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PEPTIDES

Peptides 22 (2001) 325–334

Characterization and distribution of neuropeptide Y in the brain of a caecilian amphibian

Ted J. Ebersole[a], J. Michael Conlon[b], Frederick W. Goetz[a], and Sunny K. Boyd[a,*]

[a]*Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556*
[b]*Regulatory Peptide Center, Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, NE 68178*

Received 17 May 2000; accepted 22 September 2000

Abstract

Neuropeptide Y (NPY) from the brain of an amphibian from the order Gymnophiona (the caecilian, *Typhlonectes natans*) was characterized. We cloned a 790 base pair cDNA encoding the caecilian NPY precursor. The open reading frame consisted of 291 bases, indicating an NPY precursor of 97 amino acids. Both deduced and isolated NPY primary structures were Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu[10]-Asp-Ala-Pro-Ala-Glu-Asp-Met-Ala-Lys-Tyr[20]-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu[30]-Ile-Thr-Arg-Gln-Arg-Tyr · NH2. In caecilian brain, we observed NPY immunoreactive cells within the medial pallium, basal forebrain, preoptic area, midbrain tegmentum and trigeminal nucleus. The prevalence of preoptic and hypothalamic terminal field staining supports the hypothesis that NPY controls pituitary function in this caecilian.

Keywords: Typhlonectes natans; Gymnophiona; NPY gene; reproduction; pituitary

1. Introduction

Brain neuropeptide Y (NPY) distribution has been described for representatives from each vertebrate class [hagfish [10]; lamprey [6]; elasmobranch [52]; teleost [43]; amphibians [14]; reptiles [44]; birds [3]; mammals [1]]. Within the class Amphibia, however, NPY-like immunoreactivity has been described only in the brain of four anuran species [*Rana ridibunda* [14], *Rana esculenta* [18,34], *Rana catesbeiana* [7], *Xenopus laevis* [34,50]] and a single urodele species [*Triturus cristatus*, [42]]. The third amphibian order, Gymnophiona (commonly called caecilians), contains more than 150 species but whether NPY is present in the brain of any caecilian is not known. The caecilians are unique, tropical amphibians. Most species are limbless, blind burrowers and this order diverged from the other amphibian orders at least 150 million years before the present [23]. The distribution of NPY in the caecilian brain may suggest novel functions of the peptide in this order or indicate conserved patterns of NPY function across vertebrates in general.

Functions of NPY in nonmammals are not well understood. In mammals, NPY modulates gonadotropin release, sexual behavior, memory, food intake, and cardiovascular and stress responses, for example [21,24,25,28,29]. NPY may modulate reproduction or feeding in some species of fish [35,48] and birds [20,30,45]. The only firmly-established role for NPY in amphibians is inhibition of melanocyte-stimulating hormone (MSH) release [9,15–17,19,51,54]. NPY thus functions in background adaptation behavior of frogs and toads [46,51]. NPY appears not to be involved in background adaptation in salamanders where it has been tested however [16]. It seems likely that the functions of NPY in amphibian species are many more than currently known.

The structure of NPY is well-conserved [8,32]. This 36 amino acid peptide belongs to the same family as pancreatic polypeptide (PP) and peptide tyrosine-tyrosine (PYY). The known amino acid sequences of NPY from jawed vertebrates differ at no more than five positions from the putative ancestral NPY sequence. Cloning studies also show highly conserved sequences in other regions of the NPY gene (e.g., 5). This high degree of amino acid and nucleotide sequence conservation suggests that NPY is an important regulatory peptide. Sequences used to compare amphibians with other vertebrates may not be representative of the whole class

* Corresponding author. Tel: (219) 631-5021; fax: (219) 631-7413.
E-mail address: boyd.1@nd.edu

0196-9781/01/$ – see front matter
PII: S0196-9781(01)00334-5

however. The NPY gene has been cloned in only one anuran amphibian, *X. laevis* [26,53]. Therefore, we isolated and cloned the full length NPY cDNA from the brain of one species of caecilian amphibian, *Typhlonectes natans*. In addition, we determined the structure of the brain peptide and used immunocytochemistry to map the distribution of NPY cells and fibers in *T. natans* brain.

2. Materials and methods

2.1. Cloning of NPY cDNA

A cDNA library (1×10^6 pfu) was constructed in λZAP Express with poly A^+ RNA isolated from brain tissue of *Typhlonectes natans*. A cDNA fragment (~700 base pairs) was generated from the library using a PCR-based strategy involving a vector primer, T7, and an internal gene-specific primer, 5′-CCAGC(A/G)GAGGACATGGCC A(A/G)A-3′, designed from the consensus alignment of nucleotide sequences within the +13 to +19 amino acids of known mature NPY sequences from *X. laevis*, human and rat [2,33, 38,53]. This fragment was labeled with [α-^{32}P]dATP (>3000 Ci/mmol; ICN, Costa Mesa, CA) using Klenow fragment and random primer (Prime-It II; Stratagene). The labeled fragment was used to screen the library (approximately 200,000 plaques) under high-stringency conditions to obtain the full length NPY cDNA. Positive clones were rescreened once to homogeneity and *in vivo* excised. Sequencing was performed on plasmid DNA using a modified dideoxy chain termination method (SequiTherm EXCEL II Long Read DNA Sequencing Kit-ALF; Epicentre, Madison, WI, USA) with Cy5-labeled vector primers flanking the DNA insertion site. The sequencing reactions were separated and analyzed using an ALFexpress Sequencer (Pharmacia Biotech).

2.2. Purification and characterization of NPY

Whole brains were removed from adult *T. natans* of both sexes (n = 10; mean length 35 cm; mean weight 29 g; Wilson Pet Supply, Woodale, IL) which had been sacrificed by immersion in 0.02% benzocaine. Tissues were immediately frozen on dry ice and stored at −80°C. Pooled brains

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    5′---GGCACCAGGAGAGAGGAGCGGAACGGGGTAACCGGGGCAGTAGCAGCGAGCGAGAAGCACCTCACACTCTACTTAGAGCGCG      82
-28                              -20                                  -10
Met Gln Gly Ser Met Arg Leu Trp Leu Ser Val Leu Thr Phe Thr Leu Ser Leu Leu Ile Cys Leu Gly
ATG CAG GGG AGC ATG AGG TTG TGG CTG TCT GTC CTG ACT TTC ACC CTG AGC TTG CTG ATC TGC TTG GGG      151
                                  SIGNAL PEPTIDE
                -1  1                              10
Thr Leu Ala Asp Ala Tyr Pro Ser Lys Pro Asp Asn Pro Gly Glu Asp Ala Pro Ala Glu Asp Met Ala
ACG CTG GCA GAT GCT TAT CCA TCC AAA CCG GAC AAT CCT GGA GAG GAC GCG CCG GCA GAG GAC ATG GCC      220
                                  NPY
    20                                 30                                  40
Lys Tyr Tyr Ser Ala Leu Arg His Tyr Ile Asn Leu Ile Thr Arg Gln Arg Tyr Gly Lys Arg Ser Asn
AAA TAT TAC TCG GCA CTG AGG CAT TAC ATC AAT CTC ATC ACA AGA CAG AGA TAT GGA AAG AGA TCA AAC      289
                                  50                                  60
Pro Glu Thr Met Val Ser Asp Val Trp Trp Arg Glu Ser Thr Glu Asn Ile Pro Arg Ser Arg Phe Glu
CCG GAG ACA ATG GTA TCA GAT TGC TGG TGG AGG GAA AGC ACA GAA AAT ATT CCT AGA TCT AGG TTT GAA      358
                                  CPON
                69
Asp Pro Ser Met Trp ***
GAC CCT TCT ATG TGG TGA TGGAAGCTGTGACTCATCTTCAGCCTTTTCTGATTTTTCCTTGTCCAGATTTCATTTCGATAAAACC      443

AACTGGATTTTTCTCAGTCCTCCAAACGCAGCCAGAGCATGCAGGCATTACACTGACTTCTGTAGAGTTCTTTTTCTCCATGATATTTGTA      534
CATAAATTAATTTAAATAAATTATTTGTGCATTTGAAAATGTAACATCCTGAACAAGTGGGAGGAGGGAAGCTATCCTTAGTACAGCAAGA      625
TTGGTTTTCATCTTTAAACTGAGTTTTGGTGCCCTTCCAGTCTAGTTCAATGCTAGCATGTACTGCACAGATATTCCAGTAATTCATACTA      716
TATGACTTGGGCATTTTAATGGAATAAATCCATTTATTTTCAGTTAAAAAAAAAAAAAAAAAAAAAAAAAAAA---3′              790
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Fig. 1. Nucleotide and deduced amino acid sequences of a cDNA encoding *T. natans* preproNPY. The sequences for the signal peptide, NPY and associated C-terminal peptide of NPY (CPON) are underlined with a hatched, filled and dashed line, respectively. The potential processing site is boxed, termination codon is marked with asterisks and polyadenylation signal (AATAAA) is underlined. GenBank accession number is AF167559.

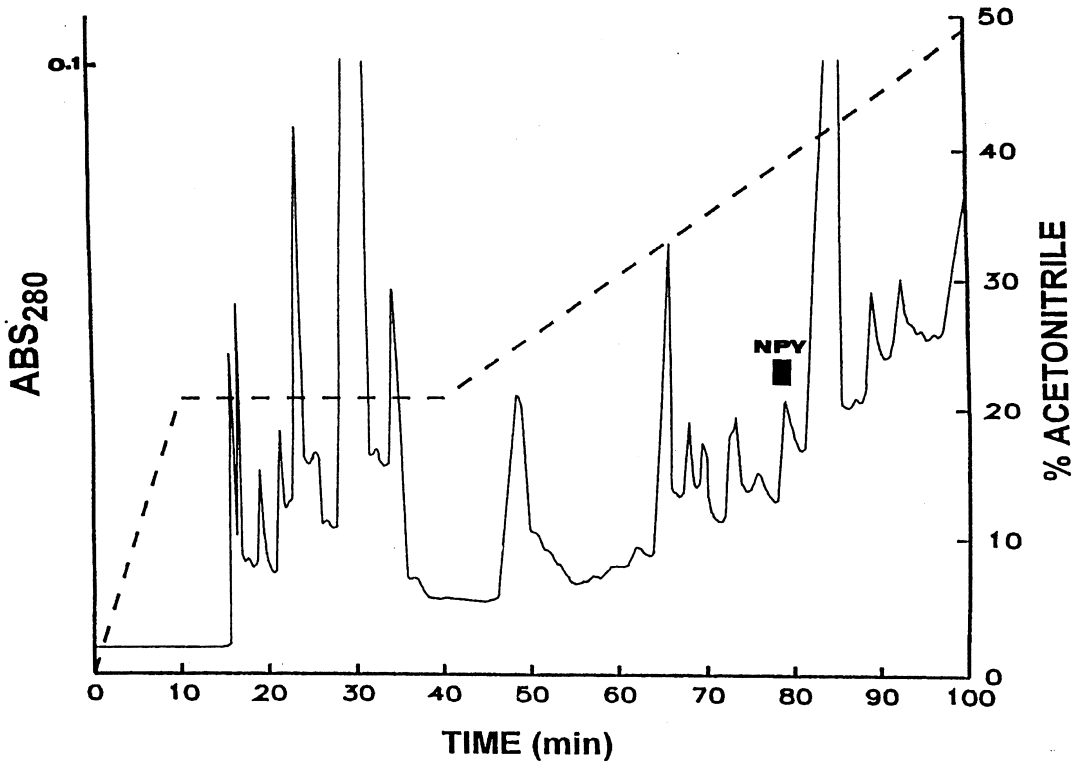

Fig. 2. Reversed-phase HPLC on a semipreparative Vydac C-18 column of an extract of *T. natans* brain after partial purification on Sep-Pak cartridges. The fraction denoted by the bar contained NPY-like immunoreactivity and was purified further. The dashed line shows the percent of acetonitrile in the eluting solvent.

(0.27 g) were boiled in 1M acetic acid (12 ml) for 5 min followed by homogenization (Polytron). After centrifugation (1600 g for 1 h at 4°C), peptides were isolated from the supernatant using Sep-Pak C-18 cartridges (Waters Associates, Milford, MA; 9). Bound material was eluted with acetonitrile/water/trifluoroacetic acid (70:29:1) and freeze-dried.

The brain extract, after partial purification on Sep-Pak cartridges, was redissolved in 0.1% trifluoracetic acid/water (5 ml) and injected onto a 1 × 25-cm Vydac 218TP510 C-18 reversed-phase HPLC column (Separations Group, Hesperia, CA) equilibrated with 0.1% trifluoroacetic acid/water at a flow rate of 2 ml/min. The concentration of acetonitrile in the eluting solvent was raised to 21% over 10 min, held at this concentration for 30 min and then raised to 49% over 60 min using linear gradients. Absorbance of peptides was measured at 214 nm and 280 nm. Fractions (2 ml) were collected and assayed for NPY-like immunoreactivity at a dilution of 1:30. The peak designated by the bar (containing NPY-like immunoreactivity; Fig. 2) was chromatographed on a 0.46 × 25-cm Vydac 214TP54 C-4 reversed-phase column equilibrated with acetonitrile/water/trifluoroacetic acid (21:78.9:0.1) at a flow rate of 1.5 ml/min. The concentration of acetonitrile in the eluting solvent was raised to 42% over 40 min using a linear gradient. Caecilian NPY was purified to near homogeneity, as assessed by peak symmetry, by sequential chromatography on a 0.46 × 25-cm Vydac 219TP54 phenyl column and a 0.46 × 25-cm Vydac 218TP54 C-18 column under the same conditions used for the C-4 column.

Caecilian NPY was detected by radioimmunoassay using antiserum 8999 which was raised against the cysteine-extended COOH-terminal hexapeptide of human NPY (Cys-Ile-Thr-Arg-Gln-Arg-Tyr-NH_2; 40). The antiserum requires the presence of an alpha-amidated C-terminal residue. Human NPY was used as standard and ^{125}I-Bolton Hunter-labeled NPY (Amersham, Arlington Heights, IL) was used as a radiolabeled tracer. Antiserum cross reacts 100% with pig PYY but shows negligible cross-reactivity with pig PP (<1%) [40].

The primary structure of caecilian NPY was determined by automated Edman degradation in an Applied Biosystems model 471A sequenator modified for detection of phenylthiohydantoin (PTH) amino acid derivatives under gradient elution conditions. Approximately 200 pmol of the peptide was used. The detection limit for PTH derivatives was 1 pmol. Mass spectrometry was performed on a Voyager RP MALDI-TOF instrument (Perspective Biosystems, Framingham, MA) equipped with a nitrogen laser (337 nm). The instrument was operated in linear mode with delayed extraction and the accelerating voltage in the ion source was 25kV. The accuracy of the mass determinations was within 0.1%.

2.3. Immunocytochemical distribution of NPY

Brains (n = 5) were fixed *in situ* (4% paraformaldehyde, 3% sucrose and 7.5% saturated picric acid in 0.1 M phosphate buffer, pH = 7.4) for 2–4 h, then removed from the skull and placed in fresh fixative overnight at 4°C. The next day, brains were transferred to 30% sucrose in buffer for storage at 4°C. Frozen frontal sections (50 μm) were placed into vials of 0.05 M tris-buffered saline (TBS; 50 mM Tris-buffer, 0.9% sodium chloride; pH = 7.6) and stored at 4°C. Sections were placed alternately between two vials. This provided two matched sets of sections from each brain.

Free-floating sections were stained using a modified procedure of Tuinhof et al. [50]. Primary antiserum (from Dr. H. Vaudry, Universite de Rouen, France) was raised in rabbit against porcine NPY [41]. Sections were treated sequentially as follows (at room temperature unless otherwise noted): 50 mM TBS with 0.3% Triton X-100 (TBST; 30 min), 1% H_2O_2 in TBST (15 min), TBST rinse (10 min), 20% normal goat serum (in TBST; 30 min) and incubation in primary antibody (in TBST) at 1:1000 dilution. After incubation for 40–48 h at 4°C, sections were rinsed in TBST (2 × 5 min), incubated with goat anti-rabbit secondary antibody (Sigma Chemical Co., 1:150) for 1 h, rinsed in TBST (2 × 5 min) and placed in peroxidase-antiperoxidase complex (ICN Immunobiologicals, 1:300) for 1 h. Sections were rinsed in TBST (2 × 5 min) and staining was visualized with 0.05% diaminobenzidine tetrachloride (Sigma Chemical Co.; in TBS, 0.012% H_2O_2 and 0.1% $NiCl_2$) for 15 min followed by a TBS rinse (5 min). Sections were mounted, dried and coverslipped with Permount (Fisher Scientific). Specificity controls included eliminating the primary antibody and preadsorption of primary antibody for 90 min with 10 μM synthetic frog NPY antigen (from Dr. M. Tonon). Previous studies with this NPY antibody have shown that preincubation of the antibody with 10 μM synthetic avian pancreatic polypeptide, avian peptide YY (PYY), or porcine vasoactive intestinal peptide had no effect on immunostaining in amphibian brain tissue [14]. Neuroanatomy was based primarily on Kuhlenbeck [31] and Hilscher-Conklin et al. [27]. Anatomical abbreviations defined in Table 1.

Table 1
Neuroanatomical Abbreviations

AOB	accessory olfactory bulb
APOA	anterior preoptic area
DH	dorsal hypothalamus
DP	dorsal pallium
H	habenula
LA	lateral amygdala
LP	lateral pallium
LS	lateral septum
MA	medial amygdala
MP	medial pallium
MS	medial septum
NA	nucleus accumbens
OT	optic tectum
POA	preoptic area
ST	striatum
TE	thalamic eminence
TEG	tegmentum
ThV	ventral thalamus
TRG	trigeminal nucleus
VH	ventral hypothalamus

3. Results

3.1. Cloning of caecilian NPY cDNA

A 790 base pair (bp) NPY cDNA was obtained from the library screening (Fig. 1). The cDNA contained 5′ and 3′ untranslated regions of 82 bp and 417 bp, respectively. The open reading frame of 291 bases presumably encoded a 97 amino acid (aa) precursor (Fig. 1). Based on sequence homology, this precursor contained a signal peptide (28 aa) and the NPY protein (36 aa). These were followed by a glycine residue that is probably involved in the C-terminal alpha-amidation of cleaved NPY and a pair of basic residues (Lys-Arg) for proteolytic cleavage during processing. The remaining open reading frame contained the carboxy-terminal peptide of proNPY (CPON; 30 aa). A polyadenylation signal was found 17 nucleotides upstream from the poly A tail.

3.2. Purification and characterization of caecilian NPY

The crude extract of *T. natans* brain contained a very high concentration (approximately 1 nmol/g tissue weight) of NPY-like immunoreactivity as measured with an antiserum raised against the conserved COOH-terminal region of human NPY. NPY-like immunoreactivity was associated with a single fraction after elution from a semi-preparative Vydac C-18 column (Fig. 2). The peptide was purified to near homogeneity (as assessed by symmetrical peak shape) by successive chromatographies on an analytical C-4 column (Fig. 3A), an analytical Vydac phenyl column (Fig. 3B), and an analytical Vydac C-18 column (Fig. 3C). The retention time of endogenous caecilian NPY was the same as that of synthetic NPY from the frog *R. ridibunda* [9]. The final yield of pure peptide was approximately 200 pmol.

As the amount of pure material was very low, approximately 90% of the peptide was subjected to automated Edman degradation. It was possible to identify without ambiguity phenylthiohydantoin-coupled amino acid derivatives for 36 cycles of operation of the sequenator. The primary structure of the caecilian NPY was established as: Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu^{10}-Asp-Ala-Pro-Ala-Glu-Asp-Met-Ala-Lys-Tyr^{20}-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu^{30}-Ile-Thr-Arg-Gln-Arg-Tyr. The presence of a C-terminal α-amidated residue in the peptide was not demonstrated chemically but its presence was

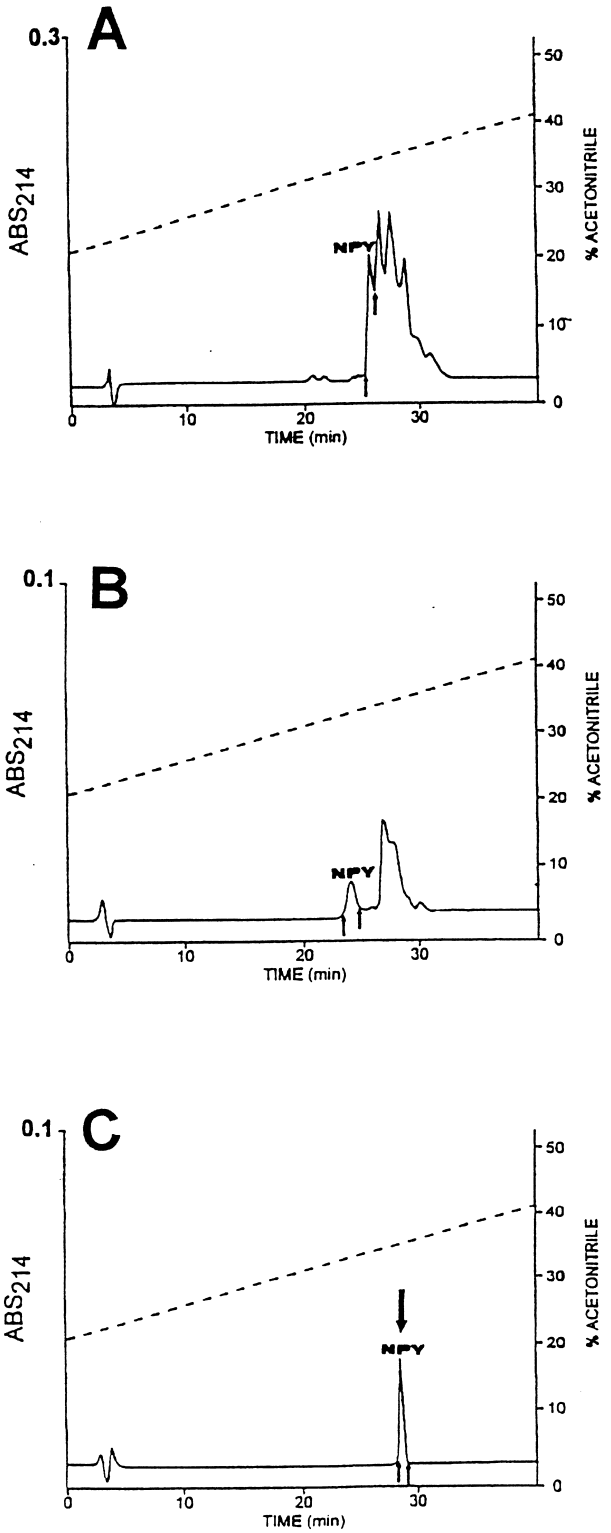

Fig. 3. Purification of *T. natans* NPY on analytical (A) Vydac C-4; (B) Vydac phenyl and (C) Vydac C-18 columns. The peaks labeled NPY contained NPY-like immunoreactivity. The arrows pointing upward show where peak collection began and ended. The arrow pointing downward on (C) indicates the retention time of NPY from the frog *R. ridibunda* [9].

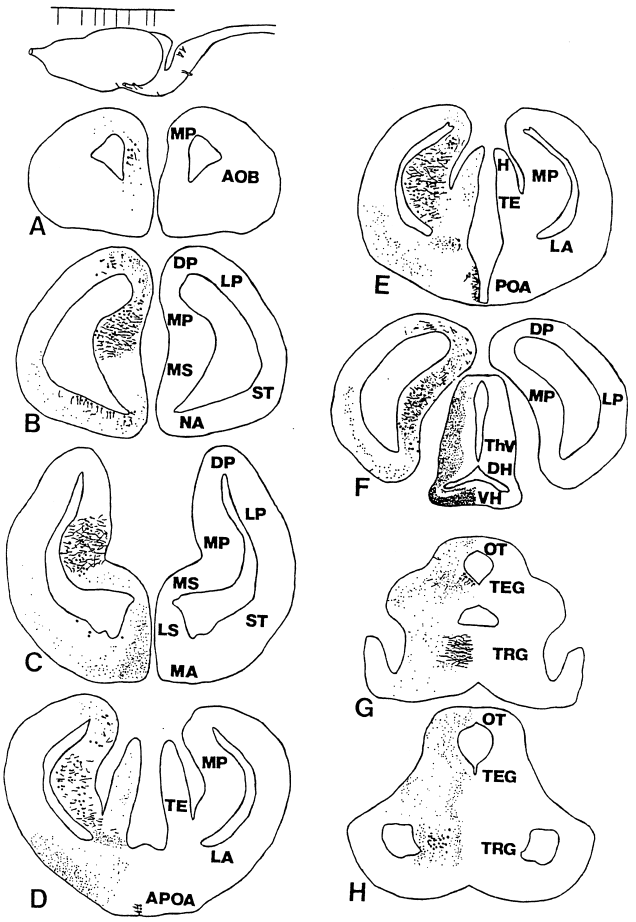

Fig. 4. Camera lucida drawings of the distribution of NPY immunoreactive cells and fibers (left side) in frontal sections through the caecilian brain (rostral to caudal); neuroanatomy defined on right side (abbreviations in Table 1). Approximate level of sections is indicated in upper left drawing of side view of caecilian brain.

clearly suggested by strong cross-reactivity with the C-terminally directed antiserum to NPY. The observed molecular mass of the caecilian NPY was 4246 ± 4 daltons compared with a calculated molecular mass of 4245 daltons.

3.3. Immunocytochemical distribution of caecilian NPY

NPY-immunoreactive (NPY-ir) cell populations were distributed throughout the entire brain of this caecilian species (Fig. 4). Five cell populations were especially prominent and always present. Some of these populations spanned multiple neuroanatomical areas. The five major NPY-ir cell body populations were found in the (1) pallium, (2) basal forebrain, (3) preoptic area (POA), (4) tegmentum, and (5) trigeminal nucleus. Staining disappeared from all brain areas when the primary antibody was preadsorbed with synthetic frog NPY (Fig. 5B) and when the primary antibody was omitted (not shown).

In the telencephalon, the pallium contained the largest population of cells that stained with the antibody to NPY.

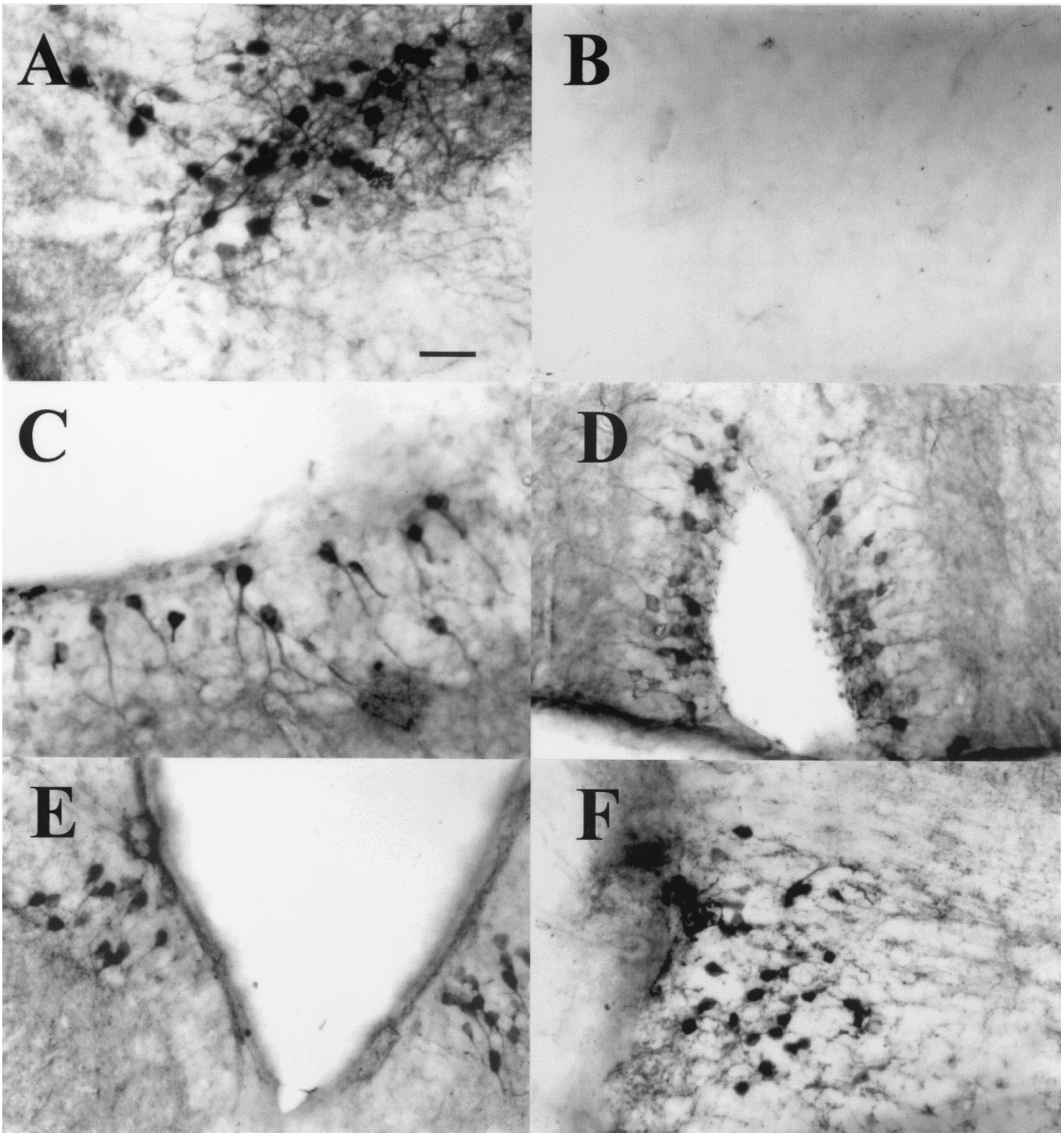

Fig. 5. Photomicrographs of NPY immunoreactive cells in the caecilian brain (frontal sections). (A) NPY-ir cell bodies in the medial pallium; (B) Staining in the medial pallium when NPY antisera was preadsorbed with synthetic frog NPY; (C) NPY-ir cell bodies in the nucleus accumbens; (D) NPY-ir cell bodies in the preoptic area; (E) NPY-ir cell bodies in the midbrain tegmentum and (F) NPY-ir cell bodies in the trigeminal nucleus. Scale bar = 50 μm.

Stained cells (approximately 20–30 cells per animal) were observed beginning in the most anterior medial pallium (Fig. 4A). This band of cells continued into the medium pallium proper which contained the greatest number of NPY-ir cells of any brain area (200 cells or more per animal). These cells were large and scattered throughout the entire medial pallium wall (Figs. 4B–F, 5A). NPY-ir fibers were dense in the medial pallium (Figs. 4B–F, 5A). These fibers were thick, pronounced and extended in many directions from cell bodies. In addition to the medium pallium,

Table 2
Comparison of known NPY primary structures from amphibians with representative structures from other vertebrate classes

Common Name	Species	NPY Structure[a]	Reference
Human	*Homo sapiens*	YPSKP DNPGE DAPAE DMARY YSALR HYINL ITRQR Y	[38]
Chicken	*Gallus gallus*	----- -S--- ----- ----- ----- ----- ----- -	[5]
Alligator	*Alligator mississippiensis*	----- ----- ----- ----- ----- ----- ----- -	[56]
Laughing frog	*Rana ridibunda*	----- ----- ----- ---K- ----- ----- ----- -	[9]
Common frog	*Rana temporaria*	----- ----- ----- ---K- ----- ----- ----- -	[37]
Clawed frog	*Xenopus laevis*	----- ----- ----- ---K- ----- ----- ----- -	[26,53]
	(Two forms)	----- ----D ----- ---K- ----- ----- ----- -	[26]
Caecilian	*Typhlonectes natans*	----- ----- ----- ---K- ----- ----- ----- -	
Goldfish	*Carassius auratus*	--T-- ----- G---- EL-K- ----- ----- ----- -	[5]
Ray	*Torpedo marmorata*	----- ----- G---- -L-K- ----- ----- ----- -	[5]

[a] Hyphens indicate amino acids identical to human.

NPY-ir cells (approximately 50–60 cells per animal) were also located in the dorsal pallium (Figs. 4B, D, F). Occasional rare cells were observed in the lateral pallium. Dorsal and lateral pallium cells were more common at caudal pallial levels. Another population of NPY-ir cells (approximately 70 cells per animal) was found in a continuum along the basal forebrain. This population thus ranged through the nucleus accumbens, striatum, and medial amygdala (Figs. 4B–C, 5C). These distinct cells had soma located very near the ventricle, in contrast to pallial cells which were displaced from the ventricular cell layers. NPY-ir fibers from basal forebrain cells extended ventro-laterally and the entire floor of the forebrain contained dense terminal field staining (Figs. 4B–E, 5C). Cell bodies immunoreactive for NPY were noticeably absent from the olfactory bulb, accessory olfactory bulb, and septum. Fiber and terminal field staining was absent from the accessory olfactory bulb and rare in the olfactory bulb. Septal fibers were also rare with the exception of the most caudal septum, immediately adjacent to the thalamic eminence (Fig. 4D).

The POA possessed a significant population of NPY-ir cell bodies. A small, clustered population of cells (approximately 30 cells per animal) was located in the anterior POA (Fig. 4D) but the posterior POA contained the most prominent population. These posterior POA cell bodies (approximately 100 cells per animal) lined the third ventricle (Figs. 4E, 5D). There were a few thin fibers that projected laterally away from the POA cells but fiber and terminal field staining in the POA was moderate overall (Figs. 4D, E, 5D). At the level of the POA (Fig. 4E), dense terminal field staining was observed in the lateral amygdala. There were no NPY-ir cell bodies in the suprachiasmatic nucleus of this caecilian. A few lightly-stained cell bodies were found in the hypothalamus. Dense fiber and terminal field staining was found in dorsal and ventral hypothalamic areas (Fig. 4F). Light fiber staining was present in thalamic areas and the habenula but immunoreactive cells were not found in either location.

In the midbrain, NPY-ir cells (approximately 50–70 per animal) were observed in the tegmentum, clustered immediately around the aqueduct of Sylvius (Figs. 4G, 5E). Fiber NPY-ir staining was moderate in the tegmentum and light in the tectum. No NPY-ir cell bodies were observed in the tectum of this caecilian. More caudally, another population of smaller, darkly-stained cells (approximately 70–80 per animal) was present within the trigeminal motor nucleus. These cells were medial to the fourth ventricle (Figs. 4H, 5F). Long, thin fibers were observed running between the trigeminal motor nuclei (Fig. 4G). Dense fiber staining was found in the solitary tract. No NPY-ir cell bodies were observed caudal to the trigeminal nucleus.

4. Discussion

Neuropeptide Y has been described in a representative species from the amphibian order Gymnophiona for the first time in this study. The amino acid sequence of the peptide has been strongly conserved during the evolution of vertebrates. Both the isolated and deduced NPY peptide sequences from the caecilian *T. natans* were identical to the peptide previously isolated from the brain of the frogs *R. ridibunda* and *R. temporaria* [9,37] and deduced from the nucleotide sequence of a cloned hypothalamic cDNA from the frog *X. laevis* [26,53] (Table 2). The nucleotide sequence for this caecilian NPY was 88%, 86%, 85%, 79%, 77%, and 66% identical to NPY nucleotide sequences from anuran amphibian (*X. laevis*), chicken, human, goldfish, ray and lamprey, respectively [5,26,38,49,53]. Pancreatic polypeptide from this caecilian shares 16 of the 36 amino acids in NPY but PP is much less conserved across vertebrates [13,32]. The C-terminal peptide extension of proNPY (CPON) also shows strong sequence conservation across vertebrates [32] and the *T. natans* peptide is 90% similar (66% identical) to the human CPON [38]. This caecilian species, however, has an amino acid motif of valine and two adjacent tryptophan residues which has been seen in the CPON of *X. laevis* but not in any other vertebrate [26,53]. Conservation of CPON structure supports the hypothesis that this peptide has an important function. The unique amino acid motif of both amphibian CPON forms may be

important in binding of the CPON to an unusual amphibian receptor.

Distribution of NPY-like immunoreactivity in the brain of this caecilian does not support the hypothesis that NPY is involved in background adaptation behavior in this species. Caecilians, which are either blind or have much reduced vision [55], have not been reported to possess this light-adaptive behavior. The caecilian *T. natans* did not have NPY-ir cells in the suprachiasmatic nucleus, optic tectum or visual processing areas of the thalamus. This is in marked contrast to reports in all other amphibians (both anuran and urodele), which have prominent NPY-ir cell populations in these brain areas [7,14,18,34,42,50]. The suprachiasmatic nucleus and optic tectum, along with the retina, control background adaptation behavior in anurans [46,51]. Thus, although background adaptation behavior is the only currently ascribed function for NPY in amphibians, it is unlikely that this is a function of NPY in this caecilian.

The prevalence of NPY-immunoreactivity in POA cell bodies and hypothalamic fibers in this caecilian brain supports the hypothesis that NPY regulates pituitary hormone secretion in this species. Distribution of NPY-ir in these areas is found in other amphibians [7,14,18,34,42,50] and also across other vertebrate classes (e.g., 4,10,11). NPY modulates the release of several pituitary hormones in mammals, including luteinizing hormone, growth hormone, adrenocorticotrophic hormone, and prolactin [21,28]. In the frog *X. laevis*, NPY inhibits the *in vitro* release of several proopiomelanocortin-derived peptides from the intermediate lobe of the pituitary [54]. NPY may function similarly in this caecilian. The caecilian diencephalon and pituitary have a structure similar to that of other amphibians and the pituitary produces immunologically similar peptide and protein hormones [22,36,57].

Location of NPY-ir cell bodies in this caecilian brain differed from locations in other amphibian brains in several additional regards. First, NPY-ir was noticeably absent from the septum and the main and accessory olfactory bulb of *T. natans*. NPY-ir cell bodies are found in these areas in two anuran amphibians [34]. These differences may be related to remodeling of the caecilian olfactory system to accommodate a unique chemosensory structure called the "tentacular organ," which is a distinctive feature of this amphibian order [47]. Second, the distribution of NPY-ir cell bodies in the hindbrain of this caecilian was much more restricted than described for other amphibians. The *T. natans* brain possessed a prominent population of cells in the trigeminal motor nucleus and NPY-ir cells are found in the same location in anuran and urodele species [7,34,42,50]. This caecilian did not have additional cells in the visceral nucleus, cerebellar nucleus, nucleus of the solitary tract, or spinal cord where such cells have been located in other amphibians [7,34,42,50].

It is unlikely that NPY in amphibians functions only as a modulator of pituitary intermediate lobe secretions. The broad distribution of NPY in this caecilian and in the brain of other amphibians suggests that this peptide has many neuromodulatory functions. Conserved patterns of NPY immunoreactivity support possible functions in control of reproduction (via POA and hypothalamus; 28,39), control of auditory processing (via the midbrain tegmentum cell population; 58) and control of the cardiovascular system (via hindbrain populations; 12).

Acknowledgments

We thank Drs. H. Vaudry and M. Tonon, University of Rouen, France for gifts of NPY antiserum (for immunocytochemistry) and synthetic frog NPY. Antiserum 8999 (for radioimmunoassay) was a generous gift from Dr. M. M. T. O'Hare, Queen's University of Belfast, N. Ireland. We thank P.F. Nielsen, Novo Nordisk, Bagrvaerd, Denmark for mass spectrometry measurements and Cold Spring Harbor Laboratory for materials used to make a caecilian brain cDNA library. This work was supported by the National Science Foundation (IBN#98-06997 and IBN#95-14305).

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