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Potentiating effect of methyltestosterone in vitellogenin synthesis in the eel, *Anguilla japonica*

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Introduction

Vitelllogenin(Vg) is a sex specific protein appearing in the blood of sexually maturing female fish as well as other oviparous vetebrates. It is synthesized in the liver in response to estradiol-17β(E_2). Although E_2 is a main inducer of Vg synthesis, other hormonal requirements have also been reported to be important in Vg synthesis(lazier et al., 1996; Peyon et al., 1997). Previous studies have indicated that the multihormonal stimulation of growth hormone(GH) and/or prolactin as well as E_2 is essential for the active synthesis of Vg in eel. In the present study, the potentiating effect of androgens in Vg synthesis was investigated *in vivo*, and *in vitro* using the primary culture of immature eel hepatocytes.

Materials and Methods

Immature eels weighing 200~250g were obtained from a local supplier and kept in freshwater tanks at 22℃. They were not fed during the experiments. Fish were given a single injection of E_2(5~500μg/kg BW) and methyltestosterone (1~5mg/kg BW) dissoloved in DMSO, alone or together. Eel hepatocytes were prepared according to the procedure of Kwon and Mugiya(1994). Cells were plated into a 60mm Petri dish(Falcon) at a density of 3×10^5 cells/dish. L-15 medium containing 0.2μM bovine insulin, streptomycin and penicillin was used for cell culture. The media containing the hormones were changed after the first 2-day culture and collected every 2 days thereafter. All incubations were replicated at least twice.

Results and Discussion

In the *in vivo* trials, injection of either GH (1μg/kg body weight, BW) or methyltestosterone (MT, 5 mg/kg BW) alone to immature eels failed to induce *in vivo* Vg synthesis. When the eel hepatocytes were cultured with either E_2 (10^{-9}~10^{-5}M), bovine GH (10~100 ng/ml), eel GH (10~100 ng/ml), or MT (10^{-9}~10^{-5}M) alone, no Vg was detected in any of the media throughout the experimental period (12days). However,

cultures with E_2 in combination with either MT or GH induced Vg synthesis and the rate of its production gradually increased in a time- and dose-dependent fashion until at least 10days. Treatment of the culture with MT+GH in the absence of E_2 did not induce Vg synthesis, whereas treatment with MT+GH+E_2 induced far more Vg synthesis than those with MT+E_2 or GH+E_2. Treatment with either testosterone, androstene or progesterone in the presence of E_2 was also effective in inducing Vg synthesis although not as potent as MT+E_2, GH+E_2 or MT+GH+E_2. In addition, in the culture of hepatocytes obtained from immature eels primed by E_2 injection, the treatment with either E_2, GH, or MT alone did not increase the synthesis of Vg, while the combination of E_2 either with GH or MT strongly stimulated Vg synthesis. Addition of tamoxifen to these cultures dramatically reduced Vg synthesis, implying that E_2 played a key role in the synthesis of Vg in the experimental cultures. Together, these results suggest that androgens and/or GH increase the effect of E_2 on the synthesis of Vg in this species.

References

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