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Effects of the aromatase inhibitor Fadrozole on reproductive steroids and spermiation in male coho salmon (*Oncorhynchus kisutch*) during sexual maturation

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Abstract

In male teleosts, plasma oestrogen levels are undetectable, or when detectable, are present in very low amounts. Several studies have shown that the testes are incapable of secreting oestrogens. In contrast, other studies have demonstrated that oestrogen can be formed in the male brain, and that oestrogens are involved with neuroendocrine and behavioral responses. Therefore, this study evaluated the effects of inhibiting oestrogen production in male coho salmon (*Oncorhynchus kisutch*) during sexual maturation using the aromatase inhibitor Fadrozole (AI). Groups of fish, which were approximately 1.5 months before natural spawning, received injections of 1.0 or 10.0 mg AI/kg or five weekly injections of 10 mg AI/kg. Plasma sex steroid hormones (17β-oestradiol, testosterone, 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P) and 11-keto-testosterone) were determined before and during 32 days after injection. Injection with AI inhibited 17β-oestradiol secretion by the brain. Fish injected with 10 mg AI/kg or 10 mg AI/kg weekly presented significantly higher plasma 17,20β-P levels (12.11 ± 1.75 and 11.98 ± 2.85 ng/ml, respectively) at 6 h after injection than before injection (0.19 ± 0.19 and 1.00 ± 0.42

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ng/ml, respectively). At 16 days after injection, the groups injected with 10 mg AI/kg or 10 mg AI/kg weekly were already spermiating at significantly higher rates than the vehicle group. Gonadosomatic index (GSI) and sperm motility were not different among the groups. This study demonstrated that Fadrozole inhibited 17β-oestradiol in the brain, and prematurely increased plasma 17,20β-P levels.

Keywords: Aromatase inhibitor; Coho salmon; 17β-Oestradiol; 17,20β-Dihydroxy-4-pregnen-3-one; 11-Keto-testosterone; Testosterone

1. Introduction

In male salmonids, the two major androgens and progestagen produced by the testis are testosterone, 11-ketotestosterone and 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P), respectively (Fostier et al., 1983; Nagahama, 1987). These androgens are believed to be involved in spermatogenesis, since plasma testosterone and 11-ketotestosterone increase during this period (Scott and Sumpter, 1989). In teleost testes, the interstitial cells (Leydig cells) are the main source of androgens (Loir, 1990a,b). On the other hand, 17,20β-P has been shown to be responsible for final sperm maturation (Miura et al., 1992). Several researchers have demonstrated that sperm cells are responsible for 17,20β-P synthesis (Ueda et al., 1984; Barry et al., 1990; Sakai et al., 1990). More recently, however, it has been demonstrated that non-flagellated germ cells from rainbow trout, *Oncorhynchus mykiss*, immature testes were able to synthesize 17,20β-P, and 20β-hydroxysteroid dehydrogenase activity has been detected in these cells (Vizziano et al., 1995, 1996).

Most of the in vitro studies that have investigated the steroidogenic ability of the testis, however, have demonstrated that it is incapable of secreting oestrogens, and therefore lacks aromatase activity (Callard et al., 1978b; Colombo et al., 1978; Depêche and Sire, 1982). On the other hand, oestrogens can be formed in the brain, and aromatase activity has been detected in the fish brain independent of the sex (female rainbow trout: Lambert and van Bohemen, 1980; male Atlantic salmon, *Salmo salar*, Mayer et al., 1991; male and female of three-spined stickleback, *Gasterosteus aculeatus*: Borg et al., 1987a,b; male African catfish, *Clarias gariepinus*: Timmers et al., 1987; male and female goldfish: Pasmanik and Callard, 1985). Indeed, aromatization of androgens in the central nervous system has been demonstrated in representatives of all classes of vertebrates (Callard et al., 1978a). Since it has been shown that the fish testis has minimal ability to synthesize oestrogens (see text above), the presence of aromatase activity in the brain suggests that the brain is the source for the low levels of plasma oestrogen (Fostier et al., 1983). Additional evidence that in males the brain is the major site of oestrogen biosynthesis and that they are released into the general circulation has been provided in African catfish (Timmers, 1988), and zebra finch (Schlinger and Arnold, 1991, 1993; Arnold and Schlinger, 1993). Since there is good evidence that the formation of oestrogens from circulating androgens in the brain is important for certain neuroendocrine and behavioral responses (Naftolin, et al., 1975; McEwen et al., 1979; Bonsall et al., 1992; Arnold and Schlinger, 1993; Zumpe et al., 1993), it is possible that

inhibition of oestrogen formation in the brain may affect the normal neuroendocrine secretion during reproductive development in fish.

Associating the significance of oestrogen in males, as discussed above, with the previous observation in this laboratory (data unpublished) that males treated with 10 mg aromatase inhibitor (AI)/kg tended to spermiate earlier, the purpose of the present study was to determine the effects of inhibiting oestrogen biosynthesis on plasma sex steroid secretion and on reproductive development.

2. Materials and methods

2.1. Experimental animals

One hundred adult coho salmon were obtained in mid-August 1996 from the Capilano Salmon Hatchery, Vancouver, BC (Department of Fisheries and Oceans) and transported to the West Vancouver Laboratory (Department of Fisheries and Oceans) and held outdoors in 3-m-diameter fiberglass tanks, which were constantly supplied with freshwater from Cypress Creek (8 ± 2°C). Before any handling procedure, fish were anesthetized by immersion in water containing 100 mg tricaine methanesulfonate (MS-222) (Syndel Laboratories, Vancouver, BC) per litre buffered 1:1 with sodium bicarbonate. Fish were individually identified with a PIT tag inserted in the ventral muscle. Since it was impossible to visually sex the fish by examining external morphology, a sample of blood was collected from each individual and the plasma 17β-oestradiol level was determined in each fish. Fish in which 17β-oestradiol was undetectable were considered to be males. At the time of capture, the weights and the lengths of the male fish were 0.8 ± 0.2 kg and 42.3 ± 4.0 cm (mean $\pm$ SEM), respectively, and they were approximately 1.5 months before natural spawning. Fish were randomly divided into groups in accordance with the experimental protocol, and each group was kept in an 800-l tank constantly supplied with fresh creek water.

2.2. AI injection and treatments

These procedures were carried out at least 3 days after fish had been transferred to the 800-l tanks. The non-steroidal AI Fadrozole (CGS 16949A) (4-(5,6,7,8-tetrahydroimidazol[1,5]-pyridin-5-yl)benzonitrile monohydrochloride), which was a gift from Novartis (Honora Cooper-Eckhardt, Summit, NJ) was dissolved in the vehicle propylene glycol (Fisher Scientific) (Steele et al., 1987). Stock solutions that contained 0.1, 1.0, 10.0 mg of AI/ml were prepared. Fish were injected intraperitonially at the base of the right ventral fin using individual 4-ml syringes fitted with an 18.5 gauge needle. Control fish were injected with the vehicle propylene glycol only (1.0 ml/kg body weight). Fish were divided in four groups: control vehicle injected ($n = 8$), a group treated with 1.0 mg AI/kg ($n = 8$), a group treated with 10.0 mg AI/kg ($n = 8$), and a group treated with 10.0 mg AI/kg on days 1, 7, 14, 21 and 28 ($n = 8$). The experimental protocol

consisted of determining the plasma sex steroid levels before injection with AI and during 32 days after the first injection.

2.3. Blood collection

A sample of blood (3 ml) was collected from the caudal vasculature using a 10-ml heparinized vacutainer (Becton Dickinson) with a 21 gauge needle, and held on ice before centrifugation at 4000 rpm for 10 min at 4°C. The plasma was aliquoted into 1.5-ml plastic microfuge tubes and stored frozen at −20°C until assayed. Blood samples were collected at 0 hour (just before injection with vehicle or Fadrozole), 6 h, 1, 2, 4, 8, 16, and 32 days after the first injection.

2.4. Organ collection

After the last blood collection, fish were killed by decapitation. The testes were removed and weighed and gonadosomatic index (GSI) was determined. Ten untreated fish were killed at 0 h, from which GSI was determined to compare with the fish after 32 days. The procedure used for brain culture was modified from Timmers et al. (1987). After decapitation, the brains were removed and immediately transferred to a container with Leibovitz L-15 culture medium (pH adjusted to 7.8) (Gibco BRL Products) on ice. From each brain, a transverse slice of approximately 2 mm (100 mg) containing parts of the diencephalon, mesencephalon and metencephalon, which have been shown to contain aromatase activity (Timmers et al., 1987), was cut and transferred to a petri dish, washed three times, placed in 24 well plates (Falcon, Becton Dickinson) containing 1 ml of L-15, and incubated in the presence of 100 ng of testosterone/ml for 18 h at 12°C.

2.5. Spermiation rate and sperm motility rate

Spermiation was checked through gentle abdominal massage, before blood sampling, at 0, 8, 16, and 32 days. During sperm collection contamination of the milt with blood, faeces, urine and water was avoided. When sperm was released, it was collected into a sterile plastic bag and held on ice at 4°C for no longer than 3 h. Whenever a fish released sperm, the average motility of a sample was determined microscopically by placing 100 μl of sperm on a slide and mixing with water. Percentage of sperm motility in each sample was assessed by the examiner through visual inspection of forward sperm movements.

2.6. Steroid extraction and hormone measurements

The steroids were extracted from the brain culture incubation medium using a similar method to that described by Campbell (1992). Aliquots of the incubation medium (500 μl) were placed in a plastic tube, and ethyl acetate (900 μl) was added, the tubes were capped and the two phases mixed by shaking. After 10-min extraction, the tubes

were centrifuged for 2 min at $3000 \times g$ to separate the two phases. Then, 200 μl of the supernatant phase was placed in 12×75 mm borosilicate tubes, evaporated to dryness overnight in a fumehood, and redissolved in 100 μl buffer. From the redissolved solution, 50 μl was removed, and dissolved 10-fold in steroid assay buffer (1:10). Plasma and culture media 17β-oestradiol, and plasma testosterone, 17,20β-P, and 11-ketostestosterone levels were measured by radioimmunoassays described and validated by Van Der Kraak et al. (1984) and Dye et al. (1986).

3. Statistical analysis

When data failed to achieve either normal distribution or equal variance, data were Log10 transformed. Time-dependent variations within each group were studied by repeated measures analysis of variance, since individual fish were sampled repeatedly, followed by a Student–Newman–Keuls test. Friedman Repeated Measures analysis of variance (ANOVA) on ranks was performed when data failed to achieve either normality or equal variance followed by a Student–Newman–Keuls test. Group-dependent variations in plasma concentrations for each hormone were detected by ANOVA followed by all pairwise multiple comparison by Student–Newman–Keuls test. The Kruskal–Wallis ANOVA on ranks was performed when data failed to achieve either normality or equal variance, followed by all pairwise multiple comparison by Student–Newman–Keuls test. ANOVA was also used to detect variations in GSI. The effects of treatments on spermiation were analyzed comparing two treatments using Fischer Exact Test. In the in vitro studies variations among the different treatments were detected by ANOVA followed by all pairwise multiple comparison by Student–Newman–Keuls test. Unless indicated, statistically significant differences were determined at $p = 0.05$.

4. Results

Two fish from the control group and three from the multiple injection group (AI 5×10 mg/kg) jumped out of the tank and died during the course of the experiment. The exact number of fish in each group ranged from five to eight, and is shown in Fig. 1.

17β-Oestradiol in the male plasma was below the detection limit (minimum detectable amount by the assay = 0.1 ng/ml) at all times. However, it was possible to detect 17β-oestradiol in the medium containing brain tissue at the end of the experiment. The results showed that only brain tissue from the vehicle injected group was able to secrete significant ($p < 0.05$) amounts of 17β-oestradiol into the incubation medium (Fig. 2), although the low dose group also secreted small amounts of 17β-oestradiol into the medium.

Plasma 17,20β-P levels in the vehicle injected group remained low and constant from 0 h to 8 days (Fig. 1), and at 16 and 32 days there was a significant increase ($p < 0.05$).

Treatment	17-20β-P (ng/ml) / days after injection							
	0	6h	1d	2d	4d	8d	16d	32d
Control	0.58	1.58	0.00	1.60	0.00	0.39	4.15	12.84
n=6	±	±	±	±	±	±	±	±
●	0.37a	0.33a/a	0.00a	0.36a	0.00a	0.39a/a	1.12b	3.21c
1.0	0,65	2.13	0.18	3.32	0.96	0.18	1.77	6.21
n=8	±	±	±	±	±	±	±	±
■	0,24a	0.42bc/a	0.18a	0.65cd	0.50ab	0.18a/a	0.44bc	1.72d
10.0	0,19	12.11	2.91	3.71	2.89	0.71	2.25	4.24
n=8	±	±	±	±	±	±	±	±
▲	0.19a	1.75c/b	0.55b	0.28b	0.51b	0.51ab/a	0.79b	1.92b
10.0 x 5	1,00	11.98	2.00	2.05	1.95	4.49	18.03	26.35
n=5	±	±	±	±	±	±	±	±
▼	0,42a	2.85c/b	0.54ab	0.21ab	0.10ab	1.26b/b	4.90c	8.91c

Treatment	11-ketotestosterone (ng/ml) / days after injection							
	0	6h	1d	2d	4d	8d	16d	32d
Control	16.06	9.62	8.74	10.42	11.12	23.94	32.02	50.59
n=6	±	±	±	±	±	±	±	±
●	2,34ab	2.03a	2.42a	4.93ab	4.7ab/a	6.21bc	6.04cd	8.08d
1.0	13.53	8.61	5.65	26.46	35.38	41.58	23.44	53.13
n=8	±	±	±	±	±	±	±	±
■	1.66bc	1.51ab	1.99a	6.98bc	10cd/b	11.46cd	6.37bc	8.64d
10.0	16.42	7.69	2.66	10.21	45.78	43.39	31.96	26.21
n=8	±	±	±	±	±	±	±	±
▲	1.42c	1.36b	1.03a	1.74b	10.3d/b	14.00d	10.3bcd	9.5abcd
10.0 x 5	16.79	14.92	8.65	15.25	63.43	29.59	64.94	47.75
n=5	±	±	±	±	±	±	±	±
▼	1.03a	3.00a	3.39a	6.19a	18.0b/b	5.33ab	18.10b	18.6ab

Treatment	Testosterone (ng/ml) / days after injection							
	0	6h	1d	2d	4d	8d	16d	32d
Control	19.93	11.78	10.56	14.88	8.81	19.28	22.43	21.81
n=6	±	±	±	±	±	±	±	±
●	4.32ac	3.02bc	2.26ab	3.74bc	1.73a/a	3.96bc	4.34c	5.07ab
1.0	23.65	13.52	9.18	54.04	13.23	27.40	10.00	24.70
n=8	±	±	±	±	±	±	±	±
■	2.18bc	2.85ab	4.13a	12.31c	15.6ab/b	5.32bc	2.18a	4.64bc
10.0	24.18	9.97	11.21	44.79	63.28	37.95	16.91	32.23
n=8	±	±	±	±	±	±	±	±
▲	1.49b	1.53a	3.20a	8.41c	10.3c/c	10.35bc	5.31ab	8.84bc
10.0 x 5	24.50	12.10	24.20	42.90	66.60	70.80	73.50	59.60
n=5	±	±	±	±	±	±	±	±
▼	4.36b	1.95a	5.42ab	10.64bc	5.15c/c	15.12bc	19.16c	23.11bc

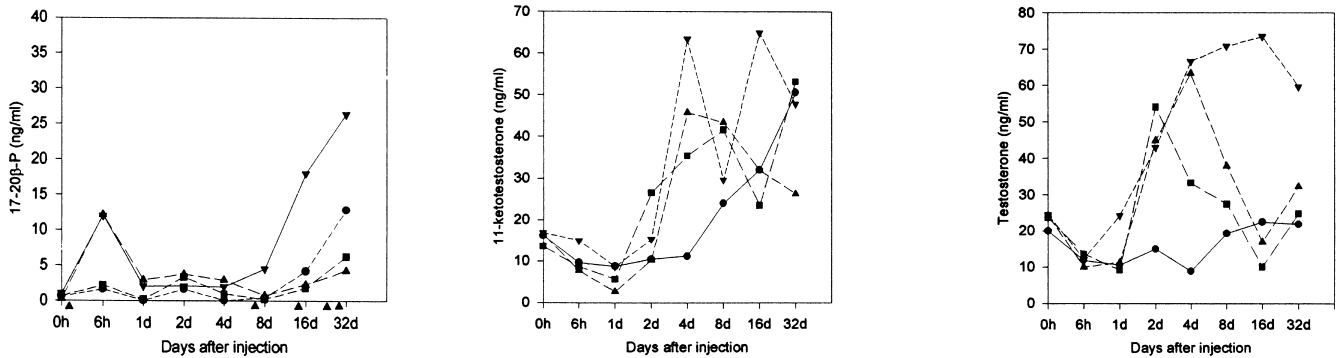

Fig. 1. Plasma concentration of 17,20β-P, 11-ketotestosterone and testosterone in male coho salmon injected or not with Fadrozole (AI mg/kg). Each value in the tables represent the mean ± SEM. Plasma hormone concentrations which were significantly different ($p < 0.05$) within each group treatment along the 0 to 32 days samplings are identified by different letters before slash. Plasma hormone concentrations, which were significantly different ($p < 0.05$) among the four treatment groups at a given sampling time, are identified with different letters after slash. *n*, indicates the number of fish per group. The symbols ●, ■, ▲, ▼ indicate the different treatments. The triangles (▲) under the *x*-axis of the first graph indicate the injection schedule for the group injected weekly.

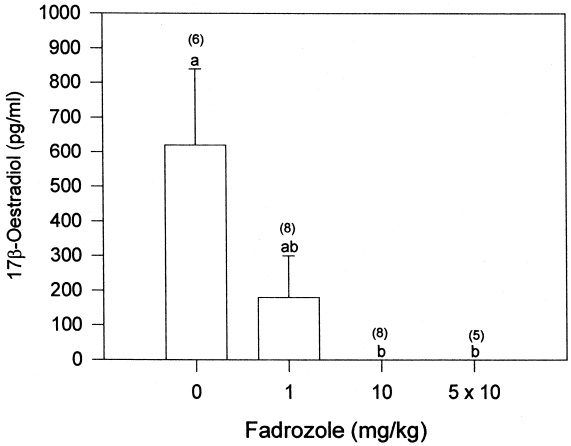

Fig. 2. In vitro 17β-oestradiol production by the brains of male coho salmon at the end of the experiment (32 days). Each bar represents the mean ± SEM from males in each treatment group. Brains were incubated with incubation medium in the presence of testosterone (100 ng/ml) for 18 h at 12°C. Amounts of 17β-oestradiol released to the medium which are similar ($p > 0.05$) as determined by Student–Newman–Keuls test, are identified by the same superscript letter. Numbers between brackets above the superscript letter indicate number of fish in each group.

In the group treated with 1.0 mg AI/kg plasma, 17,20β-P levels increased slightly but significantly ($p < 0.05$) at 6 h after injection. After that and up to 32 days, the levels were variable. In the group treated with 10.0 mg AI/kg, plasma 17,20β-P increased significantly ($p < 0.05$) 6 h after injection, and decreased at 1 day remaining almost constant throughout the period before rising significantly at 32 days. The treatment group injected weekly with 10.0 mg AI/kg showed similar hormone profiles to the group which received a single injection of 10.0 mg AI/kg from 0 h up to 4 days. At 8 days, i.e. one day after the second injection, however, plasma 17,20β-P levels slightly but significantly ($p < 0.05$) increased in relation to the levels at 0 h, increased significantly ($p < 0.05$) one day after the third injection and remained high up to 32 days.

Plasma 11-ketotestosterone levels in the control group remained constant from 0 to 8 days, and after that the levels increased significantly ($p < 0.05$) up to the end of the experiment in relation to levels observed at 0 h. In the group injected with 1.0 mg AI/kg, plasma 11-ketotestosterone also varied during the period and the level of the hormone showed a significant ($p < 0.05$) decline at 1 day after injection. At 2 days and after, plasma 11-ketotesterone levels recovered significantly ($p < 0.05$) in relation to 1 day. The profile of plasma 11-ketotestosterone levels in the group injected with 10.0 mg AI/kg showed significant ($p < 0.05$) declines up to 1 day, and recovery at 2 days and after, but peaking at 4 days. Plasma 11-ketotestosterone levels increased significantly ($p < 0.05$) 4 days after injection in the multiple injected group and showed distinct peaks after that.

In the control group, plasma testosterone remained almost unchanged during the 32-day period. It declined significantly ($p < 0.05$) at 4 days after injection in relation to levels at 0 h, but at 8 days there was a recovery, and the concentration then remained

constant throughout the rest of the experimental period. In the group treated with 1.0 mg AI/kg, plasma testosterone levels showed a significant ($p < 0.05$) decline at 1 day after injection. At 2 days, it recovered significantly ($p < 0.05$) in relation to 1 day after injection, and at 16 days, plasma testosterone levels decreased significantly ($p < 0.05$) again. In the group treated with 10.0 mg AI/kg, plasma testosterone levels showed a significant ($p < 0.05$) decline at 6 h after injection, and increased significantly ($p < 0.05$) at 2 days, peaking at 4 days and declining after that. In the group that received multiple injections, testosterone levels varied similar to the previous group up to 4 days, remaining steady after that.

Comparison among the groups demonstrated that at 6 h after injection the groups injected with 10.0 mg AI/kg presented significantly ($p < 0.05$) higher plasma 17,20β-P levels than the vehicle injected group and the group injected with 1.0 mg AI/kg (Fig. 1). At 8 days, only the group injected weekly with 10.0 mg AI/kg showed significantly ($p < 0.05$) higher plasma 17,20β-P levels when compared to the other groups. In all the treatment groups injected with AI, plasma 11-ketotestoterone and plasma testosterone levels increased significantly ($p < 0.05$) at 4 days after injection (Fig. 1).

At 16 days, fish that received 10.0 mg AI/kg weekly started to spermiate before the vehicle injected group ($p < 0.08$) and before the group which received 1.0 mg AI/kg ($p < 0.05$) (Fig. 3). Comparison between the treated groups and the fish that were

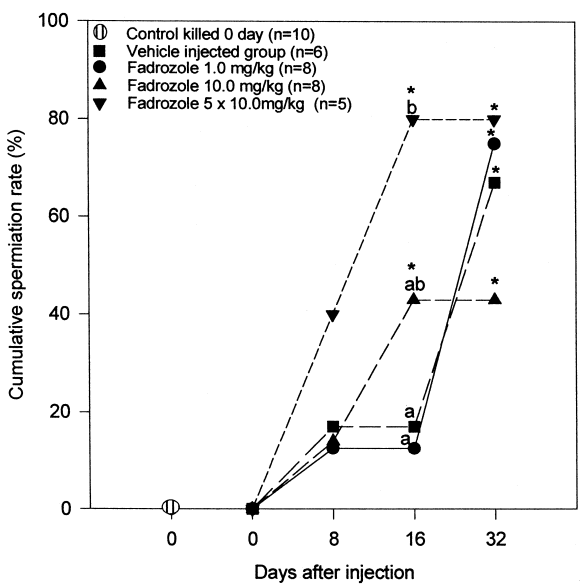

Fig. 3. Effects of Fadrozole on the cumulative spermiation in male coho salmon. Values represent the cumulative percentage of the number of fish that spermiated in each group. The proportion of observations at each checking day that are significantly different ($p < 0.05$) among the treatment groups, as determined by Fisher Exact Test, are identified by different superscript letter. The asterisk symbol (*) means that the proportion of observation at each checking day is significantly different ($p < 0.05$), as determined by Fisher Exact Test, from the control group examined and killed on day 0.

examined and killed at the beginning of the experiment showed significant spermiation ($p < 0.05$ and $p < 0.01$, respectively) at 32 days in the vehicle injected group and the group injected with 1.0 mg AI/kg. On the other hand, in the other two groups injected with 10 mg AI/kg and 10 mg AI/kg weekly, an increase in spermiation ($p = 0.077$ and $p < 0.01$) occurred at 16 days. Examination of sperm motility revealed that there was no difference among the groups ($p > 0.05$) and the mean sperm motility was 89%. The GSI was not different among the groups ($p > 0.05$) at 32 days (mean $\pm$ SEM, control group: 6.08 ± 0.46; 1.0 mg AI/kg: 6.34 ± 0.60; 10.0 mg AI/kg: 5.35 ± 0.45; weekly 10.0 mg AI/kg: 5.80 ± 0.24).

5. Discussion

It was not possible to detect 17β-oestradiol in any of the male coho salmon plasma samples. Usually, in male salmonids and other species of fish the quantification of plasma 17β-oestradiol levels by radioimmunoassay reveals low levels of 17β-oestradiol (Billard et al., 1978; Idler et al., 1981; Nagahama et al., 1982; Sower and Schreck, 1982; Fitzpatrick et al., 1986). Sower and Schreck (1982) and Fitzpatrick et al. (1986) who worked with the same species in a similar developmental stage quantified plasma 17β-oestradiol levels, using similar radioimmunoassay, which ranged from 0.1 to 0.6 ng/ml. This demonstrates that if 17β-oestradiol was present in the plasma of the fish used in this experiment it would be in amounts lower than the detection limit.

Although several tissues in the body of the fish are able to produce steroid hormones and may contribute to the overall production of sex steroid hormones, the gonads are the main source of sex steroid hormones (Fostier et al., 1983). In this study, we did not investigate the production of sex steroid hormones by the testis. Most of the in vitro studies that have investigated the steroidogenic ability of the testis, however, have demonstrated that it is incapable of secreting oestrogens, and lacks aromatase activity (Callard et al., 1978b; Colombo et al., 1978; Depêche and Sire, 1982). Oestrogens were produced, however, in small amounts by the testis of an elasmobranch fish, spiny dogfish, *Squalus acanthias* (Callard et al., 1978b).

The present study has demonstrated, by measuring the in vitro secretion of 17β-oestradiol from slices of brain incubated with the aromatizable androgen testosterone, the presence of aromatase activity in the brain of male coho salmon. Aromatase activity has also been detected in the brain of several other species of teleosts (Lambert and van Bohemen, 1980; Pasmanik and Callard, 1985; Borg et al., 1987a,b; Timmers et al., 1987; Mayer et al., 1991). There is additional evidence that in males the brain is the major site of oestrogen biosynthesis and that oestrogen is released into the general circulation (Timmers, 1988; Schlinger and Arnold, 1991, 1993; Arnold and Schlinger, 1993).

At the end of the experimental period, secretion of 17β-oestradiol was only detected in the brains of the control fish, indicating that Fadrozole was capable of inhibiting aromatase activity at the brain level. Fadrozole also inhibited aromatase activity in the brain of male Atlantic salmon parr (Antonopoulou et al., 1995), in zebra finch (Wade et al., 1994), in monkey species (Zumpe et al., 1993), and in the rat (Bonsall et al., 1992).

Since there is good evidence that the formation of oestrogens from circulating androgens in the brain is important for certain neuroendocrine and behavioral responses (Naftolin, et al., 1975; McEwen et al., 1979; Bonsall et al., 1992; Arnold and Schlinger, 1993, Zumpe et al., 1993), it is possible that inhibition of oestrogen formation in the brain would affect the normal neuroendocrine secretion during reproductive development in fish.

The present results indicated that inhibition of 17β-oestradiol production by the aromatase inhibitor Fadrozole caused a premature increase in plasma 17,20β-P levels in adult male coho salmon. Several studies have shown that in male fish, plasma 17,20β-P levels remain low during the period of testicular development (spermatogenesis) and then increase dramatically coincidentally with spermiation (Ueda et al., 1983; Scott and Sumpter, 1989; Planas and Swanson, 1995). Miura et al. (1992) demonstrated that 17,20β-P is responsible for promoting sperm motility in masu salmon by increasing the pH of the sperm duct, which in turn elevates cAMP levels in the sperm, allowing the acquisition of sperm motility. This contrasts with the situation in mammals, where the hormonal control of spermatogenesis involves mainly testosterone production (Schulster et al., 1976).

The present study also showed that the treatment group injected weekly with 10 mg AI/kg started to spermiate earlier than the control group and at 16 days, 80% (four of five fish) of the fish were spermiating in contrast with 17% (one of six fish) in the control group and 12.5% (one of eight fish) in the group injected with 1.0 mg AI/kg. It is interesting to notice that at this point the fish had received the third injection of AI and the plasma 17,20β-P levels peaked again, increasing from approximately 4 ng/ml at 8 days to 18 ng/ml at 16 and 32 days. The other groups, however, had plasma 17,20β-P levels, ranging from 1.5 to 3.0 ng/ml. The plasma 17,20β-P levels observed at the period of higher spermiation rate correlates well with those for spermiating coho salmon observed by Fitzpatrick et al. (1986) and Planas and Swanson (1995). One could raise the question that the advancement in the spermiation rate in the multiple injected group was due to handling or injection of vehicle. However, during the first 4 days, when all groups had been equally manipulated, there was a significant change in plasma sex steroid levels in all groups, except the vehicle injected group, demonstrating that the effects seen were due to AI.

Even though it was not possible to detect 17β-oestradiol in the male plasma, the results in terms of the ability of the brain to secrete 17β-oestradiol after stimulation with the aromatizable androgen testosterone was similar to that observed in females (Afonso, 1997), where only the control group was able to secrete a measurable quantity of oestrogen. Furthermore, the premature increase in plasma 17,20β-P levels observed in females during vitellogenesis or close to final maturation (Afonso et al., 1999a,b) was also observed in males at a similar magnitude, but was different in terms of duration. It is also interesting to notice that just as the females injected with AI (10 mg/kg) close to final maturation (which presented decreased plasma 17β-oestradiol concentration and increased plasma 17,20β-P levels) started to ovulate earlier, the males injected weekly (10 mg/kg) started to spermiate earlier. Therefore, this study suggests that in males the steps involved in sexual maturation, which lead to spermiation include a decrease in oestrogen production, probably at the brain level.

All groups treated with AI presented significantly higher levels of testosterone and 11-ketotestosterone than the control group at 4 days after injection. In other salmonid fish, a decrease in plasma testosterone and 11-ketotestosterone concomitantly with an increase in plasma 17,20β-P occurs during the onset of spermiation (Scott and Baynes, 1982; Scott and Sumpter, 1989; Fitzpatrick et al., 1986; Planas and Swanson, 1995). It has been suggested that a shift occurs from androgen to progestagen production in the testis prior to spermiation (Scott and Baynes, 1982; Sakai et al., 1989; Barry et al., 1990). The shift in steroidogenic pathways was not evident in this experiment. Even though Planas and Swanson (1995) observed in vivo the changes in hormone profiles, their in vitro studies did not support the proposed idea of a clear shift in steroid biosynthesis close to spermiation, mostly because there was no decrease in androgens after basal or GTH II-stimulated production of 17,20β-P. Similar to the observations in this study were the findings of Planas and Swanson (1995) that plasma testosterone and 11-ketotestosterone levels remained elevated for approximately 4 weeks after the plasma levels of 17,20β-P started to increase.

In summary, this study has demonstrated that AI inhibited 17β-oestradiol secretion in the brain of male salmon. Injection with AI in males caused a premature and transient increase in plasma 17,20β-P levels. Multiple injections caused further increases in plasma 17,20β-P, and 16 days after the beginning of the experiment the treatment groups that received the highest doses of AI (10 and 5×10 mg/kg) started to spermiate.

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