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Calcitonin-like immunoreactivity in the subcommissural organ–Reissner's fiber complex of some freshwater and marine teleosts

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ABSTRACT

The subcommissural organ (SCO) is a highly especialised circumventricular ependymal organ covering and penetrating the posterior commissure. The secretory products of the SCO condense to form Reissner's fiber (RF). Because of its extensive secretory activity and the chemical properties of its secretion, the organ functions as similar to the neurosecretory cells. Teleosts comprised of more than 20,000 extant species that show great diversity in terms of the form, habit and habitat. Affinity of calcitonin antibodies for the SCO–RF complex was used as a histochemical tool to study the morphology of some freshwater and seawater teleosts and its potential correlate to their osmotic environment. While intense to moderate calcitonin-like immunoreactivity was seen in the cells of the SCO of majority of the freshwater species viz., common carp, catfish, eel and perch; the SCO of goldfish revealed limited immunoreactivity. Like the SCO, the RF in all species was also immunostained with antibodies against calcitonin. It appeared as a single, continuous fiber that ran from SCO into the third ventricle and extended through the aqueduct, fourth ventricle and central canal of the spinal cord. In contrast to that in the freshwater fishes, the SCO–RF complex in majority of the seawater fishes, showed no calcitonin-like immunoreactivity. The data presented in this study described the comparative histomorphology of the SCO–RF complex and suggest a possiblity that the calcitonin-like immunoreactivity in the SCO–RF complex might be a feature correlated to the osmotic environment of the fish.

1. Introduction

The SCO of vertebrates is a highly specialized circumventricular organ (Keene and Hewer, 1935; Wislocki and Leduc, 1952, 1954; Bargmann and Schiebler, 1952; Gilbert, 1956; Oksche, 1969; Leonhardt, 1980; Sterba, 1969) situated in the roof of the third ventricle at the junction between di- and mesencephalon. It lies immediately behind the habenular ganglion and below the posterior commissure. Because of its extensive secretory activity and the chemical properties of its secretion, the organ functions similar to the neurosecretory cells (Knowles, 1969). In the teleost *Salmo gairdneri*, the SCO is found to be composed of compact, thin, elongated, ciliated and multirowed cells containing secretory material basally as well as apically (Schäfer and Blüm, 1988). The SCO is known to release secretory products into the ventricle to form a thread-like structure, the Reissner's fiber (RF). The RF is composed of glycoprotein material (Nualart et al., 1991; Rodriguez et al., 1987), which is discharged via apical exocytosis from the tall ependymal SCO cells. In most vertebrates, the fiber passes along the mesencephalic, fourth ventricle and the entire length of the central canal in the spinal cord. To date a large number of studies have attempted to reveal the structure and function of the SCO–RF complex (Leonhardt, 1980; Oksche, 1993; Rodríguez et al., 1987, 1990, 1992); however, the precise nature of the secretion or its functional significance have eluded our understanding.

The role of SCO in fluid electrolyte balance has been widely investigated. The SCO has been shown to be involved in various aspects of water and electrolyte homeostasis, such as volume reception, thirst, sodium excretion, diuresis and aldosterone secretion (see Rodríguez et al., 1992). Gilbert (1956) put forward the hypothesis that the SCO is actively involved in osmoregulation. The SCO–RF complex of a catfish, frog and a lizard were immunostained with antibodies against calcitonin (Subhedar et al., 1997) suggesting the occurrence of some calcitonin-like epitopes on the secretory products. Recently we have shown that the calcitonin immunorectivity in the SCO–RF complex of a freshwater teleost was modulated by GABA-ergic system (Saha et al., 2000).

Teleosts comprise of more than 20,000 extant species that show great diversity in terms of the form, habit and habitat. In the present study calcitonin antibody as a probe for immunohistochemical analysis was used to perform a comparative study of the

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SCO–RF complex of some teleosts from different osmotic environments viz. freshwater (twelve species) and seawater (six species). Present investigations will provide some important information about (1) the comparative morphology of the SCO–RF complex, (2) the occurrence/absence of calcitonin-like epitopes in the SCO–RF complex, and (3) the relationship, if any, between the occurrence of calcitonin-like immunoreactivity in the SCO–RF complex and the sea/freshwater habitat of the fish. Furthermore, the work based on fish belonging to freshwater as well as marine habitat, may provide clues to the functional significance of the SCO–RF complex.

2. Materials and methods

Twelve adult freshwater and six marine teleostean species of either sex were used in the present study (Table 1). While the freshwater species were collected from the lakes and rivers around Nagpur, marine species were collected from the coastal areas off Calcutta in November and December (coinciding with the postspawning period). Experimental procedures were performed according to the guidelines of the Institutional Animal Ethics Committee (IAEC) under the Committee for the Purpose of Control and Supervision of Experiments for Animals (CPCSEA), New Delhi, India. The number, weight, and sex of the fish were recorded. The animals were deeply anaesthetized with an overdose of ethyl *m*-amino-benzoate (tricaine; MS 222) and brain and 1-cm-long spinal cord, just behind the medulla, were dissected out and fixed in Bouin-Hollande fixative for 24 h at 4 °C. The tissues were cryoprotected with 20% sucrose in phosphate buffer (pH 7.4) at 4 °C, rapidly frozen with expanding CO_2 and cut with cryostat at 15 μm thickness. While the brain was sectioned in the transverse plane, the spinal cord was sectioned in the sagittal plane. Standard peroxidase anti-peroxidase method (Sternberger et al., 1970) and immunofluoroscence (Subhedar et al., 1996) techniques were used to localize calcitonin-like immunoreactivity in the slide mounted sections. Polyclonal antibodies against calcitonin (Incstar Cat # 20071) at dilutions of 1:1000 were used for immunocytochemical labeling (for details see Subhedar et al., 1997; Saha et al., 2000). Briefly, tissue sections were treated with 0.5% hydrogen peroxide in phosphate buffered saline (PBS, pH 7.4) for 30 min to remove the endogenous peroxide activity in the tissue and washed for 15 min. Sections were then blocked in normal goat serum (10%) for 30 min and incubated in the calcitonin antibody diluted in PBS (pH 7.4) containing 0.4% Triton X-100 and 1% normal goat serum for 48 h in a humid atmosphere at 4 °C. Following 10 min wash the sections were incubated in goat anti-rabbit IgG (National Institute of Immunology, New Delhi, India) at 1:100 dilution in PBS for 2 h at room temperature. The sections were then washed for 10 min and incubated in PAP complex (Sigma, USA) at 1:100 dilution in PBS for 2 h followed by a 10 min wash in Tris buffer (pH 7.6). The sections were then treated with 0.05% 3,3′-diaminobenzidine tetra-hydrochloride with 0.03% H_2O_2 in 0.05 M Tris buffer (pH 7.6) for 6–8 min, rinsed in Tris buffer, dehydrated through graded series of alcohols, cleared in xylene and coverslipped with DPX. For immunofluoroscence technique following incubation in the primary antiserum (1:500) the sections were washed for 10 min and then incubated in goat anti-rabbit IgG conjugated to FITC (1:100) in PBS for 4 h in dark at room temperature. The sections were then rinsed in PBS mounted in glycerin gel and observed under a fluorescent microscope (Leica, Germany) with a digital CCD camera attachment and images were captured. Qualitative analysis of the immunoreaction in the SCO–RF complex was done by a worker unaware of the teleostean species and their habitat. The degree of calcitonin-like immunoreactivity was scored on a scale of 0–3; 0 for absence, 1 for poor/negligible, 2 for moderate and 3 for an intense high degree of relative labeling. The SCO height was measured at 500× and width was measured at 250×. The height was measured at three different sites: two laterals along the edge and the medial regions of the mid coronal section of the SCO and averaged. The width of the SCO was measured in the mid coronal section of the SCO. The diameter of the RF of each fish was measured under oil immersion at 1250× at five equally spaced regions along its length in the central canal of the spinal cord; the data from total number of animals of each species were pooled and averaged (see Table 1). The width, height of SCO, RF diameter and the weight of fish were analyzed by nonlinear regression analysis using GraphPad Prism software.

Specificity of the calcitonin antibody was tested earlier (Subhedar et al., 1997; Saha et al., 2000). These include omitting one reaction step, replacing anti-calcitonin with normal rabbit or goat serum, and preabsorbing 1 ml diluted primary antibodies with 10^{-5} M salmon thyrocalcitonin (Sigma, Cat No. T 3660) for 24 h at 4 °C prior to incubation. In addition, the antibodies were tested on the sections of the ultimobranchial gland of the catfish, since the tissue is known to be the peripheral source of calcitonin.

3. Results

3.1. Subcommissural organ–Reissner's fiber complex in some freshwater teleosts

The data on the nature of calcitonin-like immunoreactivity in the SCO–RF complex and dimensions of SCO and RF of the freshwater fish used in the present study are summarized in Table 1. The SCO of the common carp *Cyprinus carpio* was found to be large, shaped like an arch in the roof of the third ventricle and revealed intense calcitonin-like immunoreactivity. While the cells in the lateral part of the SCO showed more immunoreactivity in the apical region, those in the medial SCO showed immunoreactivity in the basal as well as apical zones (Fig. 1A). In the SCO of goldfish, although the over all immunoreactivity was poor, fine immunoreactive granules were seen at the apical and basal ends.

As investigated in our earlier studies the ependymal cells of the SCO of *C. batrachus* (Subhedar et al., 1997; Saha et al., 2000) showed calcitonin-like immunoreactivity; while intense immunoreactivity was seen towards the basal as well as apical ends, moderate immunoreactivity was observed throughout the cell body (Fig. 1B).

Table 1
The data on the freshwater and seawater fish and the nature of calcitonin-like immunoreactivity in their SCO and RF are summarized in table.

Order	Family	Genus and species	No. of fishes (*n*)	Body weight (gm)	SCO			RF	
					CT-like-ir	Height (μm)	Width (μm)	CT-like-ir	Diameter (μm)
Freshwater teleosts									
Clupiformis	Notopteridae	*Notopterus notopterus*	6	200	0	17.25	88.5	0	3.5
Cypriniformis	Cyprinidae	*Cyprinus carpio*	5	700	3	52.5	7600	2	13
	Cyprinidae	*Carassius auratus*	5	40	1	57.5	350	1	3.5
	Claridae	*Clarias batrachus*	6	80	2	45	300	3	3.5
	Heteropneustide	*Heteropneustes fossilis*	6	75	3	64	348	2	3.5
	Bagaridae	*Mystus seenghala*	5	60	3	24	305	2	2
Anguilliformes	Anguillidae	*Anguilla bengalensis*	4	250	3	112.5	420	3	5
Channiformes	Channidae	*Channa punctatus*	5	75	0	17.25	270	0	3
Symbranchiformes	Amphipnidae	*Amphipnous cuchia*	3	150	0	37.5	125	0	6
Perciformes	Centropomidae	*Lates calcarifer*	10	75	2	42.5	200	3	6
	Cichlidae	*Tilapia mossambica*	8	75	0	50	200	0	4
	Anabantidae	*Anabas testudineus*	6	80	3	37.5	275	3	5
Seawater teleosts									
Perciformes	Carangidae	*Uraspis uraspis*	5	150	0	45	200	0	5
	Trichuridae	*Lepturacanthus savala*	5	150	0	47.5	100	0	3
Clupiformes	Clupeidae	*Escualosa thoracata*	5	60	0	37.5	100	0	5.5
		Ilisha megaloptera	5	500	1/0	16.25	55	0	3
		Chirocentrus nudus	6	200	2	20	112.5	3	3
Salmoniformes	Stromatidae	*Pampus argenteus*	5	150	0	25	187.5	0	3

Note: 3: intense; 2: moderate; 1/0: poor/negligible: 0: absent; CT-like-ir: Calcitonin-like immunoreactivity.