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The Influence of Immature Gonads on Onset of Gonadotropic Hormone Accumulation in the Juvenile Rainbow Trout Pituitary Gland

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Juvenile rainbow trout received gonadotropic hormone (GtH) treatment to investigate the response of the immature gonad and its effect on the onset of rising pituitary GtH levels, an early sign of initiation of sexual maturation in the teleost fishes. Brief exposure to GtH evoked increases in plasma androgens and significantly increased pituitary GtH in males; the accumulation of pituitary GtH was not observed in female trout which showed significant but smaller increases in plasma androgen resulting from GtH administration. There was no evidence of gonadal negative feedback in intact immature fish since pituitary GtH and plasma androgens remained at basal levels in castrate juvenile trout. Since the castrate male trout failed to respond to GtH injections, the data suggest that the immature gonad produces a steroid capable of triggering pituitary GtH production by hypothalmo-pituitary feedback in males.

The endocrine activity and interaction of the hypothalamus–pituitary–gonad axis (HPG) in sexually immature teleost fishes is poorly understood, and little is known about physiological events which trigger onset of sexual maturation in these animals. An early indication of the onset of sexual development in teleosts is a rising level of gonadotropic hormone (GtH) appearing in the pituitary gland. Dramatic fluctuations in pituitary GtH accompany seasonal reproductive cycles in the precocious male Atlantic salmon parr (Crim and Evans, 1978; Dodd *et al.*, 1978; Stuart-Kregor *et al.*, 1981). These studies confirm much earlier work indicating that pituitary GtH activity may be directly correlated with reproductive development in teleosts.

Pituitary GtH in juvenile trout is extremely low but the GtH level is rapidly elevated following testosterone treatment (Crim and Evans, 1979). Recently, these observations were extended by demon-

strating that one can manipulate the pituitary gonadotropin level in juvenile trout with either androgen or estrogenic steroid hormones (Crim *et al.*, 1981). The physiological significance of steroid manipulation of pituitary GtH in juvenile trout would be partly elucidated if it could be demonstrated that the level of pituitary GtH in juvenile trout is influenced by androgenic steroid hormone activity of the immature gonads. Therefore, the present study was designed to give juvenile trout exogenous GtH treatment and to investigate the feedback influence of stimulated immature gonads on pituitary GtH activity.

MATERIALS AND METHODS

Experimental design. An initial experiment was conducted using 15 ± 1 g sexually immature trout. Forty-one male trout were selected by abdominal inspection during the castration or sham castration surgical procedures. Castration was performed following anaesthesia with phenoxethanol by making an incision in the ventral body wall. The anterior portion of

the gonad near the liver was grasped with forceps and the entire length of the gonad was removed after pulling upward in an anterior direction. The wound was closed with nylon suture. No food was offered until the wound was healed. Fish were identified with a small fingerling tag installed immediately in front of the dorsal fin. One week following surgery the fish were treated with GtH (chum salmon) delivered in two perivisceral injections. A primary GtH dose ($0.1 \mu\text{g}/10 \text{ g body wt}$) was administered, followed in 2 days by a final dose ($1 \mu\text{g}/10 \text{ g body wt}$). Fourteen days after the final injection the fish were sacrificed and pituitaries were collected for GtH analysis by radioimmunoassay.

A second study was performed on a group of intact immature rainbow trout of mixed sex (16–35 g). The fish were treated with GtH ($2 \mu\text{g}/10 \text{ g body wt}$) twice a week for a period of 2 weeks (total of 4 GtH injections). Samples of blood and pituitaries were collected at the time of autopsy carried out on Days 1 and 14 after the final hormone injection.

A third experiment was carried out to determine the influence of GtH treatment on intact and castrate juvenile trout. The trout weighed 11–48 g. Nylon sutures were removed 2 weeks after surgery. GtH treatment, which began 3 weeks after surgery, again consisted of 4 perivisceral injections according to the schedule outlined in experiment 2. On Day 14 following the final GtH injection, blood and pituitaries were collected. At autopsy a gross microscopic inspection of the perivisceral cavity was conducted to determine the successful removal of gonads in castrate fish.

Hormone analysis. Pituitary and plasma GtH values were determined by radioimmunoassay methods previously described (Crim *et al.*, 1975).

Androgens were also quantified by radioimmunoassay. The antibody to 11-ketotestosterone had a titer of 1:30,000 and was prepared and used as previously described (Idler and Ng, 1979) except that 11-ketotestosterone and testosterone were isolated from plasma using paper chromatography (cyclohexane 100:toluene 70:methanol 100:water 25 for 6 hr). The antibody to testosterone was purchased from Radioassay Systems Laboratories (Carson, Calif.) and had good specificity for testosterone (Ng and Idler, 1980). High specific activity 11- ^3H ketotestosterone was prepared according to Truscott (1981) and purified along with ^3H testosterone at 3-month intervals by paper chromatography.

Preparation of GtH from chum salmon pituitaries. The Con AII F_2 GtH was prepared according to Idler *et al.*, 1975b.

Statistical analysis. The Student's *t* test was utilized to make appropriate group comparisons within each experiment.

RESULTS

An attempt was made in a preliminary experiment to determine the influence of

brief GtH treatment on the pituitary GtH level in juvenile male rainbow trout. In intact and castrate control (saline-treated) males, pituitary GtH was extremely low or not detectable in the majority of individuals (data not shown). Likewise, pituitary GtH was undetectable in 10 of 11 castrate males receiving GtH treatment (1 pituitary value 0.5 ng). In contrast, 10 of 11 intact immature males treated with GtH had a measurable pituitary GtH level but the results were highly variable (range $0.5\text{--}47.9 \text{ ng GtH/pituitary}$). Therefore, in subsequent experiments, the dose of GtH and the number of injections were increased.

In the second experiment four injections of GtH were administered and pituitaries were collected on Days 1 and 14 following the final GtH injection. The data in Table 1 show that, compared with low female pituitary GtH levels, juvenile male pituitary GtH was significantly elevated on Days 1 and 14 of collection ($P < 0.005$; $P < 0.05$, respectively).

A third study was designed to confirm the effect of GtH injections on pituitary GtH in juvenile trout specifically in males. Castrate fish were included in this experiment as a control procedure for evaluating the capability of the immature trout gonad to respond to the GtH treatment. Blood samples were obtained in some fish on Day 1, following the final injection of GtH, to allow determination of circulating GtH values achieved by exogenous hormone treatment. Plasma GtH values remained low in castrate and sham-castrate (intact) trout treated with saline (Table 2). However, cir-

TABLE 1
PITUITARY GtH CONTENT IN JUVENILE RAINBOW TROUT AT 1 AND 14 DAYS FOLLOWING TREATMENT WITH GtH

Sex	Pituitary GtH (ng)			
	Day 1		Day 14	
Female	6.26 $\pm$ 1.51	(5) ^a	6.50 $\pm$ 2.43	(5)
Male	158 $\pm$ 35	(4)	236 $\pm$ 105	(4)

^a Values are $\bar{X} \pm \text{SE (N)}$.

TABLE 2
PLASMA GtH CONCENTRATION (DAY 1) AND PITUITARY GtH CONTENT (DAY 14) FOLLOWING THE
FINAL INJECTION OF GtH IN JUVENILE RAINBOW TROUT

Sex	Treatment	Plasma GtH (ng/ml)	Pituitary GtH (ng)
Males			
Sham castrate	Saline	2.48 ± 0.30	1.02 ± 0.58 (6) ^a
Castrate	Saline	4.06 ± 0.42	0.30 ± 0.06 (8)
Sham castrate	GtH	277 ± 41	99 ± 28 (11)
Castrate	GtH	152 ± 45	2.72 ± 1.97 (10)
Females			
Sham castrate	Saline	2.86 ± 0.30	3.14 ± 3.01 (10)
Castrate	Saline	3.20 ± 0.21	0.28 ± 0.03 (10)
Sham castrate	GtH	367 ± 33	2.08 ± 0.39 (10)
Castrate	GtH	222 ± 69	0.31 ± 0.05 (9)

^a Values are $\bar{X} \pm SE (N)$.

culating GtH was greatly elevated in the plasma of both male and female (castrate and sham-castrate) fish receiving the GtH injections. No increase in pituitary GtH was observed in response to just the castration of the sexually immature male and female rainbow trout (Table 2). In agreement with the results of the above experiment, GtH treatment of intact juvenile female trout failed to increase pituitary GtH levels. However, following GtH treatment of intact juvenile males, pituitary GtH significantly increased ($P < 0.005$) compared with castrate males receiving the GtH treatment. These data suggest that the testes of sexually immature trout have the capacity to stimulate increases in pituitary GtH by hormone feedback.

The search for a presumptive immature testicular steroid secretory product was begun with steroid radioimmunoassay analyses of plasma obtained from GtH treated juvenile trout. Initially, the plasmas obtained in experiment 2 were pooled according to sex. Steroid profile determinations (data not shown) indicated that testosterone and 11-ketotestosterone were the androgens present (11 β -hydroxytestosterone not detectable); plasma estradiol-17 β was found to be low and remained unchanged with time after withdrawal of GtH treatment. In experiment 3 individual de-

terminations of androgen titers were conducted for plasmas collected on Days 1 and 14 after the final GtH injection (Table 3). In GtH treated (sham-castrate) males, in which accumulation of pituitary GtH had commenced, the levels of testosterone and 11-ketotestosterone were significantly increased ($P < 0.005$) compared with intact males receiving saline treatment. No significant increases in plasma androgens were observed in GtH treated castrate males. Plasma testosterone was detectable but low in intact (sham-castrate) female trout treated with GtH. In females 11-ketotestosterone was not detectable. Fourteen days after the withdrawal of GtH therapy, plasma androgen levels declined in males but the low testosterone level in females remained unchanged.

DISCUSSION

The life cycle of many teleosts can be divided into three periods of reproductive activity including juvenile, pubertal, and adult phases. Most of the work to date on endocrine control of fish reproduction deals with seasonal sexual development during the adult period (Billard and Breton, 1979) and therefore, little is known about endocrine interactions in the hypothalamo-pituitary-gonad axis (HPG) of sexually immature teleosts.

TABLE 3
PLASMA ANDROGEN CONCENTRATIONS (ng/ml) ON DAYS 1 AND 14 AFTER THE
FINAL GtH INJECTION IN JUVENILE RAINBOW TROUT

Sex	Treatment	N	Testosterone		11-Ketotestosterone	
			Day 1	Day 14	Day 1	Day 14
Males						
Sham Castrate	Saline	5	5.4 ± 1.7 ^a	—	2.5 ± 0.5	—
Sham Castrate	GtH	5	22.4 ± 3.7	3.5 ± 0.9 (11)	9.5 ± 1.2	<0.5
Castrate	GtH	5	3.6 ± 0.8	—	1.8 ± 0.4	—
Females						
Sham Castrate	GtH	5	4.1 ± 1.8 (3) ^b	1.8 ± 0.2 (10)	<0.5	<0.5

^a Values are $\bar{X} \pm \text{SE}$.

^b Values are $\bar{X} \pm \text{SE (N)}$.

Some observations suggest that the HPG of juvenile fish is poorly active, if at all. In Atlantic salmon parr plasma androgen levels are very low (Stuart-Kregor *et al.*, 1981). Gonadotrophs are poorly developed in immature Atlantic salmon parr (Olivereau, 1976) and also in the pituitary of coho salmon fry (Leatherland and Lin, 1975). Plasma and pituitary GtH is extremely low in the juvenile trout (Crim and Evans, 1979, 1980) and immature Atlantic salmon parr (Crim and Evans, 1978; Stuart-Kregor *et al.*, 1981). Furthermore, the present study showed that plasma and pituitary GtH remain unchanged following gonadectomy of both male and female juvenile trout. Hypersecretion of GtH in response to castration of adult rainbow trout has been reported (Billard *et al.*, 1976, 1977); however, the failure of castration to influence GtH levels in juveniles suggests that steroid feedback is lacking and the HPG of juvenile trout may be inactive.

If the HPG in juveniles is quiescent as present information seems to suggest, it is of interest to determine what triggers the onset of sexual development in fish during the pubertal period. Crim and Peter (1978) showed that testosterone treatment induces a rapid rise of GtH in pituitaries of precocious male and immature female Atlantic salmon parr. Pituitary GtH activity is also stimulated by testosterone in the immature

rainbow trout (Crim and Evans, 1979), and long-term treatment of sexually immature eels with estradiol triggers development of pituitary gonadotrophs (Olivereau and Chambolle, 1978, 1979; Olivereau and Olivereau, 1979a,b). Recent work by Crim *et al.* (1981) on steroid specificity of positive feedback agents suggests that only estrogen or aromatizable androgens are effective on GtH in the juvenile rainbow trout. Taken together, the above studies suggest that gonadal steroids may regulate the gonadotropic activity of the fish pituitary gland especially during the pubertal period of gonadal development, and also possibly gonadal recrudescence, when pituitary GtH levels are rapidly rising.

The immature gonads of juvenile rainbow trout are responsive to GtH as evidenced by stimulation of the formation of cyclic AMP by the gonad *in vitro* (Idler *et al.*, 1975a), gonad histology (Upadhyay *et al.*, 1978), stimulation of androgen production *in vivo* (Ng and Idler, 1980), and enhancement of estrogen and vitellogenin synthesis (Idler and Campbell, 1980). The immature trout used in the present study responded to GtH stimulation by increasing the plasma androgen level. In male trout the steroid response to GtH treatment was sufficient to activate GtH accumulation in the juvenile pituitary gland. Since pituitary GtH activation in males was prevented by gonadec-

tomy it appears that the immature testes is capable of evoking an increase in pituitary GtH via positive hormone feedback. Direct evidence of rising sex steroid levels during the early phases of gonad development in pubertal fish is very sparse although Stuart-Kregor *et al.*, (1981) recently reported increased plasma testosterone levels in the precocious male Atlantic salmon parr in July. In juvenile female trout, pituitary GtH did not rise following GtH injections under the conditions of the present experiment. Perhaps the androgen production from the juvenile ovary was inadequate to produce a pituitary GtH response. It is also possible that plasma binding of androgens prevented pituitary GtH feedback in females since high bound to free androgen ratios have been reported to be sex specific in trout (Ng and Idler, 1980). It is clear from previous studies (Crim and Evans, 1979; Crim *et al.*, 1981), however, that immature female pituitaries are responsive to exogenous steroid hormone treatment since the rise in pituitary GtH in juvenile males and females is very similar.

When comparing the juvenile phase of teleosts and higher vertebrates, there appears to be major differences in the endocrine activity of the HPG. In mammals gonadotropins and gonadal hormones are readily detectable in endocrine organs and also in circulation (Grumbach *et al.*, 1974). Increased gonadotropin secretion after castration indicates that negative feedback regulation of gonadotropin secretion is operational during all stages of sexual development beginning with the juvenile period. Changes in steroid hormone feedback sensitivity and target organ responsitivity are considered major factors in controlling onset of puberty in mammals. There have been a few reports of positive feedback effects of testosterone on gonadotropin secretion in mammals (Smith *et al.*, 1977) and in birds (Wilson, 1978). In fish, further work is required to evaluate HPG activity in juveniles and in adults during the period

of gonadal regression. The significance of positive steroid feedback during both of these phases of reproductive development is of great interest.

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