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Pubertal development of male African catfish, *Clarias gariepinus*. *In vitro* steroidogenesis by testis and interrenal tissue and plasma levels of sexual steroids

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Abstract

In fish, sex steroids initiate and/or accelerate the maturation of the brain-pituitary-gonad axis. In order to obtain information on the steroid milieu during the pubertal development of male African catfish, we have monitored the conversion of [^{3}H]-pregnenolone and [^{3}H]-androstenedione by testis and [^{3}H]-pregnenolone by interrenal tissue fragments *in vitro*. Pubertal development occurs between two and six months of age. Testicular development proceeds through four main stages that are characterised histologically by the presence of spermatogonia (stage I), spermatogonia and spermatocytes (stage II), spermatogonia, spermatocytes and spermatids (stage III), and all germ cells including spermatozoa (stage IV). 11β-Hydroxyandrostenedione and cortisol were the main products of testes and interrenal tissue, respectively, in all stages of the pubertal development; a change in the steroidogenic pattern was not observed during this period. The interrenal tissue displayed no significant conversion of [^{3}H]-pregnenolone to 11-oxygenated androgens. Blood plasma was analyzed for the presence of five androgens; testosterone, 11β-hydroxytestosterone, 11β-hydroxyandrostenedione, androstenetrione, and 11-ketotestosterone. 11-Ketotestosterone was the quantitatively dominating androgen in the circulation at all stages of development, which was more pronounced in stages III and IV. The obvious differences between the *in vitro* and *in vivo* results, namely 11β-hydroxyandrostenedione being the main testicular product *vs.* 11-ketotestosterone dominating in the blood, may indicate that 11β-hydroxyandrostenedione is converted to 11-ketotestosterone at extratesticular sites.

Introduction

Pubertal development leads to the acquisition of the capacity to reproduce. The hormonal regulation of puberty in human beings, as well as in other vertebrates, has received considerable attention but is still not well understood (Goos 1993). Attempts to define puberty led to the notion that this developmental process does not readily lend itself to definition (Ojeda *et al.* 1980). For example, Bronson and Rissman (1986) defined puberty as the onset of fertility and the period of rapid development that precedes it; for Marson *et al.* (1991) puberty in human beings is a period of transition between the infantile stage and adulthood; Le Bail (1986) defined the start of puberty in salmonid fishes as the appearance of the first spermatocytes; several other definitions can be found in literature (Crim and Evans 1983; Schreibman *et al.* 1986; Williamson *et al.* 1990; Goos 1993; Renaville *et al.* 1993; Schulz *et al.* 1994a). In the present study, we will consider pubertal development in male African catfish, *Clarias gariepinus*, as the period that starts with

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appearance of spermatocytes and ends with the appearance of the first spermatozoa in adolescent males. Under natural conditions, adult catfish show an annual reproductive cycle (van Oordt *et al.* 1987; Resink *et al.* 1987), which culminates in a spawning period once a year. Although under hatchery conditions neither males nor females spawn spontaneously, gonadal development proceeds normally in both sexes, until a prespawning stage is attained, in which the fish remain throughout the year (Peute *et al.* 1986; Schoonen *et al.* 1987).

The brain-pituitary-gonad axis (BPG axis) is the endocrine system of prevailing importance for the regulation of reproductive processes, and hence for puberty. Experimental evidence indicates that sex steroids initiate and/or accelerate the maturation of the BPG axis in teleost fish (*e.g.*, Crim and Evans 1979, 1982; Crim *et al.* 1981; Gielen *et al.* 1982; Dufour *et al.* 1983, 1985; Gielen and Goos 1983; Schreibman *et al.* 1986; Amano *et al.* 1994; Montero *et al.* 1995). Therefore, the hypothesis was adopted that sex steroids have stimulatory effects on the development of the BPG axis. In order to test the hypothesis in our experimental model, basic information is needed regarding the steroid milieu during the pubertal development.

In male vertebrates androgens are synthesised in the testis, but also the adrenal gland can be a source of androgens (Hyatt *et al.* 1983; Bélanger *et al.* 1990). Accordingly, Vermeulen *et al.* (1995) showed that the interrenal tissue of catfish synthesizes, in addition to cortisol (C), C19 steroids, such as androstenedione (A_2) and 5α- and 5β-androstanedione. The *in vitro* steroid metabolism in the testis of adult African catfish has been studied extensively by Schoonen and Lambert (1986a), using labelled steroid precursors. More information in adult males about the end products of testicular steroidogenesis, and about gonadal steroids in blood plasma, was obtained by gas chromatographic-mass spectrometric studies by Vermeulen *et al.* (1993, 1994). The serum levels of four androgens during advanced stages of puberty in male African catfish were reported by Schulz *et al.* (1994a,b).

The primary aim of the present study was to monitor androgens synthesis qualitatively in testis and interrenal tissue throughout pubertal development. Furthermore, the blood plasma sex steroid levels were quantified during all stages of pubertal development. This provided the possibility to compare the pattern of steroidogenesis *in vitro* with the actual androgens levels in the circulation. In order to correlate the steroidogenic pattern and the steroid blood levels with the stage of spermatogenesis, the latter was determined histologically for each tissue and plasma sample donor fish.

Materials and methods

Radioactive steroids and chemicals

[7-^{3}H]-Pregnenolone (sp. act. 925 GBq mmol^{-1}) and [7-^{3}H]-androsternedione (sp. act. 947 GBq mmol^{-1}) were purchased from NEN-Dupont (USA), and their purity was checked by thin layer chromatography (TLC). Reference steroids were obtained from either Merck, Steraloids, Sigma, or Makor Chemical. All chemicals and solvents (Baker and Merck) were of analytical grade.

Animals

African catfish were raised in the laboratory by induced ovulation and artificial fertilization as described previously (de Leeuw *et al.* 1985), except that catfish pituitary extract, instead of human chorionic gonadotropin, was used to induce ovulation. The fish were kept in a copper-free circulation system at 25 ± 2°C, exposed to 14 hours light/10 hours dark, and fed with Trouvit pellets (Trouw, Putten, Netherlands). Samples were collected between 10 and 24 weeks of age. Six to ten fish were killed weekly by an overdose of 2% aqueous phenoxyethanol (1 ml) administered into the gill chambers. The fish were weighed and the testes and interrenal tissue were removed and prepared for *in vitro* incubations. The gonadosomatic index (GSI) was calculated as gonad weight divided by (body weight–gonad weight) × 100.

Collection of blood samples

Blood samples were taken by puncturing the caudal vasculature, using 1 ml syringes which were first rinsed with a solution of 6% sodium citrate in

0.8% NaCl. Blood was collected in Eppendorf tubes containing the same solution (50 $\mu l ml^{-1}$ of blood), and centrifuged for 10 min (800 × g) at 4°C. Plasma samples were stored at –20°C until use.

Testicular histology

Testicular samples from each fish were fixed for 1 h in 0.1 M sodium cacodylate buffer (pH 7.2), containing 2% glutaraldehyde and 1% paraformaldehyde, postfixed with 1% OsO_4 (1 h) in the same buffer, and then dehydrated and embedded in epoxy resin. Semi-thin sections (1 µm), cut on a Reichert-Jung Ultracut microtome, were collected on gelatine-coated slides and stained with 1% methylene blue in 1% borax. Spermatogenesis was divided into four stages at the light microscopical level (Table 1, Fig. 1).

Table 1. Testis development in African catfish

Stage	Key word	Histological appearance
I	Spermatogonia	Lobules contain spermatogonial stem cells and spermatogonia; clusters of Leydig cells are located in the interstitium; Sertoli cells are numerous.
II	Spermatocytes	Meiosis has started, and hence spermatocytes are found next to spermatogonia
III	Spermatids	Spermatogenesis proceeds; the lobules are larger, and spermatids are present; spermatogonia have became rare.
IV	Spermatozoa	Cells at all stages of spermatogonesis are present; spermatozoa have appeared in the lumen.

Incubation and extraction procedure

Testicular and interrenal tissue was cut into fragments of *ca.* 2 mm^3 and placed in 250 µl of medium (Earle's balanced salt solution, M199 EBSS, pH 7.18, supplemented with 0.02 M HEPES, 100 units/ml penicillin G and 100 $\mu g ml^{-1}$ streptomycin; GIBCO, Paisley, UK). To the incubation medium 37 KBq of [^{3}H]-pregnenolone (12.6 ng) or [^{3}H]-androstenedione (11.2 ng), dissolved in 15 µl of propyleneglycol, was added. The incubations lasted 3h at 25°C in an air atmosphere in a metabolic shaker, and were terminated by addition of 5 ml of dichloromethane and 2 ml of water. Before extraction, the following carrier steroids were added in 200 µl propanol. To the incubations with [^{3}H]-pregnenolone 25 µg of each of the following steroids: progesterone (P_4), 17-hydroxyprogesterone ($17P_4$), 17,20β-dihydroxy-4-pregnen-3-one (17,20βP), androstenedione (A_2), testosterone (T), 11β-hydroxyandrostenedione (OHA), androstenetrione (OA), 11β-hydroxytestosterone (OHT), and 11-ketotestosterone (11KT) and 50 µg of pregnenolone (P_5), 17α-hydroxypregnenolone ($17P_5$) and dehydroepiandrosterone (DHA) were added. To the incubations with [^{3}H]-androstenedione 25 µg A_2, T, OHA, OA, OHT, 11KT, and 50 µg of 5β-androstanedione and etiocholanolone were added. Steroids were extracted with dichloromethane (3 × 5 ml) and the combined dichloromethane extracts were evaporated. The residue, dissolved in a few droplets of dichloromethane-methanol (9:1) was transferred to TLC plates.

Chromatography

TLC was carried out on precoated silica gel $60F_{254}$ (Merck) plates in saturated tanks with the following systems: I, toluene-cyclohexane (1:1); II, toluene-ethylacetate (3:1); and III, chloroform-ethanol (95:5). System I was always used as the first one to separate steroids, remaining at the base line, from apolar compounds. Detection of carrier and reference steroids was performed according to Schoonen and Lambert (1986a). Radioactive areas were located by means of Berthold automatic TLC linear analyser (LB 2842). The radioactivity in the different fractions was determined using a Packard 1900 TR Liquid Scintillation counter. Microchemical reactions (acetylation, oxidation, formylation) and final identification by recrystallization to constant specific activity were carried out as described by Schoonen and Lambert (1986a). However, recrystallizations were only done using testicular samples as incubation of interrenal tissue did not provide evidence for the production of 11-oxygenated androgens.

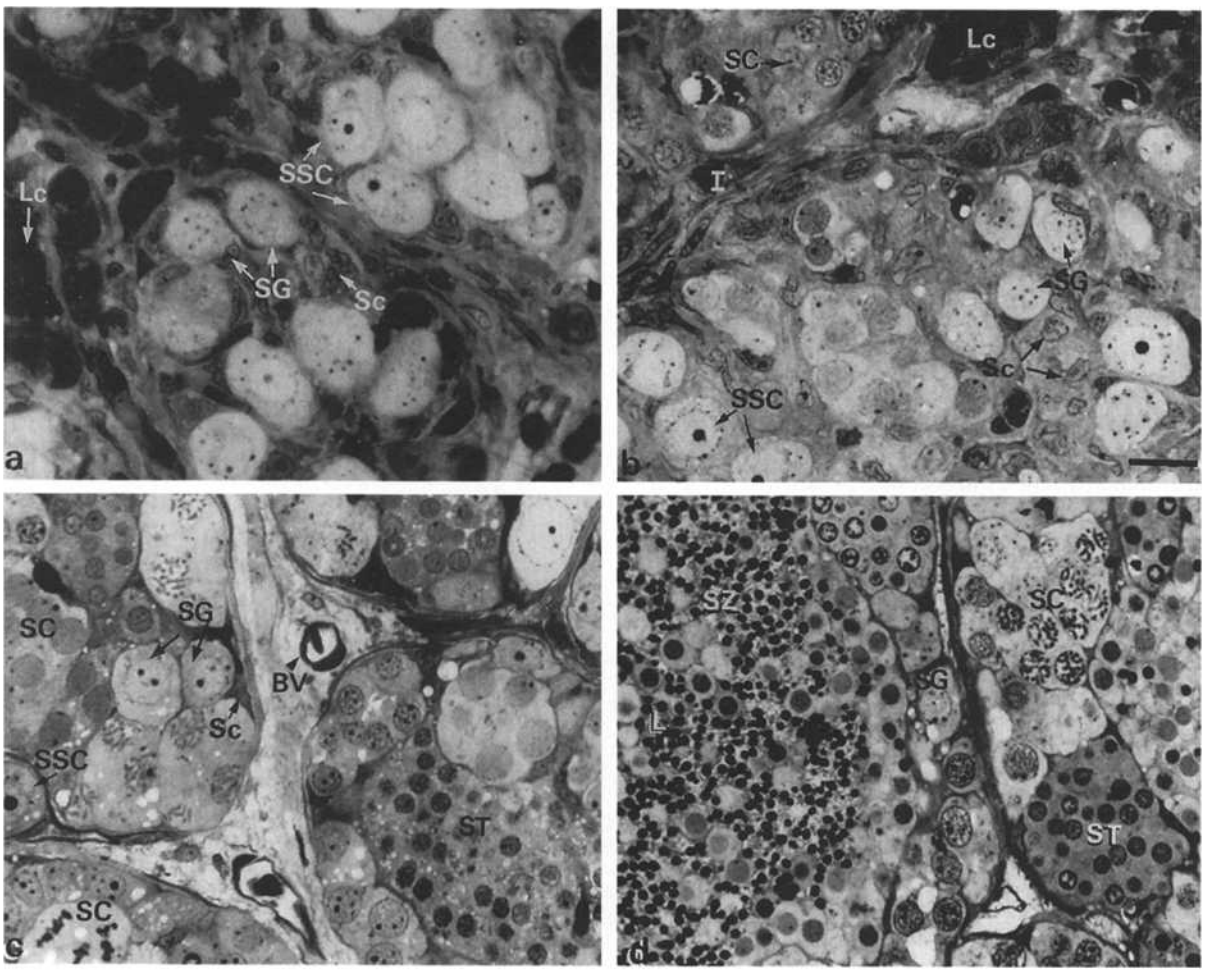

Fig. 1. 1 µm sections of African catfish testis at different stages of pubertal development. Bar = 10 µm. a – Stage I, spermatogonia; b – Stage II, Spermatocytes; c – Stage III, Spermatids; d – Stage IV, Spermatozoa (see Table 1 for details). SSC – spermatogonial stem cell; SG – spermatogonia; SC – spermatocytes; ST – spermatids; SZ – spermatozoa; SC – Sertoli cells; LC – Leydig cells; L – lumen; I – interstitium; BV – blood vessel.

Steroid radioimmunoassay (RIA)

The plasma levels of the following steroids were determined by RIA as described previously (Schulz 1984, 1985): 11KT, T, OHA, OHT and OA. These androgens were determined because OHA and OHT are the main androgens formed in the testes of the African catfish after tissue incubations using tritiated pregnenolone and androstenedione as precursors (Schoonen and Lambert 1986a); 11KT, T, and OA are the major circulating androgens in the adult catfish (Vermeulen *et al.* 1994) and other teleost fish (Fostier *et al.* 1983; Borg 1994).

Statistics

The levels of plasma steroids are given as ng per ml of plasma and expressed as mean ± SEM. Plasma steroid concentrations and GSI values in the different stages were compared by one-way ANOVA, followed by the Fisher's least significant difference test ($p < 0.05$).

Results

Changes in testicular histology, weight and GSI during puberty

A testicular tissue sample was analyzed his-

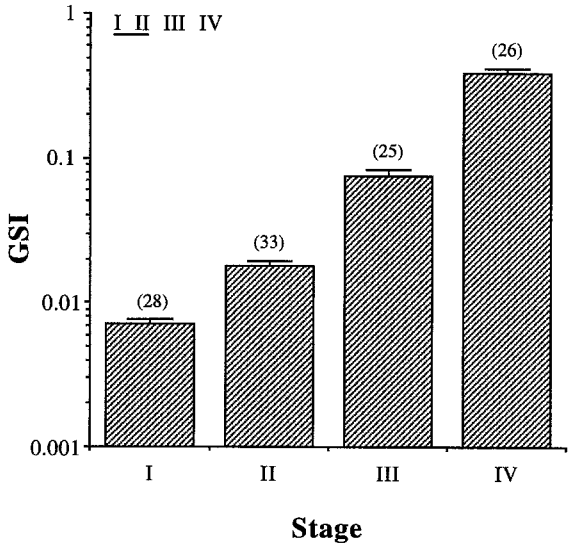

Fig. 2. Gonadosomatic index (GSI; mean ± S.E.M.; numbers in parenthesis give sample size) of male African catfish during the four stages of pubertal development; groups sharing the same underscore are not significantly different ($p < 0.05$, ANOVA, followed by the Fisher least significance difference test).

tologically from each blood/tissue sample donor. Spermatogenesis was divided into four stages as shown in Table 1 and Figure 1. In stage I and II (Figs. 1a and 1b) the interstitial area contained clusters of darkly stained Leydig cells. Numerous Sertoli cells, which often were not in contact with the germ cells were observed in the lobules. In stage III and IV (Figs. 1c and 1d) the clusters of Leydig cells became dispersed and the number of Leydig cells per section had decreased. Most Sertoli cells were now in contact with germ cells. Changes in the gonadosomatic index (GSI) during pubertal development are shown in Figure 2. The GSI was lowest in stage I and increased steadily during spermatogenesis.

Interrenal tissue incubation with [^{3}H]-pregnenolone

The pattern of steroids produced from [^{3}H]-pregnenolone by interrenal fragments was similar throughout pubertal development. After extraction, the organic fraction contained more than 95% of the total radioactivity. No significant conversion to C19 steroids was observed, although special attention was paid to the formation of 11-oxygenated androgens. Only cortisol, the intermediates $17P_5$ and $17P_4$, and 11-desoxycortisol (DOC) were detectable following TLC in systems II and III (Table 2).

Table 2. Interrenal tissue incubation with [^{3}H]-pregnenolone

Stage	Cortisol	$17P_5/17P_4$	DOC
I	65±3	32±3	3±0.6
II	67±2	29±1	4±1.0
III	70±1	24±1	5±0.9
IV	71±3	25±2	4±1.3

% yield (n = 15–20); mean ± SEM

Testicular incubations with [^{3}H]-pregnenolone

After extraction of the incubation media, 95–99% of the total radioactivity was present in the organic fraction. Separation of the steroids using TLC system II revealed three main radioactive areas (Table 3) corresponding to the carriers P_5, a mixture of $17P_5/17P_4$, and OHA. Sometimes, a small amount of radioactivity was observed at the area corresponding to OA. After a second TLC run (system III), derivatization, and recrystallization to constant specific activity, the presence of the above mentioned steroids was confirmed (Table 4). In all stages, the main product representing at least 85% of the total activity, was OHA. No significant amount of [^{3}H]T could be detected.

Testicular incubations with [^{3}H]-androstenedione

OHA was again identified (Table 3) as the main product when androstenedione was used as a precursor. In addition, small amounts of other 11-oxygenated androgens were found; a trace of T could also be identified.

Plasma levels of sex steroids

The plasma levels of five androgens (11KT, T, OHA, OA and OHT) during the four stages of pubertal development are shown in Figure 3. Androgen concentrations increased during pubertal development. In stage I and II, only low levels of 11KT (0.2–0.6 $ngml^{-1}$) and OHA (0.1–0.3 $ngml^{-1}$) were recorded. The levels of 11KT in stage I and II

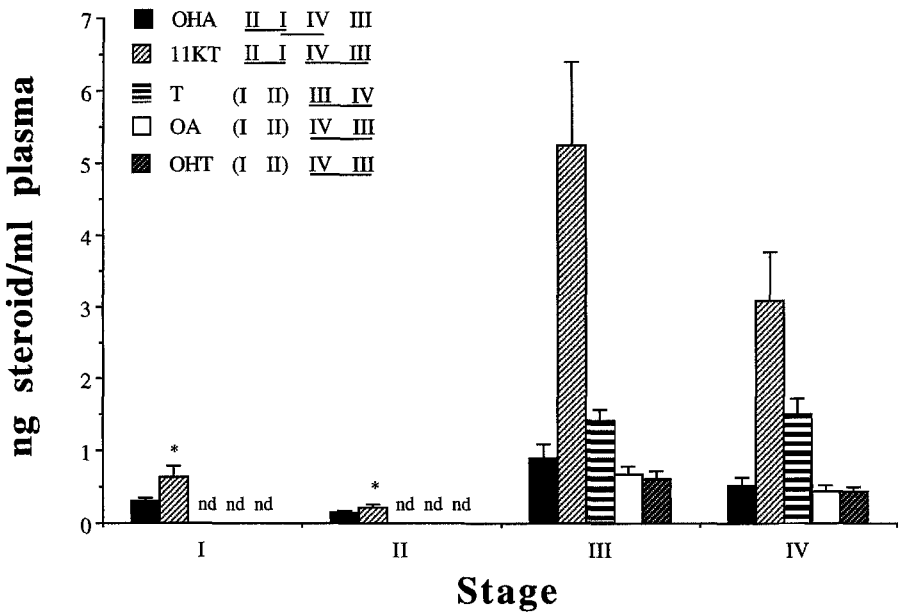

Fig. 3. Sex steroid levels (mean ± SEM, n = 10 in all stages) in blood plasma (ng/ml) at different stages of pubertal development; nd – not detectable; * stage I and II, detectable in 8 and 5 animals respectively; groups sharing the same underscore are not significantly different ($p < 0.05$, ANOVA followed by the Fisher least significance difference test).

were above the limit of detection (0.1 $ngml^{-1}$) in 8 of 10, and 5 of 10 animals, respectively, whereas OHA could always be detected. Coinciding with the appearance of spermatids (stage III), all five androgens became detectable. 11KT was the quantitatively dominating androgen, and a 10-fold increase to 5.2 $ngml^{-1}$ was measured compared to stages I and II. In stage IV, when spermatozoa were present, the steroid levels were similar to those in stage III.

Discussion

The main objective of the present study was to collect information on the steroid environment during the pubertal development of male African catfish. The pubertal development has been subdivided histologically into four stages which have been used to group all other data. Compared to the reproductive cycle of adult feral males (van Oordt *et al.* 1987), the early stages of pubertal development correspond to the preparatory phase characterized by spermatogonial multiplication. However, the GSI values in juvenile fish are lower than those in adult feral males, in part due to the presence, in the latter, of residual sperm from the previous cycle (Resink *et al.* 1987). The later stages of pubertal development correspond histologically to the prespawning period which is characterized by a strong spermatogenetic activity. The main difference between adolescent fish (stage IV) and mature (feral or hatchery-reared) males is the predominance of spermatozoa over other types of germ cells in mature fish which is associated with a further 3-fold increase in the GSI to values of *ca.* 1.2 (van Oordt *et al.* 1978). Hence, pubertal development appears to proceed normally in hatchery-reared fish, in a way similar – at least histologically – to the sequence of events during testicular recrudescence in feral males.

In fish, sex steroids can initiate and/or accelerate the maturation of the BPG axis (for references, see introduction). As sources for steroids, the gonads and the interrenal tissue have to be considered. Although the seminal vesicles are a source of steroid hormones as well in adult African catfish (Schoonen and Lambert 1986b; van den Hurk *et al.* 1987; Fishelson *et al.* 1994), the vesicles develop not earlier than from stage III onwards. Hence, it is unlikely that the seminal vesicles play a significant role as steroidogenic organs at the onset of pubertal development.

Cortisol was the main product of interrenal tis-

Table 3. Testis incubation with [^{3}H]-pregnenolone and [^{3}H]-androstenedione

	[^{3}H]-pregnenolone		[^{3}H]-androstenedione	
Stage	OHA	$17P_5/17P_4$	OHA	OHT/OA/11KT
I	87±1	4±0.5	90±1	2±0.3
II	85±2	5±1.0	88±3	4±1.0
III	92±1	3±2.0	91±2	4±0.5
IV	91±2	6±0.9	89±2	5±0.8

% yield (n = 15–20); mean ± SEM

sue incubated with [^{3}H]-pregnenolone during all stages of pubertal development. Similar results have been reported for the adult herring (*Clupea harengus harengus*), adult atlantic salmon (*Salmo salar*, Sangalang *et al.* 1972), and for adult *Oreochromis mossambicus* (Balm *et al.* 1989). The interrenal tissue of adult catfish produced some androgens, such as A_2 (Vermeulen *et al.* 1995). However, no indication for the conversion of [^{3}H]-pregnenolone into androgens by the interrenal tissue of juvenile or adolescent male African catfish was found. Thus, an effect of androgens of interrenal origin on the maturation of the BPG axis is unlikely. However, it cannot be excluded that cortisol has a weak stimulatory (van den Hurk *et al.* 1984; Dufour *et al.* 1983) or inhibitory effect (van den Hurk and van Oordt 1985) on the BPG axis in juvenile fish. In addition, these data suggest that interrenal androgen production may be a characteristic of adult male catfish. In juvenile coho salmon (*Oncorhynchus kisutch*, Patiño *et al.* 1987) cortisol was also the main product detected by HPLC in media after incubation of interrenal tissue in the absence or presence of adrenocorticotropic hormone *in vitro*.

With both [^{3}H]-pregnenolone and [^{3}H]-androstenedione as precursors, OHA was the main androgen produced by testicular tissue. Moreover, small amounts of intermediates (*e.g.* $17P_5$ and $17P_4$) and a trace of other 11-oxygenated androgens were found, namely OHT, OA, and 11KT. According to Schoonen and Lambert (1986a), testicular steroid biosynthesis in adults follows the Δ_4 route as $17P_5$ and DHA were absent while P_4 could be detected. In juvenile fish, however, the presence of both $17P_5$ and $17P_4$ and the absence of P_4 indicate a different biosynthetic pathway. Previous investigations in which testicular steroid production of adult feral or hatchery-reared African catfish was analyzed *in vitro* using three different methods (Schoonen and Lambert 1986a; Resink *et al.* 1987; Vermeulen *et al.* 1993; Schulz *et al.* 1994b), similarly showed that OHA was the main end product. This has been corroborated for all stages of pubertal development in the present study suggesting that OHA plays an important role throughout pubertal development as well as during adult life. OHA has also been found to be produced by immature testes *in vitro* in other teleosts fish. Colombo *et al.* (1972) observed that testis fragments of *Anguilla anguilla* (silver stage) converted [4-^{14}C]-A_4 and [4-^{14}C]-P_5 mainly to OHA; Eckstein *et al.* (1982) found the same using tritiated P_5 and androstenedione as substrates; testes homogenates from juvenile rainbow trout (*Oncorhynchus mykiss*, 100 days after fertilization) synthesize mainly OHA and A_2 from P_5 as a precursor (van den Hurk *et al.* 1982); and immature testis of *Jenynsia lineata* also exhibited 11β-hydroxylase activity, with OHA being the main product (Tesone and Charreau 1980).

Regarding the circulating sex steroids, pubertal development can be subdivided into two phases: an initial phase – spermatogonial proliferation, spermatocyte formation and meiosis (stages I and II) characterized by low or undetectable levels; the secondary phase – presence of haploid germ cells including spermatozoa (stages III and IV) was characterized by increased levels of all androgens analyzed. 11KT was the quantitatively dominating androgen during all phases of pubertal development; however 11KT was low or not detectable in some stage I and II samples. The strong (10-fold) increase in stage III and the maintenance of elevated levels in stage IV, suggests a more important role for 11KT in later phases of pubertal development. The same may hold true for T which first surpassed the limit of detection in stage III. The biological activity of 11KT has been demonstrated repeatedly (*e.g.*, Billard 1978; Pottinger 1987; Borg *et al.* 1993; Schulz *et al.* 1993). Similarly, T has been shown to have positive effects on the number of GnRH neurons (Amano *et al.* 1994), on the pituitary GTH II content (*e.g.*, Crim and Evans 1979), and receptor-like binding of T has been reported in the brain of goldfish (*Carassius auratus*; Pasmanik and Callard 1988) and skin of male brown trout (*Salmo trutta*; Pottinger 1987). The

Table 4. Identification of metabolites in incubation media from testis of African catfish

Substrate	Metabolite	TLC systems[1]	Crystalizations (dpm mg^{-1})			
			1	2	3	4
Pregnenolone						
	17-hydroxypregnenolone	I(1×) II(3×) III(1×) A+, III(1×)	4699	5139	5958	–
	17-hydroxyprogesterone	I(1×) II(3×) III(1×) F–, III(1×)	7219	6011	6431	6764
	11β-hydroxyandrostenedione	I(1×) II(3×) III(1×) F–, III(1×)	53109	51655	51863	–
Androstenedione						
	testosterone	I(1×) II(3×) III(1×) A+, III(1×)	3351	3354	3366	–
	11β-hydroxyandrostenedione	I(1×) II(3×) III(1×) F–, III(1×)	80205	80079	80066	–
	11β-hydroxytestosterone*	I(1×) II(3×) III(1×) A+, III(1×)				
	androstenetrione	I(1×) II(3×) III(1×) F–, III(1×)	3480	3254	3222	–
	11-ketotestosterone**	I(1×) II(3×) III(1×) O+, III(1×)	6861	7563	6938	–

[1] For explanation of roman numerals, see text; * activity too low for recrystalization; ** crystallized as androstenetrione; A+ positive acetylation; F – negative formylation; O+ CrO_3 positive oxidation

possible roles for OA and OHT are not clear, although both androgens show biological activity in male stickleback (*Gasterosteus aculeatus*; Borg *et al.* 1993). However, at least OA is probably first converted to 11KT and does not develop biological activity *per se* (Mayer *et al.* 1990a,b). Considering that OA and OHT become detectable along with the strong increase of 11KT levels in the catfish, we propose to consider them as intermediate products reflecting an increase in 11KT production. OHA was the only androgen that was clearly detectable at all stages, although at rather low levels compared with 11KT. Similar to OA and OHT, OHA may be considered as an intermediate for the biosynthesis of 11KT (see below), and the interaction of OHA with a specific receptor has yet to be demonstrated. However, OHA is the main end product of testicular steroidogenesis in the African catfish and in stage I, the testicular production per mg of tissue is remarkably high (Schulz *et al.* 1994b, 1996). Histologically, this correlates with clusters of Leydig cells in stage I and II. We therefore suggest that OHA may be involved in the stimulation of early stages of spermatogenesis in the African catfish. This suggestion will be tested experimentally.

Similar to the situation in adult male African catfish, the present results show that OHA is the major end product of testicular steroid production but is found at low levels in the blood while the major circulating androgen, 11KT, is produced only at very low levels by the catfish testis. Nonetheless, the testis appear to be the source of at least a precursor for 11KT, considering that castration drastically reduced circulating 11KT levels (Schulz *et al.* 1993; Vermeulen *et al.* 1994). One possibility to explain this discrepancy is to assume that the conversion of OHA to 11KT is impaired *in vitro*, irrespective of the technical approach chosen (Schoonen and Lambert 1986a; Resink *et al.* 1987; Vermeulen *et al.* 1993; Schulz *et al.* 1994b). However, we favour an alternative explanation which assumes that OHA of testicular origin is converted to 11KT at extratesticular sites. A similar situation has been observed in the three-spined stickleback: the main androgen produced by the breeding stickleback testis is OA, whereas 11KT is the main circulating androgen (Borg *et al.* 1989; Mayer *et al.* 1990a,b) with blood cells as a possible site of conversion. The hypothesis of an extratesticular conversion of OHA to 11KT will be tested experimentally in the African catfish.

In summary, the present results show that during all phases of the pubertal development of male African catfish OHA is the main product of testicular steroidogenesis *in vitro*, while 11KT is the main circulating androgen. A possible explanation for this discrepancy is the conversion of OHA to 11KT at extratesticular sites. Both 11-oxygenated androgens are candidates for inducing and/or accelerating pubertal development in male African catfish.

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