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In vitro Studies on Hormonal Regulation of Vitellogenin Synthesis in Tilapia Hepatocytes

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Introduction

Vitellogenin (VTG) is a precursor form of egg yolk proteins, which appear only in the blood circulation of female fish and its synthesis in the liver is considered to be regulated by several hormones. It has been reported that in addition to estradiol-17β (E2) several hormones are also involved in the production site of VTG, the liver (Peyon et al., 1996; Mori et al., 1998). The aim of the present study was to clarify hormonal regulation of VTG synthesis in the liver of tilapia, *Oreochromis mossambicus*. Simplicity of the experiment was carried out by developing a primary culture system of the hepatocytes. Also, the role of E2 and androgens in VTG synthesis was investigated using VTG, estrogen receptor (ER), vigilin and cytochrome P450 mRNA were assessed.

Materials and Methods

Tilapia (200-300 g) were collected using a casting net from rivers and maintained in concrete tank with filtered freshwater. Two types of VTG (VTG210 and VTG140) were purified from the blood of E2-injected male tilapia by combination of ion-exchange chromatography and gel filtration. Isolation and primary culture of tilapia hepatocytes were done in accordance with the method of Kim and Takemura (2002). Several hormones was added to the culture media after 2 days of pre-culture. Medium VTG was measured by

enzyme-linked immunosorbent asay (ELISA) (Takemura and Kim (2001). VTG, estrogen receptor (ER), vigilin and cytochrome P450 mRNA were assessed using reverse transcription-polymerase chain reaction (RT-PCR).

Results and Summary

Of the hormones tested in the present study for *in vitro* induction of VTG, E2, and androgens had stimulatory effect. Co-treatment of E2 and tamoxifen, an inhibitor of estrogen, resulted in drastic decrease of VTG synthesis. When mRNA expression of VTG, ER and vigilin (a stabilizer of VTG mRNA) in the hepatocytes were assessed after E2 treatment, peak of ER mRNA expression was followed by increases in VTG and vigilin mRNA. These results suggest that cooperative expression of these three genes in the hepatocytes after E2 action is needed to be induction and maintenance of VTG mRNA and/or molecule. Treatments of androgens resulted in induction of VTG in the female hepatocytes. Tamoxifen reduced VTG synthesis by the androgens, suggesting that the androgens bind ER and, consequently, exert estrogenic action. On the other hand, treatment of chlormadinone acetate (CMA), an antiandrogen reagent, increased in production of VTG with E2 and DHT. It was possible that, like androgen action to ER, E2 binds AR and exert androgen action. The present study revealed that E2 is a strong inducer of VTG in the tilapia hepatocytes. Since several hormones such as the androgens involve in synthesizing VTG in the hepatocytes, it is concluded that multi-hormonal regulation of vitellogenesis occurs in the liver.

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