'In vivo' effects of estradiol-17β in a freshwater fish, *Barbus conchonius* Hamilton

N. Khanna[1] and T. Singh

Department of Zoology, Kumaun University, D.S.B. Campus, Naini Tal 263002 (India), May 19, 1982

Summary. 'In vivo' effects of estradiol-17β were investigated in females of the teleost *Barbus conchonius* during the non-breeding phase. Fish treated with 25 µg estradiol-17 β/individual revealed significant hypoglycemia and a reduction in liver glycogen reserves. Plasma amino nitrogen, FFA, and organic PO_4 were elevated after estradiol therapy, whereas plasma cholesterol decreased. Liver cholesterol also fell in the treated fish. Estradiol-17β seems to play a crucial role in the initial stages of ovarian recrudescence by mobilizing energy reserves.

Estrogen therapy in mammals is known to induce hyperinsulinemia[2]. In fish, estrogen effects include an increase in plasma and hepatic lipid levels and a depletion of liver glycogen reserves[3,4]. In some teleosteans, the yolk granule phase of vitellogenesis is predominantly responsible for the growth and enlargement of the oocytes[5]. This particular phase of oogenesis results from oocyte accumulation of a blood-borne glycolipophosphoprotein (vitellogenin) which is produced by liver under estrogenic control[6]. The pivotal role of estrogens in sexual maturation makes it important to have as much information as possible on the metabolic effects of this hormone. This study was undertaken to quantify changes in blood and tissue metabolite levels following estradiol-17β therapy in *Barbus conchonius* Hamilton.

Material and methods. Adult females of *Barbus conchonius*, 4.5–5 g, were collected from Lake Naini Tal during October–November (early phase of oocyte growth) and held under laboratory conditions at 18±2 °C and natural photoperiod for 14 days prior to use. Experimental groups consisted of (a) fish receiving 5 µg estradiol-17β/individual/day for 5 days and killed 24 and 72 h after the last injection, and (b) fish receiving saline injections. Estradiol-17β (V.P. Chest Institute, C.S.I.R., New Delhi) was first dissolved in a small quantity of ethanol and then diluted with teleost saline to the desired concentration. Food provided ad libitum was withheld 48 h prior to killing. Blood and tissue samples were collected immediately after sacrifice from experimental and control groups for biochemical estimations. Plasma samples were analyzed for glucose[7], cholesterol[8], free fatty acids (FFA)[9], organic PO_4[10], and amino nitrogen[11]; glycogen[12] and cholesterol[8] in liver were also measured. The significance of the difference between control and experimental groups was evaluated by the t-test.

Results and discussion. Estrogen therapy in *Barbus conchonius* induced significant hypoglycemia ($p < 0.05$) 24 h after estradiol-17β administration. The fall in glycemia is probably caused by hyperinsulinemia since estrogens are known to exert an insulinotropic effect on the islet beta cells. In the golden shiner, *Notemigonus crysoleucas* and the goldfish, *Carassius auratus*, estradiol-17β treatment caused depletion of liver glycogen 'in vivo'[3,4]. In *Barbus conchonius* the liver glycogen reserve fell significantly at 24 and 72 h ($p < 0.05$). The effects of estrogens on liver glycogen could be important in mobilizing energy for reproductive processes. It appears that estradiol retards gluconeogenesis and glycogenesis by masking glycogenic effects of insulin[3].

Amino nitrogen and lipid metabolites in the blood and tissue of *B. conchonius* were distinctly altered in response to estradiol-17β treatment. Of the several parameters evaluated, a significant increase occurred in the plasma amino nitrogen ($p < 0.05$), FFA ($p < 0.01$), and organic PO_4 ($p < 0.05$) while a reduction was noticeable in the plasma ($p < 0.02$) and liver ($p < 0.05$) cholesterol.

Many investigators have reported depletion of body lipid reserves coinciding with gonadal maturation[13]. Liver seems to be the main target of ovarian steroids; estrogen treatment is known to induce liver hypertrophy[3] and increased liver and/or plasma lipid levels[14]. Considerable experimental evidence supports a role of estrogens in vitellogenesis in fishes[15,16] and pronounced effects of estradiol-17β on lipid metabolites in *B. conchonius* may be the result of an induction of vitellogenic activity. Earlier studies have also shown plasma cholesterol, phospholipid, protein, organic- and protein-bound PO_4 to be useful indices of vitellogenic activity[4]. The elevated amino nitrogen levels in the estrogen-treated *B. conchonius* reflect an increase in plasma free amino acids. This may be a prelude to incorporation of plasma amino acids into a glycoprotein which predominates during earlier phase of oocyte growth[5].

Estrogen-stimulated lipolysis in the adipose tissue might explain the elevated FFA levels in *B. conchonius*. The FFA resulting from mobilization of depot fat are then transported to other tissues in combination with serum albumin. In serveral other piscine species, lipid mobilization under estrogen control has also been reported[3,14]. Our results on plasma cholesterol contrast sharply with those reported in the goldfish where estradiol-17β caused a rise in this parameter[15]. Possibly, alterations in cholesterol level are mediated via hormones of other endocrine glands rather than by sex steroids alone. The estrogen induced hyperinsulinemia may partly account for the hypocholesterolemia in *B. conchonius*. In addition, an inhibition of de novo synthesis of cholesterol might also explain the reduction in plasma and liver cholesterol in this fish. In conclusion, the data presented here do imply that estradiol-17β is involved in regulating lipid and carbohydrate metabolism in *B. conchonius*. The hormone is essential not only in the induction of yolk precursors but also in the mobilization of energy stores to meet the requirements of the growing oocytes.

Effects of estradiol-17β on female *B. conchonius*

Treatment	Time (h)	Plasma glucose ± SE (mg %)	Liver glycogen ± SE (mg/g)	Amino nitrogen ± SE (mg %)	FFA ± SE (µM/ml)	Organic PO_4 ± SE (mg %)	Plasma cholesterol ± SE (mg %)	Liver cholesterol ± SE (mg %)
Control	–	80.6 ± 3.86	17.5 ± 1.86	15.4 ± 2.17	3.34 ± 0.03	35.8 ± 3.37	343.6 ± 16.4	312.8 ± 30.3
Estradiol-17β (25 µg/individual)[a]	24	66.1 ± 1.54[b]	7.1 ± 1.30[b]	25.6 ± 1.93[b]	7.32 ± 0.33[d]	60.1 ± 3.18[b]	207.6 ± 9.1[c]	198.2 ± 10.4[b]
Estradiol-17β (25 µg/individual)[a]	72	75.4 ± 3.61	9.9 ± 2.02	17.1 ± 1.36	4.62 ± 0.29	59.5 ± 18.8	258.8 ± 8.0[c]	252.4 ± 18.4

[a] 5 µg estradiol-17β daily for 5 days; [b] $p < 0.05$; [c] $p < 0.02$; [d] $p < 0.01$.

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The pigeon's sensitivity to ultraviolet and 'visible' light[1]

J. Emmerton and M. Remy

Ruhr-Universität Bochum, Psychologisches Institut, D-4630 Bochum 1 (Federal Republic of Germany), January 24, 1983

Summary. The pigeon's spectral sensitivity, determined behaviorally between 320 and 640 nm, was maximal at 580 nm. Sensitivity extended into the near-ultraviolet but was, in contrast with previous findings, relatively low at these wavelengths. The discrepancy in results in the UV may be based on functional differences arising from the pigeon's retinal specialization.

Not only invertebrates[2] but also aphakic humans[3] and some amphibians[4-6], reptiles[7] and birds[8-10] can detect UV-light (wavelengths below 400 nm). One avian species for which this has been demonstrated behaviorally is the homing pigeon *(Columbia livia)*[11]. The optic media of this bird, unlike those of humans and many other vertebrates[12], are also relatively transparent in the UV[13]. To date, spectral sensitivity at both UV and 'visible' wavelengths has been measured in a single pigeon using a heart-rate conditioning technique[14]. Sensitivity to UV was reported to be exceptionally high, both for this animal and others tested in less detail. Using a simple behavioral method we have now determined the spectral sensitivity of pigeons in a food-seeking situation and find lower UV-sensitivity in this context.

Four pigeons, deprived to 80% of their ad-libitum body weight, were tested in their home-cages from which they could reach a 6×11 cm^2 quartz diffusing plate that was evenly illuminated from below to provide a colored background field. Chromatic stimuli were produced by interference filters (Balzers and Schott, half-bandwidths 8–14 nm). The quartz optics included a neutral density wedge to vary stimulus energy. Two different 150 W xenon light sources were employed (Müller 1530, and Bausch and Lomb). The optics were screened so that only the illuminated diffusing plate was visible. Stimulus energy was measured with a calibrated Oriel 7062 photomultiplier. The experiment was conducted in a darkened room, lit between trials for 1 min by a 100 W tungsten lamp next to the cage in order to keep the pigeons light adapted during an approximately 25 min session.

Within each 30-sec trial a pigeon was presented with 20 grains of corn scattered on the diffusing plate, and the number of grains eaten was counted. In daily sessions, starting at a stimulus intensity at which approximately 90% of grains were located, intensity was reduced between trials by 0.1 log units until the bird's performance fell below 50%. On average 14 trials were completed per day. Data were collected at each wavelength over 3 sessions and wavelengths were presented in a quasi-random order.

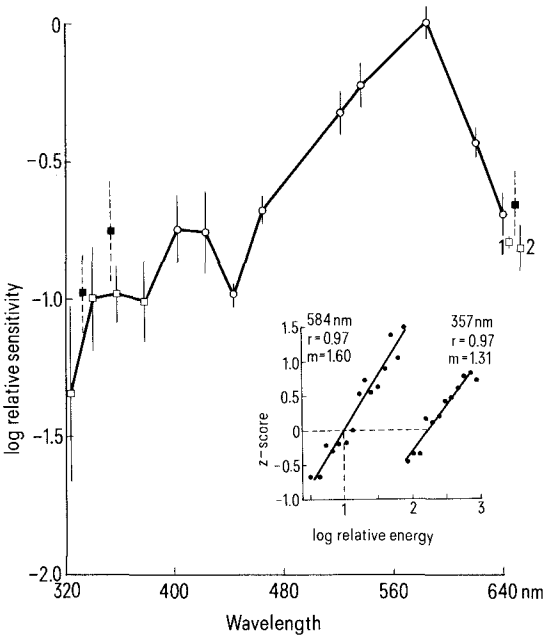

Figure 1. Means and SD of log relative sensitivity expressed on a quantal basis. Circles (○) and squares (□) refer to data obtained using Müller or Bausch and Lomb xenon lamps respectively. Black symbols indicate results using stained seeds. Points 1 and 2 are from test repetitions after exchanging the lamps and at the end of the experiment. Points at the same wavelength are displaced for clarity. Inset are 2 sets of psychophysical data, fitted by linear regression (r, correlation coefficient; m, slope), at UV and 'visible' wavelengths for 1 bird. Performance is expressed as z-scores against an arbitrary log relative energy scale on which 1 log unit corresponds to a stimulus luminance at 584 nm of 0.051 cd/m^2, measured at 6 cm above the quartz plate (the 'fixation') distance before a pigeon finally pecks[21]).