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Immunohistochemical Distribution of Urotensins I and II in the Central Nervous System of the Senegal Bichir, *Polypterus senegalus*

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ABSTRACT—The distribution of the urotensin I (UI)- and II (UII)-like immunoreactants in the central nervous system of the primitive bony fish *Polypterus senegalus* was studied by means of immunohistochemistry. Noticeably, UI-positive perikarya were present chiefly in the brain stem and the entire spinal cord with the exception of the caudalmost spinal cord segments, and their axons terminated on the wall of the adjacent blood vessels. In the cranial portion, the positive cells were found in the nucleus preopticus, the caudal diencephalon and the medulla oblongata. In contrast, the UII-immunoreactive fibers were distributed in the terminal part of the spinal cord, anterior to the 15th-to-the last vertebra; and a few UII-immunopositive perikarya occurred only in the caudalmost 5 to 6 segments. The most caudal region of the spinal cord showed a simplified or reduced neurohemal structure, where only UII-immunoreactive fibers were found. Accordingly, this area may represent the caudal neurosecretory system of the bichir, irrespective of a lack of UI-immunoreactivity. In the midregion of the spinal cord, UII-immunoreactive cerebrospinal fluid (CSF)-contacting neurons were sporadically encountered.

INTRODUCTION

In many groups of fishes, the caudal spinal cord contains neurosecretory cells with processes projecting to an extensive capillary network by which their secretory products may be released into the circulatory system [7]. Teleosts possess a distinct neurohemal organ, the urophysis, which is an enlargement of the spinal cord in the last vertebral segment. No such structure is developed in elasmobranch fishes (sharks, rays, and skates), though they have caudal neurosecretory cells (Dahlgren cells) [13]. For the holocephalan *Hydrolagus colliei*, the caudal neurosecretory elements might not be present [2]. In cyclostomes, no elements of the caudal neurosecretory system were identified [25]. Lower actinopterygians, *Acipenser medirostris* [8, 25] and *A. guldenstadti* [24], possess a caudal neurosecretory system quite similar to that of the elasmobranchs. On the other hand, in other members of primitive bony fishes, such as *Polypterus* sp. and the garpike, *Lepidosteus osteus*, it is not certain whether these forms possess a urophysis [5]. The caudal neurosecretory neurons of fishes secrete at least two major peptide hormones, urotensins I (UI) and II (UII): UI has homologous amino acid sequence with corticotropin-releasing factor (CRF) and sauvagine [9, 16], whereas UII is partially homologous with somatostatin [10, 23]. Immunohistochemical studies showed colocalization of UI and UII immunoreactants in the same cells in several fish species [13]. Moreover, intragranular colocalization of UI and UII immunoreactants has successfully been demonstrated [31]. Immunoreactivities of UI [26] and UII [14] have been detected also in other regions such as brain, hypophysis, and plasma of the sucker, *Catostomus commersoni*. Furthermore, Yulis and Lederis [32, 33] reported the presence of UII-immunoreactivity in cerebrospinal fluid (CSF)-contacting neurons in the extraurophyseal region of the spinal cord.

In polypterids, belonging to Polypteriformes or Brachiopterygii, the Dahlgren cells were demonstrated in *Polypterus* sp. [1, 6, 7], but it is not clear whether or not a real urophysis exists in these fish. In *Calamoichthys* (=*Erpetoichthys*), UII-immunoreactivity was demonstrated in the caudal spinal cord, whereas no UI-immunoreactivity was seen in this area [21].

In the light of scanty information on the polypterid caudal neurosecretory system, the present study was undertaken to elucidate the details of UI- and UII-immunoreactive structures in the central nervous system of the bichir, *Polypterus senegalus*.

MATERIALS AND METHODS

Five individuals of both sexes of the Senegal bichir, *Polypterus senegalus*, (170–245 mm in total length and 15–70 g in body weight) purchased from a local dealer were used in this study. After anesthesia with *m*-aminobenzoate-methanesulfonate (MS-222), the fish were perfused through the heart initially with heparinized physiological saline and subsequently with acid-free Bouin's fluid. The dissected spinal cords and brains were immersed in the same fixative overnight. Following routine paraffin embedding procedures, serial cross and sagittal sections were cut at an 8–10 μm thickness. Correlative sections were put on a glass slide coated with

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diluted gelatin. Immunohistochemical staining was carried out with peroxidase-antiperoxidase (PAP Dakopatts, Denmark) and streptavidin biotin (SAB-PO Nichirei, Japan) staining kits. Primary rabbit antisera raised against synthetic *Catostomus* UI [26, 27] and synthetic *Gillichthys* UII [4] were used. Controls were prepared by replacement of the specific antiserum with (1) normal rabbit serum or (2) antiserum preabsorbed with homologous antigens, synthetic UI and UII (5–18 μmol of each peptide, Sigma Chemical Company, USA) or (3) antiserum preincubated with the same amount of partly homologous antigens, human CRF (Peptide Institute Inc, Japan) and somatostatin-14 (Funakoshi, Japan). Immunostaining was absent in all sections following the first two control procedures, but was not abolished by the last one.

RESULTS

Overall distribution of UI- and UII-like immunoreactivities in the brain and spinal cord of the bichir is diagrammatically illustrated in Figure 1.

UI-immunoreactivity

Unexpectedly, UI-immunoreactivity was not detected in the caudalmost 15 spinal cord segments. Instead the distribution of UI-immunoreactivity extended more cranially from the terminal region (caudalmost 15 spinal cord segments); i.e., the immunoreactivity was found in the greater part of the spinal cord and even in the brain stem (Fig. 1). In cross sections of the spinal cord, UI-positive neurons were distributed in a scattered manner (Fig. 2). Some of them were seen adjacent to the meninges of the spinal cord (Fig. 3), whereas others were found in the subependymal layer of the central canal (Fig. 4). In the medulla oblongata, the labeled cells were found mainly in the most dorsal area (Fig. 1). These cells located in the spinal cord and the medulla oblongata were uni- or bipolar, having a length of 15–25 μm in the longer axis, and often contained a crescent-shaped or round nucleus (Figs. 3–5). Here, they often adjoined each other or were in contact with adjacent axons (Fig. 5). It was not always easy to trace the course of the axons sent from the labeled neurons, but some of them appeared to terminate around the neighboring blood vessels (Fig. 3). Accordingly, there was no concentration of the UI-immunoreactive axons in a specific portion of the spinal cord. The UI-like immunoreactive fibers and cells were also detected in the diencephalon, where the fibers showed a more extensive distribution, extending to the telencephalon and optic tectum. The labeled cells in the diencephalon were located in the nucleus preopticus (Fig. 6) and the dorsocaudal part of the diencephalon (Fig. 1).

UII-immunoreactivity

UII-immunoreactive cells were observed sporadically in the caudalmost 5 to 6 segments, ventrolateral and lateral to the central canal, however the UII-immunoreactive fibers tended to decrease anteriorly and visible even in the 15th-to-the last vertebra (Fig. 1). The cells were relatively small in size (15–25 μm in longer axis), and round to ovoid in shape, and contained a round nucleus, about 10 μm in diameter (Fig. 7). Most of the cells were uni- or bipolar, and their axonal fibers formed loose tracts that projected caudally to the filum terminale. Herring body-like droplets were often seen in the tracts. In the most caudal region of the cord, varicose fibers reacting to UII antiserum formed a neurohemal structure somewhat similar to the urophysis of the primitive teleosts (Fig. 8). Here, at least two subdivisions of the neurohemal structure were discerned (Fig. 1). The labeled fibers tended to gather in the ventral part and to merge into these subdivisions, but the large or compact swelling as seen in most teleosts could not be seen.

In the midregion of the spinal cord, a few cerebrospinal fluid (CSF)-contacting neurons immunoreactive to UII-antiserum were demonstrated. The cell bodies, 10 μm in their longer axis, were situated in the ependymal and subependymal layers ventral to the central canal (Figs. 9A, B). However, as the number of UII-immunoreactive CSF-

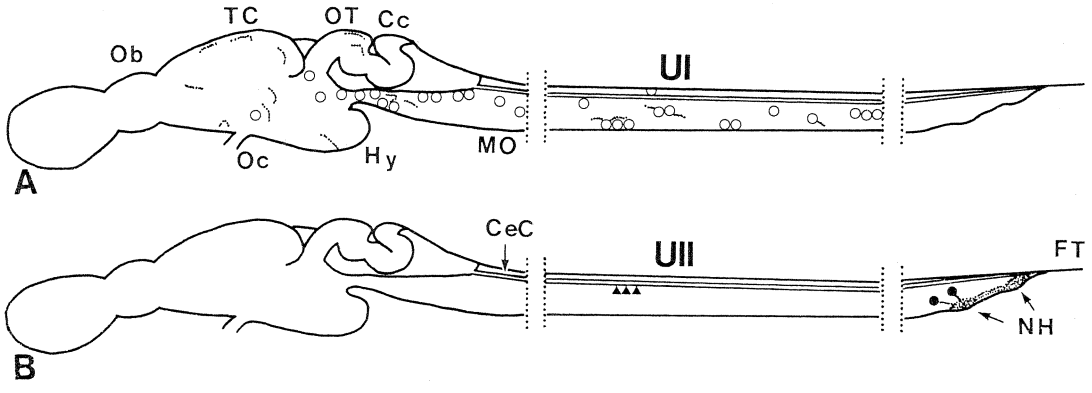

FIG. 1. Diagrammatic illustration of immunoreactivities in the central nervous system of the bichir, *Polypterus senegalus*, showing the apparently separate distribution of UI- and UII-like immunoreactivities. (A) Diagram showing UI-immunoreactive cells (○) and fibers (stippling). (B) Diagram showing UII-immunoreactivities. UII-immunoreactive cells (●) were observed only in the caudal most 5 to 6 segments, and two subdivisions of the neurohemal organ (NH) were discerned. UII-immunoreactive cerebrospinal fluid-contacting neurons (▲) were seen in the midregion of the spinal cord. Cc, corpus cerebelli; CeC, central canal; Hy, hypophysis; MO, medulla oblongata; Ob, olfactory bulb; Oc, optic chiasma; OT, optic tectum; TC, telencephalon; FT, filum terminale.

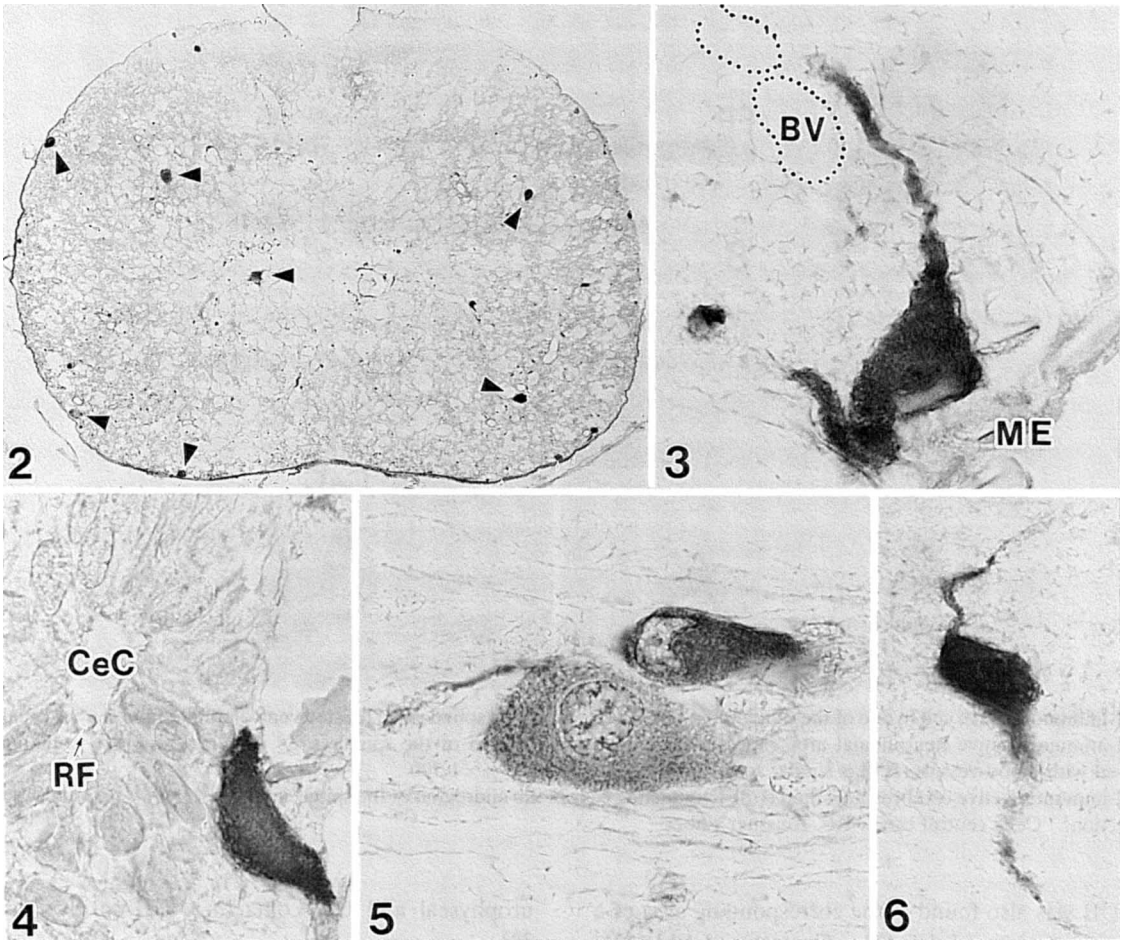

FIG. 2. Cross section of the spinal cord showing UI-immunoreactivity. Arrowheads indicate labeled cells scattered over the section. ×220
FIG. 3. Cross section of the spinal cord showing immunoreactive UI cells located in the ventrolateral portion adjacent to the meningeal tissue (ME). Note the axon sent to the adjacent blood vessel (BV). ×1,100
FIG. 4. UI-immunoreactive cell adjacent to the central canal (CeC). RF, Reissner's fiber. ×1,100
FIG. 5. Sagittal section of the midregion of the spinal cord showing two UI-immunoreactive cells adjoining each other. ×1,100
FIG. 6. UI-immunoreactive bipolar cell in the nucleus preopticus. ×1,100

contacting neurons was very small, determination of the exact location of them was difficult. No UII-immunoreactivity was detected in the brain.

DISCUSSION

The immunostained structures in the central nervous system of the bichir, *Polypterus senegalus*, showed somewhat strange features among the fish species so far examined: the UI-immunoreactivity was distributed in the greater part of the spinal cord except for the terminal region, and UI- and UII-immunoreactivities occurred separately in different areas of the spinal cord. Previous immunohistochemical findings on the caudal neurosecretory system of many species of fishes showed that UI and UII are distributed in the same area of the spinal cord and coexist in the same neurosecretory neurons [15, 19–22, 30]. *In situ* hybridization experiments also confirmed the coexistence of UI and UII [11]. Therefore, the bichir seems to be an interesting example for the distribution of UI and UII in the central nervous system. It has been pointed out that UI and UII are derived from separate precursors as a result of expression of two different genetic determinants and that they often have opposing effects with each other [12, 17]. Therefore, the physiological significance for colocalization of these two peptides in the same cells is perplexing. Apparently, separation of the two components of the UI/UII system in the *Polypterus* suggests that colocalization of both peptides may not be essential for the caudal neurosecretory system to act properly, at least in the present species. In the *Polypterus*, UI-like substance occurred extensively in the brain. However, information is still scanty to compare the localization of the UI-like molecules in piscine brains [13, 29]. Therefore, further study is needed to elucidate the chemical and/or immunohistochemical properties of the UI-like antigens in the brain, particularly in relation to CRF as a partial homolog of UI.

The presence of neurosecretory cells in the *Polypterus* caudal spinal cord was reported previously [6], and biological

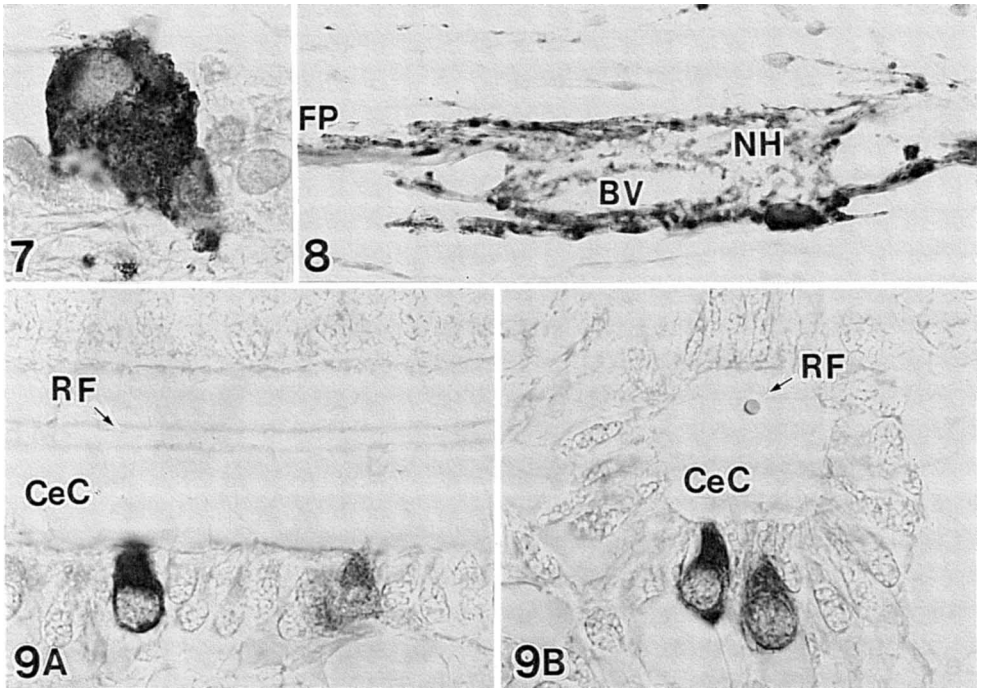

Fig. 7. UII-immunoreactive cell in one of the caudalmost 5 to 6 segments of the spinal cord, located ventrolateral to the central canal. ×1,100
Fig. 8. UII-immunoreactive neurohemal area (NH) in the most caudal region of the spinal cord. Fiber plexus (FP) containing granules associated with blood vessels (BV) is similar to the teleostean urophysis. ×1,100
Fig. 9. UII-immunoreactive cerebrospinal fluid (CSF)-contacting cells in the midregion of the spinal cord. ×1,100 (A) sagittal section. (B) cross section. CeC, central canal; RF, Reissner's fiber

activity of UII was also found in the corresponding area of a related species, *Calamoichthys* [3]. Onstott and Elde [21] reported that UII-immunoreactive structures were present in the caudal spinal cord of *Calamoichthys calabaricus*, and stated that the expanded area of UII-immunoreactivity bears a striking resemblance to that found in the teleost urophysis. The present results in *Polypterus* are generally in accord with the findings reported by them [21]. In the terminal region of the *Polypterus* spinal cord, UII-immunoreactive cells and two subdivisions of the neurohemal organs were noticed. Based on these findings, we consider that the UII-immunoreactive neurohemal area in the *Polypterus* spinal cord represents a simplified or reduced urophysis. Our previous data on the ontogenetic and phylogenetic aspects of the caudal neurosecretory system may support this view [18, 19]. However, further studies including ultrastructural analysis are necessary to determine whether this area is functionally equivalent to the urophysis.

Although small numbers of UII-immunoreactive cerebrospinal fluid (CSF)-contacting neurons were demonstrated in the spinal cord of the present *Polypterus senegalus*, extraurophyseal UII-immunoreactive neurons have already been reported in several teleosts [18, 33], ratfish [33], and white sturgeon [19]. On the other hand, such neurons have not been identified in the carp (Ichikawa, personal communication). The functional significance of these CSF-contacting neurons in the fish central nervous system is not yet fully clear, but separate functions were proposed for these urophyseal and CSF-contacting, UII-positive neurons [32, 33].

In consideration of the phylogenetic aspects of the UI/UII system, discussion should be extended to the data on the jawless vertebrates and certain protochordates. In adult lampreys and hagfish, no immunoreactive structures for UI and/or UII have been reported [21, 22]. However, in the caudal spinal cord of the *Petromyzon marinus* ammocoetes, only UI/CRF-immunoreactive fibers were observed [21]. On the other hand, in the amphioxus, *Branchiostoma belcheli*, UI-immnoreactive cells were recognized in the brain and the posterior part of the spinal cord, but there was no UII-immunoreactivity [28]. Currently, it is still difficult to deduce an evolutional trend on the UI-immunoreactive structures in the central nervous system among craniates and chordates.

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