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Androgens modulate testicular androgen production in African catfish (*Clarias gariepinus*) depending on the stage of maturity and type of androgen

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Abstract

Previous work showed that androgen treatment suppressed testicular steroidogenesis in juvenile African catfish *Clarias gariepinus*. Similar to other vertebrates, however, circulating androgen levels increase during puberty in catfish. We therefore studied if androgen-induced inhibition of androgen production decreases during sexual maturation. As in other vertebrates, testosterone (T) is found in the circulation in fish but typically, 11-ketotestosterone (11-KT) is the quantitatively dominating androgen. In previous studies with juvenile catfish, these two androgens showed different biological activities as regards spermatogenesis or pituitary hormone production, but were equally effective in suppressing testicular steroidogenesis. Hence, the second question we studied was if the two types of androgens show distinct effects on the steroidogenic system in pubertal or adult males. The inhibitory effect of 11-KT on the testicular steroidogenic capacity waned with progressing sexual maturation, while T-mediated inhibition remained strong until adulthood reducing the *in vitro* steroid production 4- to 10-fold. However, the gonadotropin responsiveness of testicular tissue was not compromised and expression of testicular gonadotropin receptors did not respond differently to the two androgens. We conclude that the selective disappearance of the inhibitory effect of 11-KT contributes to allowing the pubertal increase of the plasma level of this androgen.

Keywords: Puberty; Testis; Steroidogenesis; Gonadotropin receptors; African catfish

1. Introduction

Leydig cell androgen production is of crucial relevance for reproductive functions (Yeh et al., 2002; Pakarainen et al., 2005), rendering the regulation of androgen production an important aspect of male reproductive physiology. Androgens participate in the regulation of their own production in an apparently similar manner in different vertebrates. In rat *Rattus norvegicus* and rainbow trout *Oncorhynchus mykiss*, for example, androgen treatment down-regulated the expression of steroidogenic enzymes (Shan et al., 1995; Baron et al., 2005), and in juvenile African catfish *Clarias gariepinus*, exposure to androgens reduced the testicular androgen production capacity to 10% of control levels, and induced changes in Leydig cell ultrastructure consistent with a reduced androgen production capacity (Cavaco et al., 1999). *Vice versa*, recent pilot studies in African catfish indicated that an experimental reduction of plasma androgen levels increased the testicular androgen production *in vitro* (García-López and Schulz, unpublished observation).

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Pubertal development in African catfish (Schulz et al., 1994a), as generally observed in teleost fish (Okuzawa, 2002), is associated with increasing plasma androgen levels. Nevertheless, the *in vitro* androgen production per unit testis weight decreased during the onset of puberty in catfish (Schulz et al., 1994a). This apparent contradiction is resolved by the rapid growth of the testis during puberty (Schulz et al., 1996), also involving Leydig cell proliferation (Schulz et al., 2005), together resulting in a 10-fold increase of total testicular androgen production *in vitro* (Schulz et al., 1996). In principle, though, a decrease in androgen production per unit testis weight, which we observed along with increasing plasma androgens in pubertal catfish, is consistent with an androgen-mediated inhibition of Leydig cell activity. At later stages of puberty, after completion of the first wave of spermatogenesis at ca. 6 months of age, testis weight kept increasing in a supra-allometric manner towards full maturity at 12 months of age (Schulz et al., 1994a). During this development from pubertal stages to adulthood the testicular responsiveness increased to maximally effective luteinizing hormone (LH) stimulation (2-fold above basal in 5–8 months old fish *vs.* 3- to 5-fold in one year old fish; Schulz et al., 1994a,b). A stable or slightly increasing basal androgen production per unit tissue weight during a period of testis growth, that mainly reflects an increase in germ cell number, can be understood in the context of proliferating Leydig cells (Schulz et al., 2005), while the increased responsiveness to LH may reflect elevated expression of the LH receptor and/or other factors determining the steroidogenic capacity of Leydig cells. Altogether, the developmental processes that take place enable the rise in plasma androgen levels associated with completing puberty and reaching adulthood.

However, an increasing or high androgen production in the presence of high androgen levels—the situation found from ca. 8 months of age and in adult males—seems to conflict with the observation of androgen-induced inhibition of testicular androgen production. This apparent conflict would be resolved by assuming that the androgen-mediated inhibition of androgen production becomes less effective when approaching full maturity. Testing this hypothesis was the first objective of this study, which was approached by comparing the effects of experimentally elevated plasma androgen levels on testicular androgen release at different stages of pubertal development.

In a previous study on juvenile catfish, 11-ketotestosterone (4-androsten-17β-ol-3,11-dione; 11-KT) and testosterone (4-androsten-17β-ol-3-one; T) were equally effective in suppressing testicular steroidogenesis (Cavaco et al., 1999) while these two androgens showed distinct bioactivities as regards other processes, such as pituitary hormone production (Cavaco et al., 2001a) or spermatogenesis (Cavaco et al., 2001b). Therefore, the second objective of the current study was to test if 11-KT and T have similar or distinct effects on the steroidogenic system, or on regulatory systems targeting steroidogenesis, during different stages of pubertal development and at adulthood.

The main androgen produced by African catfish Leydig cells is 11β-hydroxyandrostenedione (4-androsten-11β-ol-3,17-dione, OHA; Vermeulen et al., 1993), which—after its release into the circulation—is quickly and effectively converted in the liver to 11-KT, the main circulating androgen (Cavaco et al., 1997). Accordingly, castration followed by OHA replacement therapy resulted in 10-fold higher plasma 11-KT levels compared to those of OHA within two hours after hormone administration (Cavaco et al., 1997). Therefore, we have treated fish with OHA to achieve an elevation of 11-KT plasma levels.

Although castration experiments showed that testis tissue in African catfish is the major source of T (Vermeulen et al., 1994), this androgen is a minor product of testicular steroidogenesis, amounting to only 1% of OHA production (Vermeulen et al., 1993). Still, T plasma levels are in the same order of magnitude as those of 11-KT (Schulz et al., 1994b), possibly reflecting the high binding affinity and capacity of T to sex hormone binding globulin (SHBG; Rebers et al., 1991). Therefore, T may be physiologically relevant for African catfish puberty (see also Cavaco et al., 2001a,b), and treatment with this androgen was included in the present study.

In order to test the *in vitro* steroid production (as measured by OHA release) of testicular tissue after androgen treatment *in vivo*, standardised short-term challenges to purified (from the pituitary) African catfish LH (cfLH; Schulz et al., 2001) were used in the present study. Previous work showed that cfLH is an efficient stimulator of testicular steroid release *in vitro* even in juvenile males (e.g. Schulz et al., 1994a, 1996; Cavaco et al., 1999). Unfortunately, African catfish follicle stimulating hormone (cfFSH) was not available for the present study. This gonadotropic hormone also has steroidogenic activity in fish (Planas and Swanson, 1995; Vischer et al., 2003), and has been suggested to stimulate the initiation of spermatogenesis via androgen release in teleosts (Planas and Swanson, 1995; Kamei et al., 2006; Ohta et al., 2007). Since cfLH activated *in vitro* both the LH and FSH receptors (cfLHR and cfFSHR, respectively; Bogerd et al., 2001; Vischer et al., 2003), cfLH is considered to induce both cfLHR- and cfFSHR-dependent steroidogenesis. Also, both FSH- and LH-induced steroid biosynthesis involve cAMP as second messenger (Planas et al., 1993). Hence, a methodological aspect of the present study was to test if non-specific activation of the adenylate cyclase (by forskolin), which should reflect maximally stimulated androgen release *in vitro*, differs from androgen release as induced by a high cfLH concentration.

2. Materials and methods

2.1. Experimental animals

Male African catfish were bred and raised as described previously (De Leeuw et al., 1985), except that African catfish pituitary extract (one pituitary equivalent per female) instead of human chorionic gonadotropin was used to induce ovulation. The fish were kept in a recirculation system under constant water temperature (25 ± 2 °C) and photoperiod