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Glycoprotein Gonadotropin in the Plasma and Its Cellular Origin in the Adenohypophysis of Sham-operated and Ovariectomized Rainbow Trout, *Salmo gairdneri*

Leonie J.A. Van Putten, J. Peute, P.G.W.J. Van Oordt, H.J.Th. Goos, and B. Breton*

Zoological Laboratory, Section Comparative Endocrinology, University of Utrecht, The Netherlands

* Laboratoire de Physiologie des Poissons, I.N.R.A., Rennes, France

Summary. Among the cells of the pituitary generally believed to produce glycoprotein gonadotropin (GTH) five forms were distinguished, based on the amount and the diameter of granules and globules and the appearance of the rough endoplasmic reticulum. In sham-operated trout so-called “globular” cells predominated, whereas after ovariectomy these were replaced by so-called “cisternal” cells, suggesting that both belong to one GTH-cell type. In addition, ovariectomy caused a strong increase in plasma GTH-levels. This indicates that the transition from globular to cisternal cells is accompanied by extrusion of GTH, and thus points to a storage of GTH in the granules and globules. It is argued that one of the five forms has the morphological characteristics of thyrotropic cells and may not produce glycoprotein GTH.

Key words: Pituitary salmonids – GTH-cells – Ultrastructure – Ovariectomy – Radioimmunoassay

Cells producing gonadotropic hormone (GTH) can be found throughout the adenohypophysis of vertebrates, although very seldom in the pars intermedia. They are basophilic, staining, e.g., with periodic acid-Schiff, alcian blue and aldehyde fuchsin. These cells are also characterized by irregular cisternae of the rough endoplasmic reticulum (RER); in many vertebrates they not only contain secretory granules but also large globules. Both granules and globules are storage forms of secretory material (see reviews by Holmes and Ball 1974; van Oordt 1979). This also holds true for teleost fishes, where such GTH-cells tend to concentrate in the ventral

Send offprint requests to: Prof. Dr. P.G.W.J. van Oordt, Zoological Laboratory, Transitorium III, Padualaan 8, 3508 TB Utrecht, The Netherlands

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border of the proximal pars distalis (p.p.d.) (for reviews, see van Oordt 1968; Ball and Baker 1969; Schreibman et al. 1973). In salmonids these "globular" basophils are not restricted to the p.p.d., but are also situated between the follicularly arranged prolactin cells in the rostral pars distalis (r.p.d.) (Cook and van Overbeeke 1972; Nagahama 1973; Boddington 1975; Oliverau 1976, 1978; Ekengren et al. 1978b,c; Peute et al. 1978; Ueda 1980). During the annual cycle of the rainbow trout the globular GTH-cells predominate some months after spawning (Peute et al. 1978). Storage of GTH within these cells could be demonstrated by means of immunohistochemical techniques, using anti-carp and anti-salmon glycoprotein GTH (McKeown and van Overbeeke 1971; Ekengren et al. 1978b,c). The contents of both the secretory granules and globules react to such anti-GTH-serum (Leunissen et al. 1980).

In addition to the globular GTH-cells, "vesicular" or "cisternal" cells can be found among the basophils of the salmonid pars distalis. Cook and van Overbeeke (1972), Oliverau (1976, 1978), and Ueda (1980) considered the latter to be a separate type of GTH-cell, whereas Ekengren et al. (1978b,c) and Peute et al. (1978) supposed the globular and the cisternal basophils to represent different stages of a single type of GTH-cell. In an attempt to resolve these conflicting views, in the present study the percentages of the globular and cisternal cells were correlated with the plasma levels of glycoprotein GTH in sham-operated and ovariectomized rainbow trout (*Salmo gairdneri*).

Materials and Methods

The fish were obtained from a hatchery in Vaassen, Holland. Nine mature females weighing 700–1000 g were used. On October 26th five of them were ovariectomized; four others were sham-operated. For ovariectomy the animals were anaesthetized in 0.1 % phenoxyethanol. The ovaries were exposed by a 5 cm ventral cut and removed in the experimental animals. The wound was closed with 4 to 5 stitches with chrom-catgut. During the operation the gills were perfused with a 0.1 % solution of the anaesthetic. Five weeks later the animals were anaesthetized with CO₂, and blood samples were taken, followed by decapitation. During dissection 8 % glutaraldehyde in 0.1 M Na-cacodylate buffer was dropped on to the pituitary. After removal of the pituitaries they were submerged in 4 % glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.2) at 0° C. After 1 to 2 h an equal volume of 2 % OsO₄ in 0.1 M Na-cacodylate buffer was added (0° C in the dark). After 45 min the tissues were postfixed in 2 % OsO₄ in 0.1 M Na-cacodylate buffer at 0° C for another hour. Subsequently, the tissues were dehydrated in graded alcohols and propylene oxide and embedded in Epon.

Sections of 1 µm thickness were cut on a Porter-Blum microtome using glass knives and stained with methylene-blue-azur II (Richardson 1960) for light microscopy. Ultrathin sections were cut on a Reichert OMU 3 ultramicrotome, collected on 200 mesh copper grids, stained with uranyl acetate and lead citrate (Reynolds 1963) and examined with a Zeiss EM 9A electron microscope.

At three times during the experimental period blood samples were taken, i.e., immediately before the operation, two weeks later and on the last day of the experiment. GTH-content of the blood samples was determined with a homologous radioimmunoassay for salmonid fishes, using *Oncorhynchus tshawytscha*-GTH (Breton et al. 1978) for label and reference, and an antibody directed against *Salmo gairdneri*-GTH (Breton et al. 1976). The antibody was raised in guinea pigs. The salmon-GTH was labeled with I¹²⁵, using NaI¹²⁵ IMS 300 from Amersham, England. Conditions of labeling and further purifications were the same as those described for carp-GTH radioimmunoassay (Breton et al. 1971). The antibody was used at a final dilution of 0.5 · 10⁻⁵, the incubation lasted 4 days at 4 °C; free and bound hormone was separated due to the second antibody method (rabbit sera directed against guinea pig globulins). Calculation was performed after logit-log transformation.

Fig1

Fig. 1. Classification of GTH-cells in the morphological categories 1–5, based on the amount and diameter of granules or globules and the presence of RER. *RER* rough endoplasmic reticulum; *gr* granule; *gl* globule

Fig2

Fig. 2. The percentage of cells in each category for each pituitary of ovarioectomized and sham-operated trout. Note the appearance of GTH-cells in the “cisternal” stage and the decrease of “globular” GTH-cells after ovarioectomy

Quantification of GTH-Cells

The putative GTH-cells were divided according to a subjective scale into five different categories on the basis of the amount and diameter of the granules and globules, and the appearance of the RER material (Fig. 1). With such a division it is possible to study the effect of ovarioectomy on the GTH-cells in a semi-standardized manner. The chosen sequence of these categories 1–5 is purely morphological and does not necessarily reflect five consecutive physiological stages.

Category 1 consists of cells with more or less strongly dilated RER cisternae and lacking any granules and globules.

Category 2-cells contain dilated cisternae of the RER and some round electron-dense granules with a diameter of 75–280 nm (average diameter 155 nm). These cells are also characterized by the presence of an active Golgi system and many mitochondria.

Category 3 consists of cells in which the area containing granules exceeds the area containing RER cisternae. These cisternae are not strongly dilated. The diameters of the granules measure between 90–280 nm (average diameter 166 nm). Most granules are round, although fusion may result in elongated forms. In cells of categories 2 and 3 globules are absent.

Category 4-cells contain some globules. These are oval or circular in shape, are less electron-dense than the granules and measure 500–1,200 nm. The granules measure between 90–500 nm (average diameter 260 nm). In these cells a considerable portion of the cytoplasm is filled with dilated cisternae of the RER. Most granules are circular in outline, but can become elongated by fusion. Coalescence of granules with globules, and of different globules with each other also occurs.

Category 5-cells are filled with granules and globules. The RER is poorly developed and consists of a few small and somewhat rounded cisternae. The globules and granules show the same characteristics as those of the category 4-cells.

For the quantification of the five categories in each pituitary, about one hundred presumptive GTH-cells were chosen from randomly selected electron-micrographs of the p.p.d., and classified. For each pituitary the percentages of cells in each category are given in Fig. 2.

Results

a) Ultrastructure of the GTH-Cells

In the sham-operated female trout at the end of the experiment $\pm 60\%$ of the presumptive GTH-cells belong to category 5 (Fig. 2), and are filled with numerous secretory granules and globules (Fig. 3). Of the remaining cells $\pm 25\%$ belong to category 3, only 2–9% to category 2, and 3–8% to category 4. It appears that in all pituitaries the cells belonging to category 3 show a tendency to be associated with branches of the neurohypophysis in the rostral region of the p.p.d., whereas the cells belonging to the other categories show a more random distribution. The pituitaries of the sham-operated trout did not contain cells in category 1, i.e., cells with cytoplasm largely occupied by dilated cisternae of the RER (Fig. 3).

On the other hand, in four out of five pituitaries of ovariectomized trout, as much as $\pm 75\%$ of the presumptive GTH-cells can be classified in category I (Fig. 4) and only 5–20% belong to category 5. Compared to the control group, the percentage of cells in category 2 had slightly increased to 7–16%, and that of cells in category 3 had decreased to 6–12%. Cells in category 4 remained at about the same percentage (Fig. 2).

The pituitary of trout nr 3123 differs from those of the other ovariectomized animals. It had a relatively low percentage (6%) of cells in category 1. Relatively high percentages of cells containing secretory granules and/or globules were found in the categories 2 (35%), 3 (22%), and 5 (35%).

In contrast to the situation in other cell types, where electron-dense vesicles are found in the Golgi area, most Golgi vesicles in the GTH-cells are electron lucent.

b) Plasma Levels of GTH (Table 1)

The plasma levels of GTH of all animals at the start of the experiment varied between 3.20 and 4.22 ng GTH per ml plasma. In sham-operated trout this level did not change during the experimental period. However, the ovariectomized animals

Fig. 3. GTH-cells in the p.p.d. of the adenohypophysis of a sham-operated trout. Most of the cells are so-called category 4- and 5-cells, i.e., predominantly containing secretory granules and globules. *GTH 3*, 4, 5 gonadotropic cells belonging to categories 3, 4 and 5; *STH* somatotrophic cell; *gr* granule; *gl* globules; *sc* stellate cell. $\times 5,400$

Fig. 4. GTH-cells of an ovariectomized trout. Most of the cells are so-called category 1-cells, i.e., in the cisternal stage. *GTH 1* gonadotropic cell belonging to category 1; *TSH?* putative thyrotrophic cell. $\times 5,400$

Table 1. GTH-content of the blood plasma immediately before the operation, two weeks later and on the last day of the experiment

Treatment	Trout nr.	GTH ng/ml 26-10-'77	GTH ng/ml 10-11-'77	GTH ng/ml 30-11-'77
Sham-operated	3102	*	3.48	*
	3103	*	4.18	2.77
	3107	*	3.20	3.58
	3112	*	4.22	3.45
Ovariectomized	3118	4.13	29.44	16.63
	3123	4.07	19.07	5.28
	3126	5.13	13.90	15.35
	3127	4.47	14.76	15.81
	3136	3.61	*	15.49

* Not measured

showed a strong, although not homogeneous, response. In the animal with the strongest rise after two weeks, the GTH-level had dropped from 29.44 to 16.63 ng/ml by the end of the experiment. Two other animals showed their highest GTH-level (15.35 and 15.81 ng) at the end of the experiment. The final GTH-level in the plasma of four ovariectomized animals was almost identical. In the fifth animal of this group (nr 3123) the GTH-level rose over a period of two weeks to a maximum (19.07), but dropped to about control levels (5.28 ng) during the last weeks of the experiment.

Discussion

The low plasma levels of glycoprotein GTH found in female trout at the start of the experiment in October and in the sham-operated controls at the end of the experiment in November correspond to the levels measured by Billard et al. (1978). During this period, preceding the profuse secretion of glycoprotein GTH necessary for the final stages of oocyte maturation and ovulation (Billard et al. 1978; van den Hurk and Peute 1979), the pituitary is probably storing GTH rather than secreting it. The high percentage of cells filled with numerous granules and globules in the control group is in accordance with this concept. Moreover, recent immunocytochemical studies have shown the presence of glycoprotein GTH inside these granules and globules (Leunissen et al. 1980). These data confirm earlier observations on the trout by Peute et al. (1978), who found a positive correlation between a high GTH-content in the pituitary and the presence of many GTH-cells with granules and globules.

Within a period of two weeks the concentration of GTH in the plasma of the ovariectomized animals rose to levels exceeding the maximum normally found during ovulation (Billard et al. 1978). This may partly be due to an abnormally high GTH-secretion by the pituitary, but also partly due to the reduction in number of GTH-binding sites after removal of the ovaries. Twenty days later the plasma GTH-concentration remained high in four ovariectomized trout and was accompanied by a strong decrease in the percentage of category 5-cells, caused by

degranulation, and by a comparable increase in the percentage of category 1-cells, representing actively secreting and/or synthesizing cells. Thus, it appears that after ovariectomy the majority of the category 5-cells transform into category 1-cells. This was not the case in one of the ovariectomized animals; it showed a relatively low percentage of category 1-cells, a higher percentage of category 5-cells, and a low plasma level of GTH. All these observations seem to confirm the conclusion of Ekengren et al. (1978b,c) and Peute et al. (1978) for *Salmo salar* and *Salmo gairdneri*, respectively, that the so-called category 1-cells are exhausted forms of the glycoprotein GTH-cells rather than a separate cell type. The presence of category 2- and 4-cells is additional evidence for the existence of only one glycoprotein GTH-cell type, since they may be regarded as intermediate forms, not essentially differing from the category 1- and 5-cells. They display similar forms of RER cisternae as in category 1-cells, and the category 4-cells show the same type of secretory granules as the category 5-cells. Moreover, the categories 4 and 5 contain identical globules.

Also in other teleosts a solitary type of GTH-cell contains large amounts of granules and globules during hormone storage and an abundance of dilated RER cisternae shortly after hormone secretion: *Gasterosteus aculeatus* (Föllénus 1968; Leatherland 1970; Benjamin 1974), *Cymatogaster aggregata* (Leatherland 1969), *Oncorhynchus nerka*, *O. keta* (Nagahama 1973), *Oryzias latipes* (Kasuga and Takahashi 1970), *Gillichthys mirabilis* (Zambrano 1971), *Carassius auratus* (Nagahama 1973; Kaul and Vollrath 1974; Lam et al. 1976), *Poecilia latipinna* (Batten et al. 1975), *Anguilla japonica* (Ueda and Takahashi 1978) and *Rhamdia hilarii* (Val-Sella and Sesso 1980). Recently Lindahl (1980) recognized four different forms of one type of GTH-cell in the Atlantic salmon (*Salmo salar*). This author also combined on the one hand the presence of granules and globules with storage and a low rate of release of secretory products, and on the other hand degranulation and vacuolization with a high level of production and release of GTH.

Other authors have also described GTH-cells possessing globules and cells containing cisternae, but have considered these to represent two different cell types. The correlation between the presence of "globular" and "vesicular" gonadotrops, respectively, and different physiological activities in the gonads of *Salvelinus leucomaenis*, together with the absence of intermediate stages, favored the suggestion that two types of glycoprotein GTH-cell exist in this species (Ueda 1980). In *Oncorhynchus nerka* Cook and van Overbeeke (1972) described in addition to globular cells "vesicular" gonadotrops, derived from cells filled with secretory granules during gonad development (Chestnut 1970).

In *Gasterosteus aculeatus* Slijkhuis (1978) found two separate types of GTH-cells, differing in electron density of the cytoplasm. At the light microscopical level, Olivereau (1976, 1978) observed two forms of GTH-cells in *Salmo salar*, *S. gairdneri* and *S. fario*. Interestingly, the second type of GTH-cell in these species differs from the first by the relatively small size of its secretory granules. A similar difference in granule size between two separate types of presumptive GTH-cells has been observed in *Zoarcetes viviparus* (Öztan 1966), *Anguilla anguilla* (Knowles and Vollrath 1966), *Carassius auratus* (Leatherland 1972), *Tilapia mossambica* (Bern et al. 1974), *Misgurnus anguillicaudatus* (Ueda and Takahashi 1977, 1980), and *Rutilus rutilus* (Ekengren et al. 1978a).

Relatively small granules also characterize the category 2- and 3-cells of *Salmo*

gairdneri. As mentioned above, the category 2-cells may belong to the glycoprotein GTH-cell type on the basis of similarities in the appearance of their RER with that of the category 1- and 4-cells. This is, however, not the case with the category 3-cells. Moreover, the category 3-cells differ from the other categories by the fact that they do not show a random distribution, and are frequently associated with branches of the neurohypophysis in the rostral region of the p.p.d. Recent immunofluorescent studies on trout pituitaries using anti-human- β -thyrotropin (TSH) resulted in a specific reaction of cells showing the same location within the p.p.d. (unpublished results). This location and the small size of the granules measuring approximately 160 nm are common characteristics of TSH-cells (Nagahama 1973, 1977; Bern et al. 1974; Benjamin 1975). This does, of course, not implicate that all category 3-cells belong to the TSH-cell type. The percentage of category 3-cells diminished after ovariectomy in four out of five experimental animals. It might be argued that category 3-cells have a double function, secreting both TSH and GTH, as has been suggested for similar cells in *Oncorhynchus kisutch* by Chestnut (1970); however, it seems unlikely that they produce glycoprotein GTH, since according to Leunissen et al. (1980) the granules of such cells do not bind anti-carp- β -GTH. A combined immunocytochemical and electron-microscopical study on the rat pituitary pointed out that also in mammals some gonadotropic cells mimic the thyrotropic cells at the ultrastructural level (Tougaard et al. 1980). According to these authors, identification of all gonadotropic cells is difficult without application of immunocytochemistry.

Recent biochemical studies point to the production of two different gonadotropic hormones in teleosts, including the salmonid *Oncorhynchus keta* (Ng and Idler 1978; Idler and Ng 1979), only one hormone being a glycoprotein. It is tempting to postulate that these two hormones originate from morphologically different cell types. However, from a comparative endocrinological point of view it seems more logical to suppose that the two gonadotropic hormones are produced by one and the same cell type (cf. van Oordt 1979). This supposition as well as the exact nature of the category 3-cells requires further clarification.

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