

Effect of Androgen and Growth Hormone on the Gene Expression of Vitellogenin and Estrogen Receptor (ER) in the Japanese eel, *Anguilla japonica*

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Introduction

Estradiol-17 β (E₂) is known to be a main inducer of vitellogenin (Vg) synthesis, but other hormones such as growth hormone (GH) and androgens have also been reported to be involved in hepatic Vg synthesis (Kwon *et al.* 2003; Kwon *et al.* 2005). In the present study, the effects of E₂, androgen and growth hormone on the regulation of estrogen receptor (ER) and Vg gene were investigated in the liver of Japanese eel, using *in vivo* and *in vitro* methods.

Materials and Methods

Immature eels (200-250 g) were given a single injection of E₂ (5-5000 μ g/kg body weight), eel recombinant GH (eGH, 1-100 μ g/kg) or methyltestosterone (MT, 1-5 mg/kg) alone, or combinations of E₂ with eGH or MT and sacrificed 10 days after hormone treatment. The *in vitro* experiments were performed using a primary culture of immature eel hepatocytes, and ER and Vg mRNA were determined by means of RT-PCR. Inhibitory effects of tamoxifen (an antiestrogen) and flutamide (an antiandrogen) on the expression of Vg mRNA by E₂, MT and eGH were also examined in the cultures of eel hepatocytes.

Results and Discussion

Treatment with E₂ (500-5000 μ g/kg) induced Vg synthesis in a dose dependent manner but injection of either eGH (100 μ g/kg) or MT (5 mg/kg) alone failed to induce Vg synthesis. In contrast, injection of MT (5 mg/kg) or eGH (1-100 μ g/kg) combined with E₂ (500 μ g/kg) increased Vg synthesis. When

eels were injected with vehicle, E₂ (5-5000 µg/kg) or E₂+MT (5 mg/kg) or E₂+eGH (10 µg/kg), ER mRNA expression was detectable in all fish, regardless of hormone treatment. Injection of E₂ increased hepatic ER mRNA expression in a dose-dependent fashion. On the other hand, MT or eGH had no additive effect on the ER mRNA expression by E₂.

Vg mRNA expression also responded to E₂ (50-5000 µg/kg) treatment in the same manner as ER mRNA expression did, showing dose-dependency. Co-treatment of E₂ (500 µg/kg) with MT (5 mg/kg) or eGH (10 µg/kg) resulted in a significant increase in Vg mRNA level compared to that of E₂ (500 µg/kg) alone, whereas no Vg mRNA expression were observed in groups treated with either eGH (10 µg/kg) or MT (5 mg/kg) alone.

Effects of E₂, MT and eGH on hepatic Vg synthesis were also examined in hepatocyte culture. In the cultures with E₂, MT or eGH alone, Vg synthesis was not induced, but the combination of E₂ either with MT or eGH strongly stimulated Vg gene expression. Addition of tamoxifen (10⁻⁵M) inhibited Vg gene expression stimulated by E₂+MT or E₂+eGH. Treatment with flutamide markedly decreased Vg mRNA stimulated by E₂+MT. These indicate that both tamoxifen and flutamide exert their inhibitory effects at the transcriptional level.

References

- Kwon, H.C., S.H. Choi, Y.U. Kim, S.O. Son and J.Y. Kwon. 2003. Involvement of androgens and growth hormone in the synthesis of vitellogenin in Japanese eel (*Anguilla japonica*). *Fish Physiol. Biochem.* 28, 351-352.
- Kwon, H.C., S.H. Choi, Y.U. Kim, S.O. Son and J.Y. Kwon. 2005. Androgen action on hepatic vitellogenin synthesis in the eel, *Anguilla japonica*, is suppressed by an androgen receptor antagonist. *J. Steroid. Biochem. Mol. Biol.* (In Press)

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