeding in other species such as mouse (Morley *et al*, 1987), squirrel (Nizielski *et al*, 1985), and swine (Parott *et al*, 1986). The action of NPY is not inhibited by α-adrenergic antagonists (Stanley and Leibowitz, 1984; Levine and Morley, 1984). While norepinephrine only acts on food intake, NPY also induces drinking behavior (see Morley, 1987, for a review). It thus appears that the orexigenic effect does not

depend on noradrenergic neurotransmission. The distribution of NPY neurons and NPY receptors suggests that the peptide may play a physiological role in the control of feeding. As a matter of fact, NPY is located in various brain areas known to be involved in the control of appetite such as the paraventricular, the dorsomedial and the preoptic nuclei, the ventral tegmental area, the amygdala, the locus coeruleus and the nucleus of the solitary tract. It should be mentioned that the nucleus of the solitary tract is particularly rich in NPY binding sites (Harfstrand *et al*, 1986; Nakajima *et al*, 1986; Martel *et al*, 1989). This population of NPY binding sites is of importance, since stimulation of feeding behavior has also been observed following injection in the fourth ventricle (Steinman *et al*, 1987). Several attempts have been to determine the precise site of action of NPY. Injection of NPY in the paraventricular nucleus and in the ventromedial nucleus of the hypothalamus stimulates both drinking and feeding, whereas only increased drinking is observed following injection in the lateral hypothalamus (Stanley and Leibowitz, 1984). The lesion of the paraventricular and ventromedial nuclei does not prevent the orexigenic action of the peptide. Stimulation of feeding is also observed following micro-injection in the medial preoptic area (Stanley *et al*, 1985a). However, no behavioral modification is observed when NPY is administered in the amygdala or thalamus (see Morley, 1987, for a review). Finally, it has been shown that NPY injections are capable of reversing the loss of body weight induced by lesions of the lateral hypothalamus (see Morley, 1987, for a review). All these data support a role of NPY in the dorsal medial, the anterior hypothalamus and the ventral tegmental area on appetite and body weight (table III).

NPY also acts on sexual behavior. In rats, intracerebroventricular injection of NPY inhibits the receptivity of females to males: a 50% inhibition of lordosis was measured after icv injection of 0.05 g of NPY. For higher doses, rejection behavior increases (Clark *et al*, 1985). Whether this action can be accounted for by the inhibitory affect of NPY on LH secretion remains unknown.

Miscellaneous actions of NPY at the brain level

NPY appears to be a peptide of prime importance in the regulation of cerebral blood flow (table III). In addition to its distribution in central arteries and in the main blood vessels of the circle of Willis, NPY has been shown to induce a powerful, long-lasting diminution of cortical blood flow (see Edvinsson, 1985, for a review). NPY causes a shift of circadian rhythms in hamster (Albers and Ferris, 1984) and modulates biosynthesis of melatonin by increasing the activity of serotonin acetyltransferase (Reuss and Schröder, 1987). NPY also affects cerebral electrical activity (Fuxe *et al*, 1983; Zini *et al*, 1984). Intracerebral injection of NPY reduces cardiac and respiratory frequencies (Fuxe *et al*, 1983; Harfstrand, 1986) and arterial pressure (Fuxe *et al*, 1983; 1986; Harfstrand, 1986). Central

Table III. Physiological effects of central administration of NPY.

Pharmacological action	*References*
Modification of circadian rhythms	Albers and Ferris, 1984
Stimulation of melatonin biosynthesis	Reuss and Schröder, 1987
Stimulation of feeding and drinking behaviour	Clark *et al*, 1984 Morley, 1987 Morley *et al*, 1987 Stanley and Leibowitz, 1984 Stanley *et al*, 1985a
Modification of arterial pressure and cardiac rhythms	Fuxe *et al*, 1983 Harfstrand, 1986
Modification of respiratory rhythms	Fuxe *et al*, 1983 Harfstrand, 1986
Modification of brain electrical activity	Fuxe *et al*, 1983 Zini *et al*, 1984
Hypothermia	Morioka *et al*, 1984
Stimulation of insulin secretion	Moltz and Donald, 1985 Kuenzel, 1988
Inhibition of libido	Clark *et al*, 1985
Modulation (mainly inhibition) of LH secretion	See table IV

administration of NPY causes hypothermia in dog (Morioka *et al*, 1984) and stimulates insulin secretion (Moltz and McDonald, 1985; Kuenzel, 1988).

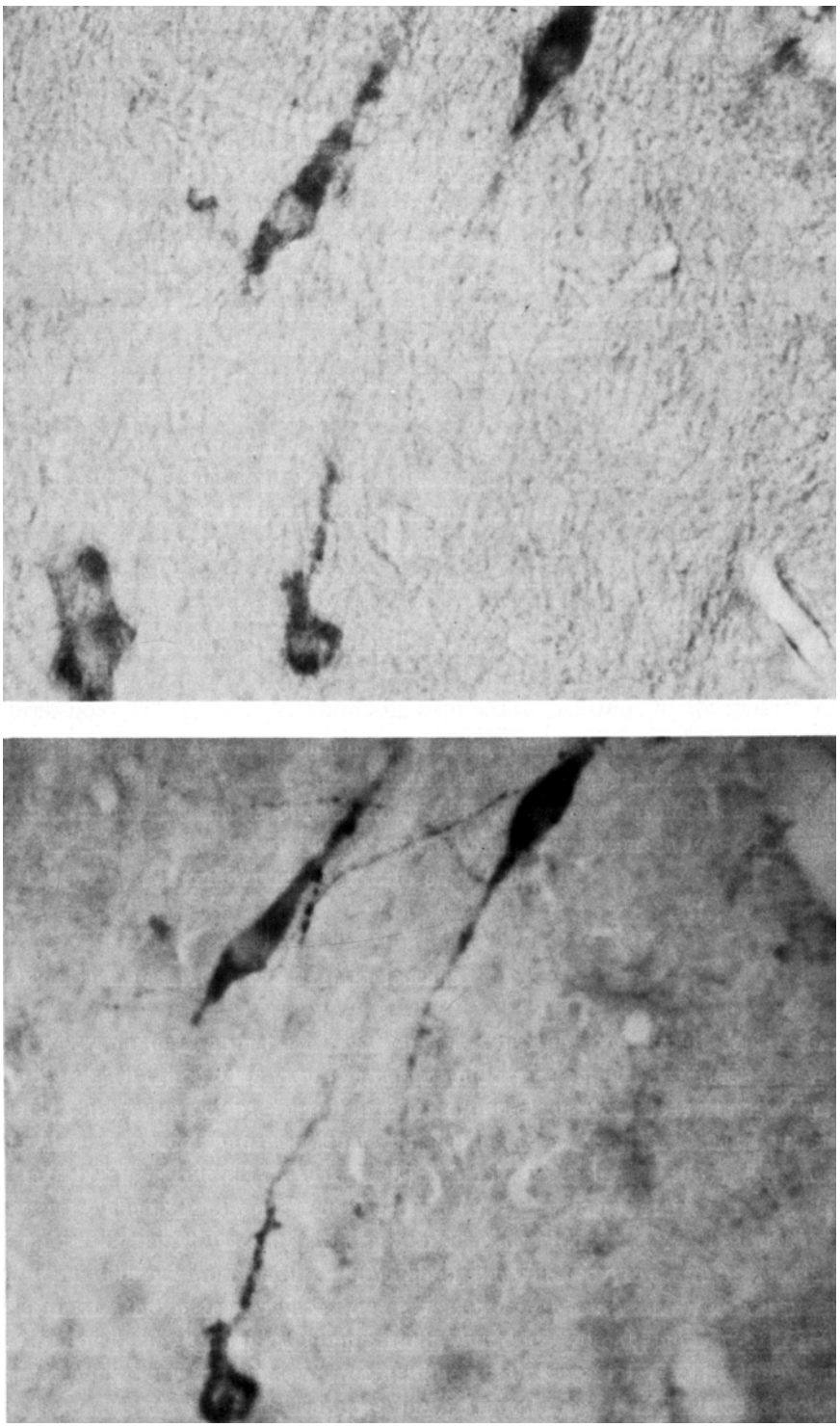
Effect of NPY on anterior pituitary hormone secretion

Intravenous administration of NPY in castrated male rats induces a marked inhibition of LH secretion (Kerkérian *et al*, 1985) without affecting the release of other pituitary hormones. These observations have been confirmed in ovariectomized female rats (Kalra and Crowley, 1984; McDonald *et al*, 1985a, b). These data, together with the location of NPY-containing neurons in the arcuate nucleus (Chronwall *et al*, 1984; Pelletier *et al*, 1984b) at first suggested that NPY could act as a neurohormone which selectively inhibits pituitary LH secretion. This hypothesis was apparently supported by the observation that intravenous injection of NPY antiserum to castrated female rats causes a robust stimulation of LH secretion (Guy *et al*, 1988a). However, several lines of evidence indicated that NPY does not exert a direct effect on pituitary gonadotrophs. *In vitro* studies showed that NPY has no effect on LH and FSH release from pituitary cells in primary culture, obtained from intact female rats (Kerkérian *et al*, 1985). Synthetic NPY has no effect either ou hemi-pituitaries from intact male or ovariectomized female rats (Rodriguez-Sierra *et al*, 1987). However, should it be mentioned that 2 groups have reported a stimulatory effect of NPY on LH release

from perifused gonadotrophs of ovariectomized rats (McDonald *et al*, 1985a; Crowley *et al*, 1987). They also showed that NPY potentiates the effect of LHRH on LH secretion from hemi-pituitaries. However, NPY appears to be totally devoid of effect on LHRH-evoked LH release from anterior pituitary cells in primary culture (Kerkérian *et al*, 1985). Moreover, autoradiographic studies using either ^{125}I-labeled PYY or ^{125}I-labeled BH-NPY show that the anterior lobe of the rat pituitary does not contain NPY binding sites (table II).

In the absence of any direct effect of NPY on anterior pituitary cells it was reasonable to postulate that NPY may exert its action at the level of the central nervous system. This hypothesis is strongly supported by experimental data. Intracerebroventricular injection of NPY stimulates LH release in steroid-primed ovariectomized rats but causes a significant inhibition of LH secretion in castrated male and female rats (Kalra and Crowley, 1984; Kerkérian *et al*, 1985). Since the central effect of NPY is mimicked by intravenous injection of this peptide (Kerkérian *et al*, 1985), it was hypothesized that NPY may affect LHRH release in 2 diencephalic regions which lack the blood–brain barrier: i), the area of the organum vasculosum of the lamina terminalis (OVLT) which contains both LHRH neurons (Pelletier, 1980) and NPY nerve fibers and exhibits a moderate density of NPY binding sites (table II); ii), the median eminence which contains LHRH and NPY nerve terminals. Double-staining immunohistochemical techniques have shown the presence of NPY fibers contacting LHRH cell bodies in the OVLT region (figure 5). In contrast, in the median eminence, the LHRH axon terminals are found in high concentrations in the external zone while NPY fibers are only observed in the internal zone, and thus contacts do not occur between the 2 types of fibers. On the basis of these morphological studies, it appears that NPY neurons may regulate LH secretion through a direct inhibitory effect on LHRH neurons of the preoptic area. In agreement with this hypothesis, Khorram *et al* (1987) using the push–pull technique have shown that NPY modulates the release of LHRH in the rabbit hypothalamus. In contrast, Crowley and Kalra (1987), using *in vitro* incubation of medial basal hypothalamus, observed a stimulatory effect of NPY on LHRH release. It is also worth mentioning that in cold blooded vertebrates NPY regulates gonadotropin release through presynaptic control of LHRH neurons (Danger *et al*, submitted).

Pharmacological studies have shown that treatment of rats with α-methyl-paratyrosine (α-MPT) did not affect the inhibitory action of NPY on LH secretion (Guy *et al*, 1988a). In rats treated with 5,7-dihydroxytryptamine (5,7-DHT), intraventricular injection of NPY causes a marked increase of LH release (Guy *et al*, 1988a). These data indicate that the effect of NPY on LH secretion is not mediated by noradrenergic neurons but suggest that NPY may act presynaptically on serotonin neurons. Several studies have shown that serotonin causes an inhibition of LH secretion (Schneider and McCann, 1979; Kordon and Glowinsky, 1972). We thus propose that NPY may have a physiological effect on LH secretion through 2 distinct pathways: i), a direct effect on the LHRH neuronal sys-



tem; ii), a stimulatory effect on serotoninergic fibers which, in turn, inhibit LHRH release (fig 6).

It should be noted, however, that a direct effect of NPY on pituitary gonadotrophs cannot be exclused. Recent experiments indicate that NPY stimulates LH release from pituitary cells of female rats (Chabot *et al*, 1988). Although NPY immunoreactive axon terminals are not observed in the external zone of the median eminence (Allen *et al*, 1983), high concentrations of NPY are found in the hypothalamo–pituitary portal blood (McDonald *et al*, 1987). Several studies support the notion that steroid hormones play a crucial role in the neuroendocrine functions of NPY (Breton *et al*, 1989). Administration of ovarian steroids elevates the concentrations of NPY in the arcuate nucleus of the rat (Crowley *et al*, 1985). NPY inhibits LHRH release by hypothalamic neurons in normal female rabbits, while in ovariectomized animals NPY stimulates LHRH release (Khorram *et al*, 1987). Similarly, Kalra and Crowley (1984) observed opposite effects of NPY on LH release, depending on whether animals were ovariectomized or not. Recent evidence also indicates that castration of male rats induces a significant decrease of NPY concentrations in the median eminence, arcuate nucleus and ventromedial nucleus (Sahu *et al*, 1987) and that testosterone treatment of gonadectomized male rats restored NPY concentrations in these hypothalamic areas (Sahu *et al*, 1989).

Possible action of NPY on secretion of other pituitary hormones has been examined (table IV). McDonald *et al* (1985a) have shown that NPY stimulates growth hormone (GH) secretion both *in vivo* and *in vitro*. However, the concentrations of NPY required to influence GH release are 100–fold higher than those used to inhibit LH release. Chabot *et al*, (1988) have also observed that NPY stimulates GH and prolactin (PRL) secretion by cultured pituitary cells. In contrast, NPY inhibits GH release from human somatotroph tumor cells (Adams *et al*, 1987). Recent studies indicate that injection of NPY in the paraventricular nucleus of conscious rats stimulates adrenocorticotropin (ACTH) and corticosterone release (Haas and George, 1987; Wahlestedt *et al*, 1987). This effect of NPY likely occurs at the hypothalamic level, since intravenous injection of NPY does not modify circulating ACTH levels (Kerkérian *et al*, 1985).

◀

Fig 5. Microphotograph of a brain section through the rat preoptic area demonstrating that NPY endings are in apposition to LHRH cell bodies. The section has been labelled by the peroxidase–antiperoxidase method using a double immunostaining technique. In the first step (top) staining for LHRH was performed and the reaction photographed. In the second step (bottom), the same section was stained for NPY. NPY fibers not detected in the first step appear in close apposition to LHRH cell bodies; (×560). (From Guy J *et al, Regul Peptides* 209–216, 1988; with permission).

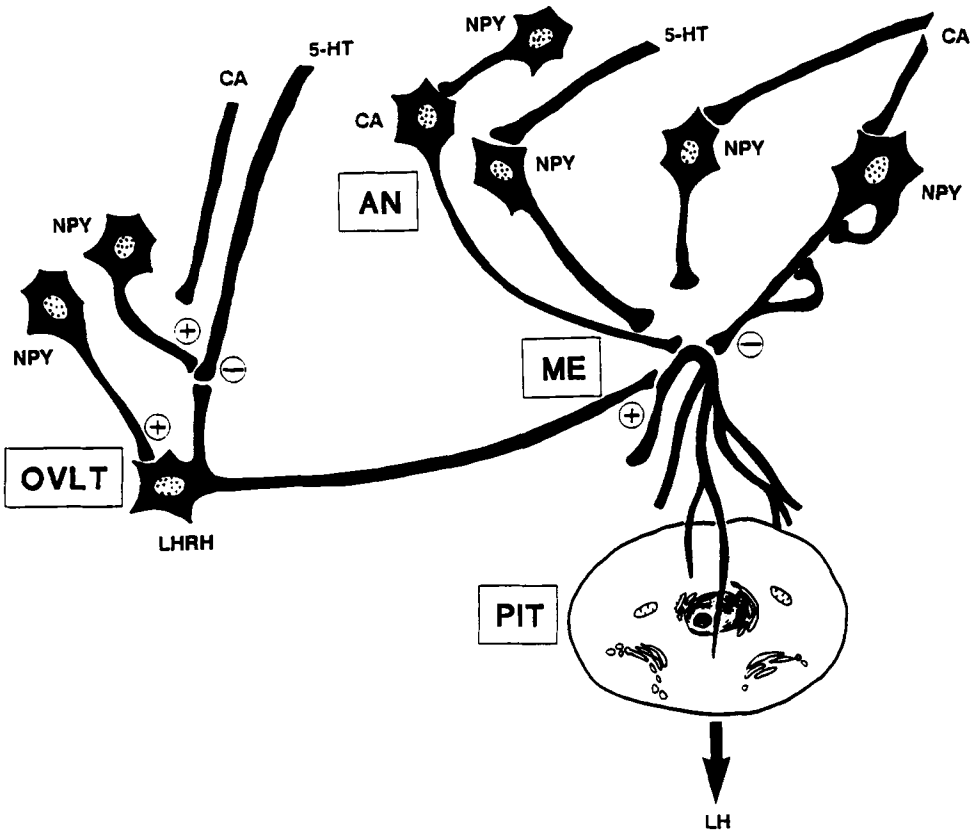


Fig 6. A diagram depicting the various neuronal systems involved in the mechanism of action of NPY on LH secretion. The–and + symbols indicate inhibitory and stimulatory effects respectively. AN, arcuate nucleus; ME, median eminence; OVLT, organum vasculosum lamina terminalis; PIT, pituitary.

Effect of NPY on posterior pituitary hormone secretion

Anatomical studies indicate that NPY-immunoreactive nerve terminals are found in the close vicinity of vasopressinergic neurons (Willoughby and Blessing, 1987). Injection of NPY into the supraoptic nucleus of conscious rats induces an increase in plasma vasopressin concentrations (Willoughby and Blessing, 1987). Electrophysiological studies have shown that NPY stimulates the activity of vasopressinergic neurons (Dray *et al*, 1986). In addition, NPY may exert an effect on vasopressin release in the neural lobe, as suggested by the presence of NPY receptors in the *pars nervosa* of rat (table II) and man (data not shown).

The possible role of NPY in the control of pars intermedia secretion has been investigated in non-mammalian vertebrates. Morphological studies indicate that in all vertebrate studies, NPY-containing neurons are widely distributed throughout

Table IV. Effect of NPY on gonadotropin and LHRH secretion.
+, stimulation; −, inhibition; 0, no effect; ?, not determined.

Animal	*Technique*	*Pharmacological action*	*References*
	in vivo		
Male rat	Sub-cutaneous injection	LH+; FSH 0	Rodriguez-Sierra *et al*, 1987
Castrated male rat	ICV or IV injection	LH−; FSH 0	Kerkérian *et al*, 1985
Castrated female rat	ICV injection	LH−; FSH 0	McDonald *et al*, 1985a, b Kalra and Crowley, 1984
Castrated female rabbit	Push–pull	LHRH−	Khorram *et al*, 1987
	in vitro		
Male rat	Hemi pituitaries	LH 0; FSH 0	Rodriguez-Sierra *et al*, 1987
Female rat	Cultured pituitary cells	LH 0; FSH 0	Kerkérian *et al*, 1985
Castrated female rat	Hemi pituitaries	LH 0; FSH ?	Crowley *et al*, 1987
	Cultured pituitary cells and perifused cells	LH+; FSH 0	McDonald *et al*, 1985a

the brain. In the amphibian hypothalamus, 3 distinct groups of NPY-ergic neurons have been visualized by immunocytochemistry in the preoptic nucleus and in the dorsal and ventral infundibular nuclei (Danger *et al*, 1985; Perroteau *et al*, 1988). These neurons contain both NPY- and C-PON-like immunoreactivity (Caillez *et al*, 1987a, b); suggesting that the structure of the pro-peptide has been highly preserved during evolution. In support of this assumption, NPY-like peptides have been localized and characterized in several non-vertebrate species (Charmantier-Daures *et al*, 1987; Rémy *et al*, 1988; Schoofs *et al*, 1988). In fish (Vallarino *et al*, 1988; Pontet *et al*, 1989) and in anurans (Danger *et al*, 1986; Caillez *et al*, 1987a, b; Verburg van Kemenade *et al*, 1987a), numerous fibers originating from the hypothalamus terminate in the intermediate lobe of the pituitary (figure 7). In the goldfish, NPY fibers also innervate gonadotrophs and somatotrophs of the adenohypophysis (Pontet *et al*, 1989). Several immunocytochemical studies have been conducted at the electron microscopic level to determine the subcellular localization of NPY (Danger *et al*, 1986; Pontet *et al*, 1989). In both fish and amphibians, NPY immunoreactive material is sequestered in small (80–100 nm) dense core vesicles. These electron microscopic studies also revealed that NPY-containing axon terminals contact melanotrophs of the pars intermedia (figure 8). It is worth mentioning that in urodeles NPY innervation is lacking in the intermediate lobe of the pituitary (Perroteau *et al*, 1988). These anatomical data represent the neuroanatomical substrate for possible neuroendocrine regulation of the pituitary melanotrophs by NPY.

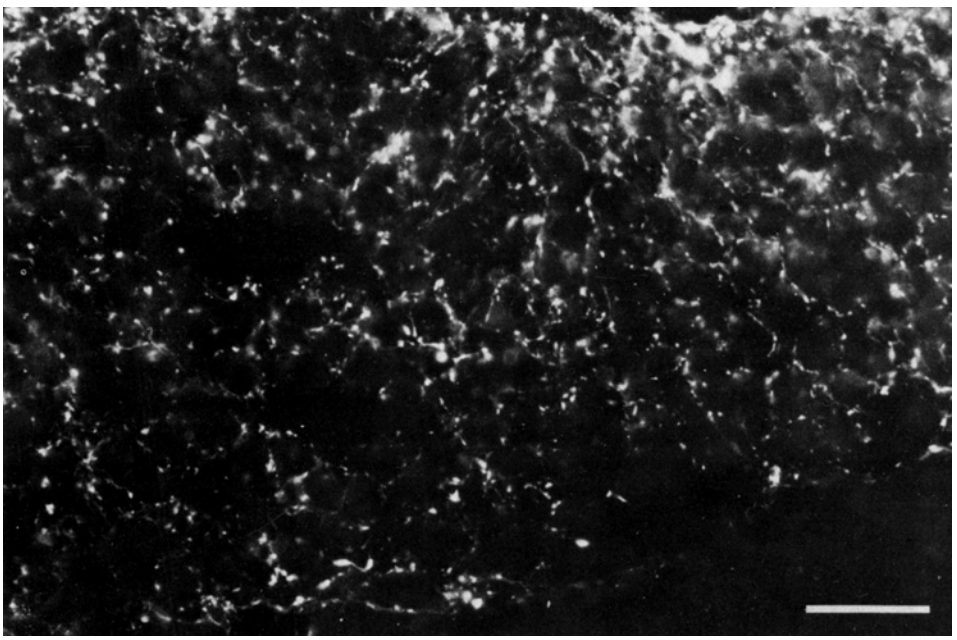


Fig 7. Immunocytochemical localization of NPY-containing fibers in the intermediate lobe of the frog pituitary. Sagittal section of the intermediate lobe stained with anti-NPY serum. White bar 25 μm. (From Danger JM *et al, Life Sci* 39, 1183–1192, 1986; with permission).

The first demonstration that NPY is actually involved in the regulation of melanotropin secretion was provided in the frog (Danger *et al*, 1986). This study was conducted by means of the perifusion technique, which is a dynamic incubation system used to investigate *in vitro* the kinetics of the responses of various tissues to exogenous secretagogues. The release of α-MSH from the pars intermedia of the pituitary was monitored using a sensitive and highly specific radioimmunoassay technique (Vaudry *et al*, 1978). Using this model, it was found that administration of graded concentrations of synthetic (porcine) NPY induces a dose-dependent inhibition of α-MSH secretion from whole pituitaries of *Rana ridibunda* (figure 9). Half-maximum inhibition was observed with a dose of 5×10^{-8} M. These results have since been confirmed in 2 other amphibian species, the frog *Rana pipiens* (Kraicer *et al*, 1988) and the toad *Xenopus laevis* (Verburg van Kemenade *et al*, 1987a). In this latter species, it was observed that NPY inhibits α-MSH release without affecting the biosynthesis or processing of the precursor pro-opiomelanocortin; in the newt *Triturus cristatus*, which lacks NPY innervation of the pars intermedia, NPY has no effect on α-MSH secretion (Danger *et al*, 1989). A comparative study has shown that while NPY inhibits α-MSH release from the pars intermedia of anurans, it has no effect on α-MSH secretion in the rat (Kraicer *et al*, 1988). This is in agreement with the lack of NPY innervation (as well as NPY binding sites) in the pars intermedia of the rat.

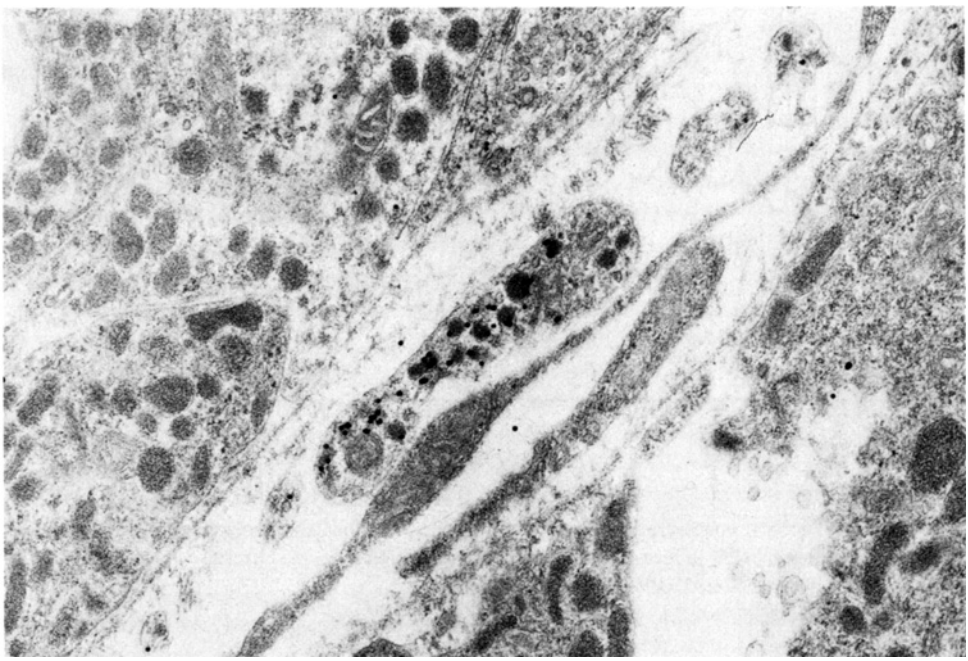


Fig 8. Immunoelectron-microscopic localization of NPY in the frog intermediate lobe. Immunostaining is restricted to a nerve fiber coursing between parenchymal cells. The gold particles are located in dense core vesicles; (×38,000). (From Danger JM *et al, Life Sci* 39, 1183–1192, 1986; with permission).

Symmetrically, in the goldfish, the intermediate lobe is richly innervated by NPY fibers (Pontet *et al*, 1989) and NPY modulates α-MSH secretion (Fryer *et al*, 1989). Using several NPY short chain analogues and other members of the PP family, the structure–activity relationship of NPY has been investigated. The results show that the bioactive determinant of NPY is located in the C-terminal part of the molecule (Danger *et al*, 1987). Two classical neurotransmitters (*ie* dopamine and GABA) are also potent inhibitors of melanotropic cell activity (Tonon *et al*, 1983; Adjeroud *et al*, 1986a, b; Verburg van Kemenade *et al*, 1986a, b, 1987b). In order to determine whether NPY acts directly on pituitary melanotrophs or presynaptically on dopamine or GABA-containing nerve fibers, the effect of NPY was investigated during prolonged administration of haloperidol and bicuculline. The inhibitory effect of NPY is not affected by the dopaminergic and GABAergic antagonists (Danger *et al*, 1986). In addition, NPY inhibits α-MSH secretion from acutely dispersed pars intermedia cells (Danger *et al*, 1990). Using the patch-clamp technique, we have recently observed that NPY induces hyperpolarization and inhibition of spontaneous action potential firing (unpublished data). All these experiments demonstrate that NPY exerts its effect

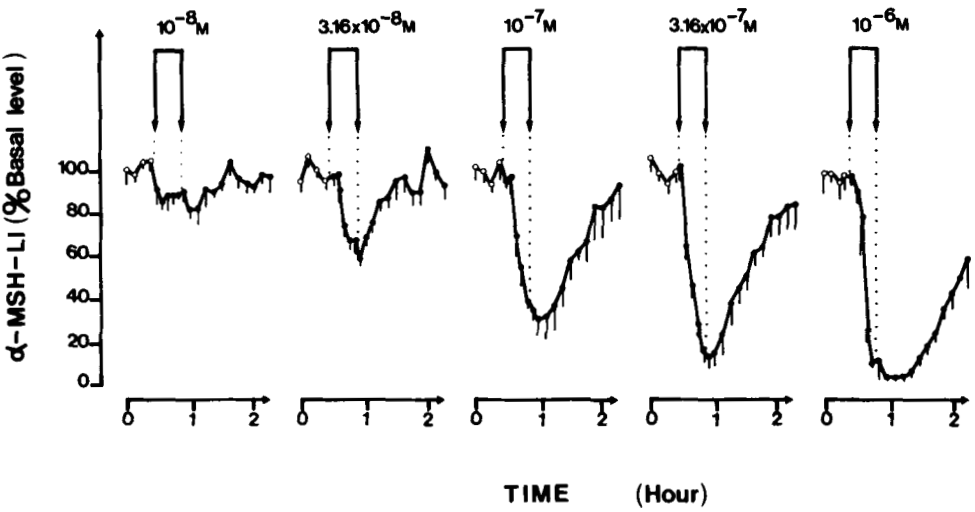


Fig 9. Effect of graded concentrations of NPY on-MSH secretion from isolated perifused frog neurointermediate lobes. NPY induces a dose-dependent inhibition of α-MSH release (From Danger JM *et al, Life Sci* 39, 1183–1192, 1986; with permission).

by acting directly on pituitary melanotrophs. *In vivo* experiments have shown that administration of NPY to freely moving *Xenopus* causes a marked melanophore aggregation in black background adapted animals (unpublished data), suggesting that NPY plays a physiological role in the regulation of the melanotropic function. These data suggest that the neuropeptide NPY may be regarded as a melanotropin-release inhibiting factor in non-mammalian vertebrates.

Conclusion

In conclusion, it now appears that the regulatory peptide NPY, which is highly concentrated and widely distributed in the brain and in the sympathetic nervous system of all vertebrates, can be regarded as a potential neurotransmitter. NPY likely mediates a number of physiological and behavioral processes both at the level of the central nervous system and in peripheral tissues. In particular, NPY is a hypophysiotropic neurohormone which plays a pivotal role in the regulation of gonadotropin secretion by anterior pituitary cells and melanotropin secretion by pars intermedia cells.

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