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Degeneration and possible renewal processes related to the interrenal cells in the head kidney of the stickleback *Gasterosteus aculeatus*

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Abstract

The ultrastructural aspect of degeneration and recovery processes involving the steroidogenic interrenal cells of the stickleback was studied. Together with the adrenergic cells, the interrenals constitute the adrenal homolog in teleosts. From our study it appears that a process of massive cell death may lead to temporary disappearance of the gland. Moreover, our E.M. observations suggest two main ways, each leading to morphological dedifferentiation of the cells, no longer recognizable as interrenals: the first way involves elimination of organelles and recovery of the nucleus surrounded by a thin rim of cytoplasm; the second involves fragmentation of the cytoplasm by other pyknotic star-shaped interrenals, together with autophagocytosis processes.

Our E.M. observations also suggest that the subsequent reconstitution of the tissue can occur in two ways. In the first, the interrenals appear mainly to differentiate from mesenchymatic-like electron-light cells, while in the second, the new interrenals appear mainly raising from some macrophagic electron-dense cells.

Some data obtained with Mallory's trichrome staining of histological sections, and localization of the enzyme 3β hydroxysteroid dehydrogenase in thin sections, support the above-mentioned results.

A hypothesis is advanced on the origin of the electron-dense differentiating interrenals, and a possible role of dedifferentiated cells in restoration of the interrenal gland is also discussed.

Keywords: Stickleback; Interrenals; Ultrastructure; Degeneration; Dedifferentiation; Differentiation

1. Introduction

In teleosts, the adrenal gland does not form a discrete body as in mammals and birds. Instead, it is composed of cords or nests of interrenal (corticosteroidogenic) and chromaffin (catecholamine-secreting) cells, more or less intermingled and often surrounding the postcardinal veins and their branches in the hemopoietic tissue of the kidney (mainly head kidney) (review by Gallo and Civinini, 2003). Our previous studies of the cytological and histochemical characteristics of the interrenal cells of females (Civinini et al., 1997) and males (Civinini et al., 2001) of the three-spined stickleback *Gasterosteus aculeatus* indicated that these cells undergo a metabolic cycle. During this cycle, some organelles, particularly SER and mitochondria, present different aspects. On the basis of the differences, in males we divided the cycle into four main phases (Civinini et al., 2001): phase 1, characterized by a slightly heterochromatic nucleus and mitochondrial proliferation; phase 2, with a roundish euchromatic nucleus and well-developed SER consisting of elongated or roundish cisternae; phase 3, with an irregularly shaped heterochromatic nucleus and enlargement of the perinuclear cisterna, disappearance of plasma membranes, disorganization of mitochondrial cristae and large vesiculization of SER; phase 4, with reorganization of the nucleus and cytoplasm and loss of some organelles and cytoplasmic fragments into the intercellular spaces.

Alternatively to phase 4 (which is usually followed by restoration of the cells to phase 1), we observed that cells might degenerate. In some cases, the degeneration was so massive that histological and histochemical observations at the L.M. failed to reveal clearly evident glandular (interrenal and chromaffin) tissue. Numerous, "dark" cells were

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associated with the degenerative processes, particularly in the reproductive and post-reproductive periods. We suggested that more or less extensive degeneration of the interrenal cells might precede renewal of this type of cell, possibly with a shift in the type of steroid production. In fact, in both females (Civinini et al., 1997) and males (Civinini et al., 2001), we also observed variability in the appearance of organelles (particularly the shape of SER cisternae and mitochondria) in different specimens, and we suggested that this might indicate different steroidogenic functions corresponding to different hormone requirements. On the other hand, in the same species, we also observed a differentiation of the interrenals from "light" cells, particularly in the pre-reproductive period (unpublished data). Moreover, no mitosis of already differentiated interrenal cells was ever observed.

In the present study, on the basis of the cytological aspects observed at the E.M., we suggest that degeneration, dedifferentiation and differentiation processes could be related to a periodic renewal of the interrenal gland. Moreover, we add some data on the ultrastructural localization of 3ß hydroxysteroid dehydrogenase (3βHSD) and on Mallory's trichrome staining of histological sections. 3βHSD is active in steroidogenic metabolism and is mainly localized on the SER membranes. Its cytochemical localization is useful to demonstrate processes of functional differentiation. Regarding Mallory's trichrome stain, Chieffi Baccari et al. (1992) found that aniline blue affinity of nuclei in the harderian gland of the green frog (in contrast to the orangiophily of other nuclei) indicates increased RNA synthesis. Therefore, we used this stain in some specimens to obtain information on the nuclear status.

2. Materials and methods

Adult specimens of the three-spined stickleback *Gasterosteus aculeatus* L. (the *leiurus* form living in southern Europe), 6–7.5 cm in length, were obtained from Posta Fibreno Lake. This lake (now a WWF oasis) at the time of sampling collection was a natural reserve of Lazio Region and permission to conduct sampling was obtained from the Manager Organization for scientific purpose. Seventy-three specimens (males and females) were collected in the quiescent (December–February), pre-reproductive (February–May), reproductive (May–September) and post-reproductive (September–November) periods for 3 years. They were housed in an aquarium and sacrificed by rapid decapitation a few days later. Forty-three of them were considered for the present research, as, in at least one of the two head kidneys, they presented a histological situation of the adrenal homolog pertaining the study in object.

2.1. Histology and electron microscopy

Head kidneys containing the adrenal homolog were dissected and fixed in 3% glutaraldehyde in cacodylate buffer 0.1 M, pH 7.4 for 3 h, postfixed in 1% osmium tetroxide in Millonig buffer for 1 h, and embedded in Araldite. Semi-thin sections stained with methylene blue were used for histological observations. Thin sections, stained with uranyl acetate and lead citrate, were observed under a Philips CM 10 electron microscope.

2.2. Mallory's trichrome stain

Head kidneys of some specimens were fixed in cold PAF mixture (4% paraformaldehyde and 0.2% picric acid in phosphate buffer 0.1 M pH 7.4) and embedded in paraffin. Histological sections were stained with the following mixture: aniline blue 0.5 g, orange G 2 g, oxalic acid 2 g, distilled water 100 ml, pH 1.5.

2.3. Ultracytochemical localization of 3βHSD

For 6 specimens, fixation and incubation of head kidneys of one side were performed according to Berchtold's technique (1977). Thick sections were fixed in a mixture of 0.25% glutaraldehyde and 1% paraformaldehyde in phosphate buffer 0.1 M pH 7.4, by two passages of 10 min. The pieces were then stored overnight at 4 °C in buffer. The incubation medium was prepared as follows: the substrate formed by etiocholane-3β-ol-17-one (2 mg in 0.6 ml of dimethysulphoxide) and NAD (10 mg in 6.4 ml of phosphate buffer 0.1 M pH 7.2) was supplemented with 1 ml of sodium citrate 0.1 M in distilled water (final concentration 10 mM), 1 ml of copper sulphate 15 mM in distilled water (final concentration 0.5 mM), 1 ml of potassium ferricyanide 5 mM in distilled water (final concentration 0.5 mM), phenazine methosulphate 0.8 mM. Sections were incubated for 1 h 30 min at 37 °C in the dark in a shaking bath, postfixed in 1% osmium tetroxide solution, dehydrated and embedded in Epon. The ultrathin sections were stained with uranyl acetate alone.

Fig. 1. First type of degeneration and dedifferentiation processes of interrenal cells. (A) Nucleus of a degenerated interrenal cell passing through an endothelial wall into the lumen of the vessel (V). Bar: 1 μm. (B) Interrenals in phase 3. Two contiguous cells are not separated by plasma membranes. Nuclei are heterochromatic and one is indented. SER is in the form of vesicles and some mitochondria are swollen. Bar: 2 μm. (C) Indented nucleus of an interrenal in phase 3. A round nucleolus is surrounded by strands of heterochromatin. Bar: 2 μm. (D) Zone with remnants of degenerated interrenals together with a cell with a nucleus similar to those shown in (B) and (C), surrounded by a thin rim of cytoplasm (arrow heads). Two apoptotic cells are also present (asterisks). Bar: 2 μm. (E) A probably redifferentiating interrenal cell, adjacent to an interrenal in phase 3 (asterisk). The nucleus is heterochromatic and the cytoplasm is poor in organelles. Bar: 2 μm. (F) Probably redifferentiating interrenals (stars) in a more advanced stage with respect to (E). Mitochondria are thin and rod-like. Typical interrenal mitochondria and fibrils (arrow heads) are interspersed among these cells. Bar: 2 μm.