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17β-Hydroxysteroid Dehydrogenase Activity in the Ovary and Head Kidney of two Teleosts, *Anabas testudineus* and *Ophicephalus punctatus*

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A procedure for assaying the activity of 17β-hydroxysteroid dehydrogenase (17β-HSDH) in the ovary and head kidney of two teleosts, *Anabas testudineus* and *Ophicephalus punctatus* is reported. Homogenates of ovary and head kidney were centrifuged at 7000g and the supernatant was used for enzyme assay by spectrophotometric measurement of the reduction of pyridine nucleotide. In the case of *A. testudineus*, 0.2 μmole the optimum concentration of 17β-estradiol for both head kidney and ovary HSDH activity whereas in the case of *O. punctatus* requirement of substrate is 0.1 μmole for ovary HSDH and 0.3 μmole for head kidney HSDH activity. Treatment with ovine LH (NIH-LH-S-19) or fish pituitary extract significantly enhanced 17β-HSDH activity in the ovaries of *O. punctatus*, whereas head kidney 17β-HSDH activity remained unaltered by the administration of fish pituitary extract, but was stimulated by LH. Present observation suggests that fish gonadotropin is specific for ovarian 17β-HSDH, ovine LH on the other hand has broader areas of action.

Introduction

Although several steroidogenic enzymes like Δ^5-3β-hydroxysteroid dehydrogenase and 11β-17α and 21-hydroxylases have been detected in the fish ovary (Bara 1965, Lambert 1966, 1970, Lance & Callard 1969, Eckstein 1970, Colombo & Bern 1971), the biochemical assay for determining the rate of activity of hydroxysteroid dehydrogenase has not yet been reported. Estradiol-17β has been identified in the ovaries of many fishes (Cedard et al. 1961, Hisaw 1963, Gottfried 1964) together with estrone and estriol, but the proportions are quite variable (Hoar 1965) and may show seasonal changes (Cedard et al. 1961). The enzyme 17β-hydroxysteroid dehydrogenase (17β-HSDH) is responsible for the conversion of 17β-estradiol to estrone, since estrogen plays an important role in vitellogenesis, it seems relevant to study 17β-hydroxysteroid dehydrogenase activity in the ovary and head kidney of teleosts. The head kidney has been included in the present study since interrenal plays a major role in the maturation of oocytes (Sundararaj & Goswami 1974). The effects of administration of ovine LH and homologous fish pituitary extract on the

activity of 17β-HSDH have also been studied.

Materials and Methods

Specimens of *Ophicephalus punctatus* and *Anabas testudineus*, were purchased from the local market during June-July and kept in glass aquaria for at least 7 days prior to initiation of experiments. The ovary and head kidney were dissected out separately and homogenized in Potter—Elvejehm homogenizer in 2 ml of 0.6% NaCl in a cold room at 7°C. Homogenization in an ice bath gave better results. The ovary and head kidney homogenates were then centrifuged at 7000 g in a refrigerated centrifuge (Janetzki, Model K 24). The residue was discarded since it did not show any 17β-HSDH activity. Method of Jarabak (1969) has been modified for the present assay. The assay system contained 440 μmoles of Na-pyrophosphate buffer at *p*H 10.2, 25 mg of crystalline bovine serum albumin, 0.2 μmole (for *A. testudineus* ovary and head kidney) or 0.1 μmole (for *O. punctatus* ovary) or 0.3 μmole (for *O. punctatus* head kidney) of 17β-estradiol in 0.02 ml of ethanol, 1.4 μmoles of NAD and appropriate quantities of enzyme in a final volume of 3.0 ml. The tissue supernatant was added last to initiate the reaction and the rate of formation of reduced nucleotide was followed at 340 nm in Beckman Model 25 UV spectrophotometer against a blank containing all ingredients except 17β-estradiol. The activity of the enzyme is expressed in unit, which is defined as the ability of 1 mg enzyme protein to reduce 1 μmole of pyridine nucleotide per minute in a cuvette of 1.0 cm light path. Protein was measured according to the method of Lowry et al. (1951) taking bovine serum albumin as the standard. In another experiment, 17β-HSDH activity in the ovary and head kidney of *O. punctatus* was assayed following administration of 400 ng/100g body wt. of ovine LH (NIH-LH—S-19) or 1 homologous pituitary homogenate per fish. Three injections were given on alternate days. Saline injected fish served as control. Fishes were sacrificed after 6 days.

Results

The rate of 17β-HSDH activity in the ovary and head kidney of *A. testudineus* and *O. punctatus* increases almost in linear fashion upto 20 seconds and then levels off (figure 1). Head kidney 17β-HSDH is more active than that of the ovary and this is true for both the fishes.

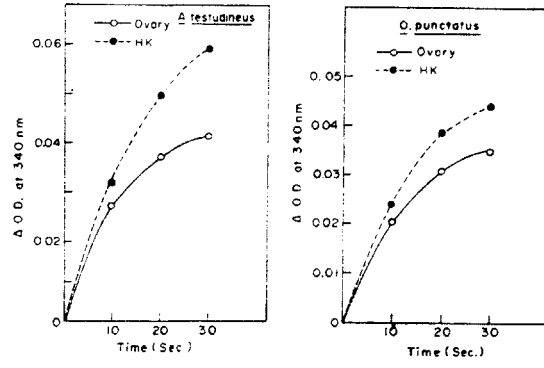

Figure 1 Rate of 17β-HSDH activity in 7000g supernatant of ovary and head kidney of *A. testudineus* and *O. punctatus*

The 17β-HSDH enzyme activity in both the cases is very much dependant on specific substrate concentration. It is evident from figure 2 that in the case of *A. testudineus* 0.2 μmole of 17β-estradiol is required to obtain the peak of enzyme activity. This peak is very sharp in the case of the head kidney enzyme whereas in the case of the ovary the enzyme activity does not decline sharply with 0.1 μmole and 0.3 μmole of

17β-estradiol. In contrast, *O. punctatus* ovary and head kidney 17β-HSDH shows peak of enzyme activity at two different substrate concentrations, 0.2 μmole for ovary and 0.3 μmole for head kidney (figure 3). The present data show that higher substrate concentration destroys 17β-HSDH activity.

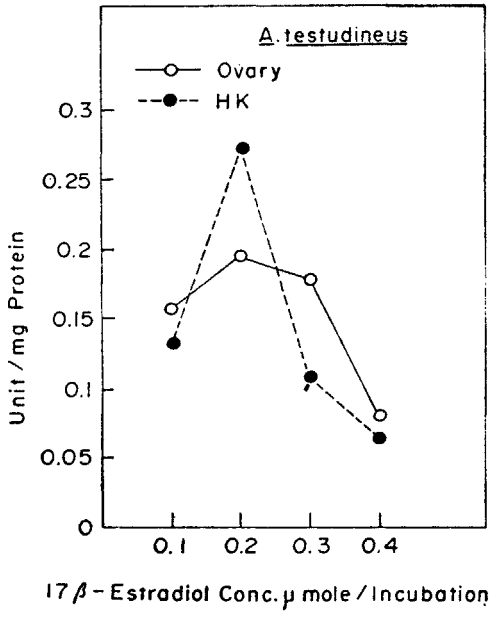

Figure 2 Effect of different substrate concentrations on 17β-HSDH activity of *A. testudineus*

Administration of ovine LH or homologous pituitary extract greatly stimulated ovarian 17β-HSDH activity (table 1). In view of the fact that the gonadotropin content of the injected pituitary homogenate is not known, comparison between the relative potencies of the two preparations was not possible. In contrast to the ovary, head kidney 17β-HSDH activity increased only after LH administration but remained unaltered following pituitary extract injection (table 2).

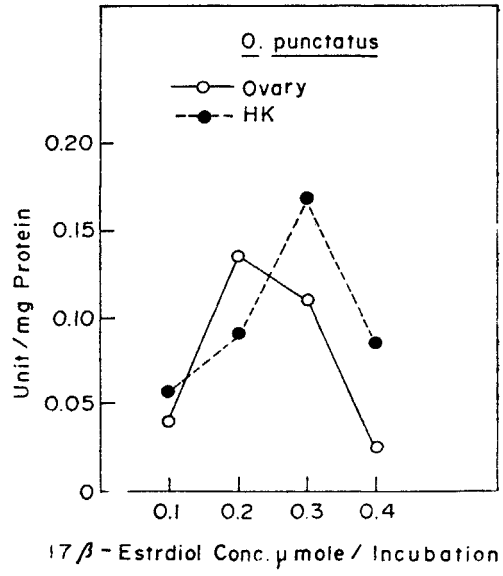

Figure 3 Effect of different substrate concentrations on 17β-HSDH activity of *O. punctatus*

Table 1 *Effect of LH & fish pituitary extract on 17β-HSDS activity of* O. punctatus *ovary*

	Unit/mg Protein
Control	0.136±0.047
LH-Treated (400 ng/100 g b.w.)	0.322±0.07
Fish pituitary extract treated	0.538±0.04

Table 2 *Effect of LH & fish pituitary on* 17β-*HSDH activity of* O. punctatus *head kidney*

	Unit/mg Protein
Control	0.163±0.02
LH-Treated (400 ng/100 g b.w.)	0.308±0.09
Fish pituitary extract treated	0.181±0.017

Discussion

The present study clearly demonstrates 17β-HSDH activity in the ovary and head kidney of *A. testudineus* and *O. punctatus*. Since this

enzyme is responsible for conversion of 17β-estradiol to estrone, its profile in different seasons may lead to a better understanding of the steroid pattern in previtellogenic, vitellogenic and postvitellogenic phases. In Atlantic salmon, estrone was found in all seasons, estriol appeared in significant amounts during spawning and estradiol was gradually disappearing in the females at spwaning (Cedard et al. 1961). This report emphasizes the need to study 17β-HSDH activity in various seasons. Lambert & Van Boheman (1978) have also reported that the proportion of estrone and 17β-estradiol in the serum of rainbow trout was different during previtellogenic and vitellogenic phases. They demonstrated that 17β-estradiol level was higher than that of estrone during previtellogenesis whereas the reverse was true during vitellogenesis. These findings suggest conversion of 17β-estradiol to estrone and vice versa and thus it may be presumed that 17β-HSDH activity varied during different seasons of the year. While ovine LH could stimulate 17β-HSDH activity both in ovary as well as head kidney, fish pituitary homogenate was effective only on the ovarian tissue. More work will have to be done to understand the significance of these findings.

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