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In vitro biosynthesis of 11β-hydroxylated neutral steroids by testes of a viviparous teleost, Zoarces viviparus (L.), during spermiogenesis

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Minced testes from the eel-pout (*Zoarces viviparus* L.) obtained at various stages of spermiogenesis from January to June were incubated with radioactive 17α-hydroxyprogesterone. The metabolites formed were identified by thin layer chromatography and by determination of their constant specific activities. 21-Deoxycortisol and 11β-hydroxyandrostenedione were the major products in all experimental groups indicating that also in this species 11β-hydroxylated androgen would appear to be important during spermiogenesis. Androstenedione was detected in low amounts. Over the study period no obvious variations in 11β-hydroxylating activity were observed. C_{21}-C_{19} Desmolase activity increased slightly, resulting in an increase in the formation of 11β-hydroxyandrostenedione to spawning. The conversion of 17α-hydroxyprogesterone to 11β-hydroxyandrostenedione seemed to occur mainly via 21-deoxycortisol.

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1. Introduction

Comparative studies of both a histochemical and biochemical nature indicate that enzymes such as steroid 11β- and 21-hydroxylases, which are adrenocortical in mammals, in teleosts have a gonadal location. In many teleost species 11-oxygenated androgens have been found to be the major testicular hormones and may be synthesized by (a) 11β-hydroxylation of androstenedione and testosterone formed from pregnenolone or progesterone (see Ozon 1972), or (b) sidechain splitting of 21-deoxycortisol (see Colombo et al. 1979) that could be derived from either 11β-hydroxyprogesterone or 17α-hydroxyprogesterone.

To evaluate the relevance of this concept to the eel-pout (*Zoarces viviparus* L.), the minced testicular tissue of this species obtained at various stages of spermiogenesis were incubated with radioactive 17α-hydroxyprogesterone The aim was also to investigate the formation of 11-oxygenated androgens in the testes of eel-pout in relation to seasonal differences that might occur during spermiogenesis.

2. Material and methods

Male eel-pouts (*Zoarces viviparus*) were caught with nets and with a bottom travel from depths of 25-40 m in the vicinity of Tvärminne Zoological Station, Gulf of Finland (Table 1). Fishes were caught at four different times of year and kept in a freerunning water aquarium for a week before sampling. The age/size and length/weight relationships of the eel-pout have been described by Kristoffersson & Oikari (1975).

Steroids

The trivial names, abbreviations and systematic names of the steroids used as follows: progesterone (Δ^4P) (4-pregnene-3, 20-dione), 17α-hydroxyprogesterone (17αOH-Δ^4P) (17α-hydroxy-4-pregnene-3, 20-dione), testosterone (T) (17β-hydroxy-4-androsten-3-one) and androstenedione (Δ^4A) (4-androstene-3, 17-dione) were obtained from Schering A.G. (Berlin, Germany); 11β-hydroxyandrostenedione (11βOH-Δ^4A) (11β-hydroxy-4-androstene-3, 17-dione) and 21-deoxycortisol (11β, 17αOH-Δ^4P) (11β, 17α-dihydroxy-4-pregnene-3, 20-dione) were obtained from Steroid Reference Collection, London. [4-^{14}C] -17α hydroxyprogesterone, spec.act. 35.9

Table 1. The males of *Zoarces viviparus* used in this investigation. GSI = gonadosomatic index.

Incub. no	Date	Length (cm)	Weight (g)	Testicular weight (g)	GSI
1	25 Jan	33.0	113.0	0.490	0.433
2	—,,—	34.5	179.0	0.964	0.538
3	13 Feb	32.5	134.5	0.925	0.687
4	—,,—	33.5	131.5	0.960	0.730
5	11 April	34.5	195.0	1.664	0.853
6	—,,—	—,,—	—,,—	—,,—	—,,—
7	9 June	31.5	151.0	2.424	1.605
8	—,,—	—,,—	—,,—	—,,—	—,,—

mCi/mmol, was obtained from The Radiochemical Centre (Amersham, England). The purity of the steroids was tested before use using thin layer chromatography (tlc).

NADP was supplied by Boehringer (Mannheim, Germany).

Solvents were analytical grade and were redistilled before use.

Treatment of the tissue and the incubation procedure

The fishes were killed under light MS-222 (1:3000, water) anesthesia. Testes were immediately removed, weighed (total weight) and minced with scissors. The minces were incubated as 500 mg portions in 5 ml Cortland saline (Wolf 1963) at 15°C under a continuous flow of oxygen for 1 hour. [4-^{14}C] -17α-hydroxyprogesterone was used as the substrate, 0.157 μCi being dissolved in 50 μl of ethanol propyleneglycol solution (1:1 v/v). NADP was used at a concentration of 1.35 mmol. Incubation was stopped by the addition of 1.5 ml of acetone. Duplicate incubations were made if enough tissue was available.

Isolation and characterization of the radioactive metabolites

For isolation, measurement and separation of the neutral metabolites of [4-^{14}C] -17α-hydroxyprogesterone, the incubated samples were further treated as described by Teräväinen & Saure (1976). The recoveries of radioactivity in ether are shown in Table 2. The neutral radioactive metabolites were further characterized and identified by rechromatography on tlc, and by determination of constant specific activity using the repetitive tlc method described by Kaartinen et al. (1971). The solvent systems used in tlc were as follows:

1. Acetone - chloroform 15:85
2. Ether - chloroform 1:3
3. Ethanol - chloroform 1:19
4. Ethylacetate - cyclohexane - ethanol 9:9:2
5. Ethylacetate - n-hexane - acetatic acid 15:4:1
6. Ethanol - chloroform 1:9
7. Ethanol - benzene 1:9
8. Ethylacetate - cyclohexane 1:1
9. Ethanol - benzene 1:5
10. Acetone - dichlormethane 1:5

3. Results

The results shown in Table 2 indicate that the bulk of the radioactivity recovered was in the form of free neutral steroids. The radioactivity remaining in the aqueous and NaOH phases was not studied.

In tlc (system 1) the radioactivity was distributed around six peaks (Fig 1) which were pooled in each experimental group. In bidimensional tlc the radioactivity of metabolites in peaks 3, 4, 5 and 6 were isopolar with authentic unlabeled steroids in the systems used, as follows:

Peak 3; 11β, 17αOH-Δ^4P, systems 4 and 6, 84 % of pooled acticity
Peak 4; 11βOH-Δ^4A, systems 6 and 7, 95 % of pooled activity
Peak 5; 17αOH-Δ^4P, systems 10 and 3, 96 % of pooled activity
Peak 6; Δ^4A, systems 8 and 9, 91 % of pooled activity

Table 2. The recoveries (% of the dose) of radioactivity extracted in ether after incubations.

Incub. no	Recovery	Incub. no	Recovery
1	85.0	5	78.7
2	82.7	6	80.2
3	78.5	7	78.6
4	86.5	8	81.8
	Mean ± *SD* = 81.5±3.1		

The identity of the radioactivity in peaks 3–6 was also confirmed by the determination of constant specific activity (Table 3). Peak 1 represent the radioactivity retained at the origin of the chromatogram and was not investigated further. Peak 2 could not be identified.

The percentage conversion of [4-^{14}C]-17α-hydroxyprogesterone to these metabolites in each experimental group is shown in Fig. 2.

The main metabolites in all groups were 21-deoxycortisol and 11β-hydroxyandrostenedione. The total amount of 11-hydroxylated compounds was highest in January (83.3 % of recovered radioactivity) decreasing towards spawning (71.1 %). The proportional amount of 11β-hydroxyandrostenedione increased from January to June. Androstenedione was detected in low amounts in all groups.

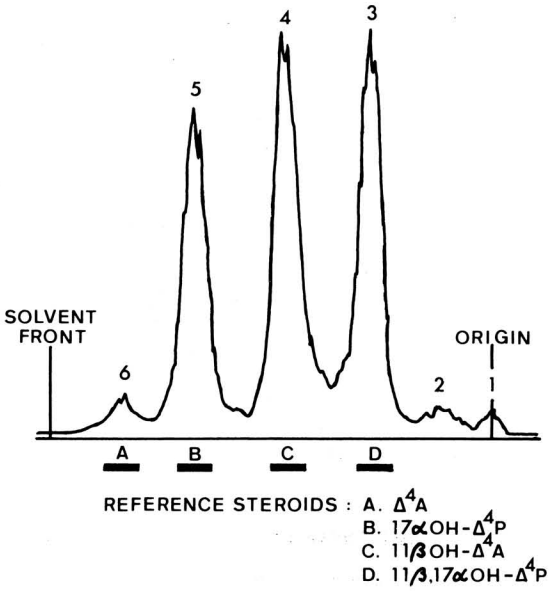


Fig. 1. A scanning record showing the separation of 17α-hydroxyprogesterone and its metabolites in tlc (system 1) (Incubation no 5, Table 1).

Table 3. Determination of constant specific activity (cpm/ug) of different steroids (Peak 3= 11β, 17αOH-Δ^4P, 4 = 11βOH-Δ^4A, 5 = 17αOH-Δ^4P, 6 = Δ^4A). Repeated tlc-method. Solvent system in parenthesis.

Chromatogr. no	Peak 3	Peak 4	Peak 5	Peak 6
Before	578	388	478	387
1	446 (3)	372 (4)	462 (1)	383 (1)
2	428 (4)	388(7)	449 (9)	369 (9)
3	418 (6)	376 (8)	433(10)	371(10)
Mean ± *SD*	431±13	379±8	448±12	374±6

4. Discussion

Numerous studies have indicated that the steroid-synthesizing cellular sites of fish testis are the interstitial Leydig cells, lobule boundary cells and Sertoli cells showing cytological and histochemical changes with the testicular cycle (see Sardul & Guraya 1979). The interstitial Leydig cells, however, are not present in the testes of eel-pout (Pekkarinen & Kristoffersson 1982).

The reproductive cycle of the eel-pout has been shown to be regular in the Gulf of Finland (Kristoffersson & Pekkarinen 1975). Spermatogenesis starts in December and lasts till June. The spawning period of eel-pout males occurs in July-August. 17β-HSD activity in the lobule boundary cells and the activities of Δ^5-3β-HSD and 11β-HSD in some cells or cell groups in the lobule walls have been detected histochemically in eel-pout testes (Pekkarinen & Kristoffersson 1982). The activity of 17β-HSD is correlated with the cyclic changes of the lobule boundary cells but no clear seasonal rhythm could be seen in the occurence of Δ^5-3β-HSD.

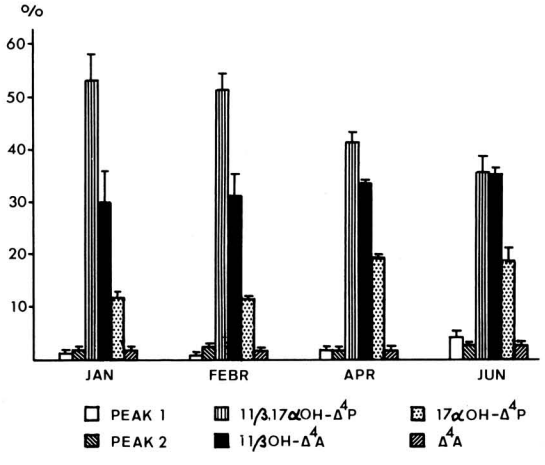
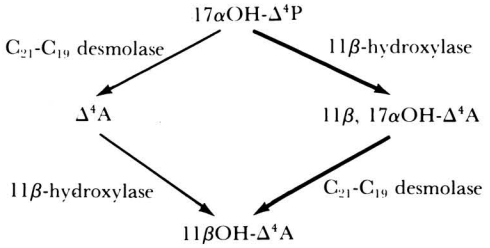


Fig. 2. Percentage conversion (mean and range) of 17α-hydroxyprogesterone to its main metabolites by testicular minces of the eel-pout at various stages during spermiogenesis.

In this investigation the major metabolites of 17β-hydroxyprogesterone by eel-pout testes were 11β, 17α-hydroxyprogesterone (21-deoxycortisol) and 11β-hydroxyandrostenedione. Minor amounts of androstenedione were also isolated.

11βOH-Δ^4A was the main metabolite also found in the incubations using progesterone as the substrate with testes of the other viviparous teleost, *Jenynsia lineata* (Tesone & Charreau 1980) and it was suggested that the testosterone possibly formed was metabolized to 11βOH-Δ^4A. Kime & Hews (1978) isolated 11β-hydroxy- and 17α-hydroxyprogesterone as the metabolites of progesterone from incubations with testes of the pike (*Esox lucius*) and the perch (*Perca fluviatilis*). This indicates that 11β-hydroxylation may also take place before loss of the steroid side chain. The results of this investigation are in agreement with this and suggest that the conversion of 17αOH-Δ^4P to 11βOH-Δ^4A occured mainly via 21-deoxycortisol, as indicated in the following scheme:



Total 11β-hydroxylating activity in eel-pout testes decreased slightly towards spawning. This is in line with the results from in vitro studies on seasonal variations in the steroid metabolism of *Pagellus acarne* (Reinboth & Latz 1982). The present results are also in agreement with the histochemical finding of Pekkarinen & Kristoffersson (1982) which showed that there was no seasonal variation in the total 11β-hydroxylating activity in eel-pout testes.

The results of this investigation suggest no remarkable variation in 11β-hydroxylating activity during spermiogenesis in eel-pout testes, but C_{21}-C_{19} desmolase activity increased from January to June, producing 11β-hydroxy-androstenedione from 21-deoxycortisol in slightly increasing amounts.

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References

Colombo, L., Colombo Belvedere, P. & Marconato, A. 1979: Biochemical and functional aspects of gonadal biosynthesis of steroid hormones in teleost fishes. — Proc. Indian Natn. Sci. Acad. B45: 391-406.

Kaartinen, E., Laukkanen, M. & Saure, A. 1971: Metabolism of dehydroepiandrosterone by rat testicular homogenates; kinetic study at different temperatures; direct effect of 17β-oestradiol. — Acta Endocrinol. (Kbh) 66: 50-64.

Kime, D. E. & Hews, E. A. 1978: In vitro biosynthesis of 11β-hydroxy and 11-oxotestosterone by testes of the pike (Esox lucius) and the perch (Perca fluviatilis). — Gen. Comp. Endocrinol. 36: 604-608.

Kristoffersson, R. & Oikari, A. 1975: Notes on the biology of the eel-pout Zoarces viviparus (L.), in the brackish water of Tvärminne, Gulf of Finland. — Ann. Zool. Fennici 12: 143-147.

Kristoffersson, R. & Pekkarinen, M. 1975: Histological changes in the testes of brackish-water Zoarces viviparus (L.) in relation to the reproductive cycle. — Ann. Zool. Fennici 12: 205-210.

Ozon, R. 1972: Androgens in fishes, amphibians, reptiles and birds. — In: Idler (ed), Steroids in nonmammalian vertebrates. Academic Press, New York.

Pekkarinen, M. & Kristoffersson, R. 1982: Histochemical study of steroid synthesizing sites in a viviparous teleost Zoarces viviparus. — Ann. Zool. Fennici 19: 135-142.

Reinboth, R. & Latz, M. 1982: In vitro experiments on seasonal variations in the steroid metabolism of testicular tissue from functional males of a protandric teleost, Pagellus acarne. — Acta Endocrinol., Suppl. 246. 99: 28.

Sardul, S. & Guraya, F. N. A. 1979: Recent advances in the morphology and histochemistry of steroid-synthesizing cellular sites in the gonads of Fish. — Proc. Indian Natn. Sci. Acad. B45: 452-461.

Teräväinen, T. & Saure, A. 1976: Changes in the testicular metabolism of dehydroepiandrosterone during the annual reproductive cycle of the hedgehog (Erinaceus europaeus L.). — Gen. Comp. Endocrinol. 29: 328-332.

Tesone, M. & Charreau, E. H. 1980: Steroid biosynthesis in the gonads of the teleost fish Jenynsia lineata. — Comp. Biochem. Physiol. 65B: 631-637.

Wolf, K. 1963: Physiological salines for freshwater teleosts. — Prog. Fish Cult. 25: 135-140.

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