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In vivo gonadotropic effects of recombinant Japanese eel follicle-stimulating hormone

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Abstract

Recombinant Japanese eel follicle-stimulating hormone (rjeFSH) produced by methylotrophic yeast was subjected to *in vivo* bioassay to assess its gonadotropic activities and availability to artificially induce maturation of this species. Intramuscular injections of rjeFSH into male eels at doses of 0.1 and 1.0 U/g body weight were repeated 3 times during 12 days. The rjeFSH significantly increased plasma 11-ketotestosterone levels, and induced both testicular growth and spermatogenesis in a dose-dependent manner. These results demonstrate that rjeFSH is effective in promoting male eel gamatogenesis *in vivo*. Instead of scarce native eel FSH, abundant recombinant eel FSH is now ready for future application to maturation induction of Japanese eel.

Keywords: Recombinant FSH; *In vivo* bioactivity; Artificial maturation; Japanese eel

1. Introduction

Japanese eel, *Anguilla japonica*, is a highly valued species in Asian aquaculture. In spite of its economical importance, the technique for artificial maturation of eels is not fully established, and seeds for eel aquaculture are still dependent on natural stockpiles. Although their life cycle and reproductive system have become increasingly clear, eels basically remain to be immature in captivity without exogenous gonadotropic hormones and sexually mature eels have never been caught from the wild so far.

Gonadotropins (GTHs), follicle-stimulating hormone (FSH) and luteinizing hormone (LH), are pituitary glycoprotein hormones responsible for gonadal development and maturation in vertebrates (Licht et al., 1977; Pierce and Parsons, 1981). In general, it has been accepted that FSH and LH have their physiological functions in early and late stages, respectively, of sexual maturation. Under rearing conditions, the pituitary content and circulating levels of GTHs remain low, and thus gonadal maturation does not proceed. Repeated administration of exogenous LH-like GTH (salmon pituitary homogenate or human chorionic gonadotropin, hCG) could induce gonadal development (Yamamoto and Yamauchi, 1974; Ohta et al., 1996; Adachi et al., 2003); however, it is still difficult to obtain fertilizable eggs of good quality constantly (Kagawa, 2003). It is well known that combined action of dual GTHs is essential for successful gonadal development and gamatogenesis processes not only in higher vertebrates (Ryan et al., 1988; Kumar et al., 1997; Ma et al., 2004) but also in

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teleosts. As demonstrated in salmonid species (Suzuki et al., 1988a,b; Nagahama et al., 1994), the presence of two distinct GTHs has been confirmed in Japanese eel (Nagae et al., 1996; Yoshiura et al., 1999). Although homologous GTH, especially FSH, is required for successful induction of gonadal maturation, the scanty of FSHs throughout vertebrates including eels has not allowed *in vivo* utilization of FSH in artificial induction of eel maturation.

To overcome this problem, we have established an expression system of recombinant Japanese eel FSH (rjeFSH) using methylotrophic yeast, *Pichia pastoris*, and *in vitro* steroidogenic activities of rjeFSH have been characterized in eel testes and ovaries (Kamei et al., 2003, 2006). Although we have revealed that the steroidogenic activity of rjeFSH is identical to that of native eel FSH (Kamei et al., 2005), it is still uncertain whether or not rjeFSH is effective in promoting eel gamatogenesis processes *in vivo*. Thus, it is essential to confirm *in vivo* bioactivity of rjeFSH for the better understanding of FSH functions and future application to aquaculture. In this study, we examined *in vivo* gonadotropic effects of rjeFSH in immature male eels.

2. Materials and methods

2.1. Expression and preparation of rjeFSH

Expression of rjeFSH was performed in methyltrophic yeast, *P. pastoris* (KM71 strain), basically according to the method described in our previous study (Kamei et al., 2003). After expression induction, rjeFSH secreted into yeast culture medium was collected by ethanol precipitation. The precipitate fraction was separated by anion-exchange chromatography on a DEAE-FF column (Pharmacia Biothech, USA), and rjeFSH was eluted with 0.3 M NaCl. The *in vitro* bioactivity of thus recovered rjeFSH was confirmed by a homologous steroidogenic assay using immature testes, as described previously (Kamei et al., 2003). In this study, the dosage of rjeFSH was expressed in a defined unit (U). This unit is defined by bioassay and is based on bioactivity of hCG in eel testis (Kamei et al., 2003, 2006). The yeast transformed with an expression vector without the jeFSH subunit cDNAs was prepared in the same manner as rjeFSH-producing yeast for a "mock" group.

2.2. In vivo rjeFSH administration

Immature cultivated male eels weighing approximately 150 g were acclimated to recirculating seawater in a 1-t tank at 12–15 °C. Prepared rjeFSH with biological activity, confirmed by *in vitro* bioassay, was utilized for *in vivo* administration to the male eels. Intramuscular injections of rjeFSH at doses of 0.1 and 1.0 U/g body weight in 300 μl of 0.9% NaCl (saline) were repeated 3 times. The injections were performed on days 0, 3 and 7, and the fish were maintained until day 12. As control experiments, fish were injected with: 1) only saline, 2) the "mock" yeast culture medium processed in the same manner as the higher dose of rjeFSH-containing yeast culture medium, and 3) hCG at a dose of 1.0 IU/g body weight. Fish were sampled on days 0 and 12. At the time of sampling, eels were anesthetized with 0.3% (v/v) 2-phenoxyethanol and weighed, and then testes were dissected out and weighed for the calculation of gonadosomatic index (GSI: gonad weight × 100 / body weight). Plasma samples were also collected for the measurement of circulating 11-ketotestosterone (11-KT) levels by radioimmunoassay according to the method reported in previous studies (Aida et al., 1984; Kobayashi et al., 1985).

2.3. Histological observations

Testes were fixed in 2% paraformaldehyde – 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 for 18 hr. They were then dehydrated through an ethanol series and embedded in Spurr's resin (Polysciences, PA). Sections were cut at 1 μm thickness with glass knives, mounted on glass slides, and stained with 1% toluidine blue. The sections were observed under a light microscope, Nikon E800 (Nikon, Japan).

2.4. Statistics

Statistical significance between control and experimental groups was determined using ANOVA, followed by the multiple range analysis of Dunnett.

3. Results and discussion

3.1. Gonadal growth induced by rjeFSH administration

In vivo rjeFSH administration induced significant testicular growth in a dose-dependent manner in seawater-acclimated eels. The GSI value of rjeFSH (1.0 U/g)-injected eels was significantly increased, whereas no significant increase in GSI was seen at the lower dose of 0.1 U/g, when compared with that before injection on day 0 (Fig. 1A). The hCG treatment also increased the GSI, but the saline and mock treatments did not alter