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Serum steroid profiles in artificially maturing female Japanese eel, *Anguilla japonica*

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Abstract

To investigate whether steroid profiles in salmon pituitary homogenate (SPH)-induced artificially maturing Japanese eel, *Anguilla japonica*, resemble those in other, naturally maturing fishes, the daily changes in 11 steroids were analyzed for a 70-day period (average time needed to reach the maturational phase). Concentrations of most steroids were low and changed on a weekly basis, with maximum values 2–5 days after an SPH injection. Thus, pregnenolone, 17α-hydroxypregnenolone, dehydroepiandrosterone, progesterone, 17α-hydroxyprogesterone, 17α,20 β-dihydroxy-4-pregnen-3-one, androstenedione and estrone levels were barely or not detectable in serum throughout the experimental period, which is largely in keeping with what is known about oogenesis-related steroids in other fishes. In contrast, serum testosterone (T) levels were high, but fluctuated considerably with each SPH injection (about 0.3–8.3 ng/ml). The serum estradiol-17β (E_2) levels increased after SPH injections and gradually rose throughout the experiment, peaking at the end of the experimental period (about 0.2–7.8 ng/ml). Serum levels of 11-ketotestosterone (11-KT) before SPH treatment were higher (approximately 2 ng/ml) than those of the other steroid hormones (less than 0.5 ng/ml). 11-KT levels increased gradually over the experimental period, and, like E_2, levels peaked towards the end of the experimental period (about 15 ng/ml). The observed patterns for T, E_2 and 11-KT are unlike those in other fishes. Furthermore, the consistent elevations in levels of 11-KT, both before and after SPH treatment, are suggestive of an important role for this steroid in controlling oocyte growth.

Keywords: Steroidogenesis; Japanese eel; *Anguilla japonica*; Oogenesis; 11-ketotestosterone; Artificial maturation

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1. Introduction

Growth and final maturation of teleost oocytes depend on pituitary gonadotropins, whose actions are mediated by steroid hormones produced by follicular tissues surrounding the oocytes (Fostier et al., 1983; Goetz, 1983). A thorough understanding of ovarian steroidogenesis is therefore central to explaining how the development of female germ cells is controlled. In many fish, such as salmonids, ovary-derived estradiol-17β (E_2) is needed to alter liver function, which then provides yolk precursor to support oocyte growth. Thereafter, during the maturational period, the salmonid ovary synthesizes 17α, 20β-dihydroxy-4-pregnen-3-one (DHP), a progestogen associated with final oocyte maturation (Nagahama et al., 1985; Nagahama, 1997).

Unlike many fish, Japanese eel (*Anguilla japonica*) do not undergo gonadal development under culture conditions. Artificial induction of gonadal maturation has therefore been pursued using injections of chum salmon pituitary homogenates (SPH: Yamamoto and Yamauchi, 1974). However, the eggs obtained by this method typically have low hatching and survival rates, and larvae cannot develop into glass eels (Tanaka et al., 2001). Are these developmental "problems" attributable to the induced spawning protocol? In SPH-induced eels, E_2 levels increased immediately prior to final maturation, whereas serum DHP was not detectable (Ijiri et al., 1995; Sato et al., 2000a). Similar findings have been reported for New Zealand longfinned eel (*A. dieffenbachii*: Lokman et al., 2001) and European eel (*A. anguilla*: Chiba et al., 1994). In our most recent report, we suggested that the high testosterone (T) levels in induced postvitellogenic eels may permit the increase in E_2 levels in spite of slightly decreasing aromatase (P450arom; the steroidogenic enzyme for E_2 synthesis) mRNA levels towards the end of oogenesis (Matsubara et al., 2003a). Therefore, steroidogenesis in artificially maturing eels appears to proceed unlike that in other teleosts, possibly causing aberrations in developing oocytes.

Changes in serum levels of 17 α-hydroxyprogesterone (17 α-OHP; the precursor for DHP), DHP, estrone (E_1; the precursor for E_2), androstenedione (AD; the precursor for E_1), T and E_2 during artificial maturation in Japanese eel have been documented (Matsubara et al., 2003a). However, to explain the unusual steroid profiles in artificially maturing eels during postvitellogenesis and in order to ascertain whether steroids are modified through the Δ4 or the Δ5 pathway, it is necessary to detail the profiles of the remaining major serum steroids, i.e., pregnenolone (P5), 17α-hydroxypregnenolone (17 α-P5), dehydroepiandrosterone (DHEA) and progesterone (P4).

In female fish, the androgens AD and T play a role as substrate for the production of estrogens, and have also been linked to hypothalamic steroid feedback (Montero et al., 1995; Lin et al., 1991). In male fish, AD and T can be converted to 11β-hydroxyandrostenedione and 11β-hydroxytestosterone by the enzyme 11β-hydroxylase. These metabolites, in turn, can be modified by 11β-hydroxysteroid dehydrogenase (Arai and Tamaoki, 1967a,b) to give rise to 11-ketoandrostenedione and 11-ketotestosterone (11-KT), traditionally considered male-specific in teleosts. In male Japanese eel, 11-KT can induce full spermatogenesis, even in vitro (Miura et al., 1991). However, 11-KT has also been found in several female fishes (Barcellos et al., 2001; Webb et al., 2001, 2002; Lokman et al., 2002), including New Zealand freshwater eels (Lokman et al., 1998), but it remains as yet unknown whether 11-KT is present in serum of female Japanese eel also. To address this issue and to comprehend the atypical E_2 and DHP levels seen in artificially maturing eels, we monitored 11 serum steroids in SPH-responsive fish for 70 days by which time females reached the maturational phase.

2. Materials and methods

2.1. Animals

Cultivated female Japanese eels (body weight 720–1200 g), feminized by E_2 administration while glass eels (Ijiri et al., 1998), were acclimated to seawater. After acclimation, blood was collected from the caudal vessels of 10 eels (initial control) by syringe after anesthesia in 0.1% benzocaine. Ninety-seven eels received weekly intramuscular injections of SPH at 40 μg/g body weight suspended in eel Ringer solution to induce vitellogenesis (Ijiri et al., 1998). Seventy-five eels responded to treatment, while the remaining 22 did not respond and therefore, were excluded from the