Post-ovulatory secretion of pituitary gonadotropins GtH I and GtH II in the rainbow trout (*Oncorhynchus mykiss*): regulation by steroids and possible role of non-steroidal gonadal factors

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Abstract

In order to determine the factors of ovarian origin which can modulate the post-ovulatory secretion of the FSH-like gonadotropin (GtH I) and the LH-like gonadotropin (GtH II), freshly ovulated female rainbow trout (*Oncorhynchus mykiss*) were divided into two groups. In the first group the fish were stripped in order to eliminate eggs and ovarian fluid from the body cavity, while in the second group the eggs were kept in the body cavity. Subsequently, fish from both groups were implanted with testosterone (10 mg/kg), 17β-estradiol (10 mg/kg) or 17,20β-dihydroxy-4-pregnen-3-one (17,20βP) (1 mg/kg) or injected every 2 days with desteroidized ovarian fluid (1·5 ml/kg).

The secretion of GtH I dramatically increased in stripped fish, reaching its maximum levels 2 weeks after ovulation. The preservation of eggs in the body cavity led to the suppression of this increase. The profiles of GtH II secretion were opposite to those encountered for GtH I because the increase of GtH II was observed only in unstripped fish.

The administration of steroids showed that testosterone is able to inhibit GtH I release and stimulate that of GtH II in stripped fish, having no effect on the release of these gonadotropins in non-stripped animals. 17β-Estradiol failed to modify GtH I secretion; however, it decreased the release of GtH II in fish containing retained eggs in the body cavity. 17,20βP had a delayed stimulating influence on GtH I release in unstripped fish. Finally, multiple injections of desteroidized ovarian fluid into stripped fish led to a significant decrease of GtH I release and to an increase of GtH II secretion.

This study demonstrates that factors which are present in ovarian fluid modulate the post-ovulatory secretion of both gonadotropins – their net action is negative on GtH I and positive on GtH II. Among the steroids, testosterone is of major importance, being able to inhibit GtH I release and to stimulate that of GtH II. We also show that non-steroidal factors present in the ovarian fluid can influence the release of both gonadotropins, which indirectly supports previous findings about the existence of inhibin/activin-like factors in fish.

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Introduction

In fish, two distinct pituitary gonadotropins (GtH I and GtH II) have been purified and characterized from several species, including salmonids (Itoh *et al.* 1988, 1990, Suzuki *et al.* 1988*a*, Kawauchi *et al.* 1989, Sekine *et al.* 1989, Swanson *et al.* 1989). From their chemical characteristics, it is assumed that GtH I and GtH II are related to the tetrapod gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) respectively (Swanson *et al.* 1989, Tyler *et al.* 1997). GtH I and GtH II are produced in different cell types in the pituitary (Nozaki *et al.* 1990, Naito *et al.* 1993) and they are differentially secreted during the annual reproductive cycle (Prat *et al.* 1996, Breton *et al.* 1998). For a long time it has been clearly established that GtH II induces oocyte maturation (Jalabert *et al.* 1973), as does LH. This is mediated through its stimulatory action on the ovarian production of the maturational-inducing steroid 17,20β-dihydroxy-4-pregnen-3-one (17,20βP) (Suzuki *et al.* 1988*b*, Kawauchi *et al.* 1989). During vitellogenesis, GtH I regulates 17β-estradiol production (Suzuki *et al.* 1988*b*). Recently it has been demonstrated that GtH I also mediates ovarian uptake of vitellogenin during vitellogenesis (Tyler *et al.* 1997). Hence, its physiological action is now considered as similar to that of FSH (Tyler *et al.* 1997).

In mammals, steroids regulate LH and FSH secretions differently. In fish this effect is not so clear, because there are only few data concerning the effects of steroids on GtH I secretion and because of the lack of specificity of GtH II

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RIAs used in previous studies (Govoroun *et al.* 1998). For example, Bommelaer *et al.* (1981) demonstrated in vitellogenic female rainbow trout that ovariectomy induced an increase of GtH II secretion that could be reversed by 17β-estradiol implantation, depending on the stage of gonadal development. This has now been shown as inconsistent. In fact in this species, during vitellogenesis, ovariectomy results in an increase of GtH I secretion without any effect on GtH II secretion (Larsen & Swanson 1997, Saligault *et al.* 1998). However, the effects of aromatizable steroids on GtH II synthesis and secretion have been confirmed in immature rainbow trout. In this case, aromatizable androgens and 17β-estradiol stimulated the expression of GtH II mRNA (Xiong *et al.* 1993, Huggard *et al.* 1996, Swanson & Dickey 1996), as well as GtH II pituitary accumulation (Crim & Evans 1982, Breton & Sambroni 1996, Breton *et al.* 1997). During a longer term, testosterone was the only steroid able to stimulate GtH II secretion (Breton & Sambroni 1996, Breton *et al.* 1997). In contrast, only the secretion of GtH I can be stimulated after steroid implantation (Breton *et al.* 1997). From recent studies done in coho salmon (*Oncorhynchus kisutch*), it has been postulated that the gonads exert negative feedback effects on the secretion of both GtH I and GtH II, although the effects varied seasonally. Hence, the nature of the specific factor(s) from the gonads that inhibits and/or stimulates production and secretion of gonadotropins remains to be clarified (Larsen & Swanson 1997). This general feedback hypothesis has never been extended to the post-ovulatory stage. Indeed, in the rainbow trout, the most important changes in GtH secretion occurred during the peri-ovulatory period, and especially after ovulation – a stage in which there are also great variations in steroid metabolic pathways and secretion (De Monès *et al.* 1989). After ovulation it has been shown that GtH II plasma levels increase drastically in fish which retain their eggs in the body cavity (Jalabert & Breton 1980, Sumpter & Scott 1989). Egg stripping reversed this phenomenon (Jalabert & Breton 1980). This was recently confirmed using a more specific RIA (Govoroun *et al.* 1998), but in addition it was shown that GtH I secretion followed profiles opposite to those of GtH II, in both stripped and unstripped animals (Breton *et al.* 1998). In all cases, the variations are of great amplitude. Thus it becomes difficult to accept as a general hypothesis a negative feedback of the gonads on the regulation of the secretion of both GtH I and GtH II. Among the gonadal factors which are involved in the regulation of GtH secretion and production there are steroids, and in addition, other factors of ovarian origin which are supposed to be implicated in this process (Tyler *et al.* 1997, Breton *et al.* 1998).

The aim of the present work was to investigate the effects on GtH I and GtH II secretion after ovulation of the main steroids secreted during the peri-ovulatory period, namely testosterone, 17β-estradiol and 17,20βP. In addition, we also studied the effects of ovarian fluid, which is abundantly produced after ovulation when eggs are preserved in the body cavity, as a possible origin of other non-steroidal factors participating in the autocrine/paracrine regulation of gonadotropin secretion as in the case of inhibins and activins in mammals.

Materials and Methods

Animals

Seventy-two 2-year-old female rainbow trout (*Oncorhynchus mykiss*) of the autumn spawning strain from INRA-SEDI fish farm (Sizun, France) were used in the experiment. Their body weight varied between 1100 and 1400 g.

Preparation of steroid implants and desteroidized ovarian fluid

Testosterone, 17β-estradiol and 17,20βP were purchased from Sigma-Aldrich Chimie (L'Isle d'Abeau Chesnes, France).

Implants were prepared using the Silastic grade elastomer, MDX4 4210 (Dow Corning, Midland, MI, USA), according to the method described by Lotz & Syllwasschy (1979). The controls were blank implants which did not contain any steroid.

The ovarian fluid was collected from freshly ovulated rainbow trout during the reproductive season in 1996. Females were stripped by hand pressure. The eggs were separated from the ovarian fluid using a draining rack. Activated charcoal was added to the ovarian fluid at a concentration of 20 mg/ml. After 2 h of continuous stirring, the solution was centrifuged for 10 min at 17 000 ***g***. The recovered supernatant was then subjected to another centrifugation under the same conditions. The resulting desteroidized ovarian fluid was aliquoted and kept at − 20 °C.

Experiment

The experiment was performed in November and December 1996. Before the experiment, fish were acclimated to a recirculated water system at 12 ± 1 °C. They were kept under a simulated natural photoperiod.

The detailed experimental protocol is described in Table 1. After anesthesia in 2-phenoxy-ethanol (0·3 ml/l), fish were checked every 2 days by hand stripping for the detection of ovulation (determined as the time when eggs can be freely stripped from the fish). Ovulated fish were tagged and treated according to Table 1 and assigned to the subsequent groups (eight fish per group). In groups 1–5, females were manually stripped in order to remove all the ovulated eggs as well as the ovarian fluid, whereas in groups 6–9, the eggs remained in the body cavity. The

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Table 1 Description of experimental approach

	Group	Treatment and way of administration	Dose
Physiological status			
Eggs stripped	1	Blank implant	—
	2	Testosterone implant	10 mg/kg
	3	17β-estradiol implant	10 mg/kg
	4	17,20βP implant	1 mg/kg
	5	Ovarian fluid injection	1.5 m/kg three times per week
Eggs kept in the body cavity	6	Blank implant	—
	7	Testosterone implant	10 mg/kg
	8	17β-estradiol implant	10 mg/kg
	9	17,20βP implant	1 mg/kg

implants were introduced into the body cavity through a very small incision in the abdomen made near the pelvic fin using a sterile surgical blade. The injections of desteroidized ovarian fluid (1·5 ml/kg body weight) were done i.p. every 2 days at a fixed time of day (0900 h).

Fish were blood sampled from a caudal vessel every 2 days at 0900 h using 1 ml heparinized syringes. Blood samples were centrifuged for 10 min at 2000 ***g***. The resulting plasma was kept at −20 °C until assayed.

Hormone assays

GtH I and GtH II plasma levels were determined using specific RIAs (Govoroun *et al.* 1998). Testosterone, 17β-estradiol and 17,20βP plasma levels were determined according to Fostier *et al.* (1978).

Statistical analysis

Data were analyzed using the non-parametric Mann–Whitney U test. The differences between groups were considered significant at $P<0·05$. All the results were expressed as mean ± S.E.M.

Results

Steroid concentrations

Before charcoal treatment, ovarian fluid contained 12·33 ng/ml testosterone, 0·83 ng/ml 17β-estradiol and 18·20 ng/ml 17,20βP. These concentrations dropped to 10·09 ng/ml for testosterone, 0·46 ng/ml for 17β-estradiol and 0·90 ng/ml for 17,20βP after charcoal extraction.

In freshly ovulated females (day 0), the mean plasma testosterone level was 150·32 ± 21·81 ng/ml. Fourteen days later it decreased to 13·25 ± 3·11 ng/ml in fish which were stripped of their eggs and to 34·10 ± 18·24 ng/ml when eggs remained in the body cavity (figure not shown). There was no statistical difference between stripped and unstripped females. In testosterone-implanted females, plasma testosterone levels increased to 344·33 ± 15·87 ng/ml in fish containing eggs and to 400·26 ± 35·23 ng/ml in stripped females. There was no significant difference between these two groups, although testosterone levels were higher ($P<0·05$) in comparison with the levels on day 0 and higher ($P<0·05$) than in non-implanted fish on day 14. The prolonged treatment with desteroidized ovarian fluid did not result in an increase of testosterone plasma levels. The average testosterone plasma level (11·73 ± 2·59 ng/ml) was similar to that of untreated stripped and unstripped animals.

On day 0, the mean plasma 17β-estradiol concentration was 2·71 ± 0·21 ng/ml. Two weeks later it had decreased to 1·00 ± 0·23 ng/ml in stripped fish and to 0·90 ± 0·30 ng/ml in animals with eggs kept in the body cavity, both being smaller ($P<0·05$) than the levels of 17β-estradiol at the beginning of the experiment (figures not shown). On the contrary, in 17β-estradiol-implanted fish, the levels of this steroid increased on day 14 to 3·76 ± 0·48 and 4·71 ± 0·58 ng/ml in stripped and non-stripped fish respectively. In this last case, the levels were higher ($P<0·05$) than the concentrations observed on the day of ovulation. In ovarian fluid-injected fish, the plasma 17β-estradiol levels on day 14 were 1·02 ± 0·11 ng/ml and did not significantly differ from the levels in stripped and unstripped control fish.

The mean 17,20βP plasma level was 385·2 ± 35·08 ng/ml on the day of ovulation. On day 14 it decreased both in fish with retained eggs and in stripped females (figures not shown), being maintained at higher levels ($P<0·05$) in fish with remaining eggs (77·86 ± 43·45 ng/ml in unstripped fish versus 6·51 ± 1·61 ng/ml in stripped animals). In fish with removed eggs, although 17,20βP plasma concentration increased to 34·71 ± 12·03 ng/ml in implanted females, versus 6·51 ± 1·61 ng/ml in non-implanted fish, this difference was not significant. A similar situation was observed in fish with retained eggs; however, the difference between fish with remaining eggs and fish with removed eggs was maintained. The prolonged treatment with desteroidized ovarian fluid did not change the plasma 17,20βP concentration, which was maintained at a similar level to that in control fish with removed eggs (i.e. 20·17 ± 6·47 ng/ml).

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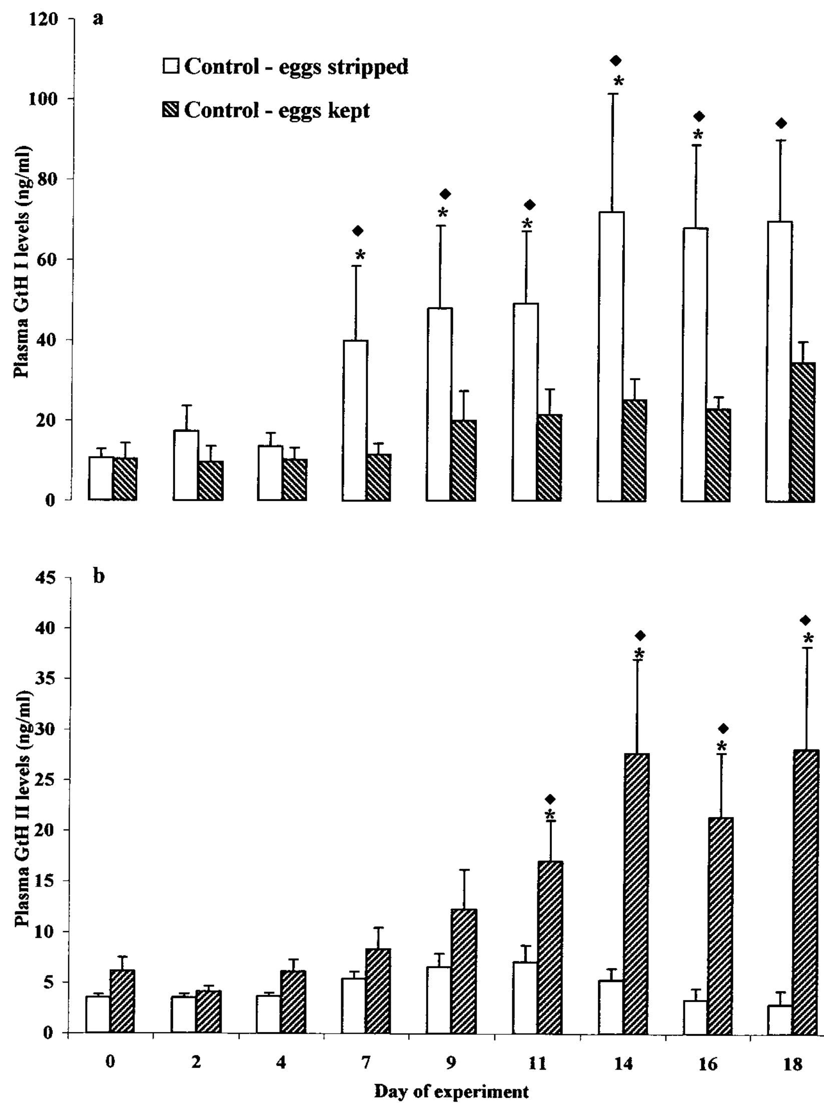

Figure 1 Profiles of GtH I (a) and GtH II (b) secretion in stripped and non-stripped fish following ovulation (day 0). One group of freshly ovulated females (n=8) was stripped in order to remove the eggs and the ovarian fluid, whereas in the other group (n=8) the fish retained eggs in the body cavity. Fish were blood sampled three times per week for GtH I and GtH II level determination. Results are mean ± S.E.M. *=significantly different from the other groups of fish at the same day. ◆=significantly different from the value at the beginning of experiment (Mann–Whitney U test, $P \leq 0{\cdot}05$).

GtH I and GtH II profiles of secretion after ovulation

On the day of ovulation (day 0), the mean GtH I plasma concentration was around 10 ng/ml both in fish which were stripped and in those in which eggs remained in the body cavity (Fig. 1a). Later, starting on day 7 until day 14, there was an increase of GtH I secretion in stripped fish, where GtH I levels reached 78·85 ± 33·86 ng/ml. Thereafter, GtH I levels remained elevated and unchanged until day 18. There was also a small, but not significant increase of GtH I plasma levels in fish which kept their eggs in the body cavity, reaching a maximum on day 18 (Fig. 1*a*).

At ovulation there was no difference between GtH II plasma concentrations in stripped and non-stripped fish (3·52 ± 0·32 versus 6·14 ± 1·36 ng/ml respectively). Thereafter, profiles of GtH II secretion were opposite to those of GtH I. No increase of GtH II plasma levels occurred in the stripped fish on days 9–11 compared with

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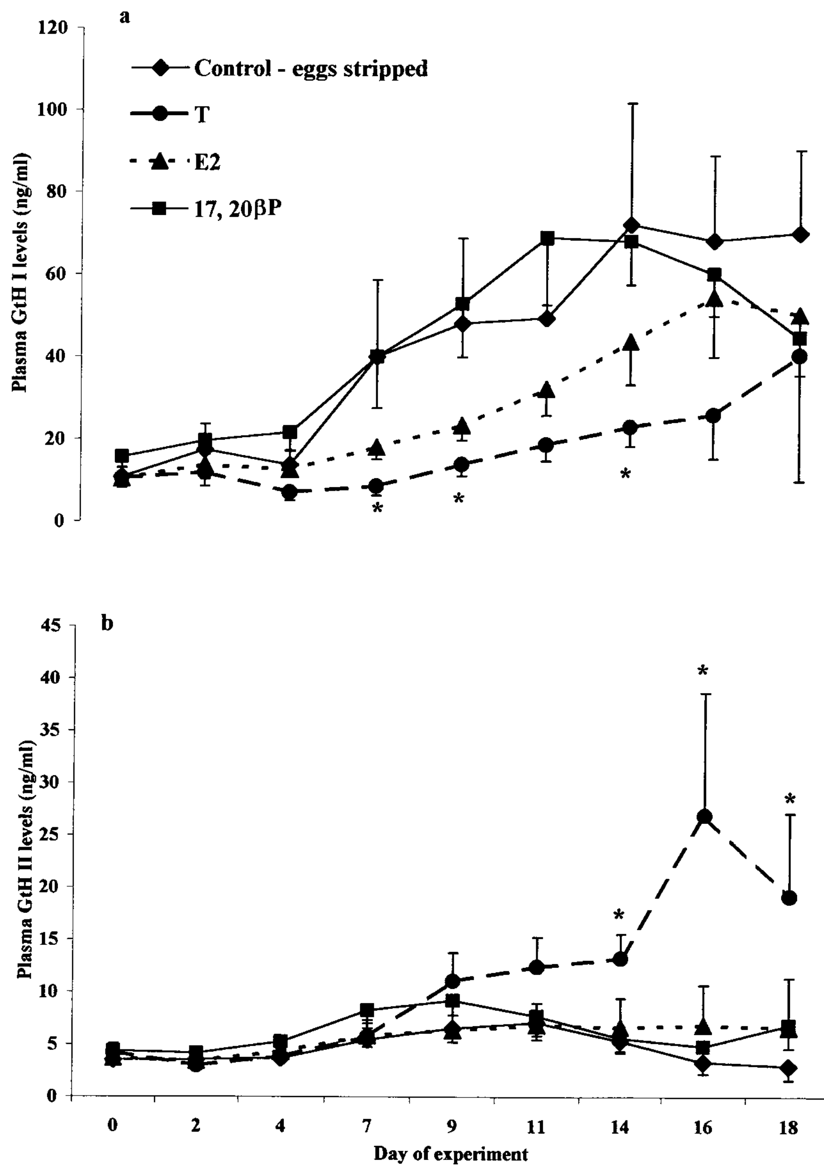

Figure 2 Effects of different steroids on GtH I (a) and GtH II (b) secretion in stripped fish. Freshly ovulated females were stripped in order to remove the eggs and the ovarian fluid. Then they were blood sampled. After allocation into groups, fish were implanted with the following: blanks, testosterone (T) (10 mg/kg), 17β-estradiol (E2) (10 mg/kg) and 17,20βP (1 mg/kg). Then fish were blood sampled three times per week for GtH I and GtH II levels determination. Results are mean ± S.E.M. *=significantly different from control (Mann–Whitney U test, $P \leq 0{\cdot}05$). For each group n=8.

the day 0 value, whereas in fish with retained eggs there was a rise of the GtH II plasma concentration peaking at 27·72 ± 9·30 ng/ml on day 14 (Fig. 1*b*).

Effects of steroids on GtH I secretion

In stripped fish, testosterone significantly inhibited the increase of GtH I secretion in comparison with the control, but this inhibition was not prevented totally (Fig. 2*a*). Although 17β-estradiol caused a slight inhibition of GtH I secretion in comparison with controls, it was not significant. 17,20βP had no effect on GtH I secretion. In the 17,20βP-implanted fish, the GtH I profile of secretion was similar to those of non-implanted stripped females. There was also no difference between the maximum values obtained in these two groups.

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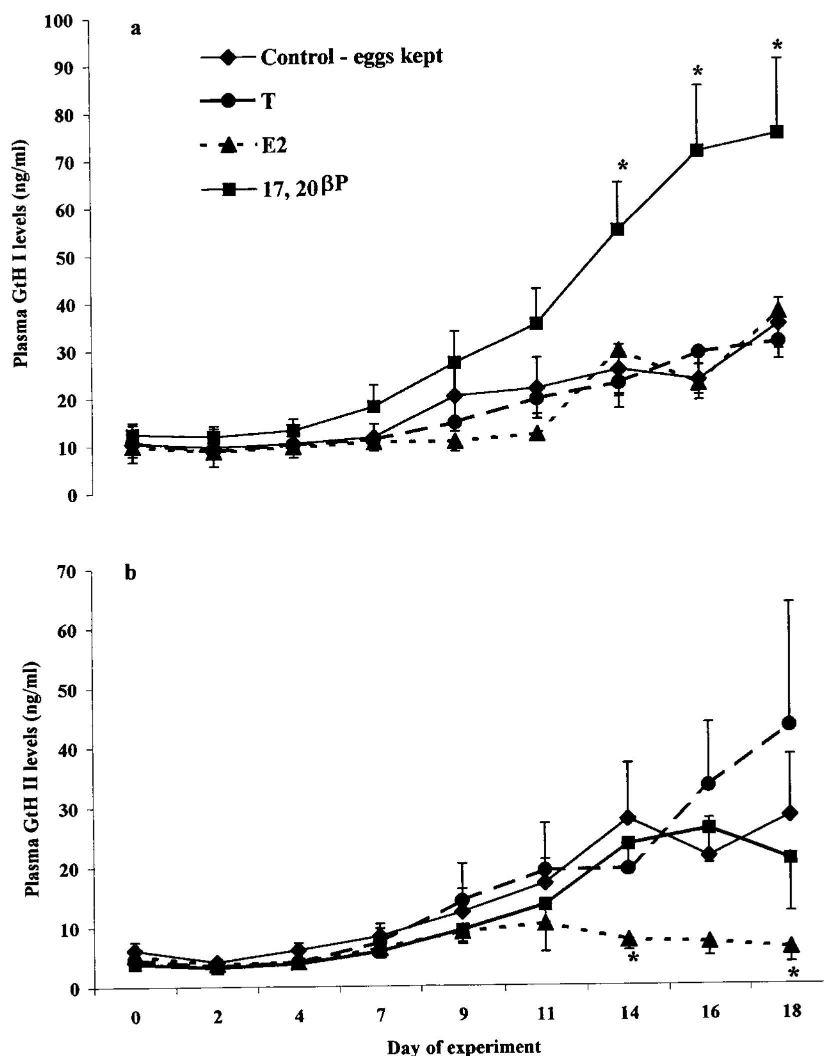

Figure 3 Effects of steroid implantation on GtH I (a) and GtH II (b) secretion in non-stripped fish. Freshly ovulated females with eggs kept in the body cavity were blood sampled, allocated to the subsequent groups and implanted with the following: blanks, testosterone (T) (10 mg/kg), 17β-estradiol (E2) (10 mg/kg) and 17,20βP (1 mg/kg). Then fish were blood sampled three times per week in order to determine their GtH I and GtH II levels. Results are expressed as mean ± S.E.M. *=significantly different from controls (Mann–Whitney U test, $P \leq 0{\cdot}05$). For each group n=8.

In fish with retained eggs, testosterone and 17β-estradiol had no effect on GtH I secretion in comparison with non-stripped controls (Fig. 3*a*). 17,20βP induced a stimulation ($P<0{\cdot}05$) of GtH I secretion, leading to a profile of GtH I secretion similar to that observed in control stripped females, but delayed in time. In this last group, GtH I plasma levels increased from 12·39 ± 2·43 ng/ml on day 0 to 74·36 ± 15·65 ng/ml on day 16. On the same day, GtH I plasma levels in the control group were only 34·41 ± 5·22 ng/ml.

Effects of steroids on GtH II secretion

In stripped fish, only testosterone stimulated GtH II secretion ($P<0{\cdot}05$) from day 14 after implantation. GtH II plasma levels reached a maximum of 26·90 ± 11·68 ng/ml on day 16, whereas in the control group it was 3·33 ± 1·15 ng/ml on the same day. 17β-Estradiol and 17,20βP were without effect (Fig. 2*b*). In fish with eggs kept in the body cavity, testosterone and 17,20βP had no effect, whereas 17β-estradiol completely abolished

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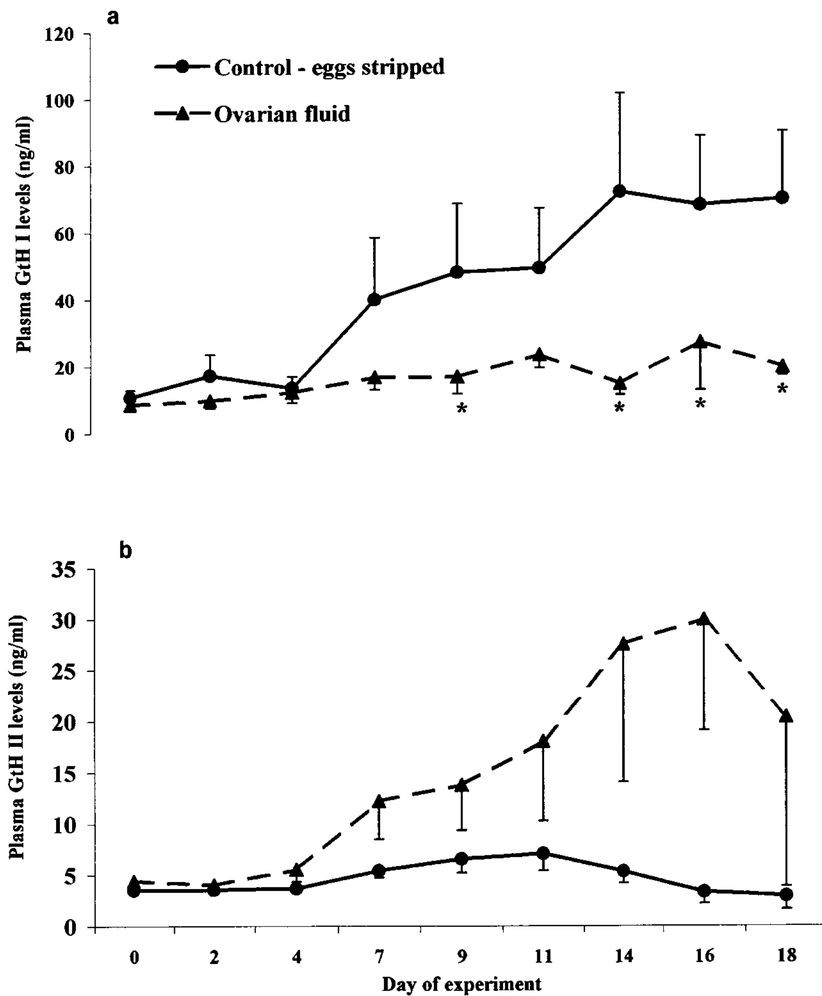

Figure 4 Effects of desteroidized ovarian fluid injections on GtH I (a) and GtH II (b) secretion in stripped fish. Ovulated fish subjected to stripping in order to eliminate the eggs and the ovarian fluid were blood sampled and injected three times per week with charcoal-treated ovarian fluid. During the experiment blood was taken three times per week (at the time of ovarian fluid injections) in order to determine plasma GtH I and GtH II levels. Results are mean ± S.E.M. *=significantly different from control (Mann–Whitney U test, $P \leq 0{\cdot}05$). For each group n=8.

the post-ovulatory increase of GtH II secretion (Fig. 3*b*).

Effects of desteroidized ovarian fluid on GtH secretion

The effect of ovarian fluid was studied only in stripped animals, in which the repeated injection of this desteroidized liquid every 2 days caused a strong inhibition of GtH I secretion (Fig. 4a). The profile of GtH I secretion did not significantly increase, similar to the occurrence in unstripped control fish. This inhibition became significant ($P<0{\cdot}05$) after 9 days of treatment, where GtH I plasma levels were 55·63 ± 30·89 ng/ml in controls and 16·81 ± 5·07 ng/ml in ovarian fluid-treated fish. The greatest difference was seen 5 days later, i.e. on day 14 (78·85 ± 33·86 ng/ml versus 14·66 ± 3·33 ng/ml).

Contrary to the effect on GtH I, the desteroidized ovarian fluid stimulated GtH II secretion from 4·43 ± 0·87 ng/ml on day 0 to 18·03 ± 7·76 ng/ml on day 11 and 29·91 ± 10·82 ng/ml on day 16 (Fig. 4*b*). Because of a great variability in the response to the treatment among individuals, the levels of GtH II were not statistically different between ovarian fluid-treated and stripped control fish.

Discussion

Since the purification of two gonadotropins (GtH I and GtH II) in fish, there are now growing, but still partial data

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on the regulation of the synthesis and the secretion of these pituitary hormones by steroids. To date, the obtained results concern mainly gonadal stages from immaturity to the beginning of gametogenesis, a period in which testosterone and 17β-estradiol exert a positive feedback on both GtH I and GtH II secretion (Breton *et al.* 1997). There is now evidence that, during gametogenesis, 17β-estradiol has a direct negative feedback on GtH I secretion (Larsen & Swanson 1997, Saligault *et al.* 1998), whereas it has an indirect positive or negative effect on GtH II by modulating a 17β-estradiol-activated dopamine inhibitory tone (Saligault *et al.* 1998). It was postulated that, prior to maturation in the goldfish, 17β-estradiol was not a trigger of GtH maturational surge (Kobayashi *et al.* 1987). However, more recent data suggest that the decrease of 17β-estradiol before ovulation is essential for the removal of the dopamine inhibitory tone, which in turn facilitates the stimulatory action of gonadotropin-releasing hormone (GnRH) on GtH II secretion (Saligault *et al.* 1998). In this process, high plasma levels of testosterone would also be an important physiological factor contributing to the ovulatory surge, as in the goldfish (Kobayashi *et al.* 1989). There is also evidence that, in the rainbow trout, testosterone evokes a stimulation of GnRH synthesis and release (Breton & Sambroni 1996).

In contrast to the above stages, the post-ovulatory regulation of GtH I and GtH II secretion has been less studied. Our previous results (Breton *et al.* 1998) have shown that, after ovulation in fish with eggs eliminated from the body cavity, GtH I levels dramatically increase, while the secretion of GtH II remains almost unchanged. Furthermore, the secretion profiles of these hormones are reversed if the eggs are maintained in the body cavity. These results are confirmed in the present work, where during 3 weeks following ovulation there were great and opposite variations in plasma levels of both gonadotropins. In salmonids, after ovulation, testosterone plasma levels decrease very rapidly and reach their lowest values 2–3 weeks later (Scott *et al.* 1983). We have found similar results. In spite of these changes, however, after ovulation this steroid remains potent in modulating gonadotropin secretion. Indeed, testosterone implantation, which causes the elevation of testosterone plasma levels, nearly completely inhibited the post-ovulatory elevation of GtH I and stimulated GtH II secretion in stripped animals, confirming its feedback action on both gonadotropins after ovulation. Following the stripping, testosterone removal led to an increase of GtH I secretion, whereas in unstripped females, where the concentrations of testosterone are much higher, this rise of GtH I was blocked, which suggests the negative feedback of testosterone on the secretion of this hormone. In contrast, this steroid had a clear positive feedback on GtH II secretion in unstripped fish, and this feedback disappeared after stripping.

In non-implanted stripped fish, the effects of testosterone withdrawal on both GtH I and GtH II secretion appear to be similar to those postulated during vitellogenesis in ovariectomized fish (Larsen & Swanson 1997). Indeed, in the female rainbow trout during vitellogenesis, GtH I plasma levels start to decrease in parallel with the rise of GtH II levels at the moment when testosterone levels reach their maximum and when 17β-estradiol levels start to decrease (Breton *et al.* 1983, 1998, Scott *et al.* 1983). This indicates the presence of negative feedback of testosterone on GtH I secretion and its positive feedback on that of GtH II. However, in unstripped animals, testosterone failed to modify such changes in the secretion of both gonadotropins. This would suggest that, in these fish, the maximum stimulatory level of GtH II secretion and the inhibitory influence of testosterone on GtH I secretion were already reached and maintained. Above these levels, testosterone would be unable to modify the release of these gonadotropins, since their secretion in implanted stripped fish mimics that of unstripped controls and testosterone-implanted animals.

It has been postulated that, in immature triploid rainbow trout, long-term testosterone implantation stimulates GtH II secretion by increasing pituitary GnRH accumulation and release (Breton & Sambroni 1996). Moreover, after ovulation GnRH was also demonstrated to be unable to stimulate GtH I secretion, whereas it had a moderate action on the stimulation of GtH II secretion (Breton *et al.* 1998). Thus, the differential action of testosterone on both GtH I and GtH II secretion would not be mediated only by GnRH, but this steroid might modify the release of gonadotropins by the direct influence at the level of gonadotropes.

Another hypothesis is that, in unstripped animals, the differential feedback of testosterone would not be preserved or would be blocked by other factors of ovarian origin, probably from ovarian fluid. We can assume that the active factors in this fluid are not of steroid origin, because the augmentation of plasma concentrations of testosterone and 17,20βP as well as 17β-estradiol after ovarian fluid injections was negligible in comparison with the plasma steroid levels observed in the experiment. For example, 1·5 ml charcoal-treated ovarian fluid contains 15 ng testosterone. If there was a 100% recovery in the blood, this would lead to an increase of plasma testosterone levels of 0·25 ng/ml against natural values of 150·32 ng/ml on the day of ovulation, 13·25 ng/ml in stripped fish 14 days after ovulation and 34·10 ng/ml in unstripped fish 14 days after ovulation. These increases are similar for other steroids.

This lack of influence of steroids contained in the injected ovarian fluid is proved by the fact that the treatment by this fluid did not change plasma steroid concentrations compared with the levels observed in the stripped control group. The existence of endogenous non-steroidal factors of ovarian origin which can actively influence GtH secretion is confirmed by the complete inhibition of GtH I post-ovulatory elevation in stripped

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females and by the stimulation of GtH II levels after prolonged treatment with desteroidized ovarian fluid. Thus the presence of eggs in the body cavity would result in the production of factors, as mentioned previously, probably of ovarian origin, which would inactivate and reverse the sensitivity of the brain–pituitary axis to testosterone action and modulate its feedback action.

GnRH is one of the factors which could be involved in this phenomenon. Indeed, the presence of a GnRH-like factor has been demonstrated in the African catfish (Habibi *et al.* 1994) and goldfish ovary (Pati & Habibi 1998). However, it is improbable that GnRH would stimulate GtH II secretion but inhibit that of GtH I. Therefore, it seems that the action of ovarian fluid shown in this work resembles the modulation of FSH and LH secretion in mammals by ovarian factors such as inhibins and activins.

Numerous data show that inhibin decreases FSH release, accumulation and synthesis, whereas activin stimulates the secretion and the production of this hormone (De Jong 1988, Rivier *et al.* 1990). Both activin and inhibin have no influence on basal LH secretion; however, they can modulate GnRH-induced LH secretion. In fish, there are studies, performed in goldfish, demonstrating that inhibin A as well as activin A stimulates GtH II secretion in a dose-dependent manner (Ge *et al.* 1991, 1992). This was later confirmed by the use of desteroidized goldfish ovarian extracts (Ge & Peter 1994). In our study, the tendency of desteroidized ovarian fluid to stimulate GtH II secretion reflects, in the rainbow trout, the same results previously obtained in goldfish. In addition, the decrease of GtH I secretion after treatment with desteroidized ovarian fluid looks similar to the inhibition of FSH secretion by inhibin in mammals. Therefore, similar mechanisms of non-steroidal gonadal retrocontrol of gonadotropin release also exist in fish. This is the case at least for GtH I – the fish FSH-like gonadotropin. However, regarding GtH II secretion, the action of the above mentioned factors on GtH II secretion appears to differ from those of inhibin and activin on LH secretion, by modulating not only GnRH-induced GtH II secretion (Ge *et al.* 1992), but also its basal secretion.

In non-implanted fish, 17,20βP plasma levels decreased more rapidly in stripped females compared with fish with retained eggs in the body cavity, in which the ovarian fluid also contained significant concentration of this steroid. Maybe this contributes to the higher levels of 17,20βP in the plasma of unstripped females by its passage from the ovarian fluid to the blood circulation. In relation to the plasma levels of the gonadotropins, these results suggest that, in stripped females, 17,20βP exerts a negative feedback on GtH I secretion and a positive feedback on the release of GtH II. This effect was reversed in unstripped animals. In this last case, the implantation of 17,20βP at a dose of 1 mg/kg resulted in an increase in GtH I secretion, similar to that recorded in control stripped animals, but delayed in time. It could be possible that in these fish the metabolic pathways of steroids would differ from those in stripped animals and the pre-ovulatory endocrine regulatory mechanisms could be preserved. In this hypothesis, 17,20βP could be one of the factors involved in the positive feedback leading to the augmentation of plasma GtH I levels just prior to ovulation.

In contrast to other steroids, changes in 17β-estradiol levels were not so important, and did not differ between stripped and unstripped animals in which 17β-estradiol reaches its minimum values. It has been demonstrated that this decrease is linked to the blockade of aromatization (De Monès *et al.* 1989). This process, however, does not seem to be modified by the egg preservation. Compared with testosterone, the implantation of 17β-estradiol induced only a moderate but significant increase of its plasma concentration, maybe because of a high rate of 17β-estradiol degradation after ovulation. In 17β-estradiol-implanted fish, the only effect was an inhibition of GtH II secretion in unstripped females, indicating a negative feedback of this steroid on GtH II secretion in these fish. As we already discussed in the results obtained with testosterone, the endocrine status of unstripped females would be more similar to that of pre-ovulating fish. Hence, the GtH II post-ovulatory surge would be under the control of the positive feedback of testosterone, and in addition our results confirm the negative feedback of 17β-estradiol on GtH II secretion, which is presumed to act through the modulation of dopaminergic inhibitory tone (Saligault *et al.* 1998).

In conclusion, this work demonstrates the pivotal role of testosterone in the control of GtH I and GtH II secretion during the peri-ovulatory period, and to a lesser extent that of 17β-estradiol in the control of GtH II secretion during the same period. In addition, it provides evidence that factors of ovarian origin may play a fundamental role by exerting a direct action on the gonadotropes as well as a possible influence on gonadotropin release through the modulation of testosterone action. Taking into consideration our results, it is suggested that ovarian fluid could be a good material for further research and for identification of these factors.

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