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Ultrastructure of Gonadotrophs in the Eel Following Oestradiol Treatment

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Male and female silver eels, in fresh water or sea water, have few gonadotrophs poorly differentiated. Oestradiol treatment induces their development and hyperplasia : a well developed Golgi complex, numerous secretory granules (200–500 nm), dilated cisterna and large globules (1.2–2.2 μm) are observed. As no macroscopic effect is discernible on the gonad, the release of the synthesized hormone remains questionable. Several hypotheses have been put forward to explain these data. In all events, oestradiol shows a positive feedback on the gonadotropic activity of immature eels without inducing sexual maturation.

Introduction

The proximal pars distalis of the eel pituitary contains two different cell types: one is typically stained with orange G and contains large granules (somatotrophs). The other one is poorly represented among somatotrophs (STH) by small groups of cells slightly stained with anilin blue mainly along the connectivo-vascular tissue; they react weakly with classical histochemical stainings characterizing the glycoproteins. The gonadotropic function of this second cell category was established in male eels whose sexual maturation was induced with prolans according to Fontaine's technique (1936) or with LH-contaminated TSH (Olivereau 1961); they enlarge considerably in mature males and contain abundant glycoprotein granules (Olivereau 1960). TSH cells are smaller and more rostrally located (Olivereau & Herlant 1961). Similarly, mature female eels have large gonadotrophs which may penetrate into the rostral pars distalis (Olivereau 1967).

Ultrastructural studies on gonadotrophs of the eel are scarce. Knowles and Vollrath (1966a) described two, then (1966b) five types of gonadotrophs in sea-water eels, although gonadal development was not stimulated. Sexual maturation induced with Synahorin in the Japanese eel promotes the development of gonadotrophs (Yamamoto & Nagahama 1973). As previously reported (see Olivereau & Chambolle 1978), oestradiol (E_2) treatment causes a regression of well developed gonadotrophs or exhibits no clear effect in various teleost species. A brief treatment with E_2 benzoate (4mg/kg) slightly modifies the granules of nervous

fibers, but no changes are observed in the gonadotrophs of eels (Vollrath 1971). However, they appear very sensitive to E_2 and may differentiate rapidly as described in the present study.

Materials and Methods

Male or female silver eels in fresh water or male silver eels in sea water were kept at 20 $\pm$1°C under a photoperiod of 12L : 12D. whey were injected daily or every other day with oestradiol (150, 200 or 300 μg/100g) for 4 to 78 days. Pituitaries were fixed in sublimated Bouin-Hollande or in 6.5% glutaraldehyde solution in cacodylate buffer (*p*H 7.4) and postfixed in 2% osmium tetroxide. They were embedded respectively in parafin or Epon and stained with uranyle acetate and lead citrate.

Results

In accordance with results previously obtained with light microscope studies, gonadotrophs of untreated eels, male or female, appear poorly differentiated: they have a small amount of cytoplasm with abundant mitochondria, but rather few granules with a moderate electron density, and an average diameter of 125 nm (80–200 nm). The Golgi complex is not conspicuous and the formation of granules is not observed. This picture does not seem to be influenced by the external salinity. The contrast with somatotrophs having numerous irregular granules of a high electron density is evident (figure 1).

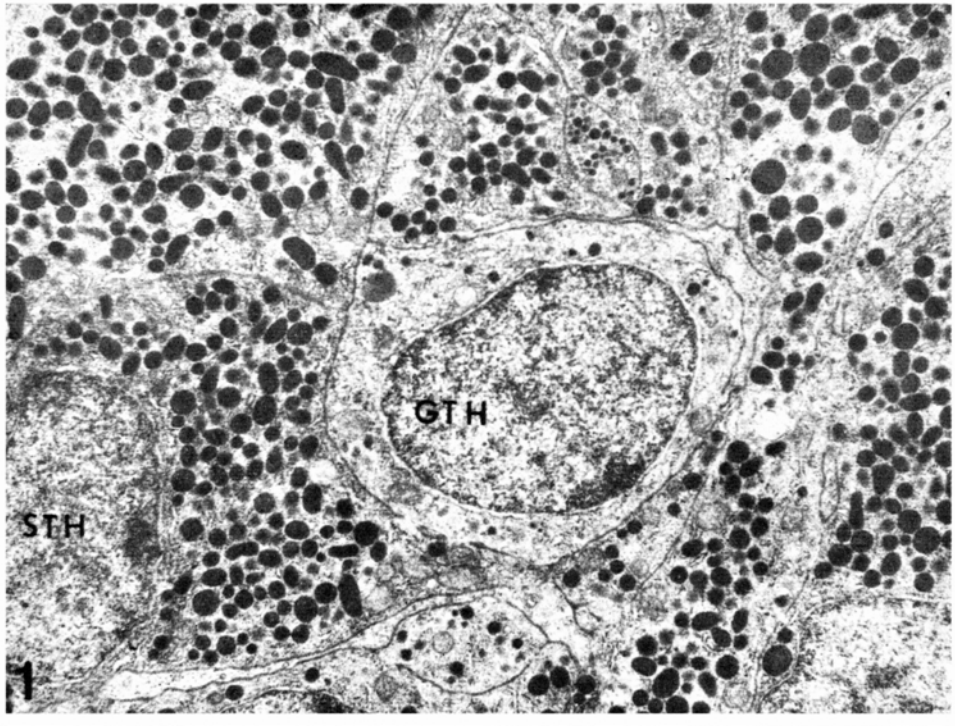
Five injections (150 μg/100 g) within 10 days are sufficient to induce the differentiation of gonadotrophs. These large cells contain numerous glycoprotein granules stained with PAS, alcian blue and aldehyde-fuchsin. Granules of various sizes and numerous cisterna with a lightly flocculent content are present, as well as a few large granules of a lesser electron density (figure 2). The size of the rounded granules varies from 200 to 500 nm, with two peaks: according to the cells, they are situated around 150 and 220 nm, or 220 and 300 nm. After 40 days of treatment, they reach 400 and 575 nm. Thus it seems that all the gonadotrophs do not differentiate simultaneously, but successively, and the first differentiated cells appear to have larger granules, the size of which increases with the duration of the treatment. The Golgi apparatus is well developed, and the dictyosomes present terminal vesicles apparently pinching off to form new electron lucent granules small in diameter. However, in several pituitary cells of eels treated for over two months, the Golgi complex is less evident. In other cells, it remains well developed, indicating again that cell differentiation is not synchronous in the pituitary. Large granules (350-450 nm) accumulate in the cytoplasm when dilated cisterna are less abundant and a few big granules, probably lysosomes, (1.2–2.2 μm) are more frequently observed in eels treated for 40 days and longer. Some gonadotrophs having a dense cytoplasm appear to be degenerating. After careful study, no exocytoses were observed.

Discussion

The presence of a large number of voluminous granulated cells with an active Golgi complex, in E_2-treated eels, contrasts with that of the small gonadotrophs, lightly granulated in control eels. A synthetic activity occurs in these stimulated cells and the light microscope picture is rather similar to that of male or female eels whose sexual maturation has been induced experimentally (see

Figure 1 Untreated freshwater male silver eel. A small gonadotroph (GTH) slightly granulated among STH cells with numerous granules (× 7800).

Figure 2 Freshwater male silver eel treated every other day with 150 µg/100 g of oestradiol for 10 days. Enlarged GTH cells with abundant granules, some larger globules, dilated cisterna and active Golgi area (g). (× 7800). —→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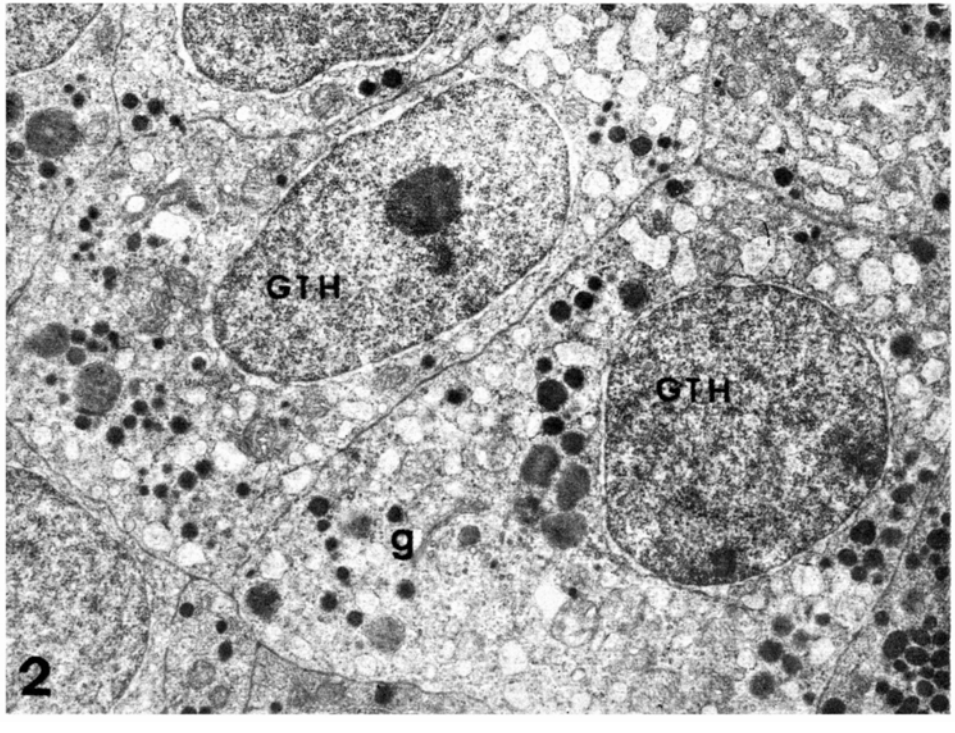
GTH
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also Sokolowska et al. 1978). However, macroscopical examination of the gonads does not reveal any stimulation. In the freshwater male, a few oocytes are observed in the organs of Syrski, whereas they are not detected in controls. The gonosomatic ratio is slightly increased (up to 2, controls 0.6 to 1.3), but this effect may be partly due to a moderate decrease in body weight. An histological study of these gonads is in progress.

The differentiation of the gonadotrophs under the influence of E_2 contrasts with the nonreactivity of the gonad. Several hypotheses may be considered:

1. The synthesized gonadotropin is not released. Exocytoses were not detected in eels killed around noon, but their occurrence in mammalian gonadotrophs is still questioned (Pelletier & Puviani 1974, Farquhar et al. 1975, Soji et al. 1976). The possible lack of release may be due to an insufficient secretion of a "LH-RH" type factor in the hypothalamus, and experiments are in progress to test this hypothesis.

2. Gonadotropin is released, but the gonad is refractory to its effect, or the stimulating effect of the gonadotropin is inhibited by the excess of circulating E_2. Doses used in this experiment are high, but lower than those injected in *Gillichthys* (100 μg/fish 20–40g/day for 19 days, Nagahama et al. 1975).

3. Recent studies by Idler et al. (1975), Ng and Idler (1978) suggest the existence of separate gonadotropins controlling vitellogenesis and maturation in two teleosts. The glycoprotein preparation fails to stimulate gonadal incorporation of the yolk which accumulates in the serum of the hypophysectomized flounder (Campbell & Idler 1976), a situation which occurs in the oestrogenized eel showing hepatomegaly and a strongly opalescent plasma (unpublished). E_2 treatment is unable to stimulate this incorporation, but it is induced by a nonglycoprotein fraction (Campbell & Idler 1976). Two cell categories were observed in salmonids (Olivereau 1976, 1978), one of which being poorly reactive with stainings demonstrating glycoproteins. This form of gonadotroph is not observed after E_2 treatment in the eel. A correspondence between the two cell types of the mature male and female eels, observed with light microscope, and the various aspects detected in electron micrographies of immature eels treated with E_2, is difficult to establish, mainly as gonadotrophs do not differentiate simultaneously.

Despite the lack of gonadal response, it is evident that E_2 exerts a positive feedback on gonadotrophs of immature eels: their ultrastructure suggests an active hormonal synthesis. However, we do not know whether this feedback acts directly on the pituitary gland or through a hypothalamic stimulation.

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