Identification and Localization of Neurohypophysial Peptides in the Brain of a Caecilian Amphibian, Typhlonectes natans (Amphibia: Gymnophiona)

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ABSTRACT

The amphibian order Gymnophiona contains more than 150 different species of caecilians. The characterization and distribution of neurohypophysial peptides, however, has not been described for any member of this order. By using high-performance liquid chromatography, radioimmunoassay, and mass spectrometry, we identified the peptide arginine vasotocin (AVT) in brain and pituitary extracts from the caecilian Typhlonectes natans. By using immunocytochemistry, we found five populations of AVT-immunoreactive (AVT-ir) cells in the brain of T. natans. AVT-ir cell bodies were located in the preoptic area, amygdala pars medialis, ventral thalamus, dorsal hypothalamic nucleus, and nucleus of the solitary tract. AVT-ir fibers and terminal fields were widespread. We also identified a mesotocin-like peptide. The distribution of this peptide in the brain of T. natans was more restricted than the distribution of AVT. Mesotocin-like-immunoreactive cell bodies were located almost exclusively in the preoptic area, with only a few other cells located in the amygdala pars medialis. This caecilian species, therefore, possesses neurohypophysial peptides that are similar in their structure and distribution to the peptides found in anuran and urodele amphibian orders. J. Comp. Neurol. 394:139–151, 1998.

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Indexing terms: vasotocin; mesotocin; vasopressin; high-performance liquid chromatography; immunocytochemistry

The class Amphibia includes three extant orders, two of which (Anura and Uroidea) have received much attention. The third amphibian order, Gymnophiona, is a little-recognized group that consists of five families and 154 known species of caecilians (Nussbaum and Wilkinson, 1989; Hedges et al., 1993). Caecilians are elongate, legless, fossorial or aquatic animals that are exclusively tropical in distribution. Their eyes serve only to detect light and are covered either by skin or by skin and bone (Fritzsch et al., 1985; Himstedt and Manteuffel, 1985; Wake, 1985; Himstedt, 1995). Although the three living orders of amphibians have similarities, their fossil histories are long and separate (Carroll and Currie, 1975). Based on morphology, caecilians are considered to be a monophyletic lineage that is separated evolutionarily from urodele and anuran amphibians by at least 200 million years (Duelman and Trueb, 1986). Caecilians differ significantly from anurans and urodeles in many regards, including body form (legless; Taylor, 1968), sensory systems (e.g., chemosensory tentacle; Wake, 1993), and reproductive physiology (e.g., viviparity; Wake, 1992). Thus, there is reason to expect that the neuroanatomy and neurochemistry of caecilians will differ from those of other amphibians, but information is scant. Because neurohypophysial peptide pathways are among the best described of neurochemical pathways in anuran and urodele brains (see, e.g., Follett and Heller, 1964; Vandesande and Dierickx, 1976; Conway and Gainer, 1987; Jokura and Urano, 1987; Nojiri et al., 1987; Gonzalez and Smeets, 1992a,b; Boyd, 1997), we investigated this system in the caecilian, Typhlonectes natans.

All vertebrates examined to date have at least two types of neurohypophysial peptides, except the primitive hagfish.
and the lamprey, which possess only arginine vasotocin (AVT; Urano et al., 1992; Acher, 1993). Due to its presence in all vertebrates, AVT is considered the ancestral precursor of peptides in this family (Acher, 1993). A duplication of the AVT gene likely gave rise to the two groups of neurohypophysial peptides (basic and neutral) found in extant vertebrates. The basic peptide lineage includes arginine vasopressin (AVP) in most mammals, lysine vasopressin in pigs and some marsupials, and AVT in all other vertebrates. The neutral lineage consists of oxytocin in mammals, mesotocin (MT) in nonmammalian tetrapods, isotocin in most teleosts, and a variety of other peptides in cartilaginous fishes (Urano et al., 1992; Acher, 1993, 1996; Hiraoa et al., 1993; Suzuki et al., 1995). A striking feature of these structural shifts is the first appearance of MT in lungfish and its persistence in anuran and urodele amphibians, reptiles, birds, and marsupials. Parts of this critical transition (between fish isotocin and tetrapod MT) are not completely documented, however. Although isolation and characterization of neurohypophysial peptides in lungfish (four species; Follett and Heller, 1964; Acher et al., 1970; Michel et al., 1993) and anuran amphibians (11 species; Sawyer et al., 1959; Nojiri et al., 1987; Rouille et al., 1989; Michel et al., 1990; Chauvet et al., 1991; Ouedraogo and Sawadogo, 1994) are broadly representative, the other two amphibian orders are either not represented at all (caecilians) or are poorly represented (urodeles; AVT found in two species and MT in only one species; Chauvet et al., 1991). One goal of this study, therefore, was to isolate and characterize the neurohypophysial peptides of a representative caecilian by using a combination of high-performance liquid chromatography (HPLC), radioimmunoassay, and mass spectrometry.

Immunocytochemistry has been used to locate cells and fibers containing neurohypophysial peptides in brains of a variety of amphibians. Distribution of immunoreactive AVT and MT has been well described for eight anurans (Rana temporaria, R. esculenta, R. ridibunda, R. catesbeiana, R. sylvatica, Bufo bufo, B. japonicus, and Xenopus laevis; Vandesande and Dierickx, 1976; J okura and Urano, 1985, 1987; Conway and Gainer, 1987; Boyd et al., 1992; Gonzalez and Smeets, 1992a; Boyd, 1994a; Mathieson, 1996) and for one urodele amphibian (Pleurodeles waltl; Gonzalez and Smeets, 1992a). The distribution of AVT and MT has never been described in any caecilian species. Therefore, anatomical locations of peptide cells and fibers in the brain of T. natans were also mapped by using immunocytochemistry.

**MATERIALS AND METHODS**

**Isolation of neurohypophysial peptides**

Peptides were isolated separately from caecilian pituitary and from brains. Pituitaries (whole glands; n = 22; weight = 39.2 mg) and brains (n = 24; weight = 1.147 g) were collected from anesthetized adults of both sexes, frozen on dry ice, and stored at -80°C. All methods were approved by the institutional animal care and use committee. Tissues were homogenized with a Polytron (Brinkmann Instruments, Westbury, NY) in 20 ml of 1 M HCl containing 15% (v/v) trifluoroacetic acid (TFA), 5% (v/v) formic acid, and 1% (w/v) NaCl. Homogenates were incubated for 1 hour at 0°C then centrifuged for 20 minutes (1,200g) at 4°C. Each supernatant was passed twice at a flow rate of 2 ml/minute through a Sep-Pak cartridge (Millipore Corp., Milford, MA) equilibrated with 0.1% TFA. For each extract, the column was washed with 4 ml 0.1% TFA and then with 4 ml acetonitrile/water/TFA (70/0.29/0.1, v/v/v) to elute peptides. Eluate volume was reduced in a refrigerated vacuum centrifuge (Savant SpeedVac, Holbrook, NY) to approximately 1 ml.

Peptides were purified by using reversed-phase HPLC. After partial purification on Sep-Pak cartridges, as described above, extracts were injected onto a Vydac 218TP510 C-18 (1/200g) at 4°C. Each supernatant was washed twice at a flow rate of 2 ml/minute through a Sep-Pak cartridge (Millipore Corp., Milford, MA) equilibrated with 0.1% TFA. For each extract, the column was washed with 4 ml 0.1% TFA and then with 4 ml acetonitrile/water/TFA (70/0.29/0.1, v/v/v) to elute peptides. Eluate volume was reduced in a refrigerated vacuum centrifuge (Savant SpeedVac, Holbrook, NY) to approximately 1 ml.

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**Neuroanatomical Abbreviations**

| APL | amygdala pars lateralis |
| APM | amygdala pars medialis |
| DH | dorsal hypothalamus |
| DP | dorsal pallium |
| H | habenula |
| LC | locus coeruleus |
| LP | lateral pallium |
| LS | lateral septum |
| M | mesencephalon |
| MO | medulla oblongata |
| MP | medial pallium |
| MS | medial septum |
| NA | nucleus accumbens |
| OB | olfactory bulb |
| OT | optic tectum |
| PAL | pallium |
| POA | preoptic area |
| S | spinal cord |
| SC | suprachiasmatic nucleus |
| SOL | solitary tract |
| ST | striatum |
| TE | thalamic eminence |
| TEG | tegmentum |
| ThD | dorsal thalamus |
| THV | ventral thalamus |
| VH | ventral hypothalamus |
| Vm | trigeminal motor nucleus |
vasopressin and was used at a final dilution of 1:75,000. Synthetic AVT (Bachem) was used as the standard in the range of 1–200 pg. Labeled AVP ([125I]-AVP; Amersham, Arlington Heights, IL; SA = 2,000 Ci/mmol) was used as the tracer. Minimum detectable concentration of AVT was 1 pg/tube. Cross-reactivity of related neuropeptidyl peptides under these conditions was 100%, 24%, and 5% for AVP, MT, and oxytocin, respectively. The antibody did not cross react with isotocin or hydrin 2, even at peptide concentrations as high as 200 pg/tube. Note that this antibody (used for the RIA only) did cross react with AVT and MT. Different antibodies that were specific for each peptide were used for immunocytochemistry.

Mass spectrometry of purified peptide was performed by Dr. Per F. Nielsen at the Novo/Nordisk Research Institute in Bagsvaerd, Denmark by using an API III LC/MS/MS system (Sciex; Thornhill, Ontario, Canada), as described previously (Conlon et al., 1992). Accuracy of mass measurement was ±0.02%.

**AVT and MT immunocytochemistry**

Sexually mature caecilians (13 of each sex; n = 26; length 25–42 cm) were obtained from Wilson Pet Supply (Woodale, IL). Caecilians were deeply anesthetized in benzocaine (0.02% solution) and perfused through the truncus arteriosus with about 100 ml 0.9% saline followed by about 150 ml 5% acrolein (Aldrich, Milwaukee, WI) in a 0.1 M phosphate buffer, pH 7.2. Brains were postfixed in situ in fresh acrolein solution for 2 hours, then removed from the skull, and placed in fresh fixative for an additional 2 hours. Brains were stored overnight in 30% sucrose in phosphate buffer at 4°C, embedded in butanol, and 75-µm Vibratome sections were cut. The Vibratome contained 0.9% NaCl in 0.05 M Tris-HCl, pH 7.6, at 0°C. Brains were sectioned in horizontal, sagittal, and frontal (transverse) planes.

Free-floating sections were processed for AVT or MT immunoreactivity with a procedure that was validated previously for amphibian brains (Boyd et al., 1992). Briefly, alternate sections from the same brain were treated with either rabbit antivasopressin serum (ICN, Lisle, IL; 1:2,000 dilution) or rabbit anti-MT serum (affinity-purified rabbit polyclonal VA-10, 1:200 dilution; donated by Harold Gainer; Conway and Gainer, 1987). The antivasopressin serum cross reacted with AVP and AVT but not with MT or isotocin (Boyd et al., 1992). After affinity purification, the VA-10 antibody was specific for MT and did not cross react with AVT (based on immunohistochemistry, differential absorption assays, and radioimmunoassays; Conway and Gainer, 1987; Altstein et al., 1988). Immunoreactivity was visualized with the peroxidase-anti-peroxidase technique and diaminobenzidine. Specificity of the antiserum and technique was also tested on alternate sections from some brains. Sections were processed with 2% normal goat serum replacing the primary antibody or with primary antiserum that had been preadsorbed with 50 µM AVT, MT, or isotocin.

**Neuroanatomy**

Cell size was determined with the NTS hardware and software system from Eutectics Electronics, Inc., Raleigh, NC. Immunoreactive cell bodies were drawn with a camera lucida, digitized by the system, and soma area was computed based on internal size calibration. All immunoreactive cell bodies in a single thick (75 µm) section were drawn for each animal in each brain area. This prevented drawing cells more than once, exclusion of large cells that might have been missed across multiple thin sections, and possible bias of the drawer toward larger cells. Determination of neuroanatomical areas was based primarily upon Northcutt and Kicliter (1980) for the telencephalon; on Kuhlenbeck (1922), Fritzsch et al. (1985), and Clairambault et al. (1980) for the diencephalon and mesencephalon; and on Gonzalez and Smeets (1994) and Wicht and Himstedt (1990) for the metencephalon and myelencephalon.

**RESULTS**

**Isolation of peptides**

The elution profile of caecilian pituitary extract from a semipreparative Vydac C-18 column contained only one immunoreactive peak (Fig. 1A). That peak coeluted with AVT. Further purification of this material on an analytical Vydac C-4 column resulted in clear separation of a single, immunoreactive peak (Fig. 1C). Mass spectrometry analysis showed a monoisotopic molecular weight of 1,049.4 atomic mass units (amu) for this extract. The theoretical value for AVT is 1,049.5 amu.

Brain extract elution profiles from a C-18 column contained two immunoreactive peaks. One peak coeluted with AVT, and the other coeluted with MT (Fig. 1B). Fractions coeluting with MT were further purified on analytical C-4 and analytical C-18 columns. On both column types, endogenous material coeluted with synthetic MT and was immunoreactive. However, we were not able to separate this peptide in a form that was sufficiently pure for edman degradation or mass spectrometry analysis.

**Immunocytochemical localization of peptides**

Distribution of AVT immunoreactivity. The most rostral AVT-immunoreactive ( -ir) cells were found in the anterior portion of the preoptic area (POA; Fig. 2, rostrocaudal orientation; immunoreactivity in Figs. 3D–F, 4A, 5A, 6A, C,E). Darkly stained cells first appeared coincident with the appearance of the third ventricle (in frontal section). This population rapidly expanded into dense, periventricular columns of cells at more caudal levels. At the same level where POA cells first appear, a lateral population of more lightly stained cells appeared in the amygdala pars medialis (Figs. 3D, 7A). The amygdala cell population extended caudally within the pars medialis and perhaps the rostral amygdala pars lateralis. AVT-ir cell bodies were not present in the caudal amygdala pars lateralis (see, e.g., Fig. 3E, F). A third population of AVT-ir cells was found in the ventral thalamus (Figs. 4B, 7B). This small population (about 10–20 cells per side) was displaced laterally from the ventricle and was separated from dorsal POA cells by a clear region that lacked immunoreactive cells. Most of these cells extended processes directly laterally. Slightly more caudally, a large compact population of darkly stained AVT-ir cells was observed in the dorsal hypothalamic nucleus (Figs. 4D, 7C). There were no AVT cells in ventral hypothalamic or suprachiasmatic nuclei. The most caudal population of AVT-ir cells was located in the nucleus of the solitary tract (Figs. 4G, 7D; location based on Gonzalez and Smeets, 1994). This small population (five to ten cells per side) consisted of large, lightly stained cells. This population was not observed in every brain.
The distribution of AVT-ir fibers in this caecilian brain was much more extensive than the distribution of MT-ir fibers. AVT-ir fibers were present at virtually every level, with the exception only of the most rostral areas. In the telencephalon, AVT-ir fibers were strikingly associated with subpallial structures and were missing almost entirely from the pallium. Rare fibers were observed in the accessory olfactory bulb (mitral layer), but significant staining first appeared in the nucleus accumbens and striatum (Fig. 3A,B). Immunoreactivity in these regions consisted of thin varicose fibers and terminal fields. As the amygdala pars medialis replaced the nucleus accumbens more caudally, the distribution and density of AVT-ir fibers continued with no obvious changes in pattern. In the rostral telencephalon of caecilians, the medial septum is the most prominent feature of the medial wall (Northcutt and Kicliter, 1980). The medial pallium is small and is not well developed dorsally, and the lateral septum has not yet appeared. Significantly, we found no AVT immunoreactivity in the well-developed medial septum of T. natans. On the other hand, dense AVT-ir fibers were found more caudally in the lateral septum (Fig. 3B). AVT-ir cells were not found in either septal area. Finally, in the most caudal telencephalon, AVT immunoreactivity was located in pallial fields for the first time. Dense terminal fields were present in the amygdala pars lateralis (Fig. 3E,F) and the lateral pallium (Figs. 3E,F, 4A,B).

The densest area of AVT-ir fibers was located in association with the POA (Figs. 3E, 4A, 6A,C,E). Most fibers were thick, were without varicosities, and coursed laterally or ventrolaterally. A few, similar thick fibers also extended dorsally into the thalamic eminence (Fig. 3D–F). The entire thalamus, in fact, contained dense AVT-ir fiber staining (Fig. 4A–D). Most fibers in the ventral thalamus were oriented in a distinct, vertical (dorsoventral) position within the neuropil (see, e.g., Fig. 4C). In the dorsal thalamus, on the other hand, thin varicose fibers and terminal fields were found. Staining in the habenula was absent in rostral regions (Fig. 3E,F) and was moderate in more caudal regions (Fig. 4A,B). Dense fiber staining was found in both dorsal and ventral hypothalamic nuclei (Fig. 4C,D).

The size of AVT-ir cell bodies. Area of AVT-ir cell somata varied significantly across different populations, with the smallest cells in the dorsal hypothalamus and the largest cells in the solitary tract of the brainstem (Table 1). Within the POA, there was no obvious bimodal distribution in cell somal area (Figs. 6A,C,E, B). Instead, cell size showed a unimodal, asymmetric distribution, and log transformation of the data resulted in a normal distribution (Kolmogorov-Smirnov Z = 1.087; P = 0.188). Although the very large POA AVT-ir cells were located in the caudal and dorsal portions of the nucleus, no nucleus directly comparable to the magnocellular POA of anurans (e.g., bullfrogs; Boyd et al., 1992) was found in T. natans. Posterior POA cells were not displaced laterally from the ventricle and were not consistently larger.

Distribution of MT-like immunoreactivity. Distribution of MT-ir cells and fibers was much more restricted than that of AVT. MT-ir cell bodies were most abundant in the anterior POA (Figs. 3D,F, 5, 6B,D,F), where they exhibited a somewhat laminar arrangement along the third ventricle. This group of cells extended laterally and graded into the amygdala (primarily the pars medialis). Thick and thin fibers were present in the POA oriented in a ventrolateral position, but they were much less dense than AVT-ir fiber projections. Scattered fibers and terminal fields...
fields were also found in the ventral thalamus, lateral suprachiasmatic nucleus (SCN), ventral hypothalamus, optic tectum, tegmentum, and dorsolateral hindbrain (Fig. 4A–G).

Control staining. Staining in tissue was abolished when each primary antibody was preadsorbed with its respective antigen (AVT antiserum + 50 µM AVT; MT antiserum + 50 µM MT). When the antiserum was adsorbed with a neurohypophysial peptide for which it was not specific (AVT antiserum + 50 µM MT; MT antiserum + 50 µM AVT), normal staining of cells occurred (Fig. 6C,D). Regional differences in staining were further evidence of antibody specificity (e.g., MT antibody stained no cells in the ventral thalamus or hindbrain). Isotocin preadsorption had no effect on staining by either antibody (Fig. 6E,F); thus, neither antibody used for immunocytochemistry would have recognized isotocin cells. Replacement of primary antibody with normal serum resulted in no specific staining.

DISCUSSION

The structure and distribution of neurohypophysial peptides in the brain of this caecilian, T. natans, support the hypothesis of a monophyletic origin for all three orders of extant amphibians. This species possessed AVT and an MT-like peptide (see below), similar to the peptides found in all anuran and urodele amphibians studied to date. In addition, AVT-ir cells in this caecilian brain are located in substantially similar neuroanatomical areas compared to other amphibians. MT-like immunoreactivity was also distributed in densities and locations similar to other amphibians. Thus, it appears that the neurohypophysial peptide systems of the order Gymnophiona, as described here for the first time, are more similar to those of anuran and urodele orders of amphibians than to those of teleost fish.

The presence of AVT in caecilians was determined unambiguously by mass spectrometry analysis. Both brain and pituitary extracts contained material that coeluted with AVT standards and was immunoreactive toward an antiserum recognizing AVT. This peptide has been found previously in 11 anuran species (including Ranids, Bufonids, and Pipids; Sawyer et al., 1959; Nojiri et al., 1987; Rouille et al., 1989; Michel et al., 1990; Chauvet et al., 1991; Ouedraogo and Sawadogo, 1994) and in two urodele species (P. waltl; and Ambystoma mexicanum; Chauvet et al., 1991). AVT has also been isolated from fish and from representatives of virtually every other vertebrate class, although the presence of AVT in mammals has been disputed (Acher and Chauvet, 1995; Acher, 1996).

Caecilian brain extracts also contained material that coeluted with synthetic MT and was immunoreactive in our RIA. MT has been identified previously in four anurans (R. esculenta, B. marinus, B. japonicus, and X. laevis; Nojiri et al., 1987; Rouille et al., 1989; Michel et al., 1990) and in one urodele species (P. waltl; Chauvet et al., 1991). It is therefore most likely that the second neurohypophysial peptide present in this caecilian brain is MT. Certain, due to differences in retention time and/or lack of immunoreactivity, this second peak is unlikely to be hydriod 2, oxytocin, or vasopressin (Fig. 1). It is also unlikely that this peak is entirely isotocin, because our RIA antibody did not recognize isotocin. It is possible, however, that this caecilian brain contains isotocin in addition to an immunoreactive peptide. Because MT and isotocin elute near each other, the peak we identified could have contained both peptides. This peak may also represent a novel peptide with a retention time similar to that of MT and cross reactivity with our RIA antibody. One candidate is a variant of MT found in the African toad B. regularis (Chauvet et al., 1995). This peptide is identical in structure to MT, but with a substitution of serine at position 5. This peptide was not available, so we could not determine its possible cross reactivity with our RIA antibody.

The brain of this caecilian contained a prominent population of intermingled AVT-ir and MT-like-ir cells in the POA. A similar population is present in all vertebrates thus examined, including anuran and urodele amphibians (e.g., R. catesbiana: Boyd et al., 1992; P. waltl: Gonzalez and Smeets, 1992a). Dense fibers from these cells likely project to the posterior pituitary and correspond to neurosecretory cells in the paraventricular and supraoptic nuclei of reptiles, birds, and mammals (see, e.g., Swaab and Pool, 1975; Stoll and Voorn, 1985; Kiss et al., 1987; Smeets et al., 1990; Proper et al., 1992). The histological structure of caecilian pituitary is similar to that of other amphibians (Schubert et al., 1977; Doer-Schott and Zuber-Vogeli, 1984; Masood-Parveez et al., 1994), and we isolated very large concentrations of AVT from the pituitary of...
T. natans. In contrast to other vertebrates, however, there was no bimodal distribution of AVT-ir cell sizes in the caecilian POA and no indication of a separate "magnocellular" nucleus. In rats (Swanson and Sawchenko, 1983) and fish (Holmqvist and Ekstrom, 1995), for example, magnocellular neurons are the specific cells that project primarily...
Fig. 4. A–G: Continuation of Figure 3 at more caudal levels. Right telencephalic hemisphere drawings are not displayed (A–C), because mesotocin (MT) immunoreactivity was never observed in these regions. For abbreviations, see list.
Fig. 5. Camera lucida drawings of the distribution of arginine vasotocin immunoreactive (AVT-ir; left) and mesotocin immunoreactive (MT-ir; right) cells and fibers in horizontal sections through the caecilian brain. Anterior is at top. The top section (A) is at a level ventral to the bottom section (B). POA, preoptic area.
Fig. 6. Distribution of arginine vasotocin (AVT) and mesotocin (MT) immunoreactivity in the preoptic area (third ventricle is to the right; horizontal sections). A: Staining with AVT antibody alone. B: Staining with MT antibody alone. C: Staining when AVT antiserum is preadsorbed with MT. D: Staining when MT antiserum is preadsorbed with AVT. E: Staining when AVT antiserum is preadsorbed with isotocin. F: Staining when MT antiserum is preadsorbed with isotocin. Scale bar = 50 µm.
to the posterior pituitary. Differences in neurosecretory cell size are also likely associated with cell activity and not solely with efferent projections, however (e.g., rats: Kallimo, 1975; chickens: Chaturvedi et al., 1994; frogs: Subhedar and Krishna, 1990). Thus, the projection from the POA to the posterior pituitary likely exists in this caecilian despite the absence of a distinct magnocellular nucleus, and smaller cell sizes may reflect less active neurosecretory cells in this aquatic speciﬁc.

Based on their location close to the median eminence and pituitary, AVT-ir cells in the dorsal hypothalamus of caecilians may also be neurosecretory. Cells in the dorsal hypothalamus did not differ signiﬁcantly in size from presumptive neurosecretory cells in the POA. Furthermore, neurosecretory cells have been identiﬁed in this region in the brains of two other caecilians (Chthonerpeton indistinctum, Ichthyophis paucisulcus) by using classic histological and enzyme histochemical techniques (Welsch et al., 1976). Anuran and urodele amphibians also possess hypothalamic AVT cells (e.g., R. catesbeiana, R. ridibunda, R. sylvatica, X. laevis, and P. waltlii; Boyd et al., 1992; Gonzalez and Smeets, 1992a,b; Mathieson, 1996). This population is more variable in other vertebrates, however. It was not found in rainbow trout, but a possible homologous population is present in the nucleus periventricularis hypothalami of the cartilaginous ﬁsh Scyliorhinus canicula (Van Den Dungen et al., 1982; Vallerino et al., 1990). In reptiles and birds, AVT-ir cell populations are present in the infundibulum or hypothalamus of several species (Viglietti-Panzica and Panzica, 1991; Propper et al., 1992; Sugita, 1994; Aste et al., 1996; and references therein). An
AVP-ir population in the dorsomedial hypothalamic nucleus of rats may also be homologous (Caffee and Van Leeuwen, 1983). AVT-ir cells were not observed in two brain regions that contained such cells in some other amphibians. First, no AVT-ir cells were found in the septal nucleus of T. natans. Septal AVT-ir cells have been found in the bullfrog (R. catesbeiana) brain (Boyd et al., 1992; Mathieson, 1996) but not in any other amphibian described so far. Second, suprachiasmatic AVT-ir cells also were not found in this caecilian. Although the eyes of caecilians are much reduced, they are functional in at least several species, including Typhlonectes (Wake, 1980, 1985; Himstedt and Manteuffel, 1985). The precise location of the caecilian SCN is difficult to discern, however, due to reduced size of the optic nerves and absence of an external optic chiasm. The location where optic nerves enter the brain of the caecilian, but AVT-ir cells and fibers were present in the presumed solitary tract of the myelencephalon. In the rhabdencephalon of R. ridibunda and P. waltlii, AVT-ir cells have been observed in the nucleus of the solitary tract and near the locus coeruleus, respectively (Gonzalez and Smeets, 1992a). AVT-ir cells are also located in the preturbineminal nucleus of the bullfrog and in a similar area in the lizard Geckcko geckco (Stoll and Voorn, 1985; Boyd et al., 1992). Determining anatomical overlap is especially difficult for caecilians, however, due to the pronounced pontine flexure in caecilian brain. An AVP-ir population in the rat locus coeruleus (Caffee and Van Leeuwen, 1983) may be homologous to the caecilian AVT-ir Hindbrain cell group. Specifically, the locus coeruleus AVP-ir cells in rats also contain noradrenaline (Caffee et al., 1985). Although coexistence of catecholamines in AVT-ir cells has not been shown in caecilians, tyrosine hydroxylase-ir cell bodies are found in the locus coeruleus and solitary tract of the caecilian T. compressicauda (Gonzalez and Smeets, 1994). These cells may be involved in the control of heart rate or blood pressure (Matsuguchi et al., 1982; Berecek et al., 1984).

These results support the hypothesis that the tetrapod pattern of neurohypophysial peptide neurochemistry and neuroanatomy first arose before divergence of orders within the Lissamphibia. Specifically, the general teleost pattern consists of cells that synthesize the peptide pair of AVT and isotocin. There are no extrahypothalamic cell populations, and extrahypothalamic fiber projections are rare. In contrast, this caecilian species possesses the general tetrapod pattern of neurohypophysial peptide production (the vasotocin and MT pair), and extrahypothalamic cells and fibers are common.

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