IN VITRO METABOLISM OF TESTOSTERONE BY GONADAL TISSUE OF A PROTANDRIC ANEMONEFISH AT VARIOUS SEXUAL STAGES.

M. Latz, P. Stahlschmidt-Allner, and R. Reinboth

Institut für Zoologie, University of Mainz, D 65 Mainz, Germany.

Summary

Gonadal tissues of the anemonefish Amphiprion frenatus, a facultative protandric teleost, were incubated with labeled testosterone. The gonads originated from animals which had been subjected to particular social stimuli by conspecifics in order to manipulate their sexual status. The 11β-hydroxylase activity is high in inverting specimens and in males and low or undetectable in females. In the incubations of inverting fishes was a total lack of 5α- and 5β-reductase activity. In female gonads no estradiol could be found.

Introduction

Fishes of the genus Amphiprion are living in tropical reefs of the indopacific region. Adult fishes inhabit sea anemones as pairs in which the male is smaller than the dominant female. They are considered to be protandric. In field experiments Fricke (Fricke & Fricke 1977) established that the change of sex in Amphiprion is socially controlled. As Amphiprion is one of the very few marine fishes that can be raised in captivity an experimental study was undertaken in order to define more clearly the gonadal development in juveniles and adults and the conditions of sex inversion of Amphiprion frenatus (P. Stahlschmidt, R. Reinboth 1988). The passage of a functional male phase is not obligatory for becoming a female and the inversion of male to female is equally not mandatory. Juveniles may develop as well to males (β) as directly to females (α). But they also retain their juvenile status (γ). The presence of females stimulates spermatogenesis in fishes in social β-position whereas the presence of males inhibits spermatogenesis in γ-animals (Fig. 1). Another peculiarity of Amphiprion is an unusual organization of the gonad of functional males: All male gonads contain from the very beginning considerable amounts of ovarian tissue which lies side by side with the testicular part without visible boundary between the two heterologous elements. (Brusle-Sicard & Reinboth 1990). For finding out wether social changes are reflected in the steroid metabolism of the gonads 15 incubations were carried out.

Methods

The tissues were taken from juveniles, females, 1 male, and from such individuals which had been placed into contact for different times (1 - 28 weeks) with a partner whose social status was known (Table 1). The incubation lasted two hours. The extraction and chromatographic separation of the steroids were carried out routinely as we described previously (Reinboth & Becker 1984).

Table 1

List of incubations

type of gonad	time of experiment
juveniles	
-male (2)	1 week
-male (2)	2 weeks
-male	3 weeks
-male	8 weeks
functional male	
-female	8 weeks
-female	16 weeks
-female (2)	28 weeks
functional females (3)	

Results and Discussion

The most striking result is a very high 11β-hydroxylase activity in most incubations (Fig. 2). The largest amount of 11-oxigenated androgens (about 90%) was observed in such juveniles which were brought together with an adult female for 1 to 2 weeks only. But the production of 11-oxigenated androgens was also high (>60%) in the gonads of those fishes that we intended to change from male to female either by isolation or by placing the male together with a juvenile over periods between 8 and 28 weeks. Even after seven months these values had not decreased markedly although the histologic examination shows that the testicular part was reduced to small islets or virtually absent.
In females 11β-hydroxylase activity was very low or not detectable.

In juveniles and in differentiating males no 5α- or 5β-reduced steroids could be observed although the presence of reduced androgens seems to be the rule in incubations with gonadal tissue of teleosts. A small amount of reduced steroids was detected in the gonads of the functional females and the male and of those specimens which had been given the chance to occupy the α-position for the longest time. It seems to be a special feature of functional gonads that they are able to reduce steroids. 11β-hydroxytestosterone is quantitatively the most important metabolite. The share of 11-ketotestosterone is usually low (< 10%) but in the females it outweighs the other three 11-oxigenated androgens. (Fig. 3). The data for the pool of juveniles and the earliest fish being under the conditions for sex inversion differ from all other incubations. In both cases nearly as much 11-ketotestosterone as 11β-hydroxytestosterone was present. The similarity of the values might be an indication for early differentiating processes, but this problem has to be clarified by future incubation. The examination of the steroidmetabolism in γ-ranking animals requires also further studies. It remains unknown in which cell type the 11β-hydroxylase is located.

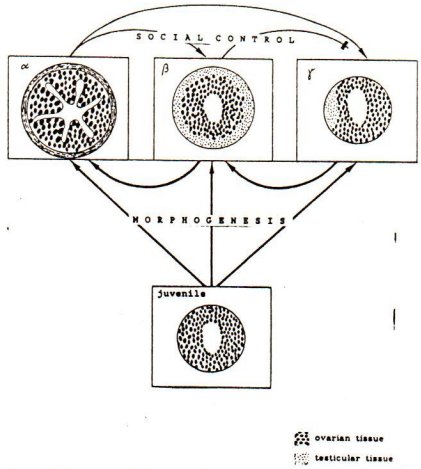

Fig. 1 Scheme illustrating morphogenetic changes which are influenced by social relations.

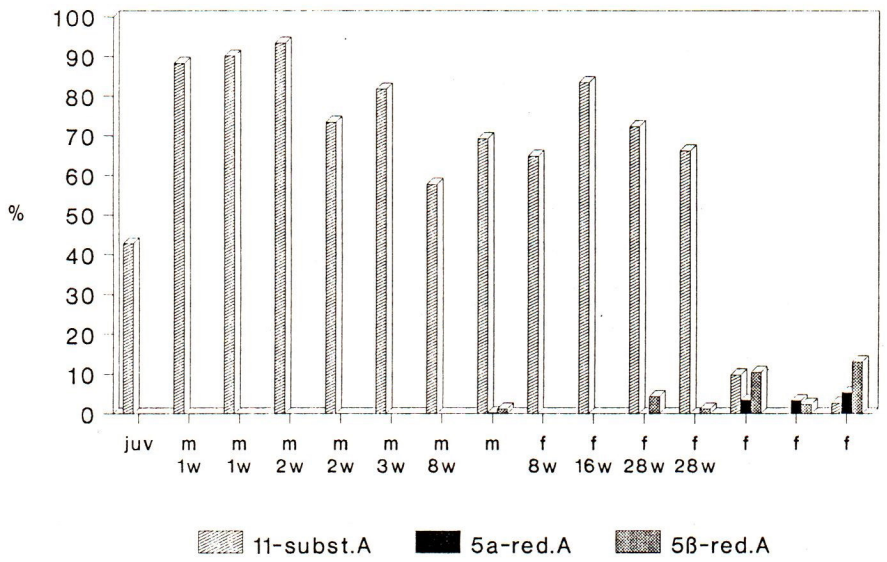

Fig. 2 Distribution of the steroid metabolites from the incubations listed in table 1.
(11-subst.A = 11 oxigenated androgens; 5α-red.A = 5α-reduced androgens; 5β-red.A = 5β-reduced androgens)

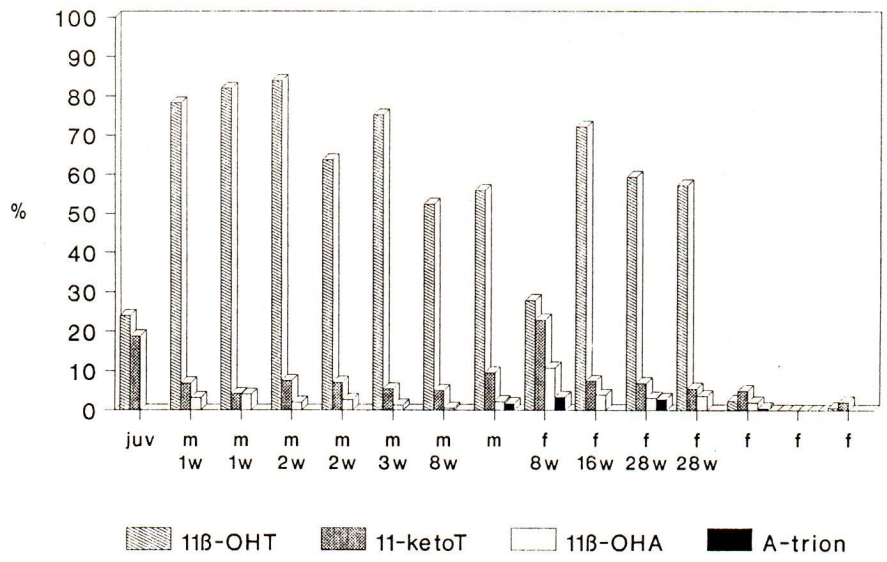

Fig. 3 Distribution of the 11-oxigenated androgens (11β-OHT = 11β-hydroxytestosterone; 11-ketoT = 11-ketotestosterone; 11β-OHA = 11β-hydroxyandrostenedione; A-trion = Androstenetrione)

References

Bruslé-Sicard, S. & R. Reinboth, 1990. Protandric hermaphrodite pecu'iarities in Amphiprion frenatus Brevoort (Teleostei, Pomacentridae). J. Fish. Biol. 36: 383-390.

Fricke, H.W. & S. Fricke, 1977. Monogamy and sex change by aggressive dominance in coral reef fish. Nature 266: 830-832.

Reinboth, R. & B. Becker, 1984. In vitro studies on steroid metabolism by gonadal tissues from ambisexual teleosts. I. Conversion of (14C)-testosterone by males and females of the protogynous wrasse Coris julis L. Gen. Comp. Endocrinol 55: 245-250.

Stahlschmidt, P. & R. Reinboth, 1988. Facultative ambisexuality in Amphiprion frenatus. Verh. DZG 82: 324.