Fish Physiology and Biochemistry **28:** 193–195, 2003.

Diurnal rhythm of steroid biosynthesis in the testis of terminal phase male of protogynous wrasse, *Pseudolabrus sieboldi*, a daily spawner

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Key words: steroid synthesis, diurnal rhythm, wrasse, *Pseudolabrus sieboldi*

Abstract

The wrasse, *Pseudolabrus sieboldi*, is a diandric protogynous labrid fish. Spawning is performed by a terminal phase (TP) male and an initial phase (IP) female between 6:00 and 9:00 h daily during two-month-long spawning season. In the present study, to investigate the roles of steroid hormones in the diurnal spermatogenesis of the *P. sieboldi* TP male, all steroid hormones produced in the testis were identified and the synthetic pathways of these steroids were determined. Furthermore, the circulating levels of the major steroids produced were analyzed throughout a day at 3-hour intervals during spawning season. In the testis, 11-ketotestosterone (11-KT), estradiol-17β (E2), 17,20β-dihydoxy-4-pregnane-3-one (17,20β-P) and 17,20β,21-trihydroxy-4-pregnen-3-one (20β-S) were synthesized as the major metabolites. *In vitro* steroid biosynthesis experiments showed similar results to the circulation profiles of the major steroids. This study is the first to clarify the complete steroidogenic pathways in the gonads of a diandric protogynous species throughout its life, when combined with the results of the steroidogenesis in the ovarian follicles. This is also the first report of a clear diurnal rhythm of the steroid production corresponding to the spermatogenic process in the testis of a male teleost.

Introduction

Pseudolabrus sieboldi, a diandric protogynous labrid fish, is distributed in shallow coastal waters off southwestern Japan, on rocky bottoms. Large TP develops from small, IP females. This fish is collected easily in large numbers. In captivity, as in nature, pairs of TP males and IP females spawn between 6:00 and 9:00 h daily during the spawning season. We have reported the diurnal rhythm of oocyte development, maturation, and ovulation, and profiles of circulating levels of several steroid hormones in females. The complete pathways of the steroid hormone synthesis in the ovarian follicles have also been clarified (Ohta et al. 2001; Ohta and Matsuyama 2002). More recently, we also succeeded in determining the social conditions that bring about both protogynous and reversed sex change in captivity (Ohta et al. 2003). These biological features of the wrasse make it a good model for studying the physiological mechanism of gametogenesis and sex change in teleost. However, there is very little information on gametogenesis and steroidogenesis in the testis of this species. In the present study, we clarified the steroid hormone synthesis pathways in the testis of TP males, and investigated the diurnal change of steroid biosynthesis in the testis of this species.

Materials and methods

After confirming the daily spawning of males reared in captivity, 4–6 male fish were sampled at each 3-hour interval throughout the day. These fishes were anesthetized and blood samples were collected for measuring serum steroid level using ELISA. They were killed by decapitation and testis was isolated for *in vitro* incubation. Testes were sampled at 6-hour intervals from the sampled males, and testicular fragments (ca. 20 mg) were incubated with 25 pmol of [^{3}H]-pregnenolone, [^{3}H]-testosterone or [^{14}C]-

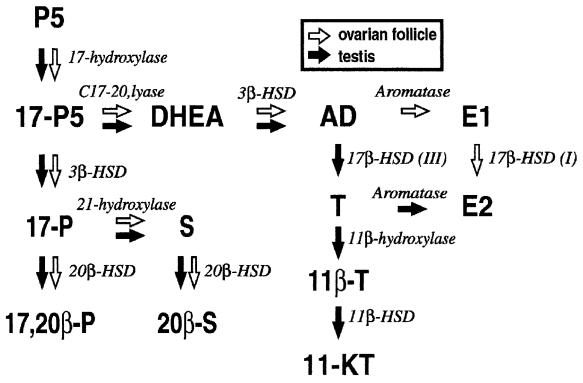

Figure 1. Complete steroidogenic pathways in the gonads of protogynous wrasse *P. sieboldi*.

androstenedione at 20 °C for 2 h. Radiolabeled steroid metabolites were analyzed by chromatographic mobility in thin layer chromatography and finally identified by recrystallization.

Results

In the testis, 11-KT, E2, 17,20β-P and 20β-S were synthesized as the major metabolites (Figure 1). 11-KT was synthesized in following sequence: pregnenolone (P5), 17-hydroxypregnenolone (17-P5), dehydroepiandrosterone (DHEA), androstenedione (AD), testosterone (T), 11β-hydroxytestosterone (11β-T), and 11-KT. 17,20β-P was converted directly from 17-hydroxyprogesterone (17-P), while, 20β-S was synthesized from 17-P *via* 11-deoxycortisol (S). In addition, an unusual finding in the testis was aromatization of T to produce E2.

The circulating levels of five steroids showed distinct diurnal profiles (Figure 2) (11-KT: 2.0–8.7 ng/ml, T: 0.9–2.4 ng/ml, E2: 0.2–36.8 ng/ml, 17,20β-P: 1.2–3.0 ng/ml, 20β-S: 0.3–0.8 ng/ml). The profiles of serum levels of androgens (11-KT and T) and progestins (17,20β-P and 20β-S) showed similar patterns respectively throughout the day. Serum 11-KT and T levels increased between 3:00 and 9:00 h and subsequently declined, although the absolute levels differed. Serum 17,20β-P and 20β-S increased between 12:00 and 18:00 h. Interestingly, there was pronounced increase in the serum E2 levels at 15:00 h.

In vitro production of five major steroids from P5 in the testis showed similar diurnal profiles to those of the circulating levels.

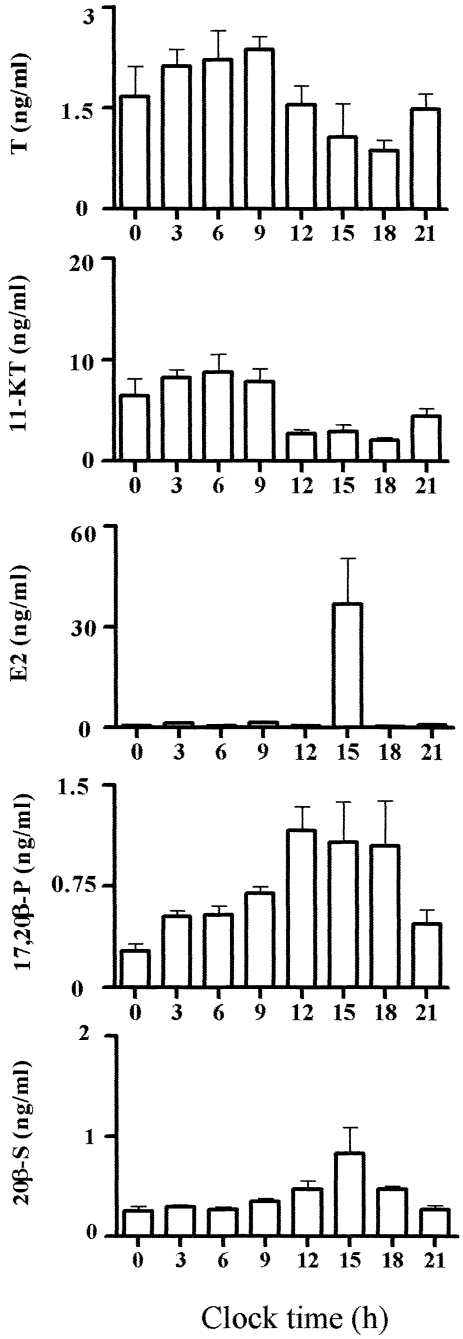

Figure 2. Changes in serum level of T, 11-KT, E2, 17,20β-P and 20β-S of TP male sampled at different times of the day during spawning season.

Discussions

In the present study, we identified the steroid hormones produced in the testis of TP male and further determined the pathways of these steroids. More recently, we have already reported the steroidogenesis in the ovarian follicles of *P. sieboldi*. Combined with these results, we clarified, for the first time, the complete steroidogenic pathways in the gonads of a hermaphrodite species throughout its life. In the ovarian follicles T is not produced, in which 17β-HSD-I synthesizes E2 from estrone (Ohta et al. 2001). In testicular tissue, 17β-HSD-III produces T from androstenedione, and T is metabolized into 11-KT and E2. In the present study, distinct diurnal rhythm of the steroid production was clarified, which is also the first report in male teleost. Serum levels of 11-KT showed increase between 3:00 and 9:00 h, corresponding to the time just prior and after the spawning time, respectively. 11-KT seems to involve in the spawning behavior as well as spermatogenesis in the testis. Serum levels of two progestins, which have been clarified as maturation-inducing steroids in female *P. sieboldi* (Ohta and Matsuyama 2002), increased between 12:00 and 18:00 h. Increases in the serum levels of 17,20β-P and 20β-S may involve in the acquisition of sperm motility, although the role of these progestins is still unknown. The most surprising aspect was extreme increase of serum E2 level at 15:00 h. This time specific production of E2 has allowed us to hypothesize that E2 may involve in diurnal rhythm spermatogonial proliferation. *In vitro* experiment to verify this hypothesis is now underway.

References

Ohta, K., Mine, T., Yamaguchi, A. and Matsuyama, M. 2001. Steroidogenic pathway to estradiol-17β synthesis in the ovarian follicles of the protogynous wrasse, *Pseudolabrus sieboldi*. Zool. Sci. 18: 937–945.

Ohta, K. and Matsuyama, M. 2002. Steroidogenic pathways to 17,20β-dihydoxy-4-pregnane-3-one and 17,20β,21-trihydroxy-4-pregnen-3-one, in ovarian follicles of bambooleaf wrasse *Pseudolabrus sieboldi*. Fish. Sci. 68: 41–50.

Ohta, K., Sundaray, J. K., Okita, T., Sakai, M., Kitano, T., Yamaguchi, A., Takeda, T. and Matsuyama, M. 2003. Bi-directional sex change and its steroidogenesis in the wrasse, *Pseudolabrus sieboldi*. Abstract, 7th International Symposium on the Reproductive Physiology of Fish, Mie, Japan. p. 49.