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Accumulation of glycoprotein gonadotropin in the pituitary of juvenile rainbow trout in response to androgens and C21-steroids, including 11-steroids

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Summary. Effects of steroids on the accumulation of glycoprotein gonadotropin (GTH) in pituitaries of juvenile trout were investigated by means of scanning cytophotometry applied to immunocytochemical preparations, and with the use of a radioimmunoassay. Effects on other aspects of GTH-cell activity were analyzed by measuring the size of the gonadotrops and their nuclei.

Progesterone added to aquarium water and methyltestosterone incorporated into the food showed a pronounced stimulatory effect on the accumulation of GTH. To a lesser extent, treatment with cortisol, cortisone, and desoxycorticosterone acetate administered to aquarium water, and 11β-hydroxy-androstenedione added to the food resulted in an increase of the hypophysial content of GTH. Steroids stimulating the accumulation of GTH in the pituitary also exhibited a positive effect on GTH-cell activity as indicated by an increase in the size of gonadotropic cells. Progesterone incorporated into the food did not influence the GTH-content and the GTH-cell activity. It is suggested that the route of administration of an exogenous steroid is essential for its effect on GTH cells in trout.

Comparison of GTH values reveals an excellent correlation between the data from the radioimmunoassay and those from the corresponding densitometric measurements. No correlation was observed between values of morphometrically determined GTH-cell activity and the densitometric values reflecting hypophysial GTH content.

Key words: Gonadotropin – Gonadotropic cells – Pituitary – Steroid hormones – Teleosts (trout)

In juvenile rainbow trout, *Salmo gairdneri,* exogenous testosterone stimulates the synthesis and storage of glycoprotein gonadotropin (GTH; Gielen et al. 1982a, b). This enhanced production of GTH is accompanied by morphological changes in the gonadotrops, such as enlargement of cells and nuclei and the increase in number of secretory granules, development of the Golgi complexes, and the appearance of large globules. Steroid hormones that exert such a stimulatory effect on the gonadotrops of juvenile fish have been shown to be aromatizable androgens and estrogens (for references, see van den Hurk 1982a; Gielen et al. 1982b). Moreover, administration of progesterone and 17α-hydroxy-progesterone results in an accelerated morphological development of the gonadotrops (van den Hurk 1982).

Progestins, androgens and estrogens can be synthesized by the gonads of juvenile trout, dependend on the time of development (van den Hurk et al. 1982b; van den Hurk and Lambert 1982). Furthermore, in juvenile trout, as demonstrated by Gielen et al. (1982a, b), the gonads respond to exogenous GTH by accelerating gametogenesis and increasing the secretion of steroid hormones. These steroids in turn stimulate the synthetic activity and morphological development of the gonadotropic cells. Gielen et al. (1982a, b), furthermore, showed that administration of GTH to castrated fish did not affect the gonadotrops, indicating that steroids of gonadal origin are involved in stimulating GTH-production and morphological development of the gonadotrops. However, gonadal steroids are not necessarily the only steroid hormones influencing the gonadotropic cells in the pituitary. Indeed, ablation of the gonads from underyearling juvenile trout did not prevent the pituitaries to accumulate GTH 18 months later, when sham-operated and control animals were in the first reproductive cycle (Gielen, unpublished results). Probably, non-gonadal steroids were responsible for the accumulation of GTH in the castrated fish. Therefore, the effects of corticosteroids should be studied in comparison with the effects of gonadal steroids on the gonadotropic cells. In doing so, the effects of steroids with and without a functional group at the 11-position have to be compared with each other since 11-steroids are non-aromatizable (Engel 1975), and there are indications that only estrogens and aromatizable androgens can stimulate GTH accumulation in the pituitary of immature trout (Crim et al. 1981).

The present study deals with the effects of cortisol, cortisone, desoxycorticosterone acetate (doca), progesterone, 11β-hydroxy-androstenedione and methyltestosterone on the accumulation of GTH and on the size of GTH-cells and their nuclei in the pituitary of juvenile rainbow trout. Analysis of gonadotropin content in the pituitary was carried out by means of scanning cytophotometry applied to immunocytochemical preparations (Terlou et al. 1983), and with the use of a radioimmunoassay. In addition, the size of gonadotrops and of their nuclei was determined by measuring the area of the cells and nuclei, respectively.

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Materials and methods

Animals

Fertilized eggs of rainbow trout were obtained from a Dutch hatchery and incubated in aquaria at ±11° C under a constant light regime of 14 L:10 D and a continuous flow of copper-free tapwater. Hatching occurred at day 28 after fertilization. Before treatment, fry were reared under similar conditions as the eggs. First feeding started at day 46, and frey were fed 4% of their body weight daily. The food was divided into three daily rations given at intervals of 3 h.

Steroid treatments

Experiments concerning the effects of steroids on the gonadotrops were carried out using batches of 200 rainbow trout. In a first series of experiments, fish were treated with steroid added to aquarium water ("water experiment"). Equimolar doses of progesterone (26 mg), desoxycorticosterone acetate (doca; 30 mg), cortisol (30 mg) and cortisone (30 mg) were suspended in 50 ml water containing 50 µl Triton X-100 as emulgator, and then administered to 100 l aquarium water from day 41 to day 69 after fertilization.

In pilot studies, demonstrating the hypophysial GTH-content and the GTH-cell activity, data from untreated fish were identical to those of Triton X-100-treated animals. Therefore, in the present study animals treated with Triton X-100 (50 µl/100 l) were used as controls. During the treatment the animals were kept in stagnant aerated water. The aquarium water was refreshed twice a week. Before refreshing the water, the aquaria were thoroughly cleaned and flushed with running water for 3 h. During the treatment the water temperature increased from 11 to 15° C.

In a second series of experiments, groups of 200 fish were treated with steroids incorporated into the food ("food experiment"). This treatment lasted for 8 weeks from the first feeding, i.e., from day 46, to day 105. Details for preparation of experimental diet and incorporation of steroids into the food are given by van den Hurk (1982). Methyltestosterone was added to the food in concentrations of 6 µg/g. From a previous study (van den Hurk 1982) it appeared that a 6 µg dose of 11β-hydroxy-androstenedione was ineffective in stimulating the GTH-cells. For this reason in the present experiments the steroid was used in a concentration of 60 µg/g. The dose of progesterone was 600 µg/g, since results of preliminary experiments did not reveal any effect on the gonadotrops when doses of 6 µg/g and 60 µg/g were used. Animals receiving a diet that differed only in the omission of steroids were used as controls. During the period of treatment each batch of experimental animals was kept in a 200 l aquarium with running copper-free water at ±11° C.

Histomorphometry and immunocytochemistry

From each experimental group five randomly selected fish were sacrificed directly after treatment by decapitation during anesthesia with 0.03% 2-phenoxy-ethanol. The lower jaw was removed and the heads were fixed in Bouin-Hollande fluid for 48 h. After rinsing in tapwater for half a day, they were decalcified for 2 days in 7% HNO_3. Following dehydration, the heads were embedded in paraffin. Five µm sagittal sections were cut and mounted on gelatin-coated slides.

For histomorphological investigation of GTH-cells and their nuclei sections were stained immunocytochemically according to the peroxidase-antiperoxidase (PAP)-method of Sternberger (1974). As first antibody an antiserum against the β-chain of carp gonadotropin (anti-β-carp GTH) was used. The antiserum was diluted 1:4,000 with 0.05 M Tris HCl (pH 7.6); incubation for 48 h at 4° C. As second antibody the donkey-antirabbit-γ-globulin (Wellcome Reagents Ltd, England) was used in a dilution of 1:25; incubation for 60 min at room temperature. PAP (Dako, Copenhagen, Denmark) was applied in a dilution of 1:200; incubation for 60 min at room temperature. The staining solution consisted of 0.05% 3,3′-diamino benzidine (DAB; BDH Chemicals Ltd, England) in 0.05 M Tris-HCl (pH 7.6) containing 0.01% H_2O_2.

To demonstrate thyrotrops and to avoid that these cells are included in the GTH-cell measurements, adjacent pituitary sections were immunostained with an antiserum against the β-chain of human thyrotropic hormone (Immuno Nuclear Corp, England) as primary antibody in a dilution of 1:4,000.

Control reactions were carried out with non-immune serum.

All DAB-positive GTH-cells, distributed in the centralmost section of the pituitary, and their nuclei were drawn at a magnification of 1800×. Total area of the cells and of the nuclei was determined with a digitizer (Hewlett-Packard 9864 A) in combination with a calculator (Hewlett-Packard 9800). After logarithmic transformation, the histomorphological values obtained were tested for statistical significance with one-way analysis of variance with subsampling (Steel and Torrie 1960).

Pituitary sections adjacent to those used for immunocytochemistry were stained with Alcian blue-periodic acid/Schiff-orange G (AB-PAS-OG; Herlant 1960) for histological examination.

Scanning cytophotometry

Every sixth sagittal section through the pituitary was selected for immunocytochemistry and subsequent quantitative study of the content of gonadotropic hormone content. The double antibody immuno-enzyme-cytochemical PAP-technique was applied for demonstration of the GTH-cells.

Since even with anti-β-carp GTH no specific immune reaction with the GTH-cells could be obtained (also the TSH-cells showed a weak but clear immune reaction), the more readily available anti-αβ-carp GTH was chosen as first antiserum (dilution 1:40000). To obtain equal immunoreaction conditions, all sections were incubated with the same batch of diluted antiserum. The sections on upside-down turned slides were in contact with a thin layer of antiserum (first, second and PAP, respectively); approximately 250 µl per slide (modification of the method of Tung 1977).

The amount of brown precipitate of oxidized DAB was measured with the scanning cytophotometrical method as described by Terlou et al. (1983). The latter study also describes the procedure to extract those densitometrical values that represent immunoreactive gonadotropin in gonadotrops and not those of cross-reacting materials in other cells. Absorbance values reflecting a GTH-content in the

pituitaries were calculated per pituitary and as the sum of the total absorbance of fish belonging to an experimental group.

Data were analyzed for statistical significance with the Student's *t*-test. Differences were considered to be significant if $p < 0.05$.

Preparation of pituitaries for measuring GTH-content

From each group of the "water experiment" and of the "food experiment", twenty or six fish, respectively, were sacrificed. After anesthesia in 2-phenoxy-ethanol 0.03%, animals were decapitated and their pituitaries removed, quickly frozen over solid carbon dioxide and stored at −80° C. Following storage, pituitaries from each experimental group of fish were pooled and then extracted in 0.2 ml phosphate-buffered saline (PBS; 0.01 M; pH 7.5) at 0° C. Samples were centrifuged for 20 min (5,000 × g), supernatants were collected and frozen, the pellets resuspended in 0.1 ml PBS and stored overnight at 4° C. The next morning these samples were shaken and recentrifuged, supernatants were collected, added to those collected the day before, and frozen. In these extracts the GTH-content was measured by means of a radioimmunoassay.

Radioimmunoassay for GTH

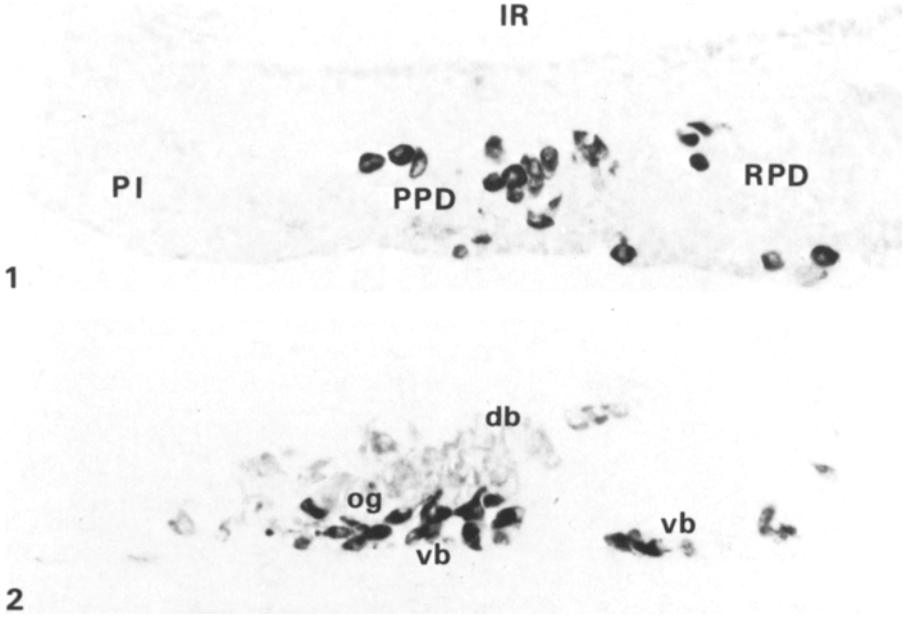
The glycoprotein gonadotropin content of pituitaries was measured with the use of the heterologous radioimmunoassay described by Gielen and Goos (1983a). Salmon GTH (Con-A II fraction, Dr. Idler, St. John's, Newfoundland, Canada) was used for labeling and as standard preparation. As primary antibody an antiserum against SG-G100 salmon gonadotropin was applied in a final dilution of $0.5 \cdot 10^{-5}$, and as second antibody the Wellcome donkey-anti-rabbit serum in a final dilution of 1:240. Since a heterologous system has been used, all data of the assay have to be considered as relative values. Therefore, the amount of GTH detected in the pituitary extracts is expressed in assay units (AU) instead of weight units (ng). Calculation was performed after logit-log transformation.

Results

Histological localization and characterization of GTH- and TSH-cells in the pars distalis of juvenile trout have recently been described (van den Hurk 1982; Gielen et al. 1982b; Gielen and Goos 1983a; van Putten et al. 1983). Cells staining equally with Alcian blue (AB) and periodic acid Schiff (PAS) are mainly distributed in the dorsal and to a lesser extend in the ventral pars distalis. These basophils show an intensive reaction with anti-β-human TSH (Fig. 1) and thus presumably are TSH-cells. Predominantly AB-positive and weakly PAS-positive cells are localized in the ventral part of the pars distalis and to a lesser extent in the rostral pars distalis. These ventral basophils apparently are GTH-cells, since they exhibit strong reactions with both anti-αβ-carp GTH (Fig. 2) and anti-β-carp GTH (Fig. 3). Dorsal basophils show a weak affinity for the carp antisera. When anti-αβ-carp GTH is used, the orange-G positive cells in the proximal pars distalis also contain some DAB precipitate.

The gonadotrops of control fish show a weak reaction with anti-β-carp GTH (Fig. 5). Addition of C21-steroids to the aquarium water results in a much stronger immunoreactivity (Figs. 3, 4). Measuring the amount of brown oxidized DAB precipitate in the gonadotrops by use of the scanning cytophotometrical method of Terlou et al. (1983), elevated mean and sum absorbance values are found in fish treated with C21-steroids (Table 1). GTH-cells are responsive to the steroids in the following order: progesterone > cortisol > doca > cortisone.

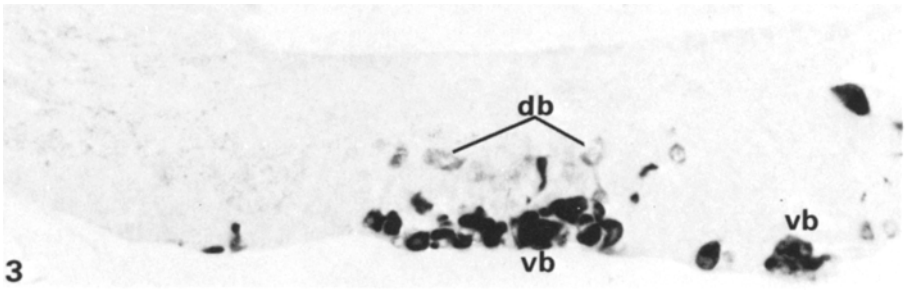
Methyltestosterone added to the food also evoked a strong immune reaction in the gonadotrops when anti-β-carp GTH was used (cf. Figs. 6, 7). In the densitometrical studies methyltestosterone shows a much more pronounced effect than 11β-hydroxy-androstenedione as indicated by mean and sum of absorbance values (Table 1). Although the mean absorbance value of fish treated with 11β-hy-



Figs. 1–7. Sagittal sections through the hypophysis of juvenile rainbow trout; *IR* infundibular recess; *PI* pars intermedia; *PPD* proximal pars distalis; *RPD* rostral pars distalis; *db* AB-PAS-positive cells, mainly located in the dorsal portion of the proximal pars distalis; *vb* AB-positive ventral basophils; *og* orange G-positive cells. × 350

Fig. 1. Dorsal basophils (*db*) reacting with anti-β-human TSH

Fig. 2. Cells reacting with anti-αβ-carp GTH. Progesterone treatment (administration to aquarium water). Weak reaction is present in dorsal basophils (*db*) and orange G-positive cells (*og*). The ventral basophils (*vb*) exhibit a strong reaction



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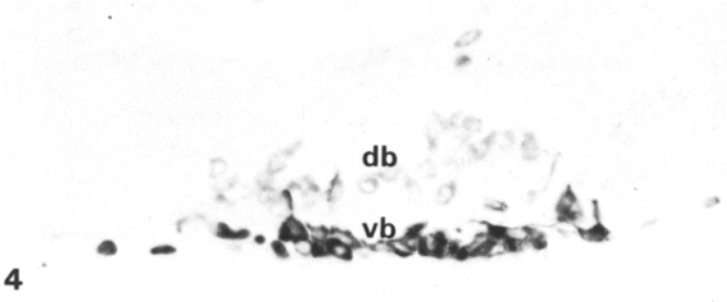

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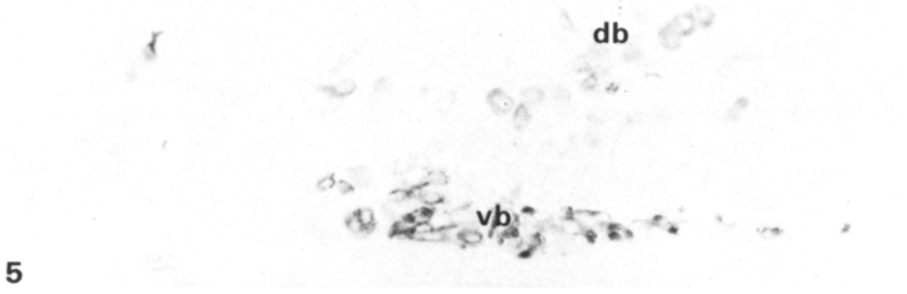

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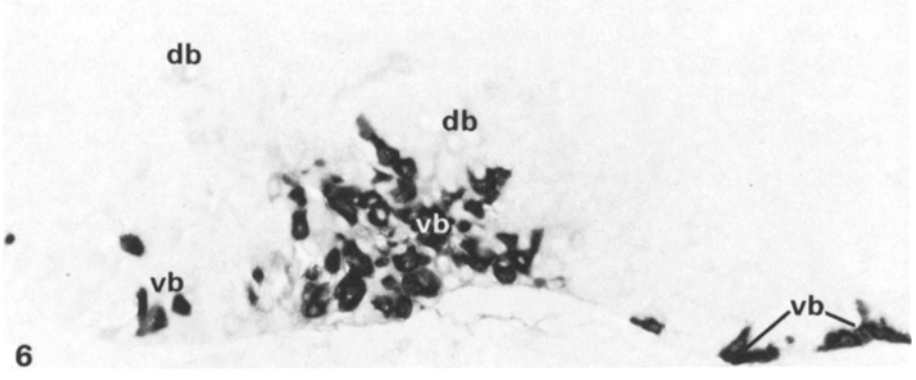
Figs. 3–5. Cells reacting with anti-β-carp GTH. Progesterone (**Fig. 3**), cortisol (**Fig. 4**), and control treatment (**Fig. 5**). Both steroids (added to aquarium water) caused an enhanced amount of immunoreactive GTH in the ventral basophils (*vb*). No change was observed in the dorsal basophils (*db*)



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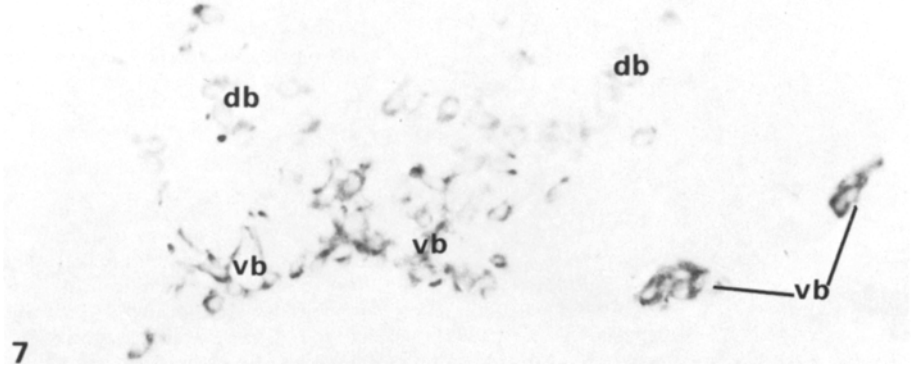

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Figs. 6, 7. Cells reacting with anti-β-carp GTH. Effects of methyltestosterone incorporated into the food (**Fig. 6**), and control treatment (**Fig. 7**). The steroid evoked a strong accumulation of immunoreactive GTH in the ventral basophils (*vb*)

Table 1. Effects of steroids on the hypophysial GTH-content determined by scanning cytophotometry

Treatment	Absorbance[a]/pituitary						
	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	SA[b]	MA+SEM[c]
"Water experiment"[d]							
control	0.40	0.00	0.40	1.35	0.82	2.97	0.59±0.23
progesterone	4.13	13.89	42.59	25.68	21.72	108.01	21.60±6.41*
cortisol	2.82	2.78	12.49	8.82	19.65	46.56	9.31±3.18*
cortisone	0.48	2.71	4.98	6.30	3.92	18.38	3.68±1.00*
doca	4.26	5.22	16.14	0.80	6.00	32.48	6.48±2.57*
"Food experiment"[e]							
control	0.92	0.00	0.40	0.00	–	1.32	0.33± 0.22
methyltestosterone	292.01	98.91	141.13	22.22	–	554.27	139.07±46.68*
11β-(OH)-adione[f]	19.90	0.90	1.05	12.29	–	34.15	8.53± 4.63
progesterone	0.00	1.28	1.47	0.46	–	3.22	0.80± 0.35

Asterisk indicates significant difference with the corresponding control group ($p<0.05$).

[a] Total absorbance of tissue spots with a light transmission lower than 40%.
[b] Sum of individual absorbance values, indicating the total absorbance values of an experimental group.
[c] Mean absorbance value ± standard error of the mean.
[d] Steroid added to aquarium water.
[e] Steroid incorporated into food.
[f] 11β-Hydroxy-androstenedione

Table 2. Effects of steroids on hypophysial GTH-content of pooled pituitaries from 20 and 6 juvenile trout ("water"- and "food experiment", respectively), measured by radioimmunoassay

Treatment	GTH-content in assay units
"Water experiment"[a]	
control	0.59
progesterone	15.75
cortisol	3.93
cortisone	2.01
doca	3.06
"Food experiment"[b]	
control	0.51
methyltestosterone	41.40
11β-hydroxy-androstenedione	2.34
progesterone	0.51

[a] Steroid added to aquarium water
[b] Steroid incorporated into food

Table 3. Effects of steroids on mean size of GTH-cells and their nuclei

Treatment	Mean size cells (μm² ± S.D.)	Mean size nuclei (μm² ± S.D.)
"Water experiment"[a]		
control	33.5±11.2	12.4±4.5
progesterone	42.5±16.4*	16.2±5.8*
cortisol	46.9±14.4*	15.6±4.7*
cortisone	42.7±14.7	15.1±5.3
doca	46.8±15.9	15.5±5.2
"Food experiment"[b]		
control	29.0± 9.4	9.4±3.3
methyltestosterone	41.4±12.9*	11.7±3.5
11β-hydroxy-androstenedione	34.1±10.6	11.6±3.8
progesterone	28.5± 9.2	9.2±3.3

Asterisk indicates significant difference compared to the corresponding control group ($p<0.05$)

[a] Steroid added to aquarium water
[b] Steroid incorporated into food

droxy-androstenedione is not significantly different from the control value, two out of four fish show enhanced absorbance values. The effect of progesterone added to the food was considerably smaller or completely absent.

In pituitary extracts from both groups of control fish GTH can hardly be detected by means of the applied radioimmunoassay (Table 2). However, the GTH-content was increased in pituitaries of fish treated with methyltestosterone and 11β-hydroxy-androstenedione added to the food, and in those of fish treated with progesterone, cortisol, cortisone and doca via the aquarium water. In the latter series of experiments GTH-cells are responsive to steroid stimulation in the same order as has been found in the densitometrical studies, i.e., progesterone > cortisol > doca > cortisone. In the "food experiment" the stimulating effect of methyltestosterone is far stronger than that of 11β-hydroxy-androstenedione, and progesterone has no effect at all on the pituitary GTH-content.

Comparison of the values from the radioimmunologically determinated pituitary GTH-content with the corresponding densitometric values revealed a clear correlation in both the "water" and "food experiment"; R = 0.979 and R = 0.998, respectively.

All steroids added to aquarium water are able to cause an increase in the mean size of the GTH-cells (Table 3). Also the mean size of the nuclei of these GTH-cells has increased. However, only fish treated with progesterone and cortisol show a mean nuclear size that significantly differs

from the control values. Among fish treated with steroids added to the food, the mean size of GTH-cells only significantly increases in those treated with methyltestosterone (Table 3). The slight increase in mean GTH-cell size of animals treated with 11β-hydroxy-androstenedione as well as the increase in nuclear size of GTH-cells from fish treated with methyltestosterone and 11β-hydroxy-androstenedione are not significantly different from these values in the corresponding control group. Administration of progesterone to the food does not result in histomorphometrical changes in GTH-cells.

In both experiments no correlation was found between the values of morphometrically determinated GTH-cell activity and the densitometrical values reflecting immunoreactive GTH in the pituitary.

Discussion

Gielen et al. (1982b) and Gielen and Goos (1983a, b) have shown that testosterone can stimulate the synthesis and storage of GTH in juvenile rainbow trout, but does not affect the limited release of GTH in these fish. The present experiments were carried out to study the effects of other steroid hormones on the synthesis and storage of GTH. Three different methods were employed: 1) morphometry of the GTH-cells and their nuclei, 2) scanning cytophotometry following immunocytochemical visualization of GTH, and 3) determination of GTH in pituitary extracts by radioimmunoassay. The morphometrical method is the oldest and most commonly used for studying changes in GTH-cell activity, such as brought about by exogenous steroids (e.g., Van den Hurk 1982; Olivereau and Olivereau 1979). However, this method has the disadvantage of being time-consuming, giving only limited information on the synthesis of the secretory products, and providing no information on the storage of the hormone. The radioimmunoassay, as employed for measuring the GTH-content in the pituitary of juvenile rainbow trout by Crim et al. (1981), Gielen and Goos (1983a) and others, cannot be applied for discriminating between the GTH-content of individual pituitaries of very young fish (Gielen and Van den Hurk, unpublished results). The densitometrical method, developed by Terlou et al. (1983), allows for the determination of differences in storage of GTH among individual pituitaries, even when the amount of radioimmunoassayable GTH is very low (cf. Tables 1, 2). In contrast to the morphometrical results of the present study, the densitometrically estimated hypophysial GTH-values correlated well with the GTH-content analyzed by the radioimmunoassay.

Methyltestosterone added to the food, like administration of the steroid via the aquarium water (Van den Hurk 1982), has a strong stimulatory effect on the size of the gonadotrops and on the accumulation of GTH. The increase in size of the cells, although not accompanied by a significant growth of their nuclei, points to an enhanced production of GTH. Indeed, in their cytophysiological studies, Gielen et al. (1982a, b, c) and Gielen and Goos (1983a) have shown that in juvenile trout testosterone treatment leads to synthesis and subsequent storage of glycoprotein gonadotropin. The increased production of GTH following a testosterone treatment could also be demonstrated after injection of radiolabeled amino acids into testosterone-treated and testosterone-untreated juvenile trout (Gielen and Goos, unpublished), i.e., immunoprecipitated GTH from pituitary extracts of fish treated with testosterone contained a much higher radioactivity than GTH from pituitary extracts of control animals.

The present data also show a strong positive effect of progesterone on the size of the GTH-cells and their nuclei, and on the GTH-accumulation in the pituitary, when it was added as a suspension to the aquarium water. This observation corresponds to previous results obtained with this steroid and with 17α-hydroxy-progesterone (van den Hurk 1982). Progesterone added to the food, however, has no effect on the GTH-cells. These observations are in agreement with data from Crim et al. (1981), who implanted progesterone intraperitoneally. Thus, in the trout, the mode of administering the steroid seems to be essential for demonstration of a possible stimulating effect on GTH-cells. Apparently, progesterone taken in by the alimentary tract or injected intraperitoneally is subjected to degradation by the liver, more so than after its uptake by the gills. This is also true for cortisol, as can be illustrated by the observations of Crim et al. (1981) that cortisol has a positive effect on GTH-accumulation when the steroid is implanted into the pituitary gland, but has no effect after perivisceral administration. The artificial androgen, methyltestosterone, does not seem to undergo rapid degradation, not even when administered via the alimentary canal.

Sex steroids with a functional group at the 11-position are not aromatizable (Engel 1975). Crim et al. (1981) suggested that only estrogens and aromatizable androgens were able to stimulate the accumulation of GTH in the pituitary of immature trout. In the same study, however, these authors also reported a limited but significant stimulatory effect of 11-ketotestosterone and 11β-hydroxy-testosterone, which they ascribed to contaminations of the preparations by aromatizable steroids, or to the very high doses used. The latter explanation could not be verified, since steroid levels were not measured. On the other hand, the present results indicate that 11β-hydroxy-androstenedione, when added to the food, may lead to an increase in synthesis and storage of GTH. Unfortunately, the effects varied and were not statistically significant. However, in gonadectomized sockeye salmon (*Oncorhynchus nerka*) activation of the gonadotrops could be induced not only by methyltestosterone but also by 11-ketotestosterone (Van Overbeeke and McBride 1971).

In the rainbow trout, 11β-hydroxy-androstenedione appears to be a male sex hormone. When added to the food it can exert a strong masculinizing effect on gonadal sex differentiation (Van den Hurk and Lambert (1982). Moreover, between day 50 and ay 100 after fertilization the testes develop the capacity to synthesize 11β-hydroxy-androstenedione (Van den Hurk et al. 1982a, b; Van den Hurk and Lambert 1982). This period coincides with the appearance of GTH-cells (Van den Hurk et al. 1982a; van den Hurk 1982). However, this is not restricted to males, and ovaries cannot produce 11β-hydroxy-androstenedione. Therefore, it is unlikely that 11β-hydroxy-androstenedione is involved in the initial differentiation of the GTH-cells. This is also true for other androgens and for oestrogens, which according to Crim et al. (1981) can be used in inducing GTH-accumulation in the pituitaries of juvenile trout. These gonadal hormones cannot be synthesized before some time after the differentiation of the gonads and the GTH-cells, and thus could only contribute to a stimulation of the production of GTH after these cells have become manifest. Proges-

tins, like progesterone, however, can be synthesized before day 50 after fertilization, and thus a role of such steroids in the initial differentiation of the GTH-cells cannot be excluded.

From the corticosteroids only cortisol has been found so far to accelerate GTH-accumulation in immature trout (Crim et al. 1981). In another teleost species, *Anguilla anguilla,* Olivereau (1972) reported slightly stimulated gonadotrops after treatment of silver eels with cortisol. In the present study not only cortisol, but also cortisone and doca were effective in stimulating the production of GTH. This effect of the corticosteroids is smaller than that of progesterone, and furthermore, the response to cortisol is somewhat more pronounced than that to the other two corticosteroids. However, the present data do not provide conclusive information on differences between the effects of the steroids used, since there is a strong variability in the morphometrical data and in the GTH-content of individual pituitaries, as measured with scanning cytophotometry.

In interrenal tissue of teleosts progesterone is not an obligatory intermediate in the biosynthesis of cortisol (Idler and Truscott 1972). Synthesis of progesterone in gonads of juvenile trout has, however, been demonstrated as an intermediate in steroid biosynthesis from day 50 after fertilization (van den Hurk et al. 1982b). It is unknown whether progesterone is present in the circulation of juvenile trout. If so, the levels apparently are not sufficient for a strong stimulation of GTH-accumulation, since control fish in the present study have low GTH-values.

Steroidogenic activity can be visualized in interrenal tissue of juvenile trout at a stage before it can be demonstrated in gonads (van den Hurk et al. 1980). Barton et al. (1980), Bry (1982), and Rance et al. (1982) demonstrated the presence of cortisol in the circulation of juveniles. Furthermore, these authors showed that the levels strongly increase under stress conditions. Cortisol also appears to be the main corticoid in adult trout (Fagerlund et al. 1968; Baker and Rance 1981; Pickering and Pottinger 1983). Apart from cortisol, Hane and Robertson (1959) found cortisone in the circulation of rainbow trout. These corticosteroids have been demonstrated to stimulate GTH-cell development in the present study. Endogenous cortisol and cortisone thus might influence this process in juvenile fish, when present in suitable concentrations or under stress conditions.

Desoxycorticosterone has been considered a minor product of the teleost interrenal tissue (Idler and Truscott 1972), and has not yet been demonstrated in circulation. The positive effect of the artificial corticosteroid doca on GTH-accumulation thus is of minor physiological importance.

In-vivo and in-vitro experiments carried out by Gielen and Goos (1983a) and Fåhraeus-van Ree et al. (1983), respectively, indicated that in juvenile trout steroid hormones can act on the GTH-cells directly. These observations, however, do not exclude an effect of steroid hormones on centers in the brain that modulate the secretion of gonadotropin. The present results leave the possibility open that steroid receptors both in the pituitary and in the brain have an affinity toward a variety of steroids. Although in juvenile rainbow trout several steroids are able to stimulate GTH-cells to a greater or lesser extent, it is unlikely that they will all express such an effect under normal conditions, since not all of them will be present in the circulation during the development of the GTH-cells.

Experimental results of Gielen et al. (1982a, b) suggested that steroids of gonadal origin stimulate the synthesis and storage of GTH in the pituitary. However, long-term castration experiments indicate that pituitaries also accumulate GTH in the absence of the gonads (Gielen, unpublished). In these experiments rainbow trout were castrated at a juvenile stage. When control and sham-operated fish entered the first reproductive cycle, they accumulated GTH in their pituitaries. A similar amount of GTH was stored in the hypophyses of the castrated fish. Considering the present results, it is likely that the stimulated production of GTH in the gonadectomized fish is caused by steroids from the interrenal tissue. On the other hand, the production of GTH may also be stimulated by non-steroid factors from the brain. Therefore, the role of brain hormones in stimulating the production of GTH will be investigated in future studies.

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