Studies on testicular steroidogenesis in Japanese eel Anguilla japonica

［課程博士・論文博士共通］

博士学位論文内容要旨
Abstract

専　攻 Major	Applied Marine Biosciences	氏　名 Name	Hiroshi Suzuki
論文題目 Title	STUDIES ON TESTICULAR STEROIDOGENESIS IN JAPANESE EEL, *Anguilla japonica*		

Japanese eel (*Anguilla japonica*) is a highly valued species in Japan and almost all eels for commercial use are raised in farms from glass eels caught in estuaries. Since glass eels for aquaculture totally depend on the natural resource, the soaring prices of eel occurred. Therefore, establishment of artificial large-scale glass-eel production, which can supply seedings stably, is strongly desired. However, Japanese eels do not mature in normal aquaculture conditions and thus many trials have been conducted to obtain their gametes by artificial induction of maturation using various heterologous exogenous gonadotropic hormones, but many problems occur. Thus, it has been believed that bioactive Japanese eel gonadotropins (Gths), as homologous gonadotropic hormones, are required for the production of gametes with stable quality and quantity. Gths, follicle-stimulating hormone (Fsh) and luteinizing hormone (Lh), play central roles in the control of gonadal development of vertebrates. It has been well known that Fsh and Lh are expressed in the pituitary gland of vertebrates including teleost. Therefore, it is desired that Japanese eel Gths are purified from the pituitary, but high-purified Gths are still not available because it is difficult to purify suitable amounts of native Gths from pituitaries. Currently, advancements in biotechnology have enabled the production of recombinant Japanese eel Gths (reGths; reFsh and reLh) using mammalian cell lines. The recombinant hormones have potential as a new tool of the artificial induction of sexual maturation, but the bioactivity of reFsh and reLh and the effects of those on Japanese eel testis have not yet been well clarified. The understanding of the testicular development by reGths is required as a clue to the development of reliable methods to induce sexual maturation using homologous hormones in Japanese eels.

In vertebrates, spermatogenesis is dependent on the Fsh and Lh stimulation through their receptors. In teleost, it is known that their actions are mediated by steroid hormones. Especially, 11-ketotestosterone (11KT) has been established in teleost as a unique and the most potent androgen that plays important roles in secondary sexual characteristics and spermatogenesis. The previous analyses indicated that the effects of Gths on testicular 11KT production were species-specific. In order to understand the physiological roles of Gths in Japanese eel, it is important to elucidate the effects of Gths on testicular steroidogenesis.

As the first step, functional analyses of reFsh and reLh were performed to confirm their bioactivities, focusing on the binding specificities to their receptors and effects on testicular steroidogenic activities. Results of reporter assay indicated that reFsh stimulated its cognate receptor, while reLh activated both receptors. Although *in vitro* incubations showed that reFsh and reLh induced testicular 11KT production in a dose and a time-dependent manner, the effective doses of reLh were apparently lower and the effects of reLh emerged faster in comparison with reFsh. The results indicated that Lh was more potent in 11-KT production. In addition, reFsh and reLh induced expression of steroidogenic enzymes related with 11KT synthesis, which suggested that Gths promoted testicular 11KT production by upregulating expression of the steroidogenic enzymes. Moreover, to identify target cells of Fsh and Lh, localization of *fshr* and *lhcgr1* transcripts was examined by quantitative real-time PCR (qPCR) using prepared Leydig cell, Sertoli cell, or germ cell fractions. Results of qPCR showed that *fshr* and *lhcgr1* mRNA were detected in both Sertoli and Leydig cells. These analyses revealed that both eel Fsh and Lh acted as steroidogenic hormones through their receptors in Sertoli and Leydig cells.

Among steroidogenic enzymes involved in the 11KT synthesis, 17β-hydroxysteroid dehydrogenases (Hsd17bs) are oxidoreductive steroidogenic enzymes that catalyze the interconversion of 17-keto and 17β-hydroxysteroids and essential for the formation of sex steroids in gonads and other tissues. Especially, Hsd17bs with 17-ketosteroid reducing activity (17KSR activity) involved in 11KT production but have not yet been well clarified in Japanese eel. Therefore, for the deeper understanding of testicular 11KT biosynthesis regulated by Gths, the present study first investigated the steroidogenic pathway for 11KT production and identified 17KSR activity crucial for 11KT synthesis in the Japanese eel testis. *In vitro* incubation of the testis with androstenedione (A4) and the subsequent analysis of the metabolites by thin-layer chromatography indicated that 11KT was synthesized from A4 via 11β-hydroxyandrostenedione (11OHA4) and 11-ketoandrostenedione (11KA4), which indicated that the steroidogenic enzyme exhibiting the 17KSR activity involved in converting 11KA4 to 11KT is crucial for 11KT production. Subsequently, cDNAs encoding three candidate enzymes, Hsd17b type3 (Hsd17b3), Hsd17b type12a (Hsd17b12a), and 20β-hydroxysteroid dehydrogenase type2 (Hsd20b2), potentially with the 17KSR activity were isolated and characterized. The Hsd17b3, Hsd17b12a, and Hsd20b2 expressed either in HEK293T or in Hepa-E1 converted 11KA4 to 11KT. In the results of Tissue-distribution analysis by qPCR, the expressions of *hsd17b12a* and *hsd20b2* were observed in the testis, while the expression of *hsd17b3* was not observed. Furthermore, the 17KSR activity and the expression of *hsd17b12a* were upregulated by the administration of Gths. These data strongly suggested that Hsd17b12a is one of Hsd17bs with 17KSR activity responsible for 11KT synthesis in the testis of Japanese eel.

A part of Hsd17bs group has been known as multifunctional proteins, which possess retinol dehydrogenase (RDH) activity, 3-ketoacyl-CoA reductase activity, 3-keto-reductase activity, and 17HSD/17KSR activity. Recently, cDNA encoding RDH11 was isolated and characterized in human. The protein has been suggested to be a novel tentative HSD17B (HSD17B15) in humans for a decade, however no definitive proof has been provided yet. Moreover, it has not been reported whether the protein is involved in the synthesis of 11KT in vertebrates including teleost. Therefore, for the deeper understanding of Hsd17bs involved in the final step of 11KT synthesis, three genes as candidate Rdh11s were isolated and characterized. The candidate genes were isolated using EST-database information and PCR-based strategy. Based on their structural features revealed by the analyses of sequence identity and phylogenetic analysis, these isolated genes are found to be comparably related to human *rdh11* and *rdh12* genes. Therefore, these genes were designated as *rdh11/12-like 1*, *rdh11/12-like 2*, and *rdh11/12-like 3*. To assess the 17KSR activity of Rdh11/12-likes, the expression vector was established and transfected into HEK293T. Three recombinant proteins expressed in HEK293T cells converted estrone to estradiol-17β and androstenedione into testosterone. Only Rdh11/12-like 1 converted 11KA4 into 11KT. Furthermore, the tissue distribution and the effects of Gths were examined using q PCR. Tissue-distribution analysis by qPCR revealed, in immature male Japanese eel, that the expression of *rdh11/12-like 1* and *rdh11/12-like 2* was observed mainly in testis and brain, while rdh11/12-like 3 is expressed ubiquitously. Moreover, *in vitro* incubation of immature testis with reFsh, reLh, and 11KT indicated that expression levels of *rdh11/12-like 1, rdh11/12-like 2* and *rdh11/12-like 3* did not change. Consequently, Japanese eel Rdh11/12-likes may belong to a group of Hsd17bs because the enzymes possess the 17KSR activity. In particular, Rdh11/12-like 1 may be one of the enzymes with 17KSR activity involved in the production of 11KT in the testis.

In conclusion, reGths were strongly suggested to be reliable hormones for the artificial induction of sexual maturation in Japanese eels. The effective doses of reLh were apparently lower and the effects of reLh emerged faster in comparison with reFsh, suggesting their differential roles in spermatogenesis. In addition, it was revealed that a final step of 11KT synthesis was catalyzed by Hsd17b12a and Rdh11/12-like 1; in particular, the expression of *hsd17b12a* gene was regulated by reGth.