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# Localization of Neuropeptide Y-like Immunoreactivity in the Saccus Vasculosus of Cartilaginous and Bony Fishes

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**ABSTRACT**—The distribution of perikarya and fibers containing neuropeptide Y (NPY)-like immunoreactant was studied in the saccus vasculosus of cartilaginous and bony fishes by immunohistochemistry using streptavidin-biotin technique. NPY-positive fibers were demonstrated in most of the 20 species examined, although the density of the fibers varied among the species. In the white sturgeon, *Acipenser transmontanus*, NPY-positive varicose fibers formed a supraependymal plexus. On the other hand, NPY-positive perikarya could be demonstrated in the ayu, *Plecoglossus altivelis*, in which an NPY-positive, presumed afferent fiber fascicle was encountered. These results suggest an involvement of NPY or related substance in the saccus vasculosus function and its regulation.

## INTRODUCTION

The saccus vasculosus (SV), a highly vascularized choroidal organ peculiar to cartilaginous and bony fishes, contains three types of cells: coronet cells, cerebrospinal fluid (CSF)-contacting neurons, and supporting cells. Its precise function is still unsettled, although sensory (receptor), secretory or cation-transporting role has been proposed or hypothesized (Altner and Zimmermann, 1972). In the teleostean SV, the CSF-contacting neurons and their fibers are cholinergic (Zimmermann and Altner, 1970; Vigh *et al.*, 1972). These fibers comprise the afferent pathway known as the tractus sacci vasculosi, which joins the nervus sacci vasculosi to enter the paired nucleus, i.e., the nucleus (ganglion) sacci vasculosi, in the hypothalamus (Vigh *et al.*, 1972). No evidence for the presence of aminergic innervation of the SV has been obtained by the fluorescent histochemical (Fremberg *et al.*, 1977; Terlou *et al.*, 1978; Watson, 1980) and immunohistochemical (Ritchie *et al.*, 1983; Ekström and van Veen, 1984; Meredith and Smeets, 1989; Roberts *et al.*, 1989; Yamanaka *et al.*, 1990) studies. So far, almost nothing is known on the peptidergic nerve elements in the SV (Chiba and Honma, 1994). In the present study, we examined immunohistochemically whether or not NPY-containing neurons might innervate the SV of cartilaginous and bony fishes.

## MATERIALS AND METHODS

Twenty species of cartilaginous and bony fishes were used in this study (Table 1). Except for the white sturgeon, *Acipenser*

*transmontanus*, and the Senegal bichir, *Polypterus senegalus*, obtained through a local dealer, all the animals were collected from different localities in Japan, the waters of Sado Island on the Sea of Japan, and Sagami Bay in the Pacific. Under anesthesia with *m*-aminobenzoate-methanesulfonate (MS-222), their brains were quickly dissected out, immersed in Bouin's solution without acetic acid, dehydrated with graded ethanols, and embedded in paraffin. The brains were cut at 8–10  $\mu$ m into sagittal or transverse sections. NPY-expressing perikarya and fibers were immunohistochemically stained by the streptavidin-biotin method with a commercial kit (Nichirei, Tokyo). Antiserum against synthetic porcine NPY (UCB Bioproducts, Belgium) was used as the primary antibody at a dilution of 1:3000. The immunoreaction was visualized by the diaminobenzidine method. The sections were washed, dehydrated, and mounted with Canada balsam. Specificity of the immunoreactions was determined by replacement of the primary antibody with (1) normal rabbit serum, (2) antiserum preabsorbed with the homologous antigen, i.e., synthetic porcine NPY (1  $\mu$ M, Peninsula Laboratories, USA), or (3) antiserum pretreated with related peptides, i.e., synthetic avian pancreatic polypeptide (APP, 10  $\mu$ M) and peptide YY (PYY; 10  $\mu$ M). The immunoreactivity was absent in all sections treated with the control procedures (1) and (2), but was not abolished in those treated with the procedure (3).

## RESULTS

NPY-like-immunoreactive (NPY-IR) fibers were demonstrated in the SV of most of the species examined, but their frequency or density showed considerable variation (Table 1). In the SV of two species of squaloid sharks, *Etmopterus brachyurus* and *Centrophorus squamosus*, no NPY-positive structures were detected, although the NPY-IR fibers and perikarya occurred in other brain areas. On the other

Table 1. Occurrence of NPY-like-immunoreactive perikarya and fibers in the saccus vasculosus of cartilaginous and bony fishes

Name of used animals	Number of specimens	Age	NPY-like immunoreactivity	
			Fibers	Perikarya
Chondrichthyes				
<i>Chimaera phantasma</i>	1	Ad	+	–
<i>Scyliorhinus torazame</i>	15	Ad	+	–
<i>Mustelus manazo</i>	4	Ad,Juv	++	–
<i>Etmopterus brachyurus</i>	2	Ad	–	–
<i>Centroscymus coelolepis</i>	2	Ad	+	–
<i>Centroscymus owstonii</i>	2	Ad	+	–
<i>Deania histricosa</i>	1	Ad	+	–
<i>Centrophorus squamosus</i>	1	Ad	–	–
<i>Raja kenoei</i>	6	Ad	+	–
<i>Dasyatis akajei</i>	5	Ad,Juv	+	–
Osteichthyes				
<i>Acipenser transmontanus</i>	3	Juv	+++	–
<i>Polypterus senegalus</i>	4	Ad	+	–
<i>Plecoglossus altivelis</i>	50	Ad	+++	++
<i>Salvelinus leucomaenis</i>	5	Ad	+	–
<i>Oncorhynchus keta</i>	23	Juv	+	–
<i>Oncorhynchus masou</i>	4	Juv	+	–
<i>Gasterosteus aculeatus</i>	7	Ad	++	–
<i>Sebastes oblongus</i>	2	Ad	+	–
<i>Cottus pollux</i>	3	Ad	+	–
<i>Aluterus scriptus</i>	1	Juv	+	–

Ad, adult; Juv, Juvenile

score: +++, frequent; ++, less frequent; +, infrequent; –, not detected

hand, the labeled fibers were common or frequent in the white sturgeon, *Acipenser transmontanus*, and the ayu, *Plecoglossus altivelis* (Table 1, Fig. 1A,B). In the *Acipenser* SV, NPY-IR varicose fibers were widely distributed as single fibers or thin strands within or beneath the epithelial (ependymal) layer, and occasionally found apposed to the vascular wall. Noticeably, the fibers running on or over the epithelial layer formed a plexus structure by joining each other or penetrated the epithelial layer (Fig. 1A). Some of the fibers in the *Acipenser* SV could be traced anteriorly to the hypophysis or dorsally to the posterior recess organ. In the *Plecoglossus* SV, NPY-IR fibers were frequently demonstrated as thin varicose fibers or branching strands of varying caliber (Fig. 1B). In some sections, these fibers and strands were grouped into a thick bundle and could be traced into the ventrocaudal portion of the posterior tubercle (Fig. 1B) including the nucleus sacci vasculosi. This definite pathway was identifiable as the tractus sacci vasculosi. On the other hand, in the SV of the gummy shark, *Mustelus manazo*, NPY-IR varicose fibers were detected in the thick nerve strand chiefly composed of NPY-negative fibers (Fig. 1C). Some labeled fibers ran into the layer of the coronet cells. In other species, NPY-IR fibers were infrequent and dispersed.

NPY-IR perikarya could be demonstrated only in the *Plecoglossus* SV. They were bottle-shaped and occurred solitarily or in loose groups within the epithelial layer (Fig. 1D). Occasionally, their apical swelling projected into the ventricular cavity, displaying a characteristic of CSF-contacting neurons

(Fig. 1E). The NPY-IR perikarya were seen more often in the animals caught in October, so far studied in 50 specimens caught during months from May to November.

## DISCUSSION

The present study demonstrated that the SV of cartilaginous and bony fishes contain NPY-IR fibers, although the density of the fibers shows a considerable variation among the species. Factors or background ascribed to this variation is currently unknown, but the following possibilities or interpretations may be possible. First, this variation may represent different degree of innervation of the SV by the NPY-IR fibers, possibly reflecting the species-specific structural design of the SV. Second, this variation may be attributed to different amounts of the tissue antigen, possibly being related to procedures of tissue preparation, particularly the condition of fixation, and/or to physiological states of the animals. These points should be further investigated.

On the other hand, both NPY-IR perikarya and fibers were exceptionally demonstrated in the *Plecoglossus* SV. These perikarya were identified as CSF-contacting neurons as demonstrated in the hypothalamus of the cloudy dogfish and the white sturgeon (Chiba and Honma, 1992, 1994). CSF-contacting bipolar neurons in the SV are generally considered to supply the afferent tract which projects toward the hypothalamus (Altner and Zimmermann, 1972). Furthermore, the anatomical localization of the NPY-IR fiber fascicle in the

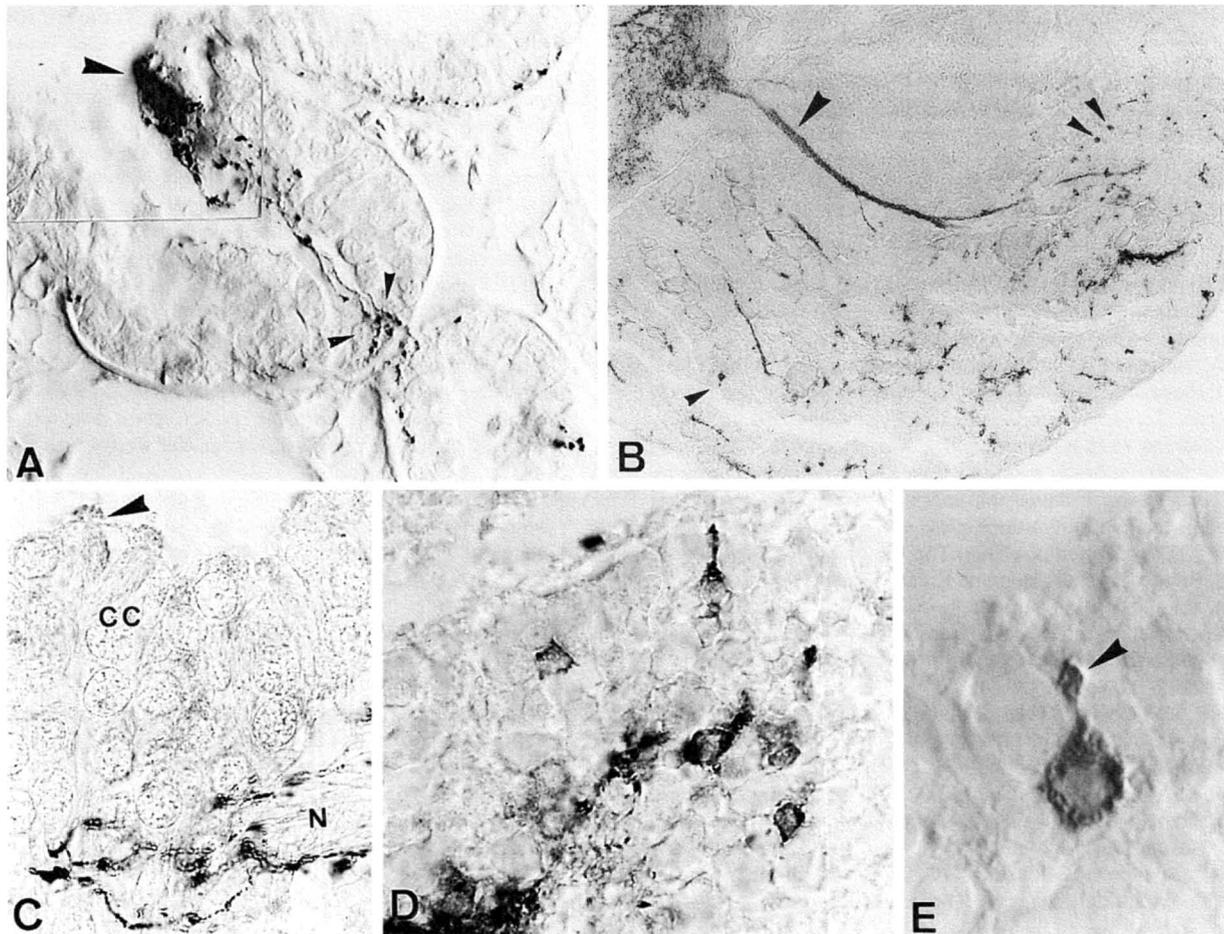


Fig. 1. (A) A part of the SV of the white sturgeon, *Acipenser transmontanus*, showing NPY-IR varicose fibers forming a supraependymal plexus (large arrowhead) or penetrating into the ependymal layer (small arrowheads).  $\times 700$ . (B) Paramedian section of the SV of the ayu, *Plecoglossus altivelis*, showing NPY-IR fibers and cells. Note a bundle of NPY-IR fibers corresponding to the tractus sacci vasculosi (large arrowhead) and immunostained cells (small arrowheads). Anterior to the left.  $\times 200$ . (C) A part of the SV of the gummy shark, *Mustelus manazo*, showing NPY-IR fibers arising from a thick bundle of nerve fibers (N). The arrowhead indicates intraventricular processes of the coronet cells (CC).  $\times 1200$ . (D) A part of the *Plecoglossus* SV showing NPY-IR cells.  $\times 1000$ . (E) An NPY-IR cell of the CSF-contacting type found in the *Plecoglossus* SV. Note the intraventricular apical swelling of the cell (arrowhead).  $\times 1300$ .

*Plecoglossus* SV appears to be identical with the tractus sacci vasculosi, the afferent tract demonstrated by silver impregnation (Altner and Zimmermann, 1972) or enzyme histochemistry (Vigh *et al.*, 1972). Therefore, we consider that in the *Plecoglossus* SV the majority of the NPY-IR fibers represent the afferent tract, although it cannot be ruled out that some of the NPY-IR fibers comprise the efferent tract. According to a review paper (Altner and Zimmermann, 1972), both tracts are common to elasmobranchs and also to teleosts. Additional experiments using the axonal tracers are required to determine whether NPY-IR fibers in the SV form the afferent and/or efferent pathway.

There is a growing body of data to show that NPY is one of the most abundant and widely distributed peptides in the central and peripheral nervous systems of higher vertebrates and acts as transmitter or modulator in the neural networks (Colmers and Whalestedt, 1993). In the light of such data, it is likely that NPY-like substance in the bipolar CSF-contacting

neurons and fibers in the *Plecoglossus* SV may also function as transmitter or modulator in the neuronal pathway of the SV. Thus, NPY-IR CSF-contacting neurons may be involved in the sensory function proposed for the SV (Altner and Zimmermann, 1972). On the other hand, apart from transmitter function of NPY, NPY-like substance in the fibers may be involved in the regulation of the SV function by hormonal action on the vascular system and epithelial (ependymal) layer of the SV. In this connection, recent information on the function of NPY, e.g., vasoconstrictory action reported in the dogfish cardiovascular system (Conlon *et al.*, 1991) and electrophysiological effects shown in the gut epithelium of teleosts (Kiliaan *et al.*, 1993) includes suggestions for future study. Further analyses are required to elucidate functional significance of NPY or related substance in the SV.

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