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Androgen Biosynthesis in Teleost and Elasmobranch Fishes

D E KIME
Department of Zoology, The University, Sheffield, S10 2TN, United Kingdom

Patterns of androgen biosynthesis in the two largest groups of fishes, the Teleostei and the Elasmobranchii, are reviewed. In elasmobranchs the major androgen is testosterone but in teleost fish, by contrast, testicular androgens are predominantly 11β-hydroxy and 11-keto derivatives of testosterone and androstenedione. Teleost ovaries, however, synthesize testosterone and in some species its 11-oxygenated derivatives from suitable precursors *in vitro*. The modes of conjugation of androgens by the testes of elasmobranchs and teleosts is also strikingly different. In the testes of the elasmobranch *Scyliorhinus caniculus*, solvolysable conjugates are formed, whereas in a number of teleosts only glucuronides are produced by the testis *in vitro*.

Environmental temperature may affect the activity of steroidogenic enzymes. Formation of 11-oxygenated androgens from testosterone is optimal at 6-21°C for trout and 11-36°C for goldfish. Glucuronide formation, which is optimal at 21-37°C in the trout and 21-41°C in the goldfish may effectively regulate the biological availability of steroid hormones as well as their precursors.

Factors, such as temperature, light and stress, which may affect testicular steroidogenesis in teleost fish are surveyed.

Introduction

In 1960, Idler and co-workers reported the presence of a new hormone, 11-ketotestosterone, in the plasma of the sockeye salmon. Since that report, the gonadal synthesis of 11-oxygenated androgens has been demonstrated in a wide variety of teleost fish, which thus exhibit a marked dissimilarity to the mammals in which 11-oxygenation is limited to the adrenal. For brevity, this paper will be limited to a review of *in vitro* androgen biosynthesis by fish gonads from suitable radioactive precursors. Complementary experiments which have isolated endogenous steroids from plasma or gonadal tissue indicate that the *in vitro* findings reflect the situation *in vivo*.

Androgen Biosynthesis by Fish Testis

11-oxygenated androgens have now been either positively or tentatively identified as products of incubations of radioactive precursors with testis tissue in 12 species of teleosts from six different orders (figure 1). Although this is only a minute proportion of existing teleost species, the diversity indicates that 11-oxygenated androgens may be the characteristic testicular hormones for bony fishes generally. In two species, *Tribolodon hakonensis* and *Gobius paganellus*, only testosterone was isolated as an *in vitro* metabolite of C_{21} precursors, but in both species the authors reported a number of unidentified polar metabolites which may have been 11-oxygenated steroids. In *Mugil*

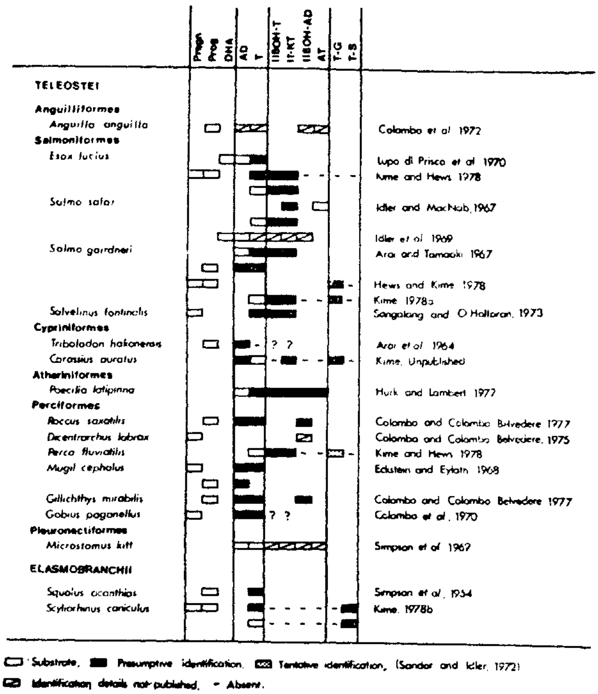

Figure 1 Androgen biosynthesis by testes of teleost and elasmobranch fishes *in vitro*

(*Pregn*, pregnenolone; *Prog*, progesterone; *DHA*, dehydroepiandrosterone; *AD*, androstenedione; *T*, testosterone; *11βOH-T*, 11β-hydroxytestosterone; 11-*KT*, 11-ketotestosterone; *11βOH-AD*, 11β-hydroxyandrostenedione; *AT*, androstenetrione; *T-G*, testosterone glucuronide; *T-S*, solvolysable conjugate of testosterone)

cephalus, testosterone but not its 11-oxygenated derivatives was isolated as a metabolite of pregnenolone. However, since 11-ketotestosterone has been reported in ovaries of the closely related species, *Mugil capito* (figure 2), the failure to find this compound in the testis is surprising and may have been due to its relative unfamiliarity. The nature of the 11-oxygenated androgens isolated from *in vitro* experiments appears to vary considerably with the species, some species forming testosterone derivatives and others androstenedione derivatives. Whether this is in fact species difference or is more a reflection of the incubation conditions is a matter of debate and can perhaps only be settled by *in vivo* experimentation.

In mammals conjugation of steroids is predominantly hepatic and although sulphates are formed in appreciable quantities by the adrenal and feto-placental unit, endocrine formation of glucuronides is unknown in the normal animal. Not surprisingly, conjugates, especially glucuronides have for long been considered as merely excretory products. In 1971, however, Idler et al. showed that in the salmon the concentration of testosterone glucuronide was higher in testicular than in peripheral plasma, indicating a testicular source of conjugates. Several papers have since appeared reporting the formation of unidentified conjugates by the teleost testis *in vitro* and we have recently demonstrated the testicular synthesis of testosterone glucuronide in three species of teleosts, the rainbow trout (*Salmo gairdneri*), the goldfish (*Carassius auratus*), and the perch (*Perca fluviatilis*). In the trout and goldfish, yields of glucuronides may be extremely high and as much as 60% of testosterone substrate may be conjugated during a 3 hr incubation.

Few elasmobranch species have been examined but results so far indicate a marked difference to the teleosts. In both *Squalus acanthias* and *Scyliorhinus caniculus*, the major androgen *in vitro* was testosterone and no evidence was found for the presence of 11-oxygenated steroids. In *Scyliorhinus caniculus*, a solvolysable conjugate of testosterone was formed in large quantities but there was no evidence for glucuronide formation. Since the number of species so far examined is very small, it is hard to ascertain whether glucuronides are characteristic of teleosts and sulphates of elasmobranchs.

Androgen Biosynthesis by Fish Ovary

Testosterone has been isolated from incubations of C_{19} and C_{21} precursors with ovaries from twelve species of teleosts belonging to seven different orders (figure 2). Whether the testosterone isolated truly represents an ovarian hormone or whether it is produced *in vivo* only as an intermediate in oestrogen synthesis is, however, unclear. In four species, 11-oxygenated androgens have also been isolated and these steroids are perhaps more likely to be of functional significance. In the

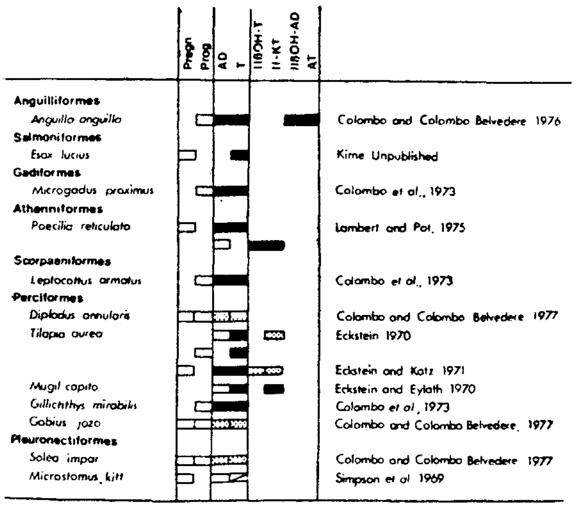

Figure 2 Androgen biosynthesis by teleost ovary *in vitro*

mullet *Mugil capito*, levels of 11-oxygenated androgens were much higher in freshwater than in sea water fish and this has been related to the infertility of this species in freshwater (Eckstein & Eylath 1970). In *Tilapia aurea*, the ratio of 11-ketotestosterone to testosterone was much higher in fish following spawning than before spawning. In the guppy, *Poecilia reticulata*, 11-ketotestosterone was apparent in 12-month-old fish but not in the 3-month-old fish and it has been suggested (Lambert & Pot 1975) that this may be the cause of the sex reversal sometimes observed in old guppies. In the eel *Anguilla anguilla*, 11-oxygenated androgens may be responsible for the long delay in sexual maturation although they could be merely the products of testicular enzymatic remnants from the long period of intersexuality of this species (Colombo & Colombo Belvedere 1976). Ovarian 11β-hydroxylase has also been related to hermaphroditism in two other species, the protogynous *Coris julis*, and the protandric *Pagellus acarne* (Reinboth 1972).

21-Hydroxylated steroids have already been shown to play an important part in the maturation of oocytes of some teleosts (Goswami & Sundararaj 1974). The role of ovarian 11-oxygenated androgens in the regulation of teleost fertility is at present far less clear.

Effect of Temperature on Testicular Androgen Biosynthesis

Both day length and temperature influence gonadal development in teleost fish. The relative importance of these two stimuli varies from species to species, but both effects have generally been considered to be mediated via the pituitary and hence by the effect of gonadotropins on the gonadal synthesis of steroids. Since many enzymes have an optimum temperature range, we considered it possible that some regulation of steroidogenesis in fish could be exerted directly by the environmental temperature acting on the gonadal enzymes. To examine this possibility we have incubated testes of two species of teleosts, the trout *Salmo gairdneri*, and the goldfish *Carassius auratus*, with $[^3H]$ testosterone at a range of temperatures from 0 to 46°C and examined the effect of temperature change on product yields. The results of these incubations (figures 3 and 4) show a number of important temperature effects. In the trout a plateau of activity is found between 6 and 21°C in the yields of 11-oxygenated androgens; a similar pattern is observed for both 11-oxo- and 11β-hydroxy-derivatives. In the goldfish which breeds at a higher temperature, the plateau is not attained until 11°C and activity diminishes significantly above 36°C. In both species the optimum temperature corresponds broadly to the favoured breeding temperature of the species. The breadth

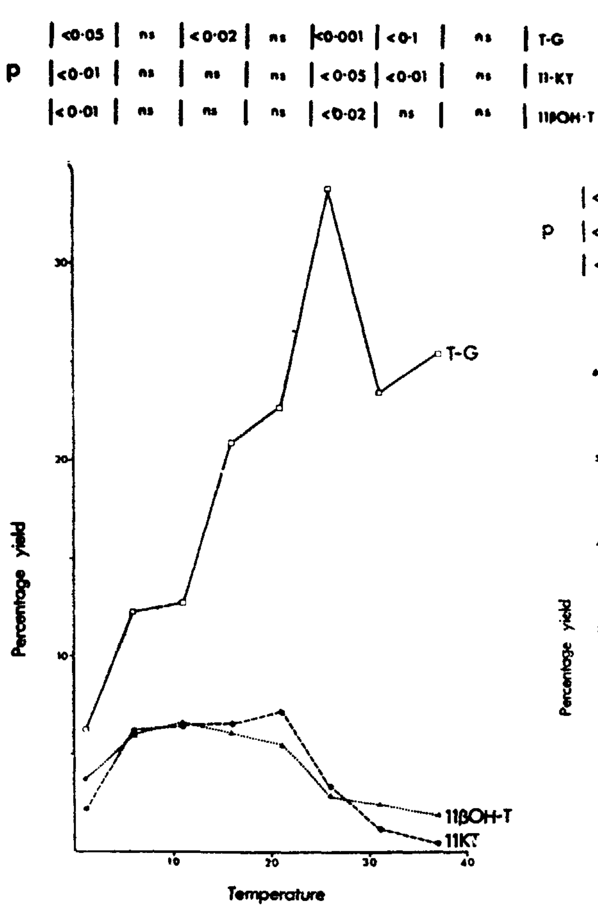

Figure 3 Effect of temperature on the metabolism of testosterone by trout testis

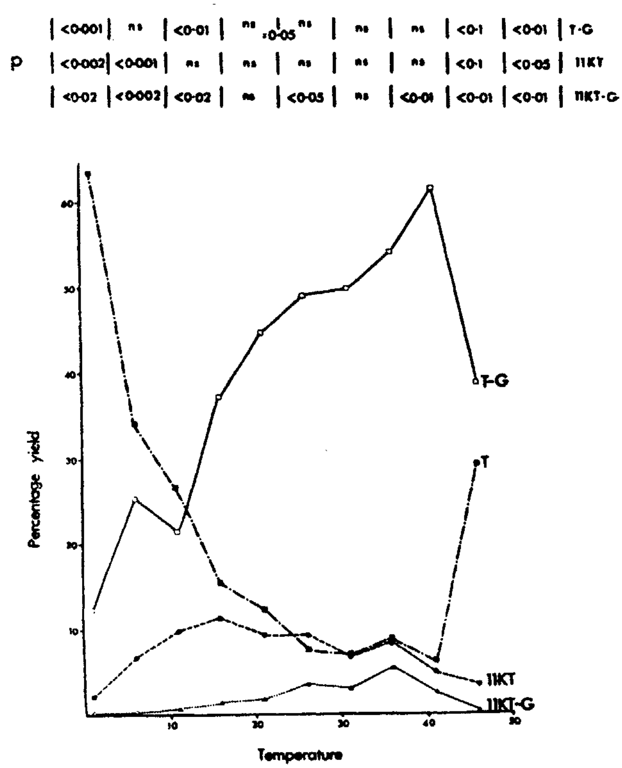

Figure 4 Effect of temperature on the metabolism of testosterone by goldfish testis

of the peaks however would indicate that control is permissive rather than regulatory and may act in conjunction with pituitary factors. The most important fact to emerge from these studies is the effect of temperature on glucuronide synthesis. In both species, the maximum yields of testosterone glucuronide are found at a higher temperature than those of 11-oxygenation. In the goldfish, 11-ketotestosterone glucuronide formation exhibits a similar temperature dependence to that of testosterone glucuronide. This indicates that the plateau in the yield of unconjugated 11-oxygenated androgens is caused by two factors: (*a*) diminishing availability of testosterone substrate due to competition by glucuronidation and (*b*) removal of 11-oxygenated products as their glucuronides. Both of these factors become increasingly important as the temperature is raised, resulting in decreased output of free 11-oxygenated androgens. In the absence of glucuronide formation it is probable that the optimal temperature for androgen formation would be significantly higher than that actually observed. Glucuronidation may thus serve an important part in limiting androgen production to the biologically preferred environmental temperature for a particular species.

Control of Androgen Biosynthesis in Teleosts

Testicular steroidogenesis and hence reproductive development may be affected by three factors: day length, environmental temperature and stress (figure 5). The relative contribution of each of these factors may be presumed to vary from species to species so as to favour reproduction at an environmentally suitable time.

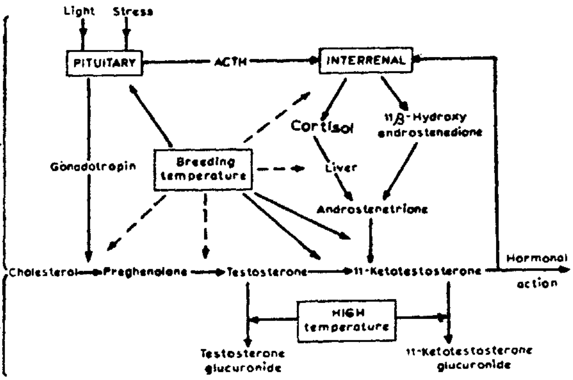

Figure 5 Factors affecting testicular steroidogenesis in teleost fish

Day length

Certain light-dark regimes may stimulate the pituitary production of gonadotropins. In mammals these hormones have been shown to stimulate the side-chain cleavage of cholesterol to give pregnenolone.

Temperature

The permissive effect of temperature on the activity of steroidogenic enzymes and the control of 11-oxygenated androgen production by competitive glucuronide formation has been described above. Temperature may also be conceived as acting on the cholesterol side-chain cleavage enzymes in a number of other ways : (*a*) It may affect gonadotropin production by the pituitary either indirectly via a thermoreceptor or directly by acting on gonadotropin synthesising enzymes which may have similar temperature characteristics to the gonadal steroidogenic enzymes, (*b*) Side chain cleavage enzymes may exhibit a similar temperature optimum to those later in the biosynthetic pathway, (*c*) Cholesterol side-chain cleavage may be more sensitive to gonadotropin stimulation at certain temperatures. As in mammals regulation of reproductive development is probably exerted mainly at the cholesterol side chain cleavage step.

Stress

Since 11-oxygenated androgens are produced by the mammalian adrenal it would perhaps not be surprising to find that in teleost fish 11-oxygenated steroids of interrenal origin may play a role in reproductive processes. Idler et al. (1969) have shown that the salmon interrenal may convert androstenedione or DHA into 11β-hydroxyandrostenedione, 11β-hydroxytestosterone and 11-ketotestosterone, and Arai et al. (1969) have demonstrated a similar conversion of androstenedione to its 11β-hydroxy derivative by interrenal tissue of the trout. More recently we have shown (Kime 1978c) that livers of the trout, the pike and the perch may convert cortisol into androstenetrione, a compound which is readily convertible by the testis into 11-ketotestosterone. It is thus conceivable that stress acting via the interrenal could result in increased 11-ketotestosterone production and thus stimulate reproductive development. This may be of particular importance in migratory species such as salmon in which gonadal development parallels the stresses of migration. Of particular interest in this respect are Fagerlund and Donaldson's finding (1969) that cortisol production could be stimulated by androgens. There may thus be a positive feedback loop, initiated by migratory stress and independent of gonadotropin control, which could lead to the observed high cortisol levels in sexually maturing salmon.

Conclusions

Relatively few of the many species of fish have so far been investigated but it is becoming increasingly clear that in the nature of the gonadal androgens, in the regulation of their biosynthesis and in the role of steroid conjugation there exist major differences between fish and mammals.

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