

**PITUITARY SOMATOTROPIC CELLS IN GOLDFISH (*Carassius auratus gibelio* Bloch)
TREATED WITH OESTRADIOL DIPROPIONATE, HCG AND LHRH**

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We studied somatotropic (STH) cells in sexually mature female goldfish in the period following spawning (September). The animals were divided into four groups and given i/p injections of hormones during 3-4 days: I. 0.1mg Oe II. 50 IU HCG; III. 0.01 mg LHRH; IV. 0.1 mg physiological saline solution. The doses were administered per 100 g body weight daily, and the animals were sacrificed 24 h after the first, second or third injection of each substance. The results obtained showed that STH cells can be stimulated with oestradiol (Oe), gonadotropin (HCG), and gonadostimulin (LHRH), and that effect is probably always through steroidogenesis, i. e. oestradiol, directly or indirectly through LHRH or HCG.

INTRODUCTION

Somatotropic cells of Teleostea have been examined using light and electron microscopy by many authors. Studies using light microscopy mostly pertain to the localization and tinctorial characteristics of these cells in relation to changes that take place during the annual sexual cycle (Dimovska, 1971; 1977; Pavlović and Pantić, 1975; Hristiforov, 1978). Studies using electron microscopy describe the ultrastructural characteristics of these cells, and certain hormones are used to follow the activities of these cells, not only by observing the appearance of certain organelles but also the size and appearance of secretory granules (Leatherland, 1972; Kaul and Vollrath, 1974; Benjamin, 1975; Pantić and Sekulić, 1978; Chambolle et al., 1981).

The effect of oestradiol on STH cells of fish can be inhibitory (Kaul and Vollrath, 1974; Pantić and Sekulić, 1978) or stimulatory (Olivereau and Olivereau, 1979). Mammalian gonadotropin (HCG) administered to carp stimulates STH cells (Pantić and Sekulić, 1978). We did not find any data on the effects of LHRH on STH cells of fish in the available literature. That is why we decided to examine the effect of gonadotropin-releasing factor, in addition to the effects of the steroid hormone oestradiol and gonadotropin (HCG).

MATERIAL AND METHODS

Sexually mature female goldfish (*Carassius auratus gibelio* Bloch) were treated with different doses of different hormones in the period following spawning (in September). There was a total of 168 treated fish, weighing 50-90 g.

We divided the animals into four groups. The following hormones were administered i/p per 100 g body weight: I. 0.1 mg oestradiol dipropionate; II. 50 IU HCG; III. 0.01 mg LHRH, IV. 0.1 ml physiological solution. The fish were sacrificed 24 hours following the administration of the first, second or third injection of each substance. The pituitaries were fixed in Bouin and Bouin-Hollande sublimate and embedded in paraffin. Sections 5-6 μ m thick were stained using the following methods: Herlant, Gomori, Gomori-Gabe, MacConail, and Cleveland-Wolfe.

Microphotographs were made using Ortolux and Opton light microscopes. Pituitary cell volumes were measured using the Tonutti formula, and the values obtained were processed using the Student t-test.

RESULTS AND DISCUSSION

Somatotropic cells of the goldfish are located in the mesoadenohypophysis, i. e. in the pars distalis proximalae. In this region, there are also gonadotropic (GTH) cells and chromophobic cells in addition to the STH cells. STH and GTH cells are distributed in separate groups (Figure 1a) and STH cells are polymorphous: round, oval or polygonal (Figure 1b). Their nuclei are polymorphous, most often oval or round with one nucleolus each (Figure 1b). These cells are acidophilic and their cytoplasm shown an affinity to phloxine, azocarmine, erythrosin and orange G. The tinctorial characteristics of these cells are similar to those of prolactin cells (Janić, 1987; Dimovska, 1971, 1977; Pavlović and Pantić, 1975). In sexually mature shads and bleaks Pavlović and Pantić (1975) established that STH cells are the most active during spawning, and that their activity is considerably decreased following this period.

We decided to administer oestradiol, HCG and LHRH in the period after spawning (in September), and we obtained the following results by observed changes in STH cells:

I. Effect of oestradiol dipropionate. Following the first and second dose of oestradiol, STH cells were increased, and after the third injection their volume was significantly increased (Table 1, Figures 2a, b). Pantić and Sekulić (1978) administered repeated doses of oestradiol to sexually mature and immature carp (*Cyprinus carpio*) and observed that the size of STH cells was decreased and of LTH cells increased. Since STH and LTH cells have similar tinctorial characteristics and subcellular organization, the authors maintained that STH cells can be transformed into LTH cells, i. e. that both hormones can be synthesized in the same type of cell. Kaul and Vollrath (1974) performed studies using the electron microscope and established that STH cells of the crucian carp contained fewer secretory granules when oestradiol was administered. Contrary to these findings, Olivereau and Olivereau (1979) observed that STH cells were stimulated after the administration of oestradiol in the eel.

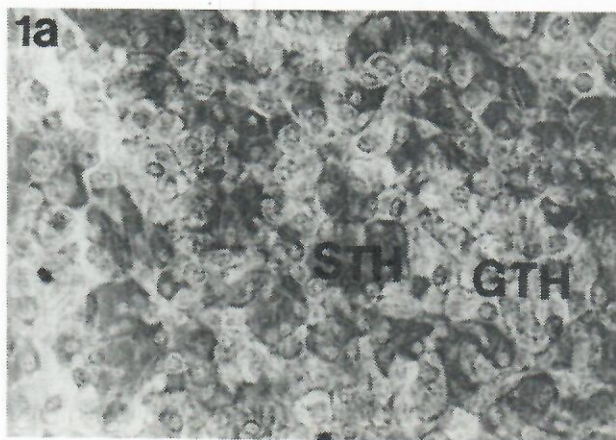


Figure 1a. Pars distalis proximalae of the goldfish hypophysis in September. STH and GTH cells, Gomori, 400 x.



Figure 1b. STH cells, Cleveland-Wolfe, 1000 x.

Table 1. The volumes of STH cells and their nuclei of goldfish in μm^3 ($\bar{X} \pm \text{SE}$) after treatment with oestradiol dipropionate

	STH cells	STH nuclei
Oe (1x)	613.71 \pm 83.61	102.15 \pm 3.32
Oe (2x)	620.18 \pm 15.94	130.47 \pm 23.09
Oe (3x)	769.29 ^p \pm 35.21	142.31 \pm 28.07
control	571.76 \pm 64.32	110.34 \pm 1.37

Values with superscripts p are statistically significant ($p < 0.05$)

II. Effect of HCG. No changes were observed in STH cells following the first injection. The second and third dose resulted in a significant increase in the cell volume (Table 2, Figure 3). Pantić and Sekulić (1978) established that

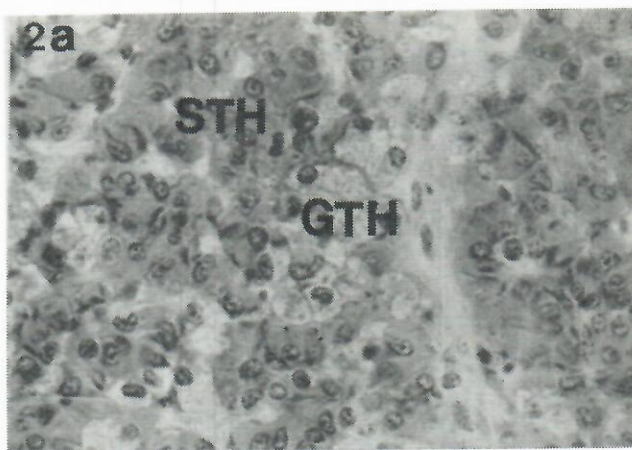


Figure 2a. Pars distalis proximalis of the goldfish hypophysis in September after the third Oe injection.
STH and GTH cells, Cleveland-Wolfe, 400 x.

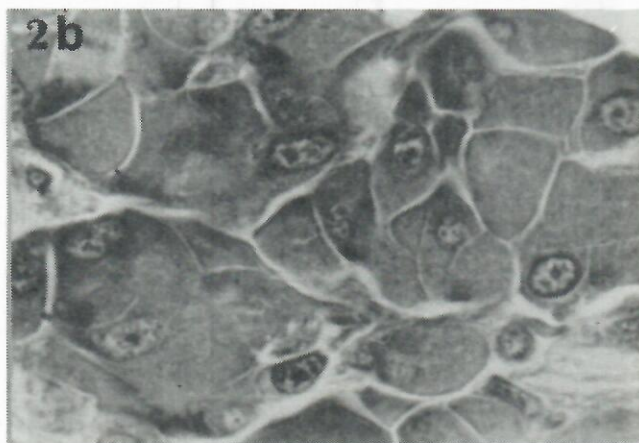


Figura 2b. STH cells, Cleveland-Wolfe, 1000 x.

STH cells of the crucian carp were changed as a result of stimulation after repeated doses of this hormone. The authors maintained that the effect is realized indirectly through steroidogenesis.

Table 2. The volumes of STH cells and their nuclei of goldfish in μm^3 ($\bar{X} \pm \text{SE}$) after treatment with HCG.

	STH cells	STH nuclei
HCG (1x)	537.64 \pm 3.88	115.60 \pm 0.62
HCG (2x)	681.54 ^P \pm 49.43	120.31 \pm 11.32
HCG (3x)	940.35 ^P \pm 35.85	141.90 \pm 23.53
control	571.76 \pm 64.32	110.34 \pm 1.37

values with superscripts p are statistically significant ($p < 0.05$)

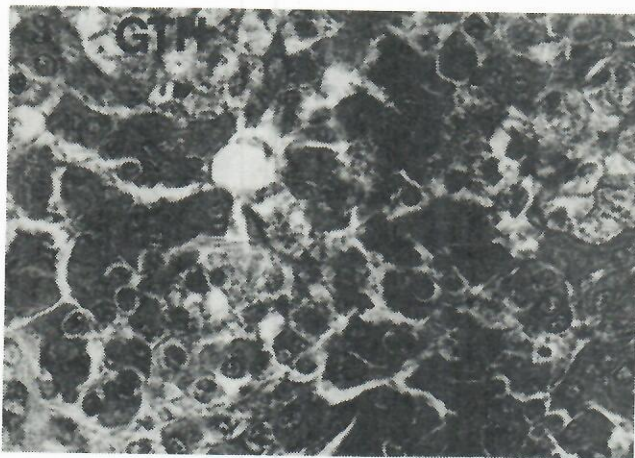


Figure 3. STH and GTH cells of the goldfish hypophysis in September after third HCG injection, Herlant, 400 x.

Table 3. The volumes of the STH cells and their nuclei of goldfish in μm^3 ($\bar{X} \pm \text{SE}$) after treatment with LHRH.

	STH cells	STH nuclei
LHRH (1x)	684.66 \pm 4.53	90.85 \pm 0.12
LHRH (2x)	720.99 \pm 31.53	116.46 \pm 34.97
LHRH (3x)	1169.11 ^p \pm 12.34	146.62 ^p \pm 2.05
control	571.76 \pm 64.32	110.34 \pm 1.37

Values with superscripts p are statistically significant ($p < 0.05$)

III. Effect of LHRH. Following the administration of a single dose of LHRH (0.01 mg), no changes were observed in STH cells compared with controls. Only after the third dose did a significant increase occur in the volume of these



Figure 4. STH cells of the goldfish hypophysis in September after third LHRH injection, Herlant, 1000 x.

cells Table 3, Figure 4). The nuclei and nucleoli of these cells became larger. Weil et al. (1980) discovered that LHRH stimulates steroidogenesis in the carp, so we can assume that in this case also, the effect of LHRH on STH cells probably takes place indirectly via steroidogenesis.

REFERENCES

1. Chambolle, P., Kah, O., Olivereau, M., Dubourg, P., Olivereau, J. 1981. Ultrastructure de l'hypophyse de *Gambusia* (poisson téléostéen) in situ et greffée a long terme. II. Pars distails proximale. *Gen. Com. Endoc.* 43, 85-95
2. Dimovska, A. 1971. Histološka struktura na dojranskata perkija (*Perca fluviatilis macedonica* Kar.). *Zavod za ribarstvo na SRM*, IV, 8, 1-31.
3. Dimovska, A. 1977. Données cytologique sur l'adenohypophyse de Perche de Doiran (*Perca fluviatilis macedonica* Kar.) dans certaines périodes du cycle sexuel. *Inv. Pesq.*, 41, 1, 15-32.
4. Hristoforov, O. L. 1978. Osobenosti stroenija i gistofiziologija sojki (*Boreogadeus soide* Lep.) Barenkova morja v godovom ciklu. *Trudi VNIRO*, CXXX, 46-60.
5. Janić, M. 1987. Prolactin cells of the pituitary gland of the Lake Doiran bleak during annual sexual cycle. *God. zbornik na PMF, Biol., Skopje*, 39-40, 129-136.
6. Kaul, S. and Vollrath, L. 1974. The Goldfish Pituitary. I. Cytology. *Cell Tiss. Res.*, 154, 211-230.
7. Leatherland, J. F. 1972. Histophysiology and innervation of the pituitary gland of the goldfish, *Carassius auratus* L.: a light and electron microscope investigation. *Can. J. Zool.*, 50, 835-844.
8. Olivereau, M. and Olivereau, J. 1979. Effect of estradiol on the cytology of the liver, gonads and pituitary, and on plasma electrolytes in the female freshwater eel. *Cell Tiss. Res.* 199, 431-454.
9. Pantić, V. and Sekulić, M. 1978. Pituitary prolactin and somatotropic cells of teleostea treated with gonadal steroids or choriogonadotropin. *Acta Veterin. (Beograd)*, 28, 2, 71-80.
10. Pavlović, M. and Pantić, V. 1975. The adenohypophysis in teleostea *Alburnus albidus* and *Alosa fallax* in different phases of sexual cycle. *Acta Veterin. (Beograd)*, 25, 4, 163-178.
11. Weil, G., Fostier, A., Horvath, L., Marlot, S., Berscenyi, M. 1980. Profiles of plasma gonadotropin and estradiol in the common carp, *Cyprinus carpio* L. as related to spawning induced by hypophysectomy or LHRH treatment. *Reprod. Nutr. Develop.* 20, 1041-1050.

**SOMATOTROPNE ĆELIJE HIPOFIZE KARAŠA (*CARASSIUS AURATUS GIBELIO* BLOCH)
TRETIRANE SA OESTRADIOL DIPROPIONATOM, HCG I LHRH**

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SADRŽAJ

Ispitivanje su STH ćelije polno zrelih ženki karaša u periodu posle mresta (septembar). Životinje smo podelili u četiri grupe i injicirali hormone i/p u toku 3-4 dana: I. 0.1 mg Oe, II. 50 IU HCG, III. 0.01 mg LHRH i IV. 0.01 ml fiziološkog rastvora. Ove doze su primili na 100 gr telesne težine svakog dana, a zatim su bile žrtvovane 24 časa nakon prve, druge, treće ili četvrte injekcije. Dobijeni rezultati su pokazali da se STH ćelije mogu stimulisati sa gonadostimulinom (LHRH), gonadotropinom (HCG) i estradiolom (Oe), a da je efekat verovatno uvek preko steroidogeneze, odnosno estradiola, ili direktnim delovanjem ili indirektnim efektom pomoću LHRH ili HCG.