Effects of gonadal steroids on gonadotropin subunit genes expression in male red seabream (Pagrus major)

SONOKO YAMAGUCHI1*, KOICHIRO GEN2, KOICHI OKUZAWA2, MICHiya MATSUyama1, AND HIROHIKO KAGAWA3

1 Faculty of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka 812-8581, Japan (syamagu@fra.affrc.go.jp), 2 Inland Station, National Research Institute of Aquaculture, Fisheries Research Agency, Tamaki, Mie 519-0423, Japan, 3 National Research Institute of Aquaculture, Fisheries Research Agency, Nansei, Mie 516-0196, Japan

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INTRODUCTION

Gonadotropins, FSH and LH, are regulators of steroidogenesis and gonadal development in vertebrates. In mammals, the synthesis and release of gonadotropins are regulated by gonadal steroids 1). Although there are two distinct gonadotropins (GTH-I and GTH-II) in teleosts, very little is known about hormonal regulation of gonadotropins, especially GTH-I. In this study, to elucidate the regulatory mechanisms of GTH-I and GTH-II synthesis by steroid hormones, we examined the effects of steroid hormones on GTH subunit genes expression in both immature and early-maturing male red seabream.

MATERIALS AND METHODS

Immature (GSI; 0.17 ± 0.02%) and early-maturing (GSI; 0.38 ± 0.09%) male red seabream, reared under natural conditions in net pens in the Gokasho bay, Nansei, Mie Japan, were used in this study. Fish were implanted with three silicone capsules containing 400 μg per capsule of 11-ketotestosterone (11-KT) or estradiol-17β (E2). One month after implantation, the pituitary glands were collected after decapitation. Total RNA was isolated from each pituitary by guanidium isothiocyanate methods. Five micrograms of total RNA per sample were subjected to electrophoresis on 1% agarose/formaldehyde gel and analyzed by Northern blot analysis using red seabream GTH-Iα and GTH-IIα probes 1). Blood samples were collected, and the serum was separated by centrifugation at 3000 rpm for 15 minutes. Serum levels of E2 and 11-KT were measured by radioimmunoassay and enzyme-linked immunosorinent assay, respectively.

RESULTS

At the end of experiments, serum levels of 11-KT and E2 in hormone treated-groups were elevated significantly compared with those in control group, and were similar to circulating levels observed in sexually mature male in the spawning season.

In immature fish, the mRNA levels of GTH-Iβ were not affected by any treatment (Fig. 1a). 11-KT, but not E2 significantly raised GTH-IIβ mRNA levels (P<0.05, Fig. 1b). The GTH-Iα mRNA levels were significantly increased by 11-KT treatment in early-maturing male fish (P<0.05, Fig. 2a). Similar to immature male, 11-KT raised GTH-IIβ mRNA levels (P<0.05, Fig. 2b). Implantation of E2 did not affect to GTH-Iα and GTH-IIβ mRNA levels in early-maturing male.

DISCUSSION

In the present study, we demonstrated that 11-KT raised the mRNA levels of GTH-IIβ in both immature and early-maturing male red seabream. These results consist with the previous study indicating that in vivo treatment of 11-KT raised GTH-IIβ mRNA levels in African catfish 3). Therefore, in male teleost, it is suggested that 11-KT is one of important regulators for GTH-IIβ gene expression. Recent study, using in vitro culture system, suggests that stimulatory effect of 11-KT on GTH-IIβ gene expression is not direct action on pituitary cells 4). Thus, it is necessary to examine whether other factors, such as GnRH, activin 5) or IGF-I 6), are involved in regulation of GTH-IIβ gene expression in red seabream.

Interestingly, GTH-Iβ mRNA levels were increased by 11-KT in early-maturing male, but not immature fish. This is, to our knowledge, the first evidence showing that 11-KT affects GTH-Iβ gene expression in the pituitary of fish. In male red seabream, GTH-Iβ mRNA levels increased in association with increase...
Fig. 1 Effects of steroid hormones on mRNA levels of GTH-I (a) and -II (b) subunit in immature male red seabream. IC, initial control; C, control; 11-KT, 11-ketotestosterone; E2, estradiol-17β. Each value represents means ± SEM. Numbers in parentheses indicate the number of fish used. An asterisk indicates significant difference in comparison with control, * P<0.05.

Fig. 2 Effects of steroid hormones on mRNA levels of GTH-I (a) and -II (b) subunit in early-maturing male red seabream. IC, initial control; C, control; 11-KT, 11-ketotestosterone; E2, estradiol-17β. Each value represents means ± SEM. Numbers in parentheses indicate the number of fish used. An asterisk indicates significant difference in comparison with control, * P<0.05.

of serum levels of 11-KT during sexual maturation (data not shown). Furthermore, in male Atlantic salmon, pituitary and plasma GTH-I levels were increased by in vivo implantation of 11-ketoandrostenedione, which is a precursor of 11-KT. Taken together, these results suggest that 11-KT also have potency of stimulating the gene expression of GTH-Iβ. In this study, we also showed that the effects of 11-KT on GTH-Iβ mRNA levels were different between immature and early-maturing male fish, suggesting that positive feedback mechanism of 11-KT on GTH-Iβ gene expression is developed during gonadal development. Further studies are necessary to clarify the mechanism underlying positive feedback of 11-KT in early-maturing male.

In conclusion, 11-KT stimulated the expression of GTH-IIβ genes in immature and early-maturing male red seabream. In contrast to GTH-IIβ, GTH-Iβ mRNA levels were increased by 11-KT in early-maturing male, but not immature male. These data suggest that 11-KT is one of the gonadal factors which stimulates both GTH-Iβ and GTH-IIβ mRNA levels in early-maturing male red seabream.

REFERENCES