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Androgen-induced changes in Leydig cell ultrastructure and steroidogenesis in juvenile African catfish, *Clarias gariepinus*

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Abstract The present report focuses on the mechanism(s) involved in the steroid-induced decrease of androgen production in immature African catfish testes that was observed in previous studies. Juvenile animals were implanted with Silastic pellets containing different 11-oxygenated androgens (11-ketotestosterone, KT; 11β-hydroxyandrostenedione, OHA; 11-ketoandrostenedione, KA), testosterone (T) or estradiol-17β (E2). Control groups received steroid-free pellets. Two weeks later, testis tissue fragments were either incubated with increasing concentrations of catfish luteinizing hormone (LH), or incubated with [^{3}H]-pregnenolone ([^{3}H]-P5) or [^{3}H]-androstenedione ([^{3}H]-A). Tissue fragments were also prepared for the quantitative assessment of Leydig cell morphology. Most of the parameters studied were not affected significantly by implantation of E2. Implantation of all androgens inhibited both the basal and the LH-stimulated androgen secretory capacity in vitro. This was associated with a reduced size of the Leydig cells and loss of half of their mitochondria. The studies on the metabolism of tritiated steroid hormones indicated that steroidogenic steps prior to 11β-hydroxylation, probably $C_{17–20}$ lyase activity, were affected by all androgens. Although the effects of 11-oxygenated androgens and T on Leydig cells were mostly similar, previous work showed that only the 11-oxygenated androgens stimulated spermatogenesis, suggesting that distinct mechanisms of action are used by 11-oxygenated androgens and T. These mechanisms, however, seem to merge on the same target(s) to impair Leydig cell androgen production. Such a negative feedback mechanism may be of relevance in the context of the decline in androgen secretion per milligram testis tissue that accompanies the first wave of spermatogenesis in pubertal African catfish.

Key words Teleost fish · Puberty · Testes · Sex steroids · Ultrastructure · Steroidogenesis · *Clarias gariepinus* (Teleostei)

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Introduction

In juvenile male teleost fish, androgenic sex steroids play an important role in the regulation of sexual maturation. Androgens act at all levels of the brain-pituitary gland-gonad (BPG) axis and may be involved in triggering puberty. Both the 11-oxygenated androgens typically found in teleost fish and aromatizable androgens can modulate the development of the gonadotropin-releasing hormone (GnRH) system (e.g. Schreibman et al. 1986; Grober et al. 1991; Amano et al. 1994). Aromatizable androgens, in particular testosterone (T) after its conversion to estradiol-17β (E2), participate in the activation of the pituitary gonadotrophs (e.g. Crim and Evans 1979; Gielen and Goos 1983; Le Dréan et al. 1996; Breton et al. 1997). 11-Oxygenated androgens have also been reported to modulate pituitary and plasma gonadotropin levels (Borg et al. 1998), but the in vivo experiments do not allow to locate the site of action of the androgens. However, a prominent aspect of the activity of the 11-oxygenated androgens, in particular 11-ketotestosterone (KT), is the direct stimulation of spermatogenesis (e.g. Miura et al. 1991, 1995).

In male African catfish (*Clarias gariepinus*), the first wave of spermatogenesis takes place between 3 and 6 months of age (Cavaco et al. 1997a). Although 11β-hydroxyandrostenedione (OHA) is the main product of

testicular steroidogenesis in juvenile and adult African catfish, the main plasma androgen is KT (Schoonen and Lambert 1986; Vermeulen et al. 1993, 1994; Schulz et al. 1996; Cavaco et al. 1997a). It is produced by conversion of OHA to KT in the liver (Cavaco et al. 1997b). In addition, despite a low testicular production, T is a fairly prominent plasma androgen, which might be related to a protection of circulating T against catabolism by binding to a sex-steroid-binding protein (Rebers et al. 1991). Hence, also in the African catfish, 11-oxygenated as well as aromatizable androgens may be relevant for pubertal development.

A previous study showed that treatment of juvenile males with the 11-oxygenated androgens KT, OHA and 11-ketoandrostenedione (KA) stimulated testicular growth and spermatogenesis, while T, 5α-dihydrotestosterone (DHT), androstenedione (A) or E2 were ineffective in this regard (Cavaco et al. 1998). In this study, however, both 11-oxygenated and aromatizable androgens attenuated the testicular OHA secretory capacity in vitro. The present study therefore aimed to shed more light on the possible mechanisms underlying the androgen-induced downregulation of the OHA secretory capacity in immature male African catfish. Particular attention was paid to possible changes in the ultrastructure of Leydig cells. Moreover, steroid treatment effects were monitored by studying basal and luteinizing hormone (LH)-stimulated androgen secretion in vitro, and by studying the testicular metabolism of radioactive steroids in vitro.

Materials and methods

Animals

African catfish (*Clarias gariepinus*) were bred and raised as described previously (De Leeuw et al. 1985), except that African catfish pituitary extract instead of human chorionic gonadotropin (hCG) was used to induce ovulation. The fish were kept in a copper-free recirculation system at a water temperature of 25±2°C, exposed to a photoperiod of 14L:10D, and fed with trout pellets (Trouw, Putten, The Netherlands). Principles of animal care and specific national laws were followed. Fish were used for experimentation at 10 weeks of age, body weight 8.8±0.4 g. At this stage the only type of germ cell in the testes (next to stem cells) is spermatogonia (Cavaco et al. 1998).

Hormones

All non-radioactive steroids were purchased from Sigma (St. Louis, MO). [7-^{3}H]-Pregnenolone ([^{3}H]-P5; specific activity 925 GBq/mmol) and [7-^{3}H]-androstenedione ([^{3}H]-A; specific activity 947 GBq/mmol) were purchased from NEN-Dupont (Brussels, Belgium), and their purity was checked by thin-layer chromatography (TLC). All chemicals and solvents (Merck, Darmstadt, Germany) were of analytical grade. The African catfish LH preparation used to stimulate testicular androgen secretion in vitro has been described previously (Schulz et al. 1997a). It was prepared from an homogenate of pituitaries of mixed sex African catfish by ethanol precipitation and column chromatography (Sephadex G100SF, Whatman DE52). Using an LH radioimmunoassay this LH preparation was found to contain at least 76% LH (w/w; some residual salt was possibly present), in comparison with highly purified African catfish LH (Koide et al. 1992).

Steroid treatment and sampling

Ten-week-old fish were implanted with Silastic pellets containing different steroids at a dose of 30 µg/g body weight as described previously (Cavaco et al. 1998). The fish received implants containing KT, OHA, KA, T or E2. Two weeks after implantation, fish were killed by decapitation. The effects of steroid treatment on testis growth, spermatogenesis, secondary sexual characteristics, and plasma sex steroid levels have been reported elsewhere (Cavaco et al. 1998). Here, we describe the treatment effects on the response of testis tissue to increasing doses of LH in terms of secretion of the main androgen produced by African catfish testis, namely OHA (Schulz et al. 1996; Cavaco et al. 1997a). Testis fragments from fish treated with KT, OHA, T or E2 were also prepared for in vitro incubations with radioactive precursors. Moreover, testis tissue fragments were used to examine the ultrastructure of Leydig cells.

11β-Hydroxyandrostenedione secretion in vitro

Testicular tissue was prepared for in vitro incubations as described previously (Schulz et al. 1994). In brief, the left and right testis of each male was weighed separately and placed in separate wells of 24-well plastic plates (Costar, Cambridge, MA) containing 0.5 ml Earle's balanced salt solution (M199 EBSS; Life Technologies-Gibco, Grand Island, NY) supplemented with hydroxyethylpiperazine ethanesulphonic acid (HEPES) (0.02 M; adjusted to pH 7.18 with NaOH), and antibiotics (100 U/ml penicillin G and 100 ng/ml streptomycin sulphate; Life Technologies-Gibco). One testis weighed 1–15 mg, the range reflecting individual variation and treatment effects (11-oxygenated androgens stimulate testis growth and spermatogenesis; Cavaco et al. 1998). The testes were cut into fragments of approximately 2 mm^3, which were rinsed once with medium. The incubation was started by replacing the medium with 0.5 ml fresh medium for the left testis of each animal (basal androgen secretion). For the right testis, 0.5 ml medium was added containing African catfish LH at increasing doses (1, 10, 100, 1000 ng/ml). The number of males found in the group of fish receiving KT pellets unfortunately was too small to examine a complete range of LH doses, so that next to basal OHA secretion only a dose of 100 ng LH/ml could be tested.

The tissue fragments were incubated in an air atmosphere at 25°C for 18 h. The medium was then removed, incubated for 1 h at 80°C, centrifuged at 16000 *g* for 30 min at room temperature, and finally the supernatants were stored at –20°C until measurement of OHA concentrations. The levels of OHA in incubation media were determined by radioimmunoassay as described previously (Schulz et al. 1996). The data are expressed as nanograms OHA secreted per milligram tissue incubated.

Leydig cell ultrastructure

Testicular fragments incubated in the absence of LH in vitro were fixed and embedded as described previously (Cavaco et al. 1997a). Ultrathin sections (60 nm) were cut using a Reichert-Jung ultramicrotome (Vienna, Austria) and stained with uranyl acetate and lead citrate. The sections were examined in Utrecht with a Zeiss EM 110 electron microscope (Oberkochen, Germany), in Guelph with a Jeol JEM100 S electron microscope (Tokyo, Japan).

The ultrastructure of the Leydig cells was analysed with an interactive image analysis system (IBAS; Kontron/Zeiss, Eching, Germany) for the cell area, the nuclear area, the percentage of the cell section area held by the nucleus, the mitochondrial area, the number of mitochondria per cell section, and the percentage of the cell section area held by the mitochondria. For each treatment group, four Leydig cells from five different tissue fragments corresponding to five individuals were analysed.

In vitro incubations with [3H]-pregnenolone and [3H]-androstenedione

Testicular tissue was prepared for in vitro incubation as described above. Fragments of approximately 2 mm^3 were placed in 250 µl medium (M199 EBSS; see above). To the incubation medium 37 KBq [3H]-P5 (12 ng) or [3H]-A (11.7 ng), dissolved in 15 µl propyleneglycol, was added. Incubation, extraction and TLC were performed as described previously (Cavaco et al. 1997a).

Statistics

Results are expressed as means±SEM. For statistical analysis, all data were $\log_{10}$ transformed and then processed by one-way ANOVA, followed by Fisher's least significant difference test ($P<0.05$). The LH concentration inducing the half-maximal response of OHA secretion (EC_{50}) was estimated by non-linear regression fits (GraphPad PRISM Software package, San Diego, CA). For the analysis of the stimulatory effect of a single dose of LH (100 ng/ml for KT-implanted animals), we used a one-tailed paired *t*-test.

Results

The basal testicular in vitro secretion of OHA was reduced significantly in response to all steroids administered (Fig. 1), with the following order of effectiveness: OHA=KT=T>KA>E2. Irrespective of the pretreatment in vivo, testis tissue always responded to LH in vitro by significant increases in the amounts of OHA secreted. Tissue from E2-treated fish showed no significant differences to the control group at all doses of LH used, while the LH sensitivity increased as indicated by a lower EC_{50} value (control: 20.1±0.2 vs E2: 3.6±0.4 ng LH/ml). Treatment with T was also associated with an increased LH sensitivity (EC_{50}: 5.7±0.2 ng LH/ml). At an LH concentration of 100 ng/ml (the only dose tested in the case of KT), KT, KA and T impaired LH-stimulated OHA secretion to a similar extent, 4–7% of the control values. Treatment with OHA had the most prominent effect, reducing LH-stimulated OHA secretion to less than 1% of the control values. The EC_{50} values for LH-stimulated steroid secretion after treatment with KA or OHA could not be estimated accurately because of the inhibitory effect of the higher LH doses (100 and 1000 ng/ml), nevertheless suggesting that the LH response pattern had changed.

Metabolism of tritiated precursors

Extraction of the media from testes after incubations with [3H]-P5 or [3H]-A showed that 95–99% of the total radioactivity was present in the organic fraction; the aqueous fraction was not further analysed. In all cases substrate was found at the end of the incubation period: for [3H]-P5 incubations 27.8–39.6%, and for [3H]-A incubations 17.4–42.5% of the respective substrates were recovered.

Separation of the [3H]-P5 metabolites by TLC revealed five main radioactive areas, corresponding to the carriers P5, A, a mixture of 17α-hydroxypregnenolone (17αP5)/17α-hydroxyprogesterone (17αP4), OHA, and finally an area (ca. 15% of the total activity) of unknown

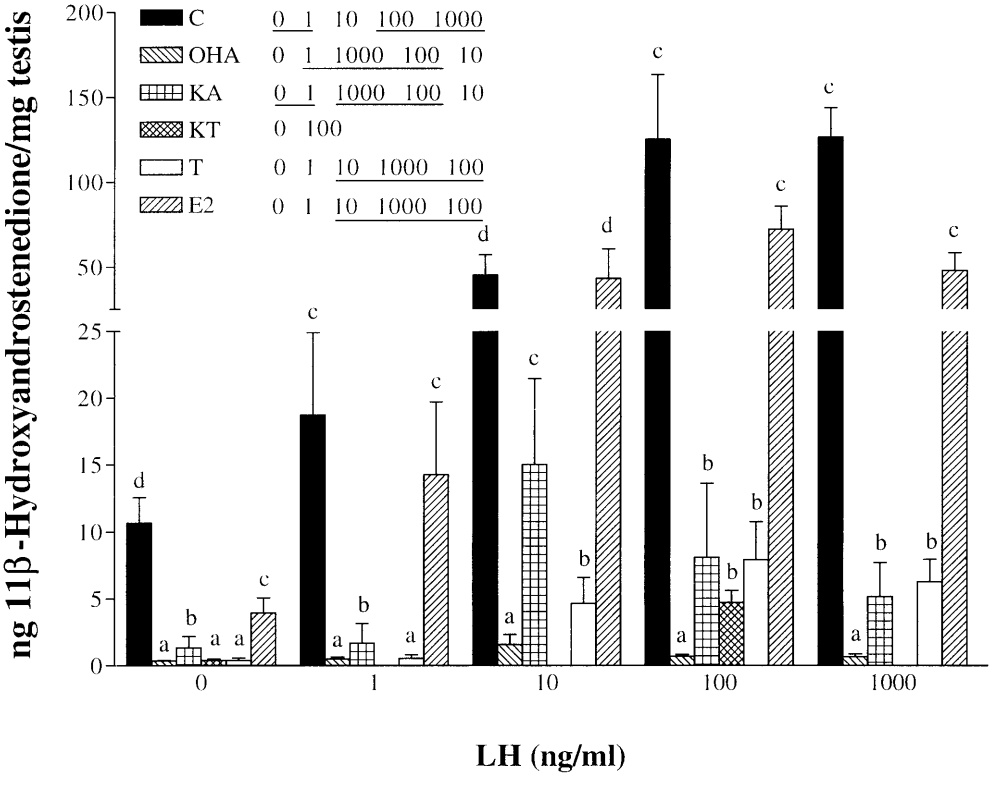

Fig. 1 In vitro secretion of OHA (ng/mg testis tissue; means±SEM) in response to increasing concentrations of LH 2 weeks after implantation of different steroids in African catfish. LH concentrations sharing the same underscore or groups labelled with the same letter at a given concentration of LH are not significantly different (*n*=6 for all incubations in the presence of LH; *n*=24 for incubations in the absence of LH, except for the KT-treated fish, where *n*=6; $P<0.05$, ANOVA, followed by Fisher's least significance difference test)

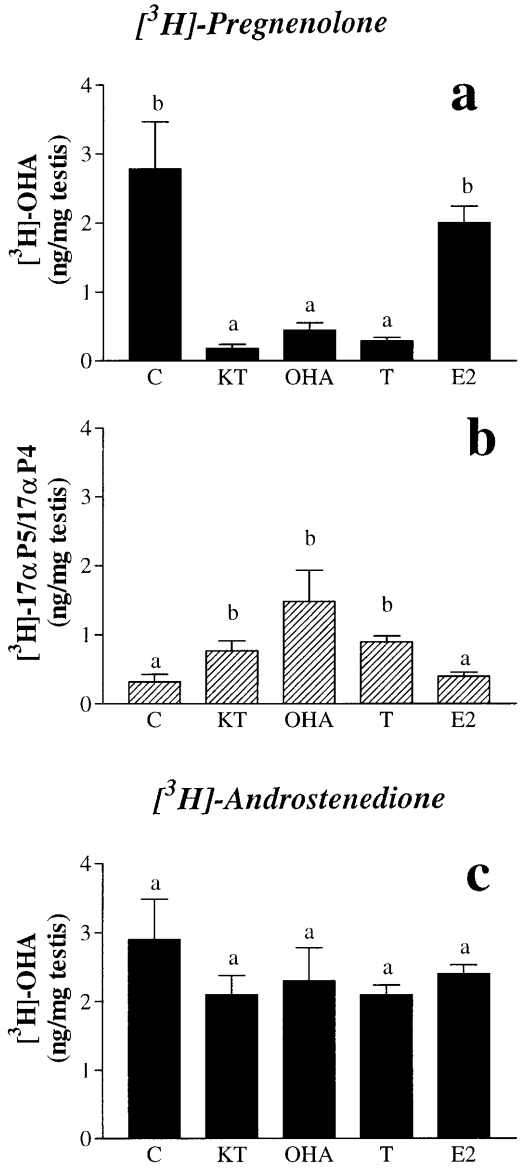

Fig. 2 Amounts of [3H]-OHA **(a)** or [3H]-17αP5/17αP4 **(b)** found after incubation with [3H]-pregnenolone, and amounts of [3H]-OHA **(c)** found after incubation with [3H]-androstenedione, of African catfish testis tissue in vitro 2 weeks after implantation of different steroids (means±SEM; n=4). Columns sharing the same letter do not differ significantly (P<0.05, ANOVA, followed by Fisher's least significance difference test)

apolar compounds; the latter area was not further analysed. Previous studies have shown that the 17αP5/17αP4 and OHA areas indeed hold these and no other compounds (Cavaco et al. 1997a). Expressing the results as nanograms [3H]-OHA found per milligram testis at the end of the incubation (Fig. 2a) revealed that treatment with KT, OHA and T reduced the amounts of [3H]-OHA to a similar degree, while E2 had no significant effect. The amounts of 17αP5/17αP4 found at the end of incubation, however, increased following androgen treatment (Fig. 2b). Similar to previous studies (Cavaco et al. 1997a), only small amounts of [3H]-T or [3H]-11-oxygenated androgens other than OHA were detected (data not shown).

Chromatographic separation of the [3H]-A metabolites revealed three main radioactive areas corresponding to the carriers OHA, a mixture of T/KA and A, with the OHA area holding the highest amount of radioactivity (ca. 70% of the total activity). Previous studies have shown that the T/KA and OHA areas indeed hold these and no other compounds (Cavaco et al. 1997a). No significant differences were found between the groups after expressing the data as nanograms [3H]-OHA found per milligram tissue (Fig. 2c).

Leydig cell ultrastructure

Leydig cells are situated in the interstitial tissue of the testis (Fig. 3a) and have a round or oval nucleus with a rim of heterochromatin adjacent to the nuclear envelope and one or more centrally located nucleoli (Fig. 3b). Mitochondria were abundant, often contained dense granules and regularly showed tubular cristae. Also, other organelles characteristic of steroid-producing cells were found such as smooth endoplasmatic reticulum (sER), lipid droplets (occasionally) and free ribosomes. After treatment with all androgens and unlike the situation in control and E2-treated groups, stretches of dilated sER were observed (Fig. 3e). Moreover, autophagosomes containing mitochondrial remnants were present (Fig. 3f). Although Leydig cells in tissue from E2-treated fish did not show quantitatively significant differences from the control group (see below and Table 1), some cells exhibited an elaborately folded nu-

Table 1 Quantitative analysis of some ultrastructural characteristics of Leydig cells 2 weeks after implantation of different steroids (30 µg/g body weight). Data are means±SEM (n=5)

	Cell	Mitochondria			Nucleus	
	Area (µm²)	N per cell section	Area (µm²)	Rel. area (% of cell section)	Area (µm²)	Rel. area (% of cell section)
C[1]	32.1±1.4[c]	24.0±2.6[b]	4.6±0.5[b]	14.2±1.0[b]	11.6±0.6[b]	36.1±1.7[a]
OHA[2]	20.8±1.2[a]	11.4±1.0[a]	1.8±0.2[a]	8.4±0.3[a]	9.8±0.8[a,b]	47.4±1.4[b]
KA[3]	25.7±1.6[b]	14.6±1.8[a]	2.3±0.9[a]	8.6±1.1[a]	9.7±1.1[a,b]	38.2±4.1[a]
KT[4]	24.3±1.2[b]	9.5±0.7[a]	2.2±0.3[a]	8.3±0.9[a]	9.6±0.8[a,b]	47.1±1.7[b]
T[5]	26.4±2.3[b]	10.9±2.2[a]	2.4±0.5[a]	8.5±1.2[a]	9.3±0.8[a]	35.0±1.9[a]
E2[6]	34.9±3.3[c]	26.4±7.9[b]	5.7±1.1[b]	15.3±2.2[b]	11.6±0.8[b]	34.9±3.4[a]

Means sharing the same superscript letter in the same column do not differ significantly (P<0.05, ANOVA, followed by Fisher's least significance difference test) (*1* control, *2* 11β-hydroxyandrostenedione, *3* 11-ketoandrostenedione, *4* 11-ketotestosterone, *5* testosterone, *6* estradiol-17β)

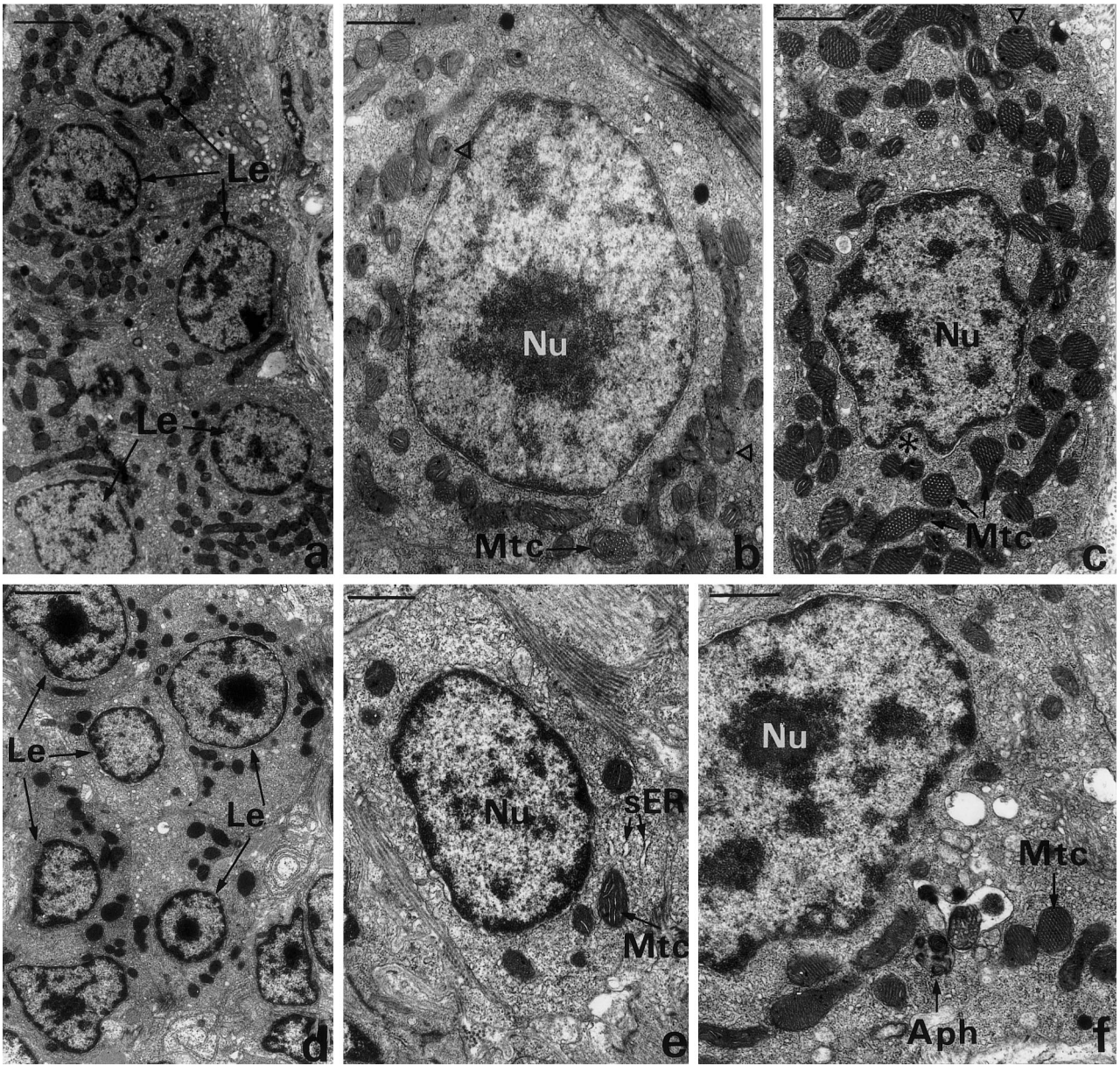

Fig. 3 Electron micrographs of Leydig cells from African catfish testis fragments after in vitro incubation in the absence of LH 2 weeks after implantation of steroid-free control pellets **(a, b)**, E2- **(c)**, T- **(d, e)** or KA- **(f)** containing pellets. **a** Cluster of Leydig cells (*Le*) in the interstitial area (control group). **b** Leydig cell (detail from control group) showing numerous mitochondria with tubular cristae (*Mtc*); intramitochondrial dense granules (*open arrowheads*) are observed regularly (*Nu* nucleus). **c** Leydig cell after treatment with E2. Note the invaginated nucleus (*) and the high number of mitochondria with tubular cristae (*Mtc*). **d** Cluster of Leydig cells after treatment with T. The number of mitochondria per Leydig cell section is reduced (cf. **a**). **e** Leydig cell (detail from T-treated group) showing dilatations of the smooth endoplasmic reticulum (*sER*). Treatment with KT, OHA and KA had effects similar to that observed after T treatment. **f** Leydig cell after treatment with KA, showing an autophagosomal structure (*Aph*) containing mitochondrial remnants. *Bars* 2 µm **(a, d)**; 1 µm **(b, f)**; 0.7 µm **(c, e)**

clear envelope (Fig. 3c) and also displayed the highest number of mitochondria recorded with up to 80 per cell section.

Quantitative analysis of some ultrastructural characteristics (Table 1) showed that treatment with all androgens led to a reduced Leydig cell surface area, OHA having the strongest effect. Both the number of mitochondria per cell section and the area held by mitochondria decreased to 44% of control values, indicating that the size of the (remaining) mitochondria had not changed. Considering that the changes in nuclear size were limited (Table 1), and that mitochondria at maximum held 15% of the cellular area, the androgen-induced reduction of the Leydig cell area by 25–40% is only in part due to a reduced number of mitochondria but also reflects a loss of cytoplasm.

A qualitative analysis of the Sertoli cells did not provide evidence of obvious changes in ultrastructural characteristics, and Sertoli cells were not analysed further.

Discussion

The present study was undertaken to gain more insight into the mechanism(s) involved in the steroid-induced modulation of androgen production in juvenile African catfish testes. In fish, as in other vertebrates, sex steroids exert feedback effects on the GnRH system and the pituitary gonadotrophs (Kah et al. 1993; Amano et al. 1994; Trudeau and Peter 1995; Le Dréan et al. 1996). It is important to discuss, therefore, whether the present treatment effects reflect direct actions of steroids on the testis or indirect effects via modulation of the gonadotrophs' activity.

Treatment with T and E2, but not with 11-oxygenated androgens, reduced circulating LH levels in juvenile male African catfish (Cavaco et al. 1995), suggesting that the inhibition of Leydig cell activity following treatment with 11-oxygenated androgens is not mediated by changes in LH blood levels. Considering moreover that T and E2 had distinct effects on Leydig cells but that both steroids reduced LH blood levels to a similar extent, we assume that reduced LH levels are of limited relevance for the effects of T on Leydig cells as well. However, the decreased basal OHA production seen after treatment with E2 may be related to the reduced LH blood levels. Reduction of circulating LH may also have led to an increased LH responsiveness as seen after treatment with E2 (and T). Metabolism of T into E2 or DHT does not seem to be needed to induce the reduction in OHA production, as both E2 (present study) and DHT (Cavaco et al. 1998) had much weaker effects than T on testicular OHA production. Also the follicle-stimulating hormone (FSH)-like GTH I stimulates steroid secretion from testes of immature or maturing Coho salmon (Planas and Swanson 1995). It is not known yet whether this is a direct effect of FSH on Leydig cells, perhaps mediated by the gonadotropin receptor type I, which binds both FSH and LH (Yan et al. 1992; Miwa et al. 1994), or whether this is related to a Sertoli-cell-mediated, paracrine stimulation of Leydig cells, such as described for FSH in rat (Matikainen et al. 1994) or man (Levalle et al. 1998). In the African catfish an FSH-like gonadotropin (protein or cDNA) has not been isolated yet (Koide et al. 1992; Rebers et al. 1997). In the Coho salmon, however, FSH plasma levels decrease in response to both T and E2 administration (Dickey and Swanson 1998). Assuming that African catfish FSH, if present in the circulation of juvenile males, responded at the onset of spermatogenesis in a way similar to FSH in Coho salmon, the distinct effects of T, but not of E2, on Leydig cells in catfish would again indicate that the effects of T were not primarily related to circulating gonadotropin levels. Work using a testicular organ culture system may be suitable to answer the question of direct versus indirect effects of androgens on steroidogenesis.

Previous experiments showed that 11-oxygenated androgens induced precocious spermatogenesis while aromatizable androgens (androstenedione and T), E2 but also non-aromatizable DHT did not display these biological activities (Cavaco et al. 1998). However, all androgens except for DHT reduced testicular OHA production. The present study confirmed and extended this finding. Leydig cell function and morphology was profoundly changed and treatment with 11-oxygenated and aromatizable androgens resulted in largely similar responses. This suggests that either the same mechanism of action was activated, or that different mechanisms of action merged on the same cellular target(s), both leading to a reduction in OHA secretory capacity. We favour the second option because the differential effects of 11-oxygenated androgens versus T as regards spermatogenesis may be taken to indicate that distinct mechanisms of action and perhaps also distinct types of androgen receptors are involved (discussed in detail in Cavaco et al. 1998; see also Sperry and Thomas 1999). It is unlikely, however, that KT/T interconversion is responsible for the similarity of effects. Conversion of KT to T is not possible biochemically; conversion of T to KT is possible but is unlikely to occur to a noteworthy extent in juveniles considering that treatment with T did not stimulate spermatogenesis and did not increase KT plasma levels (Cavaco et al. 1998). However, it cannot be excluded that an androgen receptor is present in Leydig cells that responds to both T and KT.

Androgen receptors of the nuclear steroid receptor family apparently are not expressed in male germ cells (e.g. Sar et al. 1990), so that testicular somatic cells are considered to mediate the effects of steroids on spermatogenesis. No obvious changes in Sertoli cell ultrastructure have been recorded, neither in the present study following steroid treatment (data not shown), nor in a previous study examining Sertoli cells during the first wave of spermatogenesis (Schulz et al. 1996). This is in contrast to the situation in the immature Japanese eel where ultrastructural characteristics of Sertoli cells did change during induced spermatogenesis. In this species, gonadotropin-mediated Leydig cell activation precedes the KT-mediated Sertoli cell activation (Miura et al. 1991). In the African catfish, on the other hand, Leydig cells are active before the beginning of spermatogenesis (Schulz et al. 1996), and it is possible that also Sertoli cells have been heretofore in an at least partially activated condition in the African catfish testis. In such a case morphological changes possibly related to the steroid treatment might not be very conspicuous.

Although KT may have induced changes in Sertoli cell activity that are not reflected in changes in their ultrastructure, Leydig cell ultrastructure did change in response to androgen treatment and these cells apparently are an important direct or indirect target for androgenic steroids in African catfish. It is therefore possible that the distinct effects on spermatogenesis of 11-oxygenated (stimulatory) and aromatizable androgens (no effect) are mediated – at least in part – by Leydig cells. This may involve one or more of the many paracrine factors and/or their receptors active in the testis (Saez 1994; Schlatt et al. 1997). Finally, a previous study (Cavaco et al. 1997b) and the similarity of the effects of OHA, KA, or KT on

spermatogenesis (Cavaco et al. 1998), pituitary gonadotrophs (Cavaco et al. 1995), or testicular OHA production (present study) suggest that the 11-oxygenated androgens either exert their effects via a common mechanism of action, or that KT is the biologically active androgen after bioconversion of OHA or KA. We favour the latter possibility since this bioconversion is very effective in African catfish (Cavaco et al. 1997b) and since the intratesticular concentration of OHA is in any case high in immature fish (see below). Hence, we consider KT as the physiologically most relevant androgen in male African catfish, in line with data in many other teleost fish (Borg 1994).

Two main changes have been recorded in Leydig cells. Morphologically, the reduction in the cell surface area and the loss of mitochondria were the most prominent observations. Physiologically, a steroidogenic lesion as regards $C_{17–20}$ lyase activity was recorded. The qualitative observation that autophagosomes were predominantly present in the androgen-treated groups may indicate that the mechanism to decrease the number of mitochondria is by autophagy.

The crucial event in the acute stimulation of steroidogenesis is the mobilization of cholesterol and, more importantly, the transfer of cholesterol to the inner mitochondrial membrane where the cholesterol side-chain cleavage enzyme and hence pregnenolone (P5) production is localized (Stocco and Clark 1996). The loss of half of the mitochondria may contribute to a reduced production of P5, and hence a diminished OHA production. However, OHA production was not halved but reduced to one-tenth or less of control values, while the conversion of A to OHA, catalysed by the mitochondrial enzyme 11β-hydroxylase, was similar in all treatment groups. This suggests that the activity of at least mitochondrial 11β-hydroxylase is not a limiting factor for OHA production, and is not compromised by loss of half of the mitochondria. After androgen treatment, on the other hand, 17α-hydroxylated intermediates accumulated in incubations with [^{3}H]-P5. This may indicate a reduction of the $C_{17–20}$ lyase activity which is associated with a single enzyme protein that catalyses two distinct, successive reactions: (1) the 17α-hydroxylation of P5 or progesterone (P4) and (2) the cleavage of the bond between the carbon atoms 17 and 20 to yield the androgens dehydroepiandrosterone or A, respectively. Both are intermediate precursors of T production in mammals (Hall 1994), or of OHA production in African catfish (Schoonen and Lambert 1986). In mouse Leydig cells, treatment with T decreased the steady-state levels of 17α-hydroxylase/$C_{17–20}$ lyase mRNA (Payne and Youngblood 1995). If androgen treatment had a similar effect on African catfish Leydig cells, this may explain in part the low yield of androgens. In particular the $C_{17–20}$ lyase activity appeared to be inhibited while increased levels of 17α-hydroxylated compounds may reflect the accumulation of intermediates in front of the $C_{17–20}$ lyase activity bottleneck. A possible explanation of a reduction in $C_{17–20}$ lyase activity with apparently normal 17α-hydroxylase activity is based on the observation that cytochrome b_5 awakens human $C_{17–20}$ lyase activity by protein-protein interaction (Lee-Robichaud et al. 1998) while the enzyme's 17α-hydroxylase activity may not require the more stringent conformational restrictions needed for the lyase activity (Auchus et al. 1998). If a similar regulation of the African catfish testicular $C_{17–20}$ lyase activity was active, androgen treatment may lead to a decreased expression of cytochrome b_5 and hence specifically attenuate the lyase activity.

What is the physiological relevance of the inhibitory effect of androgens on OHA production during pubertal development? One possibility is that androgens participate in regulating the local androgen levels in the testes. At the start of puberty (beginning of spermatogenesis) the African catfish testes produces large amounts of OHA per milligram testis (Schulz et al. 1996; Cavaco et al. 1997a; present study), probably resulting in high local OHA levels. However, the testes are small in juveniles so that the total testicular output of OHA (Schulz et al. 1996) and OHA plasma levels (Cavaco et al. 1997a) is low despite a high production of OHA per milligram testis tissue. As the testes grow, their total OHA output rises (Schulz et al. 1996) such that the hepatic conversion of OHA to KT becomes relevant (Cavaco et al. 1997b; Lambert et al. 1998), and KT plasma levels increase along with the appearance of spermatids (Cavaco et al. 1997a). Concomitantly, however, OHA production per milligram testis tissue drops, although the total testicular androgen output is further increased due to the strong testicular weight gain (Schulz et al. 1996). Since KT inhibits the steroidogenic capacity of Leydig cells but also stimulates germ cell development, KT may be important in the regulation of testicular OHA production and hence influence the synthesis of its precursor, thereby also controlling its effect on spermatogenesis.

Next to KT, T also appears to be an important androgen. Testosterone is produced in much smaller amounts than 11-oxygenated androgens by the catfish testis (Vermeulen et al. 1993). Nonetheless, plasma levels of T and KT are not that different (Cavaco et al. 1997a), and T is able to induce strong increases in the pituitary LH content (Cavaco et al. 1995); T may fulfill a similar function during natural sexual maturation in African catfish (Schulz et al. 1997b). As testicular T production increases during puberty (Schulz et al. 1996), an autocrine inhibitory feedback loop may be activated in the typically clustered Leydig cells in the immature testis. Interestingly, Leydig cells become dispersed in the rapidly growing testis during the first wave of spermatogenesis and Leydig cell clusters disappear soon after the onset of rapid testicular growth (Schulz et al. 1996). In the light of the results of the present study, the dispersal of Leydig cell clusters during normal puberty may be needed to (1) prevent too strong an autocrine inhibition of steroidogenesis and (2) to allow the further morphological and functional differentiation of Leydig cells during puberty (Schulz et al. 1996) and from adolescence to adulthood

(Schulz et al. 1997a). Future work will have to show the respective roles of KT and T in this process.

In summary the present study shows that the inhibitory effect of androgens on the steroidogenic capacity of the testis is accompanied by morphological changes in Leydig cells that are compatible with a reduced activity of the steroidogenic system. Since the inhibition is also apparent at the level of the $C_{17–20}$ lyase activity, the resulting reduced production of androstenedione and hence a shortage of substrate for the 11β-hydroxylase activity may explain the tenfold decrease in 11β-hydroxyandrostenedione production. Treatment with 11-oxygenated androgens or with testosterone led to similar effects, though possibly mediated via different mechanisms of action. It is proposed that the 11-oxygenated androgens exert their effect through their common derivate 11-ketotestosterone, while testosterone may act directly without being further metabolized (reduction or aromatization). The androgen-induced changes in Leydig cell ultrastructure and steroidogenesis may reflect a steroid-mediated feedback mechanism to control testicular steroid production.

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