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Sex Steroids and Pituitary Extract Increase Gonadotropin II Content in the Pituitary of Aquacultured Japanese Eel *Anguilla japonica*

Abstract

The effects of sex steroids and pituitary extract on the development of gonads as well as gonadotropin-II (GTH-II) pituitary content in the aquacultured Japanese eels (*Anguilla japonica*) were investigated. Gonadosomatic index (GSI) was not significantly stimulated in females after 3 injections over 6 weeks, nor in males after 6 injections over a 12-week period of steroids (estradiol-17β, E2; testosterone, T; 5 α-dihydrotestosterone, DHT; for each steroid with 2 doses, 0.75 and 3.75 mg/kg body weight). Salmon pituitary extract (10 mg/kg body weight) induced a progressive increase in GSI after 3-, 6-, 9- and 14-injections over a 14-week period. E2 and pituitary extract significantly increased pituitary GTH-II content in a dose-dependent manner. Androgens also increased pituitary GTH-II content but the effects were much weaker than E2. Pituitary GTH-II content were significantly increased in maturing eels (GSI > 20%) injected with salmon pituitary extract. It is suggested that the salmon pituitary extract effects on GSI in the cultured yellow eels was a direct effect on ovaries, and a multiple effect of sex steroid on various levels of reproduction.

Key words: Eel, Sex steroids, Gonadotropin II, Maturation

Eels *Anguilla spp.* are fish of great economic importance. Although the artificial maturation of eels has been obtained under experimental conditions by exogenous gonadotropins or pituitary extract since 1936 (*A. anguilla*[1,2]; *A. japonica*[3,4]), the artificial propagation of eel larvae is not yet fully successful. The resource of eels for aquaculture exclusively relied on nature. Further understanding of the endocrine characteristics of eels is important to the practical manipulation of eel maturation[5].

A lack of pituitary gonadotropin synthesis and

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release results in the blockage of gonadal development in the eels[6]. Dopamine blockage probably prevents the release and synthesis of pituitary gonadotropin II GTH-II[7-9]. Therefore, maturation of eels relies exclusively on artificial induction. Sex steroids and gonadotropins are the most likely hormones to apply for the manipulation of artificial maturation in eels. The regulation of pituitary GTH-II by sex steroids has been studied in wild silver eels. Estradiol-7β (E2) could stimulate significant high levels of pituitary (GTH-II) in female wild European silver eels[10]. Testosterone (T) could not stimulate significant levels of pituitary GTH-II in wild European silver female eels[10] but could stimulate ovarian development in wild Japanese silver eels[8,11]. Androgen-specific stimulation of *in vitro* GTH-II content in pituitary cells was demonstrated in the wild European eels [12]. Studies on the regulation of pituitary GTH-II have mostly been conducted in wild silver eels. Less information is available on cultured eels; however, they can be the major source for studies on inducing maturation[4]. The aim of this study was to investigate the effects of sex steroids or salmon pituitary extract on the GSI and GTH-II content in cultured Japanese eels.

Materials and methods

Eels

Japanese eels were obtained from aquaculture ponds in Taiwan. Three-year-old female eels (n = 64, body weight 458 ~ 914 g, body length 65 ~ 86 cm) and male eels (n = 56, body weight 270 ~ 500 g, body length 56 ~ 74 cm) were used. In addition, eight-year-old females (n = 14, body weight 1090 ~ 2080 g, body length 83 ~ 93 cm) received long-term treatment with salmon pituitary extract for induced maturation. Eels were transferred to the aquarium facility of the University and kept in 2.5-ton fiberglass tanks in a seawater system (seasonal ranges of water temperatures were 23 – 27 °C).

Hormone Treatments

(1) Sex steroids

E2, T and 5 α–dihydrotestosterone (DHT) were obtained from Sigma Co. (USA). Female eels (n = 40) were divided into 5 groups (n = 8): low dose of E2 (0.75 mg/kg body weight); high dose of E2 (3.75 mg/kg body weight); low dose of T (0.75 mg/kg body weight); high dose of T (3.75 mg/kg body weight); and control (coconut oil). The steroid was dissolved in coconut oil, and the intraperitoneal (ip) injections were given 3 times at 2-week intervals. Male eels (n = 56) were divided into 7 groups (n = 8): low dose (0.75 mg/kg body weight) of E2, T and DHT; high dose (3.75 mg/kg body weight) of E2, T and DHT; and control (coconut oil). The steroids were dissolved in coconut oil and injected ip at 2-week intervals for 6 times.

(2) Salmon pituitary extract

Aceton dried salmon pituitaries were from commercial company (Taipei, Taiwan). Two experiments were conducted for the induction of gonadal growth in female eels. For the first experiment, three-year-old females (n = 24) were injected ip weekly with 10-mg salmon pituitary extract in saline/kg body weight, while the control fish were injected with saline. For the second experiment, eight-year-old females were injected weekly with 10-mg salmon pituitary extract/kg body weight for 14 weeks (n = 8) or with saline alone (n = 6).

Radioimmunoassay of GTH-II

Fish were sacrificed one week after the last injection. GSI was measured, pituitary was collected and stored at –80 °C until radioimmunoassay (RIA) of GTH-II. GTH-II in pituitary was assayed by RIA previously established for the β-subunit of carp GTH-II[13] and validated for the measurement of eel GTH-II[14]. Iodination of GTH-II was performed by the iodogen (Pierce) method according to Salacinski et al.[15].

Statistical analysis

Mean was given with standard error of mean (± S. E. M.) and were compared ($P < 0.05$) by analysis of variance followed by multiple comparison test.

Results

Effects of exogenous hormone treatments on gonadal development

GSI in the controls was 1.7 ± 0.2 % and 1.3 ± 0.2/0.9 ± 0.01 % in the 8-year-old, and 3-year-old females, respectively (Table 1, Fig. 1). No significant increase of GSI was observed in the sex steroid-treated females as compared to the control (Fig. 1). The range of GSI in the sex steroid-treated females was 0.9 – 1.5 in low E2, 0.9 – 1.8 in high E2, 1.3 – 2.0 in low T and 1.3 – 1.6 in high T as compared to the control (0.7-1.3) (Fig. 1). Also, no significant difference but with increase of variation in males under the effects of androgens was found in the group of T or non-aromatizable DHT (0.08 – 1.58 in low T, 0.06 – 1.49 in high T; 0.06 – 1.12 in low DHT and 0.12 – 1.82 in high DHT) but not E2 (0.10 – 0.26 in low E2 and 0.07 – 0.15 in high E2) as compared to the control (0.06 – 0.2) (Fig. 1). A significant increase in GSI was detected in 3-year-old female eels after 6 weeks treatment with salmon pituitary extract in female and a further increase was observed after 9 weeks treatment (Fig. 1). GSI reached up to 20 % after 14 weeks injection in 8-year-old female eels (Table 1).

Effects of exogenous hormone treatments on pituitray GTH-II levels

Both E2 and T could stimulate the increase of pituitary GTH-II content in females in a dose-dependent manner (Fig. 2). Pituitary GTH-II levels increased 109-fold and 5.5-fold in the high dose of E2 and T as compared to the control, respectively (Fig. 2). E2, T and DHT also had stimulatory effects on the pituitary GTH-II levels in males in a dose-dependent manner (Fig. 3). Male pituitary GTH-II levels increased 1023-fold, 17-fold and 12-fold in the high dose of E2, T and DHT, respectively, as compared to the control (Fig. 3). E2 had a higher significant effect than T in females, T and DHT in males (Figs. 2 and 3). Pituitary GTH-II levels were significantly increased in pituitary extract-treated females along the period of the weekly injection with pituitary extract for 3, 6 and 9 injections as compared to the control (Fig. 4). GTH-II levels in pituitary were significantly higher in the group which received 14-injections with pituitary extract than in the control (Table 1).

Discussion

Our data indicate that sex steroids have no significant effects on gonadal development in female or male eels. However, sex steroids (E2 or T) slightly increased GSI in females as compared to the control. Also, androgens (T or DHT) but not E2 slightly increased GSI in males and with larger variation as compared to the control, indicating that

Table 1. The endocrine profiles in the control and induced maturation groups. Salmon pituitary extract was injected weekly into 8-year-old female eels for 14 weeks. The symbol "*" represents a significant difference ($P < 0.05$) between the control and hormone-treated groups.

	Control (n = 6)	***Induced Maturation (n = 8)***
Gonadosomatic index (%)	1.7 ± 0.15	20.4 ± 3.3*
Pituitary GTH-II (ng/pituitary)	293.9 ± 44.9	72259 ± 23.4*

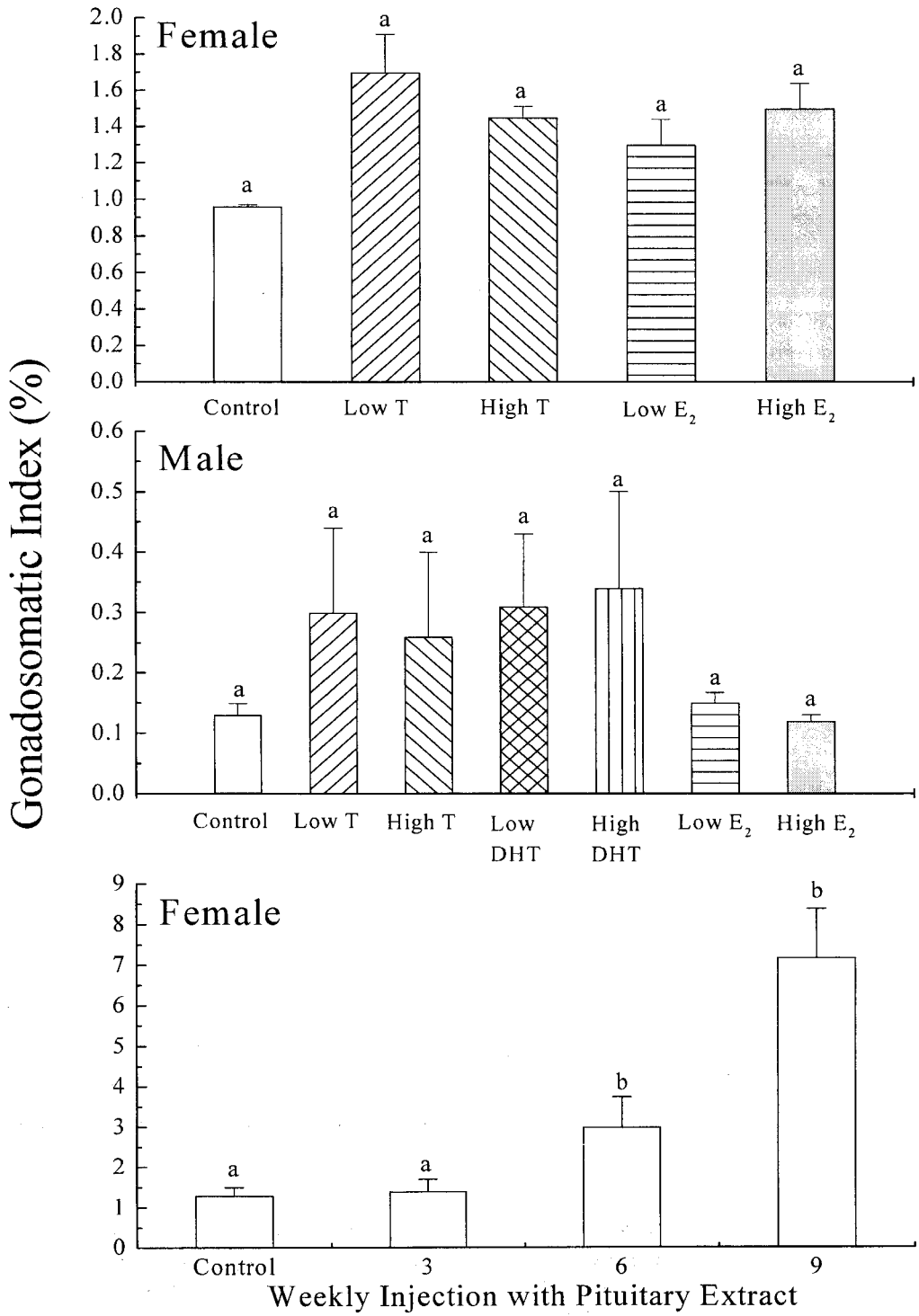

Fig. 1. Gonadosomatic index in cultured female eels (A) control, low dose (0.75 mg/kg body weight) and high dose (3.75 mg/kg body weight) of testosterone (T) or estradiol-17β (E2); male eels (B) control, low dose (0.75 mg/kg body weight) and high dose (3.75 mg/kg body weight) of T, E2 or 5 α-dihydrotestosterone (DHT), and (C) female eels weekly injected with salmon pituitary extract (10 mg/fish per week, for 3, 6, or 9 weeks). Values with different characters differ from each other ($P < 0.05$).

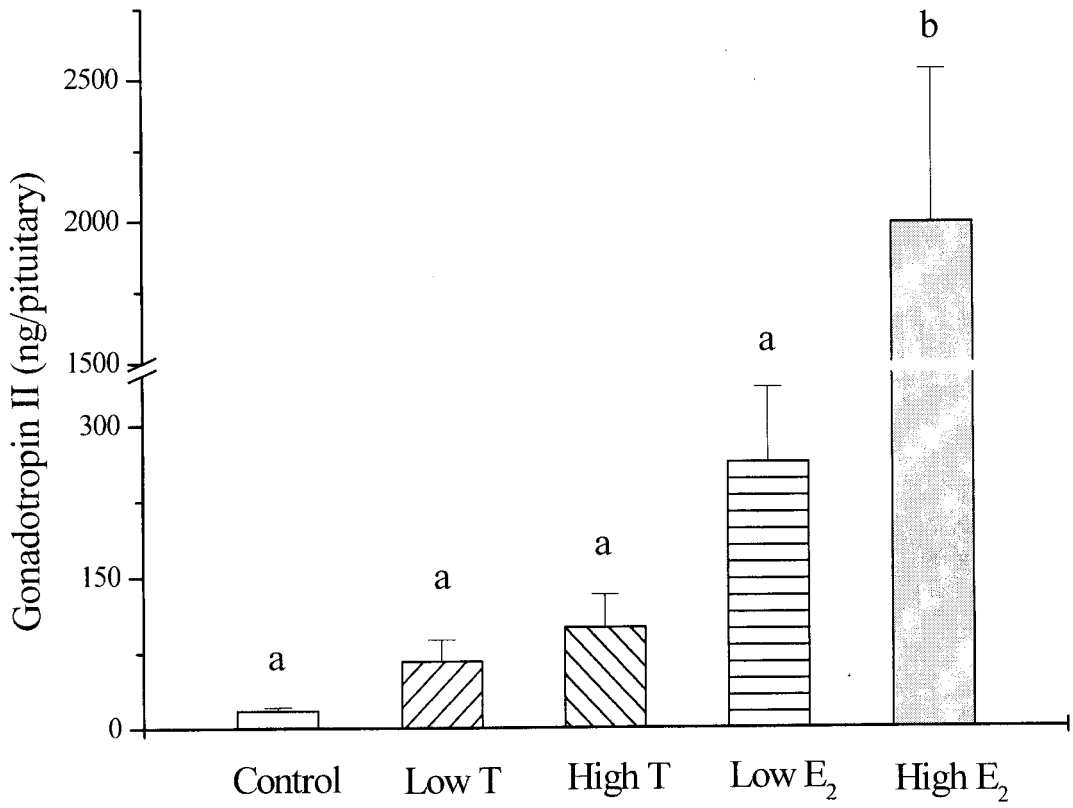

Fig. 2. Pituitary gonadotropin (GTH) II levels in cultured female eels; control, low dose (0.75 mg/kg body weight) and high dose (3.75 mg/kg body weight) of testosterone (T) or estradiol-17β (E2). Values with different characters differ from each other ($P < 0.05$).

the treatment did induce gonadal development in some of the experimental fish. Implantation with a high dose of T (75 μg/g body weight) for a long time (75 days) significantly induced gonadal development and maturation in wild silver Japanese eels, *A. japonica*[8]. The lower response to sex steroids in the present study was possibly due to the difference in duration and dose of the treatment, and also the stage of the experimental eels. In this study, the initial GSI was higher in older eels. Three-year-old female eels with ~ 1.0% GSI were still either at the yellow stage or intermediate stage, while 8-year-old female eels with 1.7% GSI were considered to have reached the silver stage[16]. Indeed, wild silver eels may be more sensitive to stimulation by sex steroids[5]. Gonadal develpment was observed in female eels treated with 6- and 9-injections of salmon pituitary extract. Further induction of gonadal development was detected in 8-year-old females treated for 14 weeks with salmon pituitary extract. Similar responses after treatment with pituitary extract were observed in Japanese[3,4] and European eels[1,2,7].

The increase of pituitary GTH-II was significantly stimulated in the pituitary extract-treated cultured eels in a number of injection-dependent profile that is consistent with the increase of GSI. Our data are in agreement with the previous studies in European [17] and Japanese silver eels[8]. The injected pituitary extract may directly act to stimulate ovarian development. The effects of injected pituitary extract to induce the increase of pituitary GTH-II content were likely due to positive feedback by endogenous

gonadal factors which were stimulated by pituitary extract treatment as previously demonstrated in an experiment with ovariectomized and intact European silver eels[17]. Induction of pituitary GTH-II content was observed only in the sham operated female silver eels but not in the ovariectomized eels treated with carp pituitary extract[17]. Other stimulatory factors in addition to gonadotropins in the pituitary extract may also contribute to the ovarian growth and the increase in pituitary GTH-II levels.

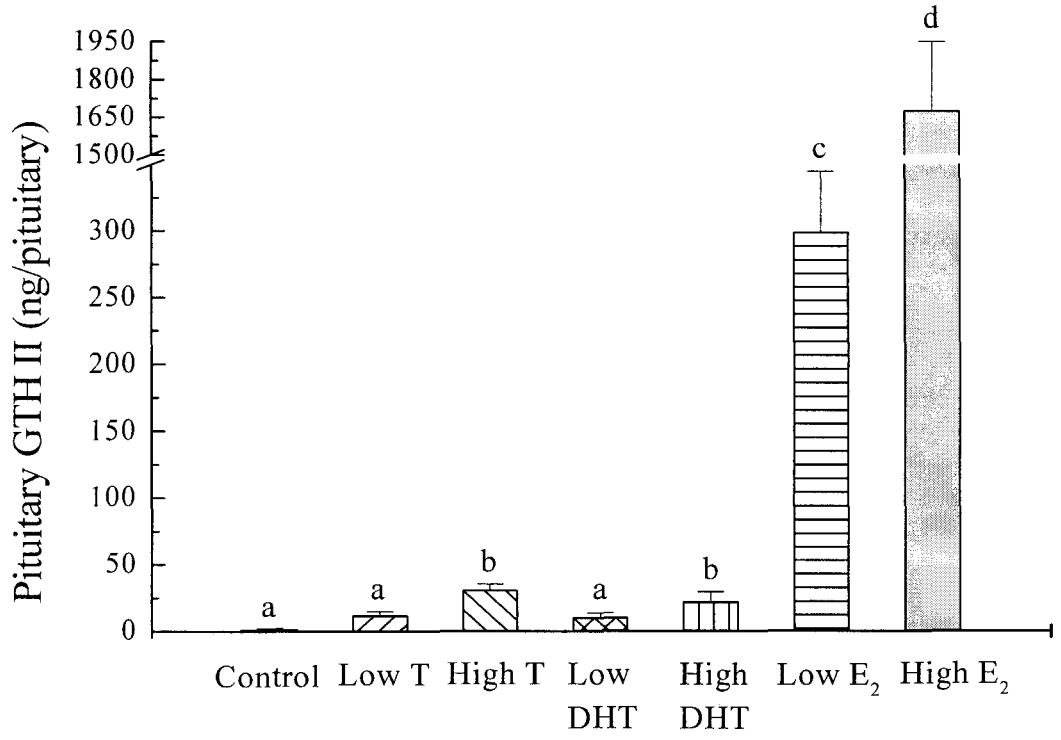

Fig. 3. Pituitary gonadotropin (GTH) II levels in cultured male eels; control, low dose (0.75 mg/kg body weight) and high dose (3.75 mg/kg body weight) of testosterone (T), 5 α-dihydrotestosterone (DHT) or estradiol-17β (E2). Values with different characters differ from each other ($P < 0.05$).

Pituitary GTH-II content was significantly increased in the E2-treated females and males in a dose-dependent manner. There was no apparent difference between female and male eels in responding to stimulation by sex steroids. This is the first study to demonstrate the regulation of pituitary GTH-II by sex steroids in cultured yellow eels. Estrogen-specific stimulation (strong estrogen- but weak androgen-effects) of pituitary GTH-II levels was, therefore, demonstrated in yellow Japanese eels. E2 is the physiological gonadal steroid and it is active in inducing pituitary GTH-II in both silver European female eels[10,17] or yellow cultured Japanese eels (in this experiment). The responsive sensitivity of pituitary GTH-II to E2 stimulation is similar between eels at the silver and yellow stage. In contrast, the

response of pituitary GTH-II to the stimulation of androgens is much higher in the wild silver eels[8,11] than in the cultured yellow eels (in the present experiment).

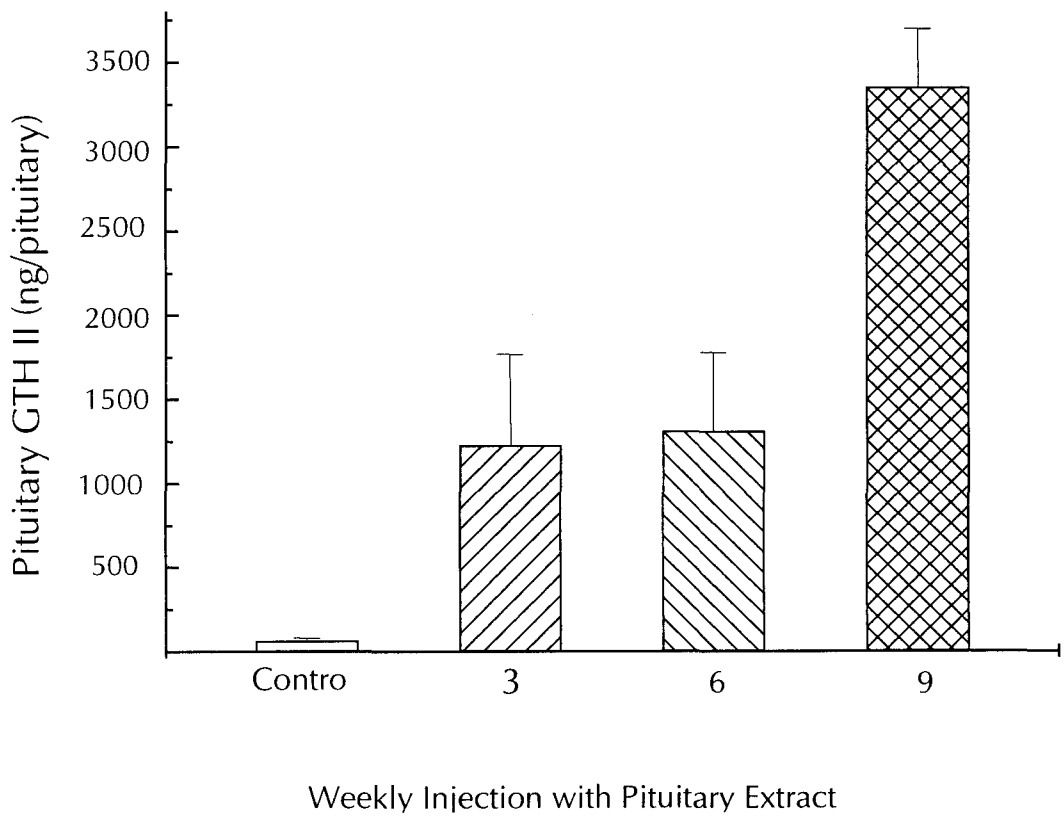

Fig. 4. Pituitary gonadotropin (GTH) II levels in cultured female eels injected weekly with salmon pituitary extract (10 mg/kg body weigh per week, for 3, 6, or 9 weeks). Values with different characters differ from each other ($P < 0.05$).

Weekly injection of E2 at 2 μg/g body weight for 3 months could stimulate high levels of mGnRH in brain and pituitary in European eel, *A. anguilla*[18]. However, T and non-aromatazible androgen (androstenedione) did not have any effects on the levels of mGnRH[18]. Therefore, E2 stimulation of pituitary GTH-II is suggested to act through GnRH pathway in European silver eels[18]. In this study, we did not follow the mGnRH level. Therefore, the significance of the direct involvement of GnRH pathway by the sex steroids treatment in eels cannot be ruled out.

In summary, our data provide some information on the regulation of GTH-II in cultured yellow eels, *A. japonica*. E2 and pituitary extract could significantly stimulate pituitary GTH-II in a dose-dependent manner in the cultured yellow eels. Androgens only weakly stimulated pituitary GTH-II levels as compared to E2. A different effects of sex steroids on pituitary GTH-II content were observed. These suggested that the salmon pituitary extract effects on GSI in the cultured yellow eels was a direct effect on

ovaries, and a multiple effect of sex steroid on various levels of reproduction.

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