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IMMUNOCYTOCHEMICAL AND ULTRASTRUCTURAL CHARACTERIZATION OF SOMATOLACTIN CELLS IN THE PITUITARY GLAND OF THE CARP FISH (*CYPRINUS CARPIO*)

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ABSTRACT

Somatolactin (SL) cells were immnuocytochemicaly identified and localized using Avidin-biotin peroxidase complex (ABC), as well as ultrastructurally characterized in the pituitary gland of the carp fish (Cyprinus carpio). The results indicated that SL-immunoreactive (ir) cells showed strong and specific cytoplasmic granular immunoreactivity to antiserum for goldfish SL, but were negative to periodic acid Schiff reagent. The distribution of SL-ir cells were restricted to the area of pars intermedia (PI) in carp fish adenohypohysis and were organized in two forms 1) discontinuous cell cords surrounding the neurohypophyseal branches and 2) small groups or isolated cells in between the parenchyma of the PI. The SL-ir cells were polygonal, triangular or spindle-shaped with many cytoplasmic processes. The nucleus of SL cells was large in size, spherical and contained a prominent nucleolus. Ultrastructurally, SL cells were irregularly outlined with elongated processes directed towards the neurohypophysis or blood vessels. The secretory granules were heterogeneous in shape and size, with high or medium electron-dense material. Some granules were fused to form polymorphic or irregular mass characteristic of SL cells. The SL cells showed highly developed rER with parallel cisternae and euchromatic nucleus indicated high active protein secreting cells.

Keywords: Pituitary gland, carp fish, immunocytochemistry, somatolactin, ultrastructure.

INTRODUCTION

The pituitary gland is one of the most important endocrine organs in vertebrates. It has two distinctive regions, the neurohypophysis consisting on neurosecretory terminals from the hypothalamus and other parts of the brain, and the adenohypohysis constituted mainly by secretory cells. In fish, these endocrine cells are arranged in groups that produce different hormones and are placed at specific regions of the adenohypohysis. In some species of fish the adenohypophyseal cell types were identified by histological, histochemical and immunocytochemical methods ***(Farbridge and Leatherland 1986; Yan and Thomas 1991; Garcia Hernandez et al. 1996; Vissio et al. 1996, 1997)***.

Somatolactin (SL) is a pituitary hormone, structurally related to both growth hormone and prolactin ***(Ono et al. 1990; Rand-Weaver et al. 1991a; Chen et al. 1994)*** that has evolved as a result of duplication in the growth hormone/prolactin gene family ***(Ono and Kawauchi 1994)***. It has been considered specified fish hormone ***(Rand-Weaver et al. 1991b; Rand-Weaver and Kawauchi 1993; Olivereau and Rand-Weaver 1994a, b)***. Although the biochemical and physicochemical properties of SL are becoming increasingly clear, its physiological significance is still largely unknown. It has been suggested that SL is involved in adaptation to environmental changes ***(Ono and Kawauchi 1994)***, adaptation to background and decreased illumination ***(Zhu and Thomas 1995, 1998)***, stress responses ***(Kakizawa et al. 1995; Johnson et al. 1997)***, the control of some physiological aspects of reproduction ***(Planas et al. 1992; Rand-Weaver et al. 1992; Rand-Weaver and Swanson 1993; Mousa and Mousa 2000)***, acid-base balance ***(Kakizawa et al. 1996)***, and the regulation of calcium, phosphate ***(Kakizawa et al. 1995; Lu et al. 1995)*** and sodium ***(Zhu and Thomas 1995)*** metabolism.

SL is mainly synthesized by cells of the pars intermedia (PI) of fish pituitary glands, which are periodic acid-Schiff (PAS)-positive in all teleosts, except in salmonids ***(Rand-Weaver et al. 1991b; Olivereau and Rand-Weaver 1994a, b)***. Immunocytochemical demonstration of SL cells have been occurred at the light microscopical level in some teleost species ***(Rand-Weaver et al. 1991a, b; Kaneko et al. 1993a, b; Olivereau and Rand-Weaver 1994a, b; Parhar and Iwata 1994; Dores et al. 1996; García Hernández et al. 1996; Villaplana et al. 1997; Mousa*** and ***Mousa 1999; Saga et al. 1999)***. However, it has been ultrastructurally characterized only in rainbow trout ***(Kaneko et al. 1993b)***,Mediterranean yellowtail ***(García Ayala et al.1997)*** and gilthead sea bream ***(Villaplana et al. 2001)***.

The aim of the present study is immunocytochemical identification and distribution, as well as ultrastructural characterization of the SL cells in the pituitary of adult carp, Cyprinus carpio.

MATERIALS AND METHODS

2.1. Materials: Twenty adult carp fish, *C. carpio*, of both sexes (0.9 – 1.5 kg B.Ws.) were obtained from fish farms in Damro, Sedi Salim City, Kafr El-sheikh governorate. Fourteen fish were utilized for light microscopical and immunocytochemical studies and six fish for transmission electron microscopy.

2.2. Tissue preparation: Immediately after decapitation of the fish, the pituitary gland was gently separated from the sella turcica and fixed in 10% neutral-buffered formalin for 24 hr, dehydrated in a graded series of ethanol solutions, and embedded in paraffin. Sagittal sections 5 μm thickness were cut and mounted on slides coated with poly-*L*-lysine (50μg/ml). Some sections were stained with PAS for histochemistry (***Bancroft and Gamble, 2002***).

2.3. Immunocytochemical technique: After dewaxing and dehydration, the sections were washed in 0.01 M phosphate-buffered saline (PBS) pH 7.4, and immunocytochemically stained using ABC method ***(Hsu et al., 1981)***. Immunolabelling was performed using a rabbit antiserum raised against goldfish somatolactin, working dilution (1:4,000). The sections were treated with the antiserum overnight at 4°C, washed in PBS, incubated for 30 min at 37°C with anti-rabbit second antibody (Vectastain, Vector Laboratory, Burlingame, CA, U.S.A.), and washed in PBS. They were then incubated with 0.02% 3.3′-diaminobenzidine tetrahydrochloride (DAB) solution containing 0.005% H_2O_2 for about 10 min, counter-stained with hematoxylin, and then observed with an optical microscope. The specificity of the reaction was verified by omission of the first antiserum and by substitution of normal rabbit serum for the specific antiserum.

2.4. Ultramicroscopic technique: The specimens were fixed for 2 h at 4°C in a mixture of l% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.2) and embedded in Epon after postfixed for 1 h at 4°C in 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.2). The sections were obtained with SEO-Sumy ultramicrotome and contrasted with uranyl acetate and lead citrate (***Drury and Wallington, 1980***) for conventional electron microscopic study. The specimens were processed at Army Veterinary Hospital, Nasser City, Egypt.

RESULTS

3.1. Light microscopy:

The pituitary gland of carp fish, *Cyprinus carpio*, consisted of two distinctive regions, adenohypophysis and neurohypophysis. The adenohypophysis was subdivided to the rostral pars distalis (RPD), the proximal pars distalis (PPD) and pars intermedia (PI) (Fig. 1a). The SL cells in the PI showed PAS-negative reaction (Fig. 1a & b)

3.1. Immnuocytochemistry:

SL-immunoreactive (ir) cells showed strong and specific cytoplasmic granular immunoreactivity to antiserum for goldfish. It was localized only in PI of the carp adenohypophysis, non in RPD or PPD (Fig. **1**c, d).The SL-ir cells were organized in two forms 1)discontinuous cell cords lying close to or surrounding the neurohypophyseal branches (Figs. **1**c-f & **2**a) and 2) small groups or isolated cells in between the parenchyma and blood vessels of PI (Figs. **1**c-f & **2**b-d). The SL cells had different shapes; it was polygonal, triangular or spindle-shaped with long cytoplasmic processes (Fig. **2**c, d). The nucleus of SL-ir cells were large in size, spherical in shape, centrally or accentrally located and had prominent nucleolus (Fig. **2**c, d).

3.2. Ultrastructure of SL cells:

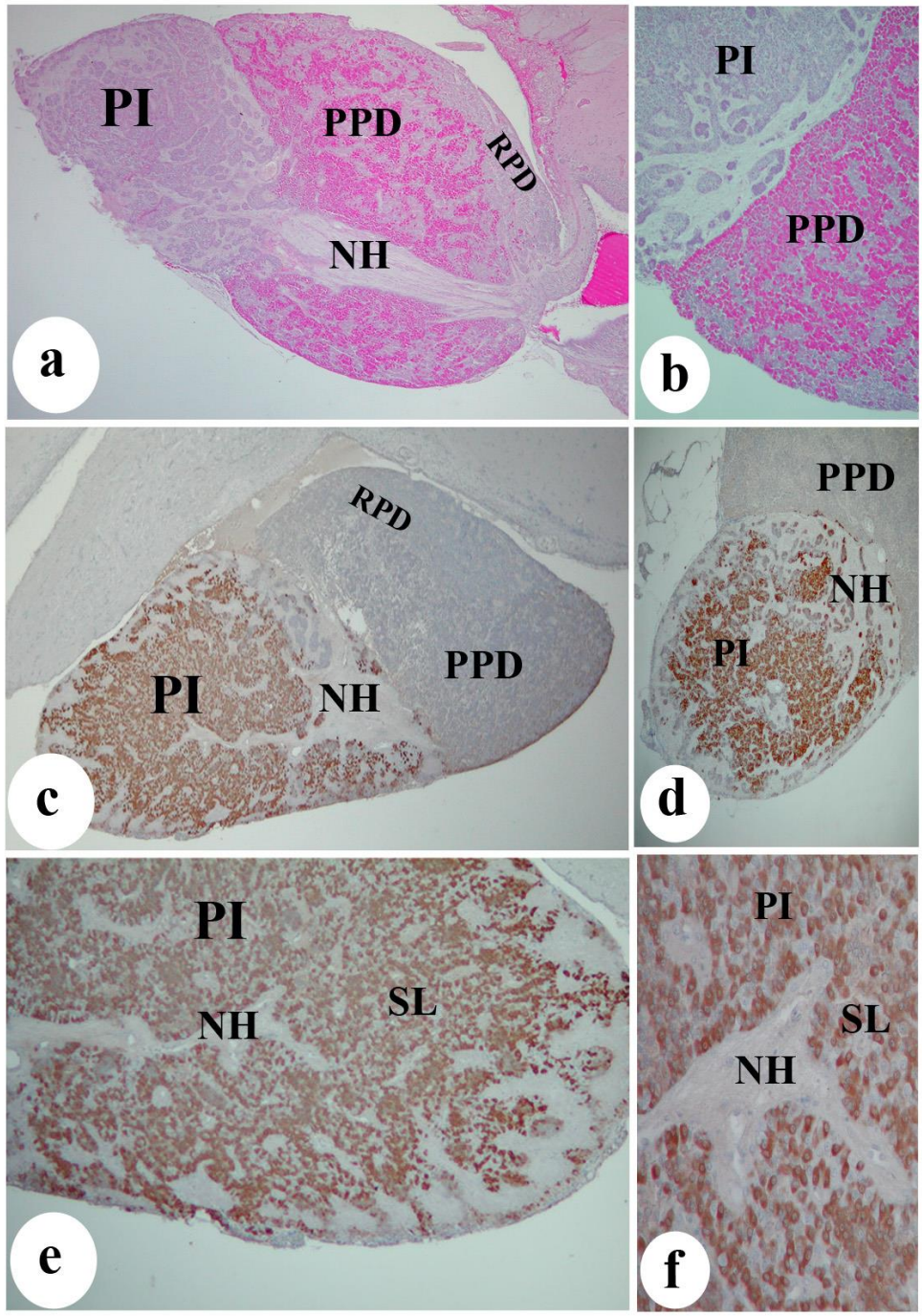
The SL cells were irregularly outlined, elongated, triangular or polygonal, and showed processes directed towards the blood vessels and neurohypophysis, or located among other adenohypophyseal cells (Fig. **3**a). SL cells had large euchromatic nucleus with a conspicuous nucleolus (Fig. **3**a, c). It had numerous secretory granules that were heterogeneous in shape, size and electron density (Fig. **3**a-e). These secretory granules were scattered throughout the cytoplasm, being more numerous in the outer areas and the cytoplasmic processes (Fig. **3**a). The secretory granules were mostly round, although some granules were oval, bilobate or pear-shaped, with a homogenous high or medium electron-dense content (Fig. **3**c-e). Some irregular and polymorphic secretory granules were found that seemed to arise from the fusion of various secretory granules, which also give the large irregular electron-dense masses appeared in cytoplasm (Fig. **3**e, f). The secretory granules varied in size; 1) large more numerous secretory granules and 2) small less frequency granules (Figs. **3**a-e). The SL cells were characterized by a well-developed rough endoplasmic reticulum (rER) organized in stacks of parallel cisternae and Golgi apparatus (Fig. **3**e).

LEGENDS

Fig. (1): Sagittal sections of the pituitary glands of carp, *C. carpio***,** stained with PAS (**a** & **b**) and immunostained with anti-goldfish SL (**c-f**), the SL-ir cells restricted only PI. Nuclei are stained with hematoxylin NH; neurohypophysis, PI; pars intermedia, PPD; proximal pars distalis, RPD; rostral pars distalis, SL; somatolactin cells. (**a, c** &**d** ×40; **b** & **e** ×100 and **F** ×200)**.**

Fig. (2): SL-ir cells in the PI of the pituitary glands of carp, C. carpio, distributed in the form of discontinuous cell cords **a**) or in groups **b**). They are polygonal (⌦), triangular (➧) or spindle-shape (➡) with cytoplasmic processes. (a×400; b×200 and c&d×800).

Fig. (3): Ultrastructural photographs of SL cells showing **a**) nucleus (N), cytoplasmic process (➡) small (⇨) and large size (➧) secretory granules; **b)** direct contact of the SL cells with the neural tissue (NH), BV: blood vessel; **c-e**) the polymorphic secretory granules, irregular mass (☆) and well-developed rER. Bars 1 μm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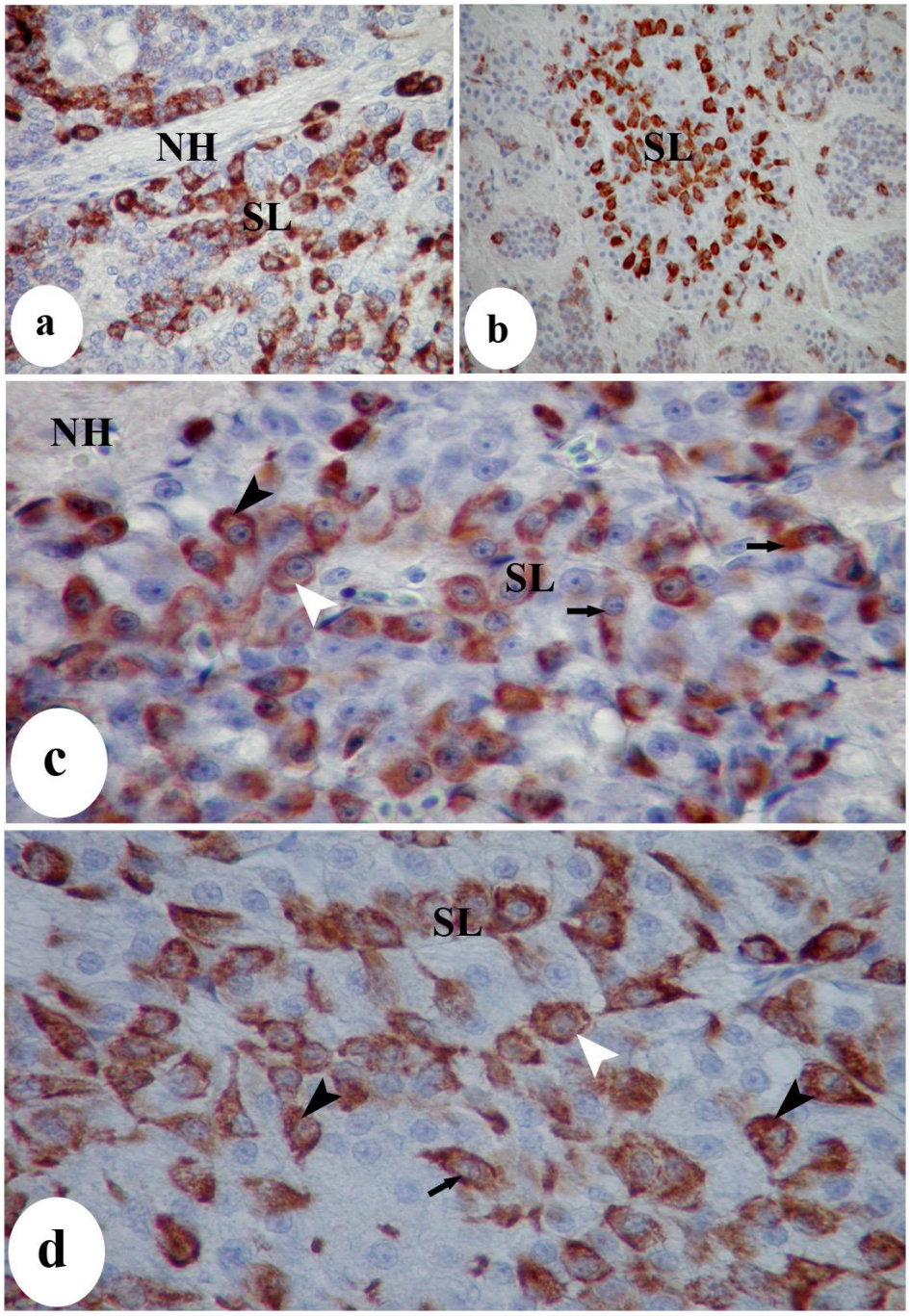
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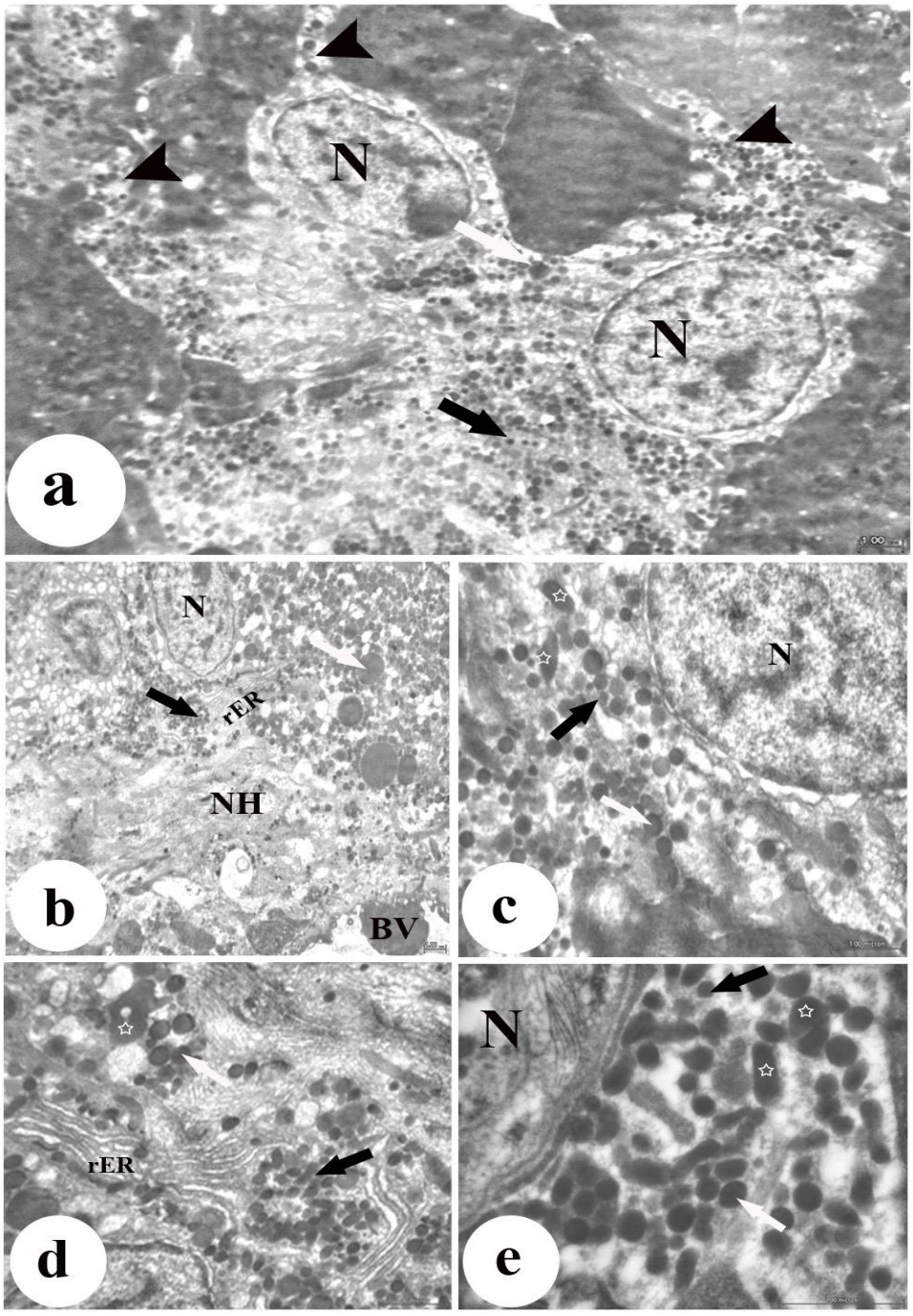
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DISCUSSION

The SL cells have been immunocytochemical identified in many teleosts (***Rand-Weaver et al. 1991a, b; Kaneko et al. 1993a; Parhar and Iwata 1994; García Hernández et al. 1996; Villaplana et al. 1997; Mousa and Mousa 1999***) and ultrastructurally characterized in other teleosts (***García Ayala et al. 1997***; ***Villaplana et al. 2001***), however this is the first report to clarify the distribution and ultrastructural features of SL cells in the carp fish pituitary, *C. carpio*.

According to (***Rand-Weaver et al. 1991b, Villaplana et al. 2001***) there were two forms of SL, glycosylate and non-glycosylate, in teleosts fish. The glycosylated form gave the PAS-positive as reported in most of the teleosts species examined to date (***Dores et al. 1996; García Hernández et al. 1996; Villaplana et al. 1997; Mousa and Mousa 1999***), however the non-glycosylated form gave negative reaction with PAS as in salmonids (***Rand-Weaver et al. 1991b; Olivereau and Rand-Weaver 1994a, b***). The results of this study showed that the SL cells in *C. carpio* were PAS-negative; this indicated that the SL cells were of the non-glycosylated form.

SL-ir cells were localized in the PI of carp fish adenohypophysis, as has been described in other teleosts (***Parhar and Iwata 1994; Amemiya et al., 1999; Saga et al., 1999***; ***Pandolfi et al., 2001***), however it has been also found in other regions of adenohypophysis, the PPD and RPD (***Olivereau and Rand-Weaver 1994a, b; García Hernández et al. 1996***; ***Villaplana et al. 1997***) and occasionally in brain (***Mousa and Mousa, 1999***).

The SL cells of *C. carpio* were generally polygonal, triangular or elongated, as in *O. mykiss* **(*Kaneko et al. 1993b*)**, while only round cells were reported in *S. dumerilii* **(*García Ayala et al. 1997*)**. It showed processes directed towards the neurohypophysis, similar results were also reported in other teleosts **(*Kaneko et al. 1993b; Olivereau and Rand-Weaver 1994a*)**. The location of *C. carpio* SL cells close to the neural tissue, as in other teleosts **(*Moons et al., 1989; Parhar and Iwata 1994*)**, suggested that its secretion were regulated by neurotransmitter substances from the hypothalamus **(*Parhar et al., 1995*)**. The secretory granules of SL cells in carp fish showed a variation in shape and size, these in accordance with that in Mediterranean yellowtail **(*García Ayala et al. 1997*)** and gilthead sea bream **(*Villaplana et al. 2001*)**. In the SL cells of *C. carpio*, bilobate, pear-shaped and irregular secretory granules seemed to arise from the fusion of various secretory granules. The fusion of secretory granules has also been reported in the PAS-positive cells **(*Batten et al. 1975*)** and in the SL cells **(*García Ayala et al. 1997; Villaplana et al., 2001*)**.

In the *C. carpio*, SL cells a well-developed rER, numerous secretory granules as well as large, euchromatic nuclei were indicated an intense protein synthesis and storage, these ultrastructural feature were also reported in SL cells of *O. mykiss* **(*Kaneko et al. 1993b*)**, *S. dumerilii* **(*García Ayala et al. 1997*)** and *S. aurata* **(*Villaplana et al., 2001*)**.

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