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The effects of gonadal development and sex steroids on growth hormone secretion in the male tilapia hybrid (*Oreochromis niloticus* x *O. aureus*)

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Abstract

Profiles of plasma growth hormone (GH) in male tilapia hybrid (*Oreochromis niloticus* x *O. aureus*) were measured and compared at different times of the year. The profiles did not appear to be repetitive, however, differences in their nature were observed at the different seasons; the most erratic profiles were seen in the height of the reproductive season (July), while the peaks were more subdued in the spring and disappeared in the autumn. Peaks in male fish were more prominent than in the females when measured in July. Perifused pituitary fragments from fish with a high GSI responded to salmon gonadotropin-releasing hormone (sGnRH) analog (10 nM−1 μM), while those from fish with a low GSI barely responded to even the highest dose. Exposure of perifused pituitary fragments from sexually-regressed fish to carp growth hormone-releasing hormone (cGHRH; 0.1 μM) or sGnRH (1 μM) stimulated GH release only after injection of the fish with methyl testosterone (MT; 3 injections of 0.4 mg kg^{-1}). The same MT pretreatment did not alter the response to dopamine (DA; 1 or 10 μM). GH pituitary content in MT-treated fish was lower than in control fish, which may be explained by the higher circulating GH levels in these fish, but does not account for the increased response to the releasing hormones. Castration abolished the response of cultured pituitary cells to sGnRH (1 fM−100 nM) without altering either their basal rate of secretion or circulating GH levels. Addition of steroids to the culture medium (MT or estradiol at 10 nM for 2 days) enabled a GH response to sGnRH stimulation in cells from sexually regressed fish. Pituitary cells which had not been exposed to steroids failed to respond to sGnRH, although their response to forskolin or TPA was similar to that of steroid-exposed cells. It would appear, therefore, that at least one of the effects of the sex steroids on the response to GnRH is exerted proximally to the formation of cAMP, or PKC, presumably at the level of the receptor. An increase in the number of receptors to the GH-releasing hormones, following steroid exposure, would explain also the changing nature of the GH secretory profile in different stages of the reproductive season.

Introduction

The growth promoting effects of gonadal steroids, particularly androgens, have long been recognized, and their possible applications in aquaculture have been studied in an attempt to discover the most effective type of androgen, the optimal dose and the stage of development most receptive to treatment (*e.g.*, Higgs *et al.* 1977, 1982; Lone and Matty 1980; Rothbard *et al.* 1988; Berglund *et al.* 1992; Lewis

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and Sower 1992). Although the application of androgen treatment for growth enhancement is currently limited due to health and environmental restrictions, their effects have been shown quite clearly. Administration of 17α ethynyl testosterone to tilapia hybrids or 17α methyl testosterone to *Oreochromis mossambicus* increased weight by 25% and as much as 180%, respectively (Guerrero 1976; Rothbard *et al.* 1988). The androgens' effect on growth is attributed to an increase in food conversion, a direct effect on gene expression of muscle cells and activation of other endogenous anabolic hormones (Lone and Matty 1980; Higgs *et al.* 1982); however, a precise relationship between these increased growth rates and changes in secretion of the growth hormone has not been established.

One of the possible mechanisms by which the gonadal steroids alter growth rates may be by modification of the profiles of pulsatile GH secretion. The secretory pattern of GH characteristic to the male rat (high infrequent pulses with low trough levels) is more stimulatory to growth than the relatively tonic level occurring in females (Jansson *et al.* 1982, 1985). At the onset of sexual maturity, there is a notable growth spurt which is clearly dependent on the gonadal steroids (Kerrigan and Rogol 1992). However, in hypophysectomized rats, administered androgens do not have the same growth promoting effects, suggesting that the steroid effect on growth is indirect, possibly *via* alteration of the GH secretory patterns (Jansson *et al.* 1984, 1985; Jansson and Frohman 1987). In mammals, the profiles of GH secretion become established at puberty (Kerrigan and Rogol 1992; Wehrenberg and Giustina 1992) and, in rats, they can be reversed by exposure to the gonadal steroid of the opposite sex (Jansson *et al.* 1985; Painson *et al.* 1992). The effect of the gonadal steroids on the secretory profile of adult rats was shown by gonadectomy which reduced by half the amplitude of GH peaks; these were restored by exposure to estradiol in females (Painson *et al.* 1992) or testosterone in males (Jansson *et al.* 1985; Jansson and Frohman 1987).

These patterns of cyclic GH secretion in mammals are controlled by the interactions of somatostatin (SRIF) and the growth hormone-releasing hormone (GHRH), which are also secreted rhythmically, but are slightly out of phase with each other (Tannenbaum *et al.* 1990). Thus the pulsatile secretion of GH is determined by the concurrence of GHRH peaks with troughs in SRIF. Studies in teleosts have shown that the secretion of GH also has a pulsatile nature (Leatherland *et al.* 1974; Marchant and Peter 1986; Bates *et al.* 1989; Le Bail *et al.* 1991; Boujard and Leatherland 1992). In trout, these profiles are related to feeding times (Reddy and Leatherland 1994) although the exact mechanisms governing them have not been fully established.

The hypothalamic control of GH release in teleosts appears to be through the inhibitory SRIF and a number of stimulatory hormones including growth hormone-releasing hormone (GHRH), gonadotropin-releasing hormone (GnRH), and dopamine (DA) (*e.g.*, Marchant and Peter 1989; Chang *et al.* 1990; Vaughan *et al.* 1992). In tilapia, the finding that GnRH-containing nerve fibres are in close proximity to the somatotrophs (Parhar and Iwata 1993), and demonstration of the stimulatory effect of sGnRH on GH release, may indicate the peptide's role in the control of GH in this fish (Melamed *et al.* 1995). The stimulatory effect of DA was considerably weaker as was that of two forms of GHRH (carp GHRH or human $GHRH_{1-29}$) whose homology with the putative tilapia GHRH is unknown (Melamed *et al.* 1995).

Studies in goldfish (Murthy *et al.* 1993; Wong *et al.* 1993a,b,c,d) and trout (Le Gac *et al.* 1993) have shown differences in response to these GH-releasing hormones at different stages in reproductive development, suggesting that the gonads may modulate the sensitivity of the somatotrophs to these stimuli.

The aim of the present study was to determine the effects of sex steroids on basal and stimulated GH secretion, and to relate these effects to changes in GH profiles in different seasons.

Materials and methods

Fish

The fish used in these experiments were male tilapia hybrids (*Oreochromis niloticus* x *O. aureus*). They

were collected from the fish farms of local kibbutzim, brought to the laboratory and housed in 600 litre plastic tanks in a greenhouse under a natural photoperiod. Fish were fed once a day between 9 and 11 a.m. Each tank contained 100–200 fish weighing 60–200 g. The water was aerated and filtered through a biological filter at a rate of 400 l/h. Temperatures ranged from 18°C in the winter to 28°C in the summer.

Experiments in vivo

Profiles of GH secretion

In order to examine the possibility that GH is secreted in a cyclic or pulsatile fashion, blood was sampled over 24 or 48h at different times of the year. Samples were taken every 3 or 4h, with each fish being sampled no more than twice during the 24h period, or 3 times over 48h. Blood was taken from the caudal vessels within 3 min of capture and after centrifugation, the plasma was stored at −20°C until assayed.

Steroid administration

17α Methyl testosterone (MT) (Sigma, St. Louis, MO) was injected i.p. three times at 2–3 day intervals, at a dose of 0.4 mg kg^{-1} for each injection. Subsequent measurements were made of GH levels in plasma and pituitary, or, on the tenth day, the pituitaries were removed for perifusion studies. The vehicle (dimethyl sulfoxide; DMSO) alone was administered in an identical fashion to the control group.

Castration

Sexually recrudescing or mature fish (100–150 g bw) were anaesthetized in tricaine methanesulfonate (MS-222; Sigma). Scales were removed in a line between the pectoral and anal fins, where the incision (30–40 mm) was made. Both testes were removed and the incision was sutured using 4/0 silk thread. Immediately following the operation, nitrofurazone (Abic, Israel) was added to the tanks and the fish were given 3–6 weeks to recover fully before commencement of experiments. Post-operation mortality did not exceed 5%.

Experiments in vitro

Perifusion experiments were carried out as in Melamed *et al.* (1995); briefly, each channel was composed of pituitary fragments from 3 fish which were suspended in Biogel (Bio-Rad, Richmond, CA) and rinsed for 14–16h, after which the stimulant was added in a pulse of 5 to 15 min. The effluent medium was collected over 15 min intervals throughout the experimental period. In each channel, the average secretion rate during the last 3h of the rinse period was used to calculate the basal secretion rate for that channel.

Primary cultures of pituitary cells were prepared as in Levavi-Sivan and Yaron (1992); briefly, trypsinized pituitary cells were plated (250,000 cells per well) and cultured for 4 days in an atmosphere of 95% O_2 and 5% CO_2 at 28°C. After two days, cells were rinsed and fresh medium containing or lacking the steroid was added. On the fourth day the cells were rinsed and incubated for 15 min before addition of the stimulant with or without the relevant steroid. The medium was collected after 3h incubation and stored at −20°C until assayed.

Hormones and drugs used in the *in vitro* experiments were: salmon gonadotropin-releasing hormone (sGnRH), dopamine (DA), 1-o-tetradecanoyl phorbol-13-acetate (TPA), forskolin, 17α methyltestosterone (MT), estradiol (E_2; Sigma, St. Louis, MO), [D-Arg^6,Pro^9]-NEt-sGnRH (sGnRHa; Bachem, Torrance, CA) and carp growth hormone-releasing hormone (cGHRH, a gift from The Salk Institute, San Diego, CA). The peptides and DA were dissolved directly in the medium while the steroids and forskolin were dissolved in ethanol, and TPA in dimethylsulfoxide. The final concentration of these solvents in the perifusion or culture medium did not exceed 0.1% which does not affect GH secretion.

Radioimmunoassay for tilapia GH

The radioimmunoassay employed to measure GH levels was based on the recombinant growth hormone (rtiGH) of *Oreochromis niloticus* (Rentier-Delrue *et al.* 1989), and a rabbit polyclonal anti-

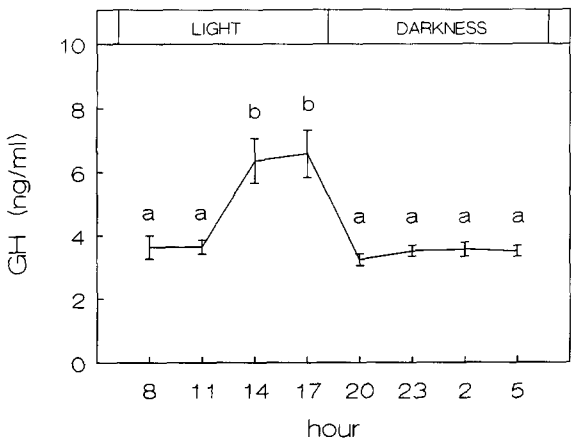

Fig. 1. A profile of circulating GH levels in male fish in April. Blood was sampled from 12 fish every 3h. Mean ± SEM. Means marked with the same letter do not significantly differ from each other ($p > 0.05$).

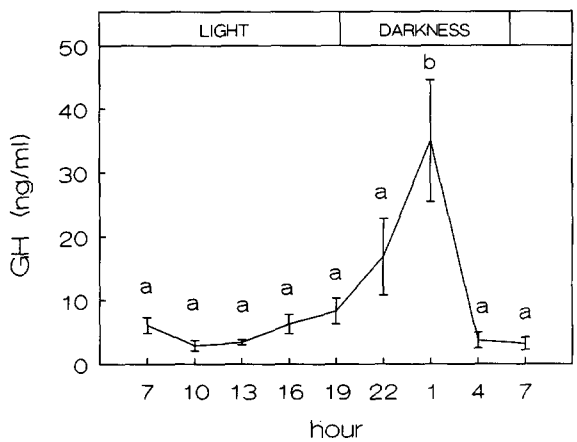

Fig. 2. A profile of circulating GH levels in male fish in July. Blood was sampled from 12 fish every 3h. Mean ± SEM. Means marked with the same letter do not significantly differ from each other ($p > 0.05$).

body produced against this hormone. Details of the assay are as in Melamed *et al.* (1995).

Statistical analysis

The significance of differences between group means was examined by ANOVA, followed by the Least Significant Difference Test (LSD). When variances differed, data were log-transformed before statistical analysis.

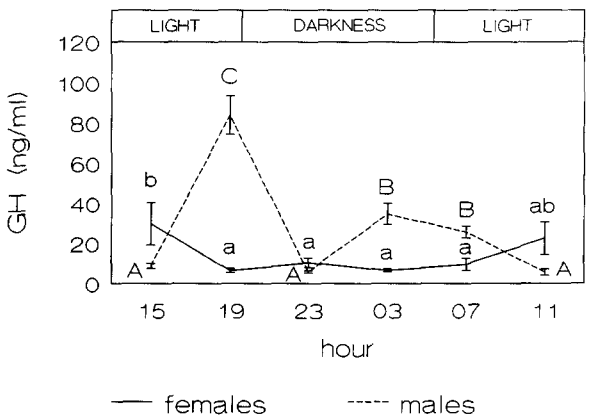

Fig. 3. Plasma GH levels of male and female fish in July of the following year. Blood was sampled from 6 females and 6 males every 4h. Mean ± SEM. The statistical analysis was done separately for male and female fish.

Results

Profiles of GH secretion

In April, 10 male tilapia had blood sampled every 3h over a 21h period. Circulating GH remained very stable at a level of 3.2–3.6 ng ml^{-1} for most of this period, although during the latter half of the photophase, the GH levels doubled ($p < 0.05$); these had returned to the basal level by sunset (Fig. 1).

A similar study on male fish was carried out in July over a 24h period. In this profile (Fig. 2) GH was at basal level of 2.8–8.3 ng ml^{-1}, but rose to a peak of 35 ± 9.5 ng ml^{-1} during the scotophase. By the following dawn, GH levels had returned to the initial level.

The aim of the third profile study, in the following July, was to compare patterns of GH secretion in males and females sampled every 4h over a 20h period. Although the basal levels were similar in both sexes (6–10 ng ml^{-1}), the males showed a marked peak (84 ± 9.5 ng ml^{-1}) in the early evening, and a smaller but more prolonged peak during the night (34.8 ± 5.3 ng ml^{-1}). The female fish showed no such drastic peak, but did show some increased GH levels (25–30 ng ml^{-1}) during the middle of the day (Fig. 3).

The fourth profile study was designed to check whether the peaks in circulating GH levels seen previously, are repeated over a 48h period. This ex-

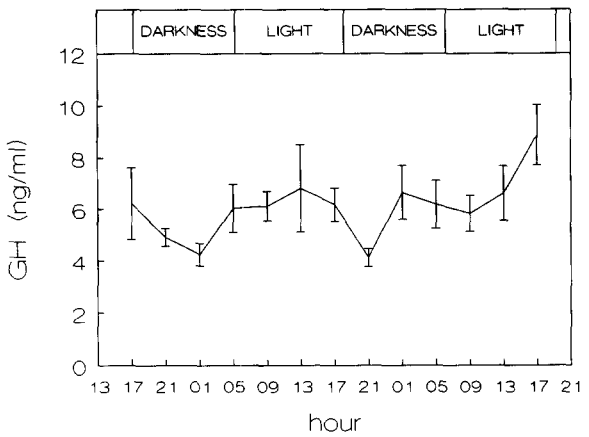

Fig. 4. A 48h profile of GH levels in male fish in September. Blood was sampled every 4h. Mean ± SEM, n = 14. Statistical analysis showed no differences in GH levels at any of the times tested ($p > 0.05$).

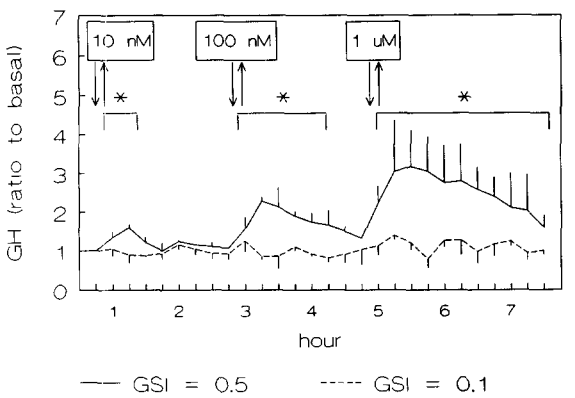

Fig. 5. GH release in response to sGnRHa from perifused pituitaries of fish with different GSI. Pituitaries collected from two groups of fish with different GSI values (GSI was 0.5 ± 0.05 or 0.1 ± 0.01, n = 9) were rinsed for 14h and a further 3h basal secretion was collected. Consecutive 15 min pulses of sGnRHa (10 nM–1 μM) were administered to all 6 parallel channels. GH secretion rates were expressed as a ratio to the basal secretion of the same channel. Statistical analysis compared GH levels secreted in response to each sGnRHa pulse between the two groups (*–$p < 0.05$). Mean ± SEM.

periment was carried out in late September on the same male fish from the previous experiment. GH levels remained similar to the basal level (4–10 ng ml^{-1}) of the previous profiles but no peak could be discerned ($p > 0.05$; Fig. 4).

Gonadal development and the response to sGnRHa

A perifusion study was carried out in the spring, using fish which had been subject to identical conditions but differed in the extent of their gonadal development. Pituitaries from two groups of nine fish with high (0.5 ± 0.05) or low (0.1 ± 0.01) GSI values were grouped together and perifused. The six parallel channels were exposed to consecutively increasing doses of sGnRHa (10–1000 nM) for 15 min each. The amount of GH secreted before stimulation was similar in both groups ($p > 0.05$; not shown). However, the pituitaries of fish with the higher GSI responded in a dose dependent manner, while pituitaries from fish with the lower GSI barely responded even to the highest dose (Fig. 5).

The effect of MT administered in vivo on subsequent in vitro stimulation of GH

These experiments were carried out to test whether MT administered *in vivo* affects the responsiveness of perifused pituitary fragments to various GH-releasing hormones. Three perifusion experiments were performed, one for each releasing hormone. For each experiment nine sexually regressed fish (taken mid-winter from water 18°C) were injected with 0.4 mg MT kg^{-1} three times during five days; a similar group of fish received the vehicle only and served as a control. On the tenth day, the fish were sacrificed and the pituitaries were removed, and exposed in perifusion for 10 min to 1 μM DA, or for 5 min to 1 μM sGnRHa or 0.1 μM cGHRH. The amount of GH secreted before stimulation from the pituitaries of either MT-treated or control fish did not differ ($p > 0.05$; not shown). Exposure of the pituitary fragments from untreated fish to the cGHRH or sGnRHa had no effect on GH secretion. However, pituitaries from MT-injected fish did respond to these stimuli. The pretreatment with MT did not alter the stimulatory effect of DA on GH secretion. Figure 6 shows the maximal response to each releasing hormone.

The perifusion experiment comparing the effect of MT on the GH response to DA was repeated on a similarly treated group of fish using a 15 min pulse of 10 μM DA. Also in this experiment MT did

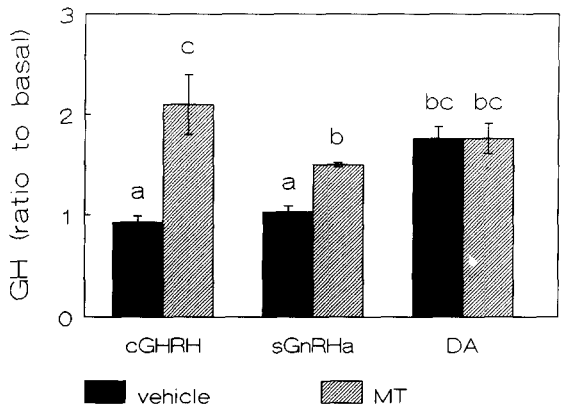

Fig. 6. The effect of MT *in vivo*, on GH release from perifused pituitaries in response to cGHRH, sGnRHa or DA. Fish were injected 0.4 mg MT kg^{-1} in DMSO, or the vehicle alone 3 times over 5 days, and on the tenth day, pituitaries were removed for perifusion experiments. cGHRH (0.1 μM) and sGnRHa (1 μM) were given for 5 min and DA (1 μM) for 10 min. The maximal response in each of these experiments is summarized; means marked with the same letter do not differ ($p > 0.05$). Perifusion details are as in Fig. 5. Mean ± SEM, n = 3.

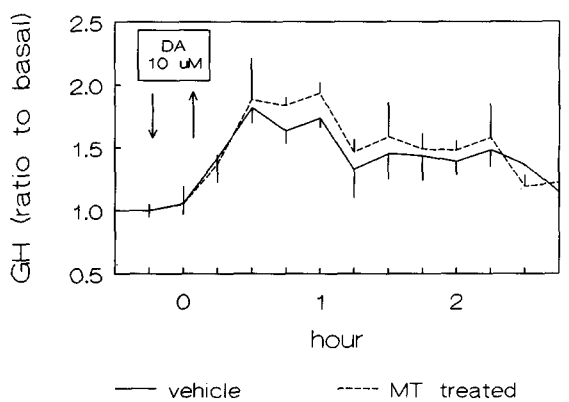

Fig. 7. GH release in response to DA from pituitary fragments of MT-treated and vehicle-injected fish. Perifused pituitary fragments were exposed to a 15 min pulse of 10 μM DA. Perifusion details as in Fig. 5. Mean ± SEM, n = 3.

not alter GH secretion in response to the amine (Fig. 7).

The effect of MT on circulating GH and pituitary content

Seventeen sexually-regressed fish were injected i.p. with 0.4 mg MT kg^{-1} three times over 5 days.

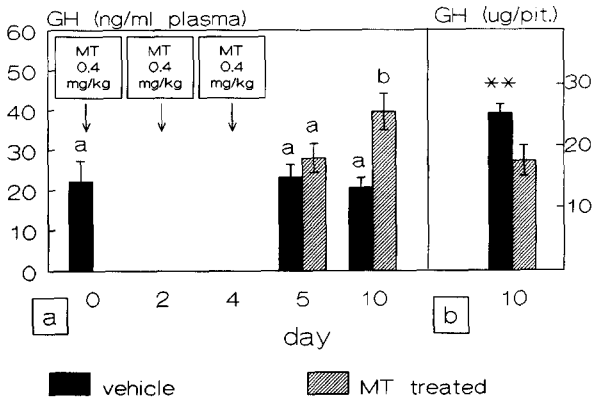

Fig. 8. The effects of MT on GH levels *in vivo*. Seventeen fish were injected MT as marked; blood was sampled before the start of treatment and 5 and 10 days thereafter. (a) GH level in plasma; means marked by the same letter do not significantly differ from each other ($p > 0.05$; n = 13). (b) pituitary content of GH on day 10. (**–$p < 0.01$). Mean ± SEM, n = 17.

Blood samples were taken at the same hour before the initial injection, and 5 and 10 days thereafter. Initially, and on the fifth day after the start of treatment, circulating GH levels were similar in both groups, but on the tenth day, the MT-treated fish showed nearly twice the GH levels as compared to the controls (39.5 ± 4.45 ng ml^{-1} *vs.* 20.56 ± 2.55 ng ml^{-1}: Fig. 8a). However, the content of GH in the pituitaries of MT-treated fish was significantly lower than in the controls ($p < 0.01$; Fig. 8b).

The effect of castration on basal and sGnRH-stimulated GH release

Pituitaries from sham-operated sexually mature and from castrated fish were dispersed and, on the fourth day of culture, sGnRH (1 fM–100 nM) was added to the incubation medium for 3h. Basal levels of GH secretion were similar ($p > 0.05$) in cells from sham-operated (459 ± 24.7 ng $well^{-1}$) and castrated fish (498.34 ± 24.8 ng $well^{-1}$). Pituitary cells from the sham-operated fish responded to 100 fM sGnRH and showed a dose-related response to concentrations up to 10 pM; at higher concentrations, the GH levels did not differ from the controls. Cells from castrated fish failed to respond to

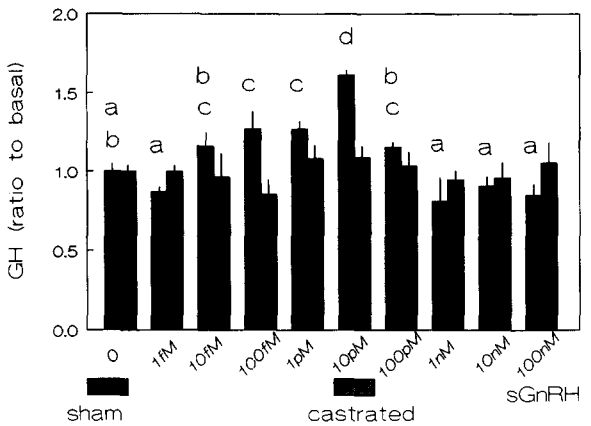

Fig. 9. GH response to sGnRH in culture of pituitary cells from castrated or sham-operated sexually mature fish. Pituitary cells were dispersed by trypsinization and cultured for 4 days before addition of sGnRH (1 fM–100 nM) for 3h. GH secretion is expressed as ratio to the basal rate. Mean ± SEM, n = 4. Groups designated by the same letter are not significantly different ($p > 0.05$). Cells from castrated fish showed no significant differences at any of the sGnRH doses administered ($p > 0.05$). The plasma GH levels, measured prior to sacrifice, were similar in both groups (sham-operated; 13.08 ± 1.51 ng ml^{-1} *vs.* castrated: 12.95 ± 1.04 ng ml^{-1}).

sGnRH at any of the doses used (Fig. 9). Plasma GH levels, measured prior to sacrifice, were similar in both castrated (12.95 ± 1.04 ng ml^{-1}) and sham-operated fish (13.08 ± 1.51 ng ml^{-1}; n = 20).

The effect of steroid administration in vitro on sGnRH-stimulated GH secretion

Pituitary cells of sexually regressed fish were exposed on the second day of culture to 10 nM MT or E_2. On the fourth day sGnRH (10 fM to 100 pM) was added to the incubation medium for 3h. Cells not exposed to the steroids failed to respond to the sGnRH, but both MT- and E_2-exposed cells showed a dose-related response. The basal level of secretion in cells treated with either steroid did not differ from the control ($p > 0.05$; Fig. 10).

A similar experiment was performed in which control and steroid-treated cells were also exposed to forskolin (10 μM) or TPA (12.5 nM). As in the previous experiment, sGnRH (10 or 100 pM) failed to stimulate GH secretion from cells which had not been exposed to steroids, however the response to

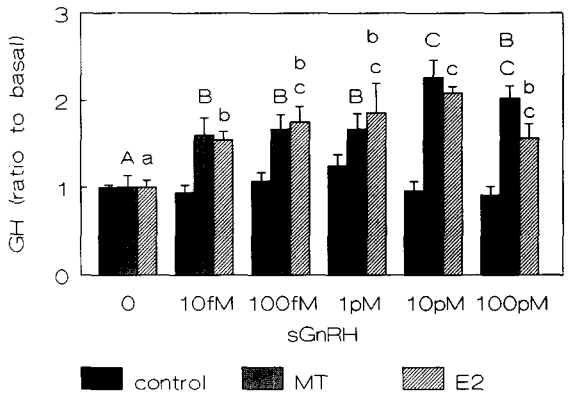

Fig. 10. The effect of steroids administered *in vitro* on sGnRH-stimulated GH secretion. Pituitary cells from sexually regressed fish were cultured as in Fig. 9, and 10 nM MT or E_2 was added to some of the wells on the second day of culture. sGnRH (10 fM–100 pM) was added on the fourth day for 3h. Data presentation is as in Fig. 9; statistical analysis was done separately for MT- and E_2-treated cells. Mean ± SEM, n = 6.

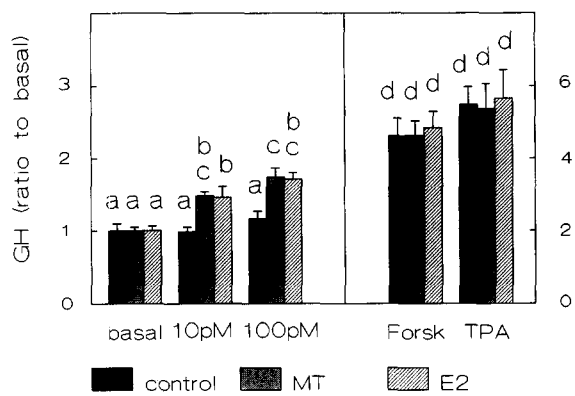

Fig. 11. The effect of MT or E_2 given *in vitro* on GH release in response to sGnRH (10–100 pM), forskolin (10 μM) or TPA (12.5 nM). Pituitary cells from sexually regressed fish were cultured as in Fig. 9, and treated with steroids as in Fig. 10. On the fourth day the cells were exposed for 3h to the agonists. The means of all groups were analyzed simultaneously, groups designated by the same letter do not differ ($p > 0.05$); mean ± SEM, n = 6. The left Y-axis refers to basal and GnRH-stimulated cells, the right Y-axis refers to forskolin and TPA-stimulated cells.

forskolin and TPA was similar in all treatment groups ($p > 0.05$; Fig. 11).

Discussion

Studies on the GH secretory profile in tilapia have consistently shown variations in the plasma GH lev-

els throughout a 24h period; these showed no apparent correlation with the photophase, scotophase, nor to feeding times or handling stress. In spite of the limitations associated with experiments of this type, a pattern of GH levels was noted in which a peak occurred at least once a day, whose magnitude and duration were most pronounced in summer when measured in two consecutive years. In autumn and early spring, the peaks were considerably lower and less pronounced (Figs. 1–4). Similar changes in the nature of the GH profile were also noted in goldfish of mixed sex; the most distinct peaks were seen in March and June (Marchant and Peter 1986). A comparison of the present results with Figs. 1–5 from the study by Marchant and Peter (1986) reveals that the leveling of the GH profile in both tilapia and goldfish coincides with the end of the breeding season.

In the present work, the difference in GH profiles of males and females seen at the height of the reproductive season (Fig. 4) suggests that the gonads may modulate these profiles. The difference between the sexes could be attributed to the distinct influence of the male and female gonadal steroids, or to the varying levels of circulating steroids in the two groups at the time of the experiment. This possibility is corroborated by the disappearance of the peaks when the same male fish were reexamined in the autumn (Fig. 3).

A positive relationship between the reproductive state of female fish and GH secretion has been noted in other teleosts. The somatotrophs of *Poecilia latipinna* showed ultra-structural changes, indicative of increased secretory activity at early and mid-vitellogenesis; their activity was reduced by late vitellogenesis and pregnancy (Young and Ball 1983). In the same species, E_2 injected into ovariectomized fish, caused hypertrophy and hyperplasia of the somatotrophs, which had become scarce and inactive after the removal of the ovaries (Young and Ball 1979). Circulating GH increased at ovulation in *Catostomus commersoni* (Stacey *et al.* 1984), and in goldfish it increased from February, when ovarian recrudescence begins, through June after which it steadily declined (Marchant and Peter 1986; Trudeau *et al.* 1992). GH levels in goldfish were particularly elevated at the time of ovulation and spawning (Yu *et al.* 1989). In rainbow trout GH increased in maturing diploid females, but not in their unmaturing, or triploid, counterparts (Sumpter *et al.* 1991).

The GH response to sGnRHa was seen to be related to the GSI (Fig. 5). When held together, male tilapia establish a hierarchical system of dominance in which only some of the males exhibit sexual activity and full testicular development. This enabled the comparison of sGnRHa-stimulated GH secretion by the pituitaries of fish from the same group which showed different levels of gonadal development. From this perifusion experiment, it would appear that the ripe, or ripening, gonad is virtually essential for the GH response to sGnRH. Such a difference in GH-response to sGnRH between fish of different reproductive states has been noted, although to a lesser degree in goldfish (Murthy *et al.* 1993; Wong *et al.* 1993c).

Injections of MT increased plasma GH by the tenth day after commencement of treatment (Fig. 8a). In sexually regressed female goldfish, implantation of testosterone capsules for 5 days did not have any effect on circulating GH levels, whereas E_2 increased plasma GH, regardless of the reproductive state of the fish (Trudeau *et al.* 1992). The same *in vivo* treatment of sexually regressed female goldfish, increased subsequent GnRH-stimulated GH secretion in perifusion studies, although this appeared to be largely a result of higher basal levels of secretion in the E_2-treated fish (Trudeau *et al.* 1992). In the present study, basal levels of GH secretion *in vitro* were unaltered by the MT injection. A comparison of the response to sGnRHa, carp GHRH or dopamine, in pituitaries from sexually regressed fish which had been injected MT, or the vehicle alone, showed that sGnRHa (1 μM) and cGHRH (0.1 μM) were effective in stimulating GH secretion only after the MT treatment.

The maximal response to dopamine (1 or 10 μM) was unaltered by MT treatment *in vivo*, as were the kinetics of the response (Figs. 6,7). In contrast, the presence of recrudescing or mature gonads, presumably producing high levels of sex steroids, appeared to reduce the response to dopamine in both male and female goldfish (Wong *et al.* 1993a,c).

Castration of mature fish did not alter either GH

levels or the subsequent basal release of the pituitary cells after dispersion, however this rendered the cells unresponsive to sGnRH at doses of 1 fM–100 nM (Fig. 9). Pituitary cells from sham-operated fish showed a dose-dependent response to this peptide over the range of 1 fM to 10 pM. Similarly, the presence of steroids, when added *in vitro* to the cell culture, facilitated the response to sGnRH (Fig. 10). Cells which had not been exposed to either endogenous steroids, or those administered in culture, failed to respond to any of the sGnRH doses given.

In mammals, both androgens and estrogens influence synthesis and release of the hypothalamic GH-controlling factors SRIF and GHRH (*e.g.*, Devesa *et al.* 1991; Kerrigan and Rogol 1992; Wehrenberg and Giustina 1992). It has been proposed that estrogen inhibits SRIF release, thereby elevating basal circulating GH levels, while androgens have the opposite effect (Devesa *et al.* 1991). The characteristic male GH secretory profile in the rat (high peaks and low troughs) appears to result from an increase in SRIF secretion, coupled with an enhanced GH response to GHRH (Cronin and Rogol 1984; Wehrenberg and Giustina 1992). If such a mechanism exists also in tilapia, it could explain some of the changes in GH secretory profiles seen at different stages in reproductive development. However, the enhanced response to the GnRH was seen also in dispersed pituitary cell culture in which no neurosecretory tissue is present. It would appear, therefore, that at least a part of the modulating effect of these steroids on GH secretion is direct, altering the response of the somatotrophs to specific, but not all, secretagogues.

The possibility that gonadal steroids also have a direct effect on the synthesis of GH must be considered, although reports of the effect of E_2 on baseline GH level or pituitary content in mammals are contradictory (summarized in Jansson *et al.* 1985; Wehrenberg and Giustina 1992; Kerrigan and Rogol 1992). Although the steroids may have stimulated GH synthesis in the present study, the increased response to the GH-releasing hormones after steroid treatment was not a direct result of increased GH amount in the pituitary which was in fact lower than in control fish (Fig. 8b).

Differences in the response of pituitary hormones to GnRH at different reproductive stages in both teleosts and mammals have been associated with changes in the numbers of pituitary GnRH receptors. In sheep, there is a correlation between GnRH receptor mRNA and the levels of follicular E_2 (Brooks *et al.* 1993), while GnRH receptor number (Gregg *et al.* 1990) and mRNA (Wu *et al.* 1994) increased following incubation of ovine pituitary cells with estradiol. Also in the goldfish, the number of GnRH receptors in the entire pituitary increased during gonadal recrudescence (Habibi *et al.* 1989). However, in fish, this effect cannot be attributed to any one cell type as GnRH receptors are present on both gonadotrophs and somatotrophs (Cook *et al.* 1991).

Pituitary cells which had not been exposed to endogenous steroids (taken from castrated or sexually regressed fish), and were not treated with steroids in culture, did not respond to sGnRH but responded to TPA, or forskolin. Protein kinase C (PKC) is involved in the signal transduction of GnRH in goldfish somatotrophs while cAMP is involved in the DA stimulation through D_1 receptors (Chang *et al.* 1993), and also mediates the GHRH signal in mammals (Frohman *et al.* 1992). The effect of the steroids on the GH response to the releasing hormones, and their lack of effect on the response to the intracellular mediators, would indicate that the steroids act proximally to PKC or cAMP, probably at the receptor level.

It is suggested that one of the mechanisms by which the gonadal steroids alter GH secretion in tilapia may be *via* changes in the response of the somatotrophs to the GH-releasing hormones (GnRH and possibly GHRH). This could be partially mediated by an increase in the number of their receptors on the somatotrophs. The increased response to GnRH in intact sexually mature fish (as compared to sexually regressed or castrated fish), and the ability to mimic this response by administration of steroids *in vitro*, suggest that the endogenous gonadal steroids have similar effects on the response of the somatotrophs to GnRH. The amplified response of GH to the endogenous releasing hormones may explain some of the changes in the nature of the secretory profiles at different stages during reproductive development in this teleost.

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