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CHANGES OF SEX STEROID CONCENTRATIONS IN FEMALE AND MALE AYU (*PLECOGLOSSUS ALTIVELIS*) STIMULATED BY A LUTEINIZING HORMONE-RELEASING HORMONE ANALOG

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Lian-Tien Sun, Hung-Jen Hu, Hung-Chi Tang, Chia-Fu Huang and Ching-Fong Chang (1992) Changes of sex steroid concentrations in female and male ayu (*Plecoglossus altivelis*) stimulated by a luteinizing hormone-releasing hormone analog. *Bull. Inst. Zool., Academia Sinica* **31**(1): 57-64. The objective of this study was to investigate changes in plasma concentrations of testosterone (T), estradiol-17β (E_2), and 17α-hydroxyprogesterone (17α-OH P) following a single injection of a luteinizing hormone-releasing hormone (LH-RH) analog in female and male ayu, *Plecoglossus altivelis*. Maturing females (n=144) and males (n=115) were divided into three groups and injected with saline, LH-RH analog 30 μg/kg wt, and 60 μg/kg wt, respectively. Levels of plasma, T, E_2, and 17α-OH P increased significantly within 6-12 hours following injection in the male and non-ovulating female ayu. Levels of the measured sex steroids in the stimulated female and male ayu declined to control group levels within 24 hours, with the exception that levels of 17α-OH P still increased in stimulated male until 48-72 hours following injection. High levels of plasma 17α-OH P also occurred in the ovulating female ayu. The LH-RH analog could induce ovulation in some ayu.

Key words: Testosterone, Estradiol-17β, 17α-hydroxyprogesterone, LH-RH Analog, Ovulation, Ayu.

Superactive analogs of mammalian luteinizing hormone-releasing hormone (LH-RH analog) have been widely used to induce ovulation and spermiation in a number of teleosts (reviewed by Abraham, 1988; reviewed by Zohar, 1989). These teleosts include ayu, *Plecoglossus altivelis* (Hirose and Ishida, 1974; Aida, 1983; Hirose *et al.*, 1991; Chang *et al.*, 1991); black porgy, *Acanthopagrus schlegeli* (Yueh *et al.*, 1990; Chang and Yueh, 1990a; Chang *et al.*, 1991); walleye, *Stizostedion vitreum* (Pankhurst *et al.*, 1986); catfish, *Clarias macrocephalus* (Ngamvongchon *et al.*, 1986); carp (Ngamvongchon *et al.*, 1987); and Atlantic salmon, *Salmo salar* (Weil and Crim, 1983; Crim and Glebe, 1984).

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Increases of plasma gonadotropin, 17α-hydroxyprogesterone (17α-OH P), and 17α, 20β-dihydroxy-4-pregnen-3-one (17α, 20β-diOH P) were found in female ayu following LH-RH analog injection (Hirose *et al.*, 1983). Plasma estradiol-17β (E_2), 17α-OH P, and 17α, 20β-diOH P increased prior to ovulation in ayu (Hirose *et al.*, 1985). Little information is available on time course changes in plasma concentrations of sex steroids during LH-RH analog-induced spawning in female ayu. Profiles of sex steroid levels in male ayu stimulated with LH-RH analog have also not been investigated. Therefore, our objectives were to study changes in plasma concentrations of testosterone (T), E_2, and 17α-OH P after a single injection of LH-RH analog in female and male ayu.

MATERIALS AND METHODS

Fish

Maturing one-year-old ayu (65.6±2.2 g in body weight) were collected from the Mar-Lin culture station in Taiwan. Non-ovulating females (n=144) with maturing oocytes (diameter>0.6 mm) and spermiating males (n=115) were selected for study by an experienced technician. All fish were fed with commercially-formulated feed, and reared in a pond (24.5×6.5×0.6 m) under natural photoperiod conditions.

Experimental Design

Maturing female and male ayu were divided into three groups; they were then injected once with either saline (n=42 females and 42 males; 5 ml/kg wt), LH-RH analog 30 μg/kg wt (n=42 females and 40 males), or 60 μg/kg wt (n=40 females and 33 males). The LH-RH analog (D-Ala^6, des-Gly^{10}, LH-RH ethylamide) was purchased from the Sigma Chemical Co. Female and male ayu in the control group were killed and bled 0, 24, 48, 72, and 96 hours following injection. Female and male ayu in the LH-RH analog group were killed and bled 0, 6, 12, 24, 48, 72, and 96 hours following injection. Ovulation in each group was checked once every day before bleeding from day 0 to day 4. Blood samples were collected whenever an individval ayu ovulated. The fish were anesthetized in a bath of 0.4 ml/1 of 2-phenoxyethanol prior to handling. About 1 ml of blood from the caudal vasculature was collected in heparinized tubes. Plasma was separated by centrifugation and stored at -20°C for later steroid analysis.

Assays

E_2, T, and 17α-hydroxyprogesterone (17α-OH P) in plasma following solvent extraction were all measured by radioimmunoassays as as described by Chang *et al.* (1990b). Intra-assay and inter-assay ranges in variation for the three steroids were 12.6-19.0% and 15.5-23.4%, respectively. The radioactivity of the bound ^{3}H-steroid in supernatant was measured using a liquid scintillation counter (Beckman 5801) with counting fluid (NE 266, Nuclear Enterprises, Edinburgh, Scotland). [2, 4, 6, 7-^{3}H] estradiol-17β (85-110 Ci/$m$mol), [1, 2, 6, 7-^{3}H] testosterone (80-105 Ci/$m$mol), and 17α-hdroxy [1, 2, 6, 7-^{3}H] progesterone (55-85 Ci/$m$mol) were purchased from the Amersham Co. (Arlington Heights, Illinois).

Data Analysis

Standard error of mean (SEM) was calculated. Analysis of variance followed by Duncan's multiple range test was used to compare the difference (Steel and Torrie, 1980).

RESULTS

None of the female ayu in the control group ovulated; on the contrary,

Table 1
Concentrations of plasma testosterone, estradiol-17β, and 17α-hydroxyprogesterone in non-ovulating female ayu following injection with saline (control) and LH-RH analog

	Hours following injection						
	0	6	12	24	48	72	96
Testosterone (ng/ml)							
Control	32.19±6.45[a] (11)	—	—	24.75±8.44 (7)	32.63±8.65 (8)	39.26±10.50 (8)	13.15±6.09 (8)
LH-RH analog							
30 μg/kg wt	32.19±6.45 (11)	97.30±24.95* (7)	51.17±21.30 (7)	56.16±23.37* (6)	28.39±5.46 (5)	30.15±7.96 (5)	65.89±25.90* (4)
60 μg/kg wt	32.19±6.45 (11)	61.03±12.00* (7)	33.33±7.25 (6)	30.31±6.14 (5)	17.07±3.48* (7)	10.97±7.60* (3)	19.16±10.60 (5)
Estradiol-17β (ng/ml)							
Control	1.11±0.14	—	—	1.05±0.10	1.04±0.01	1.04±0.10	0.74±0.12
LH-RH analog							
30 μg/kg wt	1.11±0.14	2.91±0.35*	0.49±0.04	0.63±0.64	1.16±0.22	1.17±0.72	0.63±0.20
60 μg/kg wt	1.11±0.14	1.84±0.29	2.00±0.03*	0.44±0.21	0.37±0.06	0.52±0.03	0.34±0.09
17α-hydroxyprogesterone (ng/ml)							
Control	0.93±0.31	—	—	1.80±0.73	1.36±0.42	1.19±0.11	0.71±0.19
LH-RH analog							
30 μg/kg wt	0.93±0.32	7.52±1.01*	7.79±0.97*	4.92±0.87*	1.15±0.30	1.34±0.52	1.16±0.13
60 μg/kg wt	0.93±0.32	3.63±0.87*	5.18±1.12*	1.42±0.61	0.78±0.16	2.31±0.93	0.90±0.30

[a] Means±SEM.
Numbers in parentheses indicate numbers in the samples. Sample numbers in the plasma estradiol-17β and 17α-hydroxyprogesterone of the control and LH-RH analog groups were the same as those groups in the plasma testosterone.
* Values differed ($p<0.05$) from those of the control group at respective times, except for those values sampled 6 or 12 hours after injection in the LH-RH analog group which were compared with the control group at 0 hour.

Table 2
Concentrations of plasma testosterone, estradiol-17β, and 17α-hydroxyprogesterone in male ayu following injection with saline (control) and LH-RH analog

	Hours following injection						
	0	6	12	24	48	72	96
Testosterone (ng/ml)							
Control	18.00±2.83[a] (11)	—	—	22.04±4.99 (9)	18.64±7.04 (8)	22.95±3.37 (7)	18.38±4.23 (7)
LH-RH analog							
30 μg/kg wt	18.00±2.83 (11)	30.67±0.55* (7)	67.51±11.22* (7)	52.84±8.38* (7)	17.81±5.20 (7)	13.36±4.10 (6)	27.33±9.47 (6)
60 μg/kg wt	18.00±2.83 (11)	21.14±2.83 (6)	34.54±10.05* (6)	65.45±11.90* (6)	22.54±4.33 (5)	15.25±3.05 (5)	16.05±2.72 (5)
Estradiol-17β (ng/ml)							
Control	0.28±0.03	—	—	0.16±0.02	0.20±0.04	0.13±0.01	0.10±0.01
LH-RH analog							
30 μg/kg wt	0.28±0.03	0.38±0.07	2.61±0.47*	0.23±0.02	0.32±0.02	0.16±0.01	0.08±0.01
60 μg/kg wt	0.28±0.03	0.26±0.02	0.63±0.04*	0.24±0.01	0.16±0.02	0.07±0.00	0.08±0.00
17α-hydroxyprogesterone (ng/ml)							
Control	0.52±0.31	—	—	1.52±0.43	0.62±0.24	1.02±0.35	1.36±0.42
LH-RH analog							
30 μg/kg wt	0.52±0.31	15.51±6.44*	6.12±0.98*	3.89±1.10*	15.86±0.60*	1.62±0.26	—
60 μg/kg wt	0.52±0.31	1.29±0.17*	4.71±2.47*	10.52±3.24*	8.04±4.68*	3.38±1.20*	4.42±2.91

[a] Means±SEM.
Numbers in parentheses indicate numbers in the samples. Sample numbers in the plasma estradiol-17β and 17α-hydroxyprogesterone of the control and LH-RH analog groups were the same as those groups in the plasma testosterone.
* Values differed ($p<0.05$) from those of the control group at respective times except for those values sampled 6 or 12 hours after injection in the LH-RH analog groups which were compared with the control group at 0 hour.

Table 3
Concentrations of plasma testosterone, estradiol-17β, and 17α-hydroxyprogesterone in the pooled control, ovulating, and non-ovulating female ayu groups following treatment

	No. fish	Concentration (ng/ml)		
		Testosterone	Estradiol-17β	17α-hydroxyprogesterone
Control[a]	42	28.39± 4.44[b]	1.00±0.06	1.20±0.19
LH-RH analog (30 μg/kg wt)				
Ovulating	8	18.05± 6.22	1.29±0.19	4.06±1.12*
Non-ovulating	34	54.85±10.40*	1.16±0.37	3.98±1.30*
LH-RH analog (60 μg/kg wt)				
Ovulating	7	23.63±11.93	1.39±0.23	6.65±1.21*
Non-ovulating	33	29.64± 7.32	0.92±0.78	2.37±0.61*

[a] None of the female ayu in the control group ovulated.
[b] Means±SEM.
* Values differed ($p<0.05$) from those of the control group.

19% and 18% ayu in the low- and high-dose LH-RH analog groups ovulated during the 4-day period, respectively.

Levels of plasma T, E_2, and 17α-OH P increased significantly ($p<0.05$) within 6-24 hours following injection in the non-ovulating female ayu after injections of 30 μg and 60 μg of LH-RH analog/kg wt in comparison to the control group (Table 1). Levels of plasma T also increased significantly ($p<0.05$) within 6-24 hours after injection of LH-RH analog in male ayu (Table 2). Plasma E_2 concentrations in the LH-RH analog-treated male ayu only increased 12 hours following injection (Table 2). Plasma T levels in non-ovulating females (30 μg/kg wt group) were higher than those in ovulating females (Table 3). Levels of plasma 17α-OH P increased in both ovulating and non-ovulating ayu (Table 3). Different levels of plasma E_2 were not observed among various groups (Table 3).

DISCUSSION

In agreement with previous observations (Hirose and Ishida, 1974; Chang *et al.*, 1991), an LH-RH analog dose of 30 μg or 60μg/kg wt could induce ovulation in some, but not all treated ayu. Single injections were insufficient to induce ovulation in females that might not have yet reached the final stages of vitellogensis. Two sequential doses of hormonal stimulation should be more effective in inducing ovulation. However, single injection of either dosage of LH-RH analog did stimulate high levels of plasma T, E_2, and 17α-OH P in both female and male ayu.

Our data indicated that responses of sex steroid levels to LH-RH analog stimulation were very fast in both female and male ayu. These increases of sex steroid levels should be mediated by the elevation of gonadotropin concentrations following treatment with LH-RH analog in ayu (Hirose *et al.*, 1983). Peak levels of plasma T, E_2, and 17α-OH P occurred in female ayu 6, 6-12, and 12 hours following LH-RH analog treatment, respectively. In male ayu, peak levels of plasma T, E_2, and 17α-OH P appeared 12-24, 12, and 6-48 hours following LH-RH

analog treatment, respectively. Various degrees of maturity in female and male ayu may be responsible for the different peak level timing.

Peak concentrations of plasma E_2 and 17α-OH P in female ayu in this study were consistent with other studies (Hirose *et al.*, 1983, 1985). Due to the lack of available information, no comparison could be made for levels of plasma T, E_2, and 17α-OH P in male ayu, and T in female ayu. Sex steroid levels following injections of high doses of LH-RH analog were intended to be lower than those of low doses. Down regulation of hormonal receptors with a high dose of LH-RH analog might be indicated by this experiment.

T is the major sex steroid in both female and male ayu before and after hormonal stimulation, on the contray, we observed that E_2 and T were the major sex steroids in female and male black porgy, respectively (Chang and Yueh, 1990a; Chang *et al.*, 1990; Yueh *et al.*, 1990). T plays an important role in spermatogenesis in male teleosts, and in positive feedback regulation of ovulatory gonadotropin surge in female teleosts (Pandey, 1969; Kobayashi *et al.*, 1989). T also acts as a precursor of E_2; E_2 stimulates the vitellogenesis of oocytes in teleosts (de Vlaming *et al.*, 1980). Lower levels of E_2 may be important for the occurrence of ovulation in teleosts (Billard and Peter, 1977; Chang and Yuen, 1990a; Chang *et al.*, 1991).

Only 17α-OH P levels were measured in this experiment, although 17α, 20β-diOH P is known to be involved in final oocyte maturation and spermiation in female and male teleosts (Young *et al.*, 1983; Levavi-Zermonsky and Yaron, 1986; Kobayashi *et al.*, 1986). 17α-OH P and and 17α, 20β-diOH P may also induce *in vitro* germinal vesicle breakdown in ayu (Nagahama *et al.*, 1983). High levels of 17α-OH P and 17α, 20β-diOH P occur in stimulated or naturally spawning female ayu (Hirose *et al.*, 1983, 1985). Our data also indicated that high levels of 17α-OH P are present in stimulated male and female ayu. 17α-OH P is considered to be a precursor for 17α, 20β-diOH P synthesis in ayu.

High levels of plasma 17α-hydroxyprogesterone were observed in ovulating female ayu, which is consistent with results reported by Hirose *et al.* (1985). No increases in plasma E_2 levels were observed in ovulating ayu. Plasma E_2 levels decreased prior to ovulation, then increased after spawning and remained at high levels for at least several days (Hirose *et al.*, 1985). The interval between bleeding and ovulation should be less than 24 hours, as we bled and checked ovulation every day.

In conclusion, LH-RH analog at a dose of 30 μg/kg wt or 60 μg/kg wt can induce ovulation in ayu. Levels of plasma T, E_2, and 17α-OH P increased significantly in both female and male ayu within 6-12 hours following injection. Levels of most sex steroids measured in stimulated ayu in our study declined to control group levels within 24 hours.

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性釋素提高香魚血液性類固醇激素之濃度變化

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本研究之目的在探討香魚注射性釋素後，血液之睪固酮、雌二醇及 17α-hydroxyprogesterone 之濃度變化。雌性與雄性香魚各分爲三組，分別注射一次生理食鹽水、30 μg/kg wt 性釋素或 60 μg/kg wt 性釋素。雌雄香魚血液之睪固酮、雌二醇及 17α-hydroxyprogesterone 在注射性釋素 30 μg 或 60 μg/kg wt 後 6-12 小時均有顯著上升。除雄性香魚之 17α-hydroxyprogesterone 外，雌性與雄性香魚之其他性類固醇激素在注射 24 小時以後，卽降低至相當於對照組之濃度。性釋素並可促進部份香魚產卵。