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Evidence for Binding and Action of Growth Hormone in Trout Testis[1]

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ABSTRACT

Growth hormone (GH) binding to testis tissue and GH action on trout testicular cells were studied in vitro. Labeled salmon GH (sGH) was able to bind to a trout testis membrane preparation. Binding sites showed high affinity (K_a = 1–2 × 10^9 M^{-1}) and low capacity (11 fmol/g fresh tissue) for ^{125}I-sGH. Salmon GH and bovine GH, but not salmon gonadotropin, could compete with ^{125}I-sGH for site occupancy. The binding characteristics were similar to those of trout liver GH receptors that we previously described.

Salmon GH (0.1 and 1 μg/ml) and bovine GH (10 μg/ml) could modulate steroidogenesis in cultured testicular cells: 17α-hydroxy, 20β-dihydroprogesterone (17α20βOHP) accumulation in culture medium was stimulated by GH addition, and this effect increased with duration of culture and/or stimulation; 11-ketotestosterone accumulation tended to be inhibited in the presence of GH at the beginning of culture. These effects were dependent on GH concentration and were observed both in the absence and presence of gonadotropin. The amplitude of the sGH effect varied between experiments, probably according to the physiological state of the cells used.

In vivo, GH and 17α20βOHP plasma levels increased at the beginning of spermiation (sperm production) and decreased at the end of spermiation. This relationship suggests that, at the end of the reproductive cycle, high GH levels are associated with the production of 17α20βOHP, a progestin necessary for efficient spawning in this species. We conclude that GH may play a role in testicular physiology, at least at certain stages of spermatogenesis.

INTRODUCTION

A role for growth hormone (GH) in gonadal physiology has been suggested by studies in mammals showing that GH deficiency is associated with delayed puberty and perturbed gonadal responsiveness to gonadotropin (GtH) and that GH is involved in the maintenance of LH receptors [1–3]. Numerous authors have reported the presence of somatomedin in testis and semen [4–6], as well as production of insulin-like growth factor-I (IGF-I) [7–10], and IGF-I binding and action [11–19] in the male gonad.

In vivo, GH treatment has been shown to synergize with LH to increase testosterone secretion [20] and is able to maintain IGF-I binding in Leydig cells from hypophysectomized rats [13]. However, few direct effects of GH on testicular tissue have yet been demonstrated. In mammals, Tres et al. [8] showed a modest and highly variable effect of human GH (hGH) on IGF accumulation in rat Sertoli cell cultures, while Chatelain et al. [10] found no effect of hGH on IGF-I secretion by immature pig Sertoli cells. A recent study by Closset et al. [21] clearly showed a positive action of bovine GH (bGH) injections on testicular IGF-I mRNA expression and testis development in hypophysectomized immature rats. A direct role of GH in the regulation of gonadal steroidogenesis has mainly been documented in the rat ovary [22–24].

In fish, Singh et al. [25] reported that GtH and recombinant salmon GH (sGH) could respectively increase androgen secretion by hypophysectomized *Fundulus heteroclitus* and intact trout gonadal tissue. Van der Kraak et al. [26] found that carp GH, but not carp prolactin, could directly affect in vitro ovarian steroidogenesis in goldfish by potentiating the action of GtH on testosterone and estradiol secretion.

This potential effect of GH on steroidogenesis is of particular interest in the final stages of the reproductive cycle in male salmonids. In these species, initiation of sperm production (spermiation) is associated with high levels of an androgen (11-ketotestosterone, 11KT); the following amplification of spermiation is in turn associated with increasing levels of a progestin (17α-hydroxy,20β-dihydroprogesterone, 17α20βOHP) in blood [27–30] and testicular tissue (Le Gac and colleagues, unpublished data). We found that this shift in the gonadal steroidogenic pathways does not seem due only to changing levels in circulating GtH or testicular GtH receptivity [31]. Furthermore, high levels of circulating GH have been described in the final stages of the reproductive cycle in goldfish [32], *Catostomus* [33], male trout [34], and male salmon (Le Gac and Le Bail, unpublished data).

Taken together, these facts prompted us to investigate whether purified sGH was able to interact with specific binding sites in trout testis membrane preparations, and to study the effect of sGH on the steroidogenic activity of cultured testicular cells obtained from mature trout testis as described earlier [35]. The physiological relevance of the results obtained in vitro is discussed in relation to the changes in GH and steroid hormone concentrations that take place around the spermiation period.

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Since we began this investigation, low levels of GH receptors/binding protein mRNA have been detected in rat testis by Mathews et al. [36], and GH receptor/binding protein immunological activity has been described by Lobie et al. [37] in rat female and male gonads.

MATERIALS AND METHODS

Hormones

A highly purified sGH preparation was obtained as previously described [38] from chinook salmon pituitary. Contamination was ≤ 0.06% prolactin, ≤ 0.3% gonadotrophin, and < 0.01% ACTH. Salmon GtH was kindly provided by Dr. B. Breton [39], and bGH B1 was a gift from Dr. S. Raiti from NIH, NIAMDD (Baltimore, MD). Steroids, 11KT (17α-hydroxy-4-androstene-3,17-dione), and 17α20βOHP (17α,20β- dihydroxy-4-pregnene-3-one) were from Sigma Chemical Co. (St. Louis, MO).

Binding Studies

Salmon GH was labeled with ^{125}I using the chloramine-T method described by Yao et al. [40] (sp. act. about 60 μCi/μg). Enriched membrane preparations were obtained from fully mature testis tissue essentially as previously described [41]. Briefly, tissues were homogenized in 20 mM Tris + 5 mM $MgCl_2$ + 5 mM $CaCl_2$ (pH 7.8) containing 0.3 M sucrose and inhibitors of proteolytic enzymes (1 mM PMSF, 50 μg/ml soya bean trypsin inhibitor, 1 mM paraaminobenzamidine) at 40 ml/10 g of tissue weight. The homogenate was subjected to a first centrifugation (600 × *g*, 30 min) to eliminate undisrupted tissue, unbroken cells, cell nuclei, and most of the spermatozoa, which represent more than 90% of this tissue. The supernatant was centrifuged again (45 000 × *g*, 45 min). The resulting pellet was resuspended in assay buffer (20 mM Tris, 10 mM $MgCl_2$, 0.3% BSA, pH 7) and kept at −25°C until used (from 1 to 4 days in these experiments). Liver membrane preparation was obtained using the same protocol.

A binding assay was performed on a total volume of 350 μl of assay buffer, including 100 μl of membrane preparation, 50 μl of ^{125}I-GH, and 50 μl containing, if required, unlabeled hormone as described in *Results*. Incubation lasted 16 h on ice (0–4°C) and was terminated by rapidly washing the membranes twice with 2 ml of chilled assay buffer followed by centrifugation. The pellet-associated radioactivity was counted in a Packard gamma counter (75% efficiency). Specific binding was calculated as the difference between total binding obtained in the presence of ^{125}I-GH alone and nonspecific binding obtained in the presence of an added excess of unlabeled hormone (250 ng/tube). Obtention of ^{125}I-GH as well as incubation medium has been shown to be adequate for in vitro characterization of trout liver GH receptor [40].

Cell Cultures

Rainbow trout were used at the end of their first or second reproductive cycle. Testicular cells were separated as previously described in detail by Loir [35]. Briefly, testes were perfused via both the testicular vein and the deferent duct for 1 h with a mixture of pronase and collagenase in Hanks' Balanced Salt Solution (HBSS), then for 4 h with collagenase only, and finally for 1 h with HBSS to wash away the enzymes.

The dispersed tissues were incubated for one night in HBSS + 0.1% BSA before final mechanical dispersion. Most of the spermatozoa were removed by one or two successive centrifugations in an isotonic Percoll solution (density 1.065 g/ml).

The recovered suspension consisted of individual or clumped cells containing a mixture of interstitial and tubular somatic cells and contaminating spermatozoa. (In some cultures, spermatogonia and/or a few efferent duct epithelial cells were present.) These testicular cells were seeded at about 1.5 million somatic cells per well (500 μl) in 24-well tissue culture plates coated with fibronectin. The culture medium consisted of modified Leibovitz L15 medium (pH 7.7), complemented with 4 mM sodium lactate + 2 mM glutamine + 10 μM retinol acetate and 5 μg/ml ascorbic acid + 100 μg/ml streptomycin + 100 IU/ml penicillin + 2.5 μg/ml kanamycin. Culture media and added hormones were replenished every 2 days. The viability of the cells before seeding was 99–95%; it was similar from experiment to experiment, and no cell death was observed during culture. At the end of each culture period, the media were centrifuged and kept frozen at −25°C until used.

In Vivo Experiment

Three-year-old rainbow trout of a spring spawning strain were acclimatized in our recycled water unit at 13°C under artificial light mimicking the natural photoperiod. At intervals from February to July, 10 individual fish were anesthetized in 0.03% phenoxyethanol and rapidly stripped to evaluate milt production. One milliliter of blood was sampled from the caudal vessel with a heparinized syringe, and plasma was stored at −25°C.

Hormone Measurements

For steroids, blood plasma and culture media underwent a double extraction with ethyl acetate/cyclohexane (1:1), and 17α20βOHP and 11KT were measured in the extract by RIA as described by Fostier et al. [27, 42]. Plasma GH levels were determined by RIA as previously reported [43].

Statistics

The effects of GH and GtH on in vitro steroid production were analyzed by ANOVA followed by the Kruskal-Wallis test.

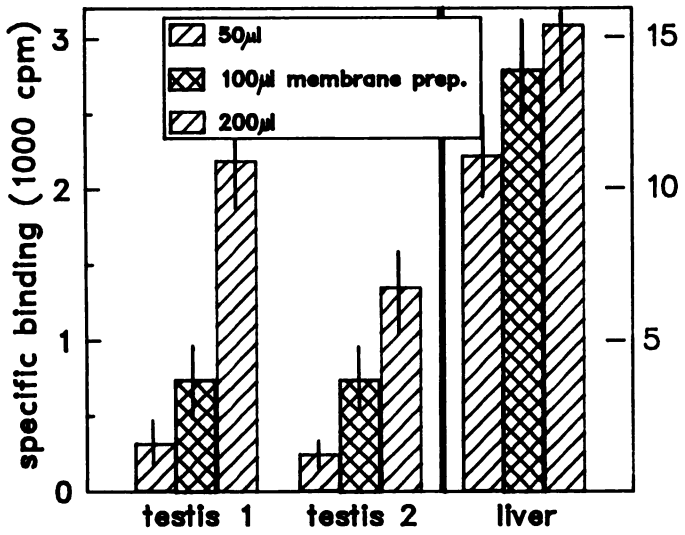

FIG. 1. Binding of ^{125}I-sGH to membrane preparations from trout testis in the spermiating stage and from trout liver (mature but nonspermiating stage). Incubation of increasing amount of membrane (1.5 mg protein/100 µl of membrane preparation) with 35 000 cpm of ^{125}I-GH (sp. act. 60 µCi/µg). Testis 1 and testis 2 are from 2 different fish.

RESULTS

Bindings of ^{125}I-sGH to Testicular Membrane Preparation

Specific binding of sGH to enriched membrane preparations obtained from testes at the time of spermiation was compared to sGH binding to a (nonspermiating) trout liver membrane preparation obtained and studied simultaneously. Labeled sGH bound specifically to testis preparations as a function of the amount of membrane added, and in these experiments the amount of specific binding reached 6% of total added hormone (approximately 45% of the labeled hormone was able to bind to excess liver membrane; maximum binding activity = 45%; Fig. 1).

When increasing concentrations of ^{125}I-GH were incubated with a fixed amount of membrane, specific binding tended to reach a plateau. This indicates that the binding sites were saturable (Fig. 2, a and b).

The Scatchard plot analysis [44] of the binding data (Fig. 2, c and d) provides evidence for a single population of binding sites in each tissue, with high affinity for sGH. The apparent affinity constants (K_a) were similar in the two tissues (K_a testis = 1–2 × 10^9 M^{-1}, K_a liver = 2 × 10^9 M^{-1}). Testicular membranes prepared as described earlier contained fewer binding sites (16 fmol/mg protein) than the liver membranes prepared simultaneously (200 fmol/mg protein). The concentration of binding sites, reported per gram of fresh tissue, appeared to be 125 times smaller in the mature testis (11 fmol/g fresh tissue) than in the liver of a nonspermiating fish (1 400 fmol/g). This last difference is linked to the fact that 1 gram of mature trout testis tissue, cleared of most spermatozoa and processed as described, provided 10–20 times less membrane protein than 1 gram of trout liver.

Competition studies (Fig. 3) showed that specific binding of ^{125}I-GH was inhibited in the presence of unlabeled sGH concentrations between 2 and 125 ng/tube (ED_{50} = 25 ng/ml). A large amount of bGH (5 µg) also totally inhibited specific binding, while addition of 500 ng of purified salmon GtH (sGtH) was without effect.

In Vitro Effects of GH on Steroidogenesis

Figures 4–6 show the effects of sGH and bGH on the accumulation of 17α20βOHP and 11KT in the culture medium of testicular cells obtained from fish in the spermiating stage. The addition of a large concentration of sGH (1 000 ng/ml) from the beginning of culture induced an increase of 17α20βOHP accumulation in the testicular cell culture medium (Fig. 4). In this experiment, the apparent stimulatory effect of GH on 17α20βOHP became evident only on Days 4 and 6 of culture. In other experiments, the stimulatory effect was small but significant on Day 2 and increased on Days 4 and 6 (Figs. 5 and 6). In fact, GH tended to maintain levels of this steroid, which normally decreased with time in the absence of GH (Figs. 4 and 5).

Addition of GH often had an inhibitory effect on 11KT accumulation in culture medium. This effect on 11KT, when observed, was maximum during the first 48-h period of incubation with GH and then tended to disappear as the spontaneous release of 11KT declined rapidly with culture duration. Analyses of variance on the 11KT data reported in Figures 5 and 6 show an overall significant inhibitory effect of GH on Day 2 ($p < 0.01$) but not on Days 4 and 6. Both the increase in 17α20βOHP and the decrease in 11KT were dependant on the concentration of sGH added to the culture (Fig. 5), and 50 ng/ml of sGH was already active.

As previously shown, addition of GtH to the incubation medium increased the production of steroids by testicular cells, particularly during the first 2 days of culture (Figs. 4 and 6). GH effects were observed whether the cells were cultured in the absence of GtH or in the presence of one large concentration (100 ng/ml; Fig. 4) or increasing concentrations (25–200 ng/ml; Fig. 6) of salmon GtH.

In one experiment, 10 µg of bGH induced effects similar to those of 1 µg of sGH on both 17α20βOHP and 11KT (Fig. 4).

As can be seen when comparing Figures 4 and 6, the amplitude of the response to sGH varied considerably between experiments; in some cases, no effect of GH was observed.

Table 1 shows the variations of GH relative response (stimulated 17α20βOHP/basal 17α20βOHP) between cultures in five different experiments and presents elements suggesting that this variation is linked to changes in the physiological state of testicular cells during the spermiation period.

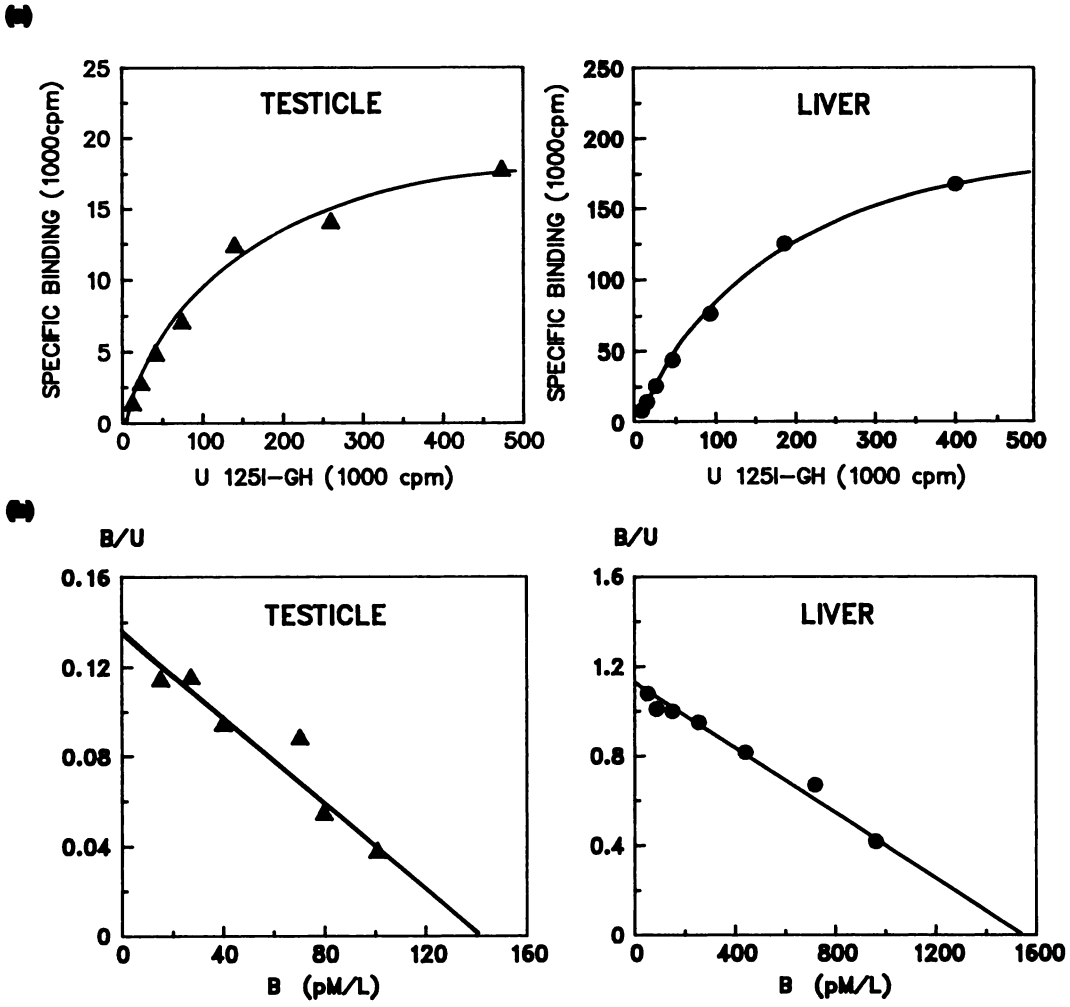

FIG. 2. Binding of ^{125}I-sGH to membrane preparations from trout testis in the spermiating stage and from trout liver (mature but nonspermiating stage). a) Effect of increasing concentrations of ^{125}I-sGH; specific binding is plotted as a function of free ^{125}I-GH. b) Scatchard plot transformation of the data. Testicular preparations from 3 fish were pooled and used at 3 mg protein/tube. The liver preparation was used at 2 mg protein/tube.

We have shown before [45] that the testicular responsiveness to GtH, in terms of androgen production, decreases during spermiation, which lasts from several weeks to four months in trout. Therefore, sperm production at the time of killing and 11KT response to GtH during the first 48 h of culture were used to classify the animals and to tentatively define the stages of spermiation. The highest 11KT response to GtH was obtained with a fully mature male not yet producing sperm (before spermiation), and the lowest response was obtained with a gonad in regression (end of spermiation).

It appears that the stimulatory effect of GH on 17α20βOHP increased at the beginning of spermiation, was maximal when the cells were obtained from a fully spermiating fish, and was minimal when the cells were isolated from a regressing gonad. Therefore, the different relative responses to GH could be a consequence of the precise physiological stage of the gonads used in our experiment.

GH and Steroid Levels during the End of the Reproductive Cycle

Changes in GH and steroid hormone concentrations in the blood of spring-spawning male trout were monitored before, during, and after their spermiation period. Plasma levels of 17α20βOHP are known to be low during the first stages of the gametogenic cycle; in our experiment, they remained low until the end of spermatogenesis (0.50 ± 0.15 ng/ml, mean ± SD in February), rose abruptly during early spermiation (maximum individual values 4–25 ng/ml), and returned to low values after spermiation (0.10 ± 0.04 ng/ml, mean ± SD in July). During the same period, 11KT plasma concentrations decreased progressively from April (56.6 ± 16.3 ng/ml) to July (0.57 ± 0.21 ng/ml).

GH levels have been shown to be low during gonadal development [34] and in this experiment were still low at the end of spermatogenesis (0.62 ± 0.06 ng/ml). The blood plasma concentrations of this hormone increased during

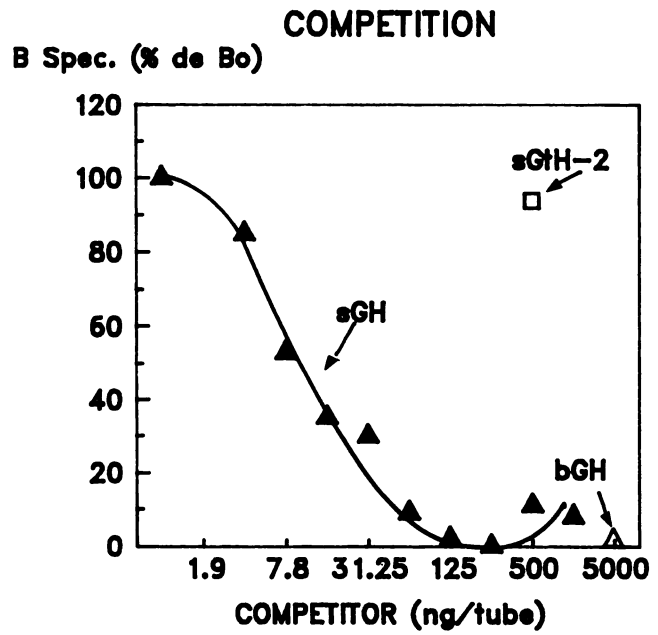

FIG. 3. ^{125}I-GH specific binding to testicular membranes (same preparation as in Fig. 2): competition studies with increasing concentrations of sGH, 5 μg bGH, or 0.5 μg sGtH.

the spermiation period (individual maximum values 5–18 ng/ml) and tended to return to low values after spermiation (1.07 ± 0.68 ng/ml).

DISCUSSION

These results show that purified sGH is able to interact with high-affinity binding sites in trout testicular tissue. These sites can recognize bGH and are distinct from the maturational GtH receptor. Previous work in our laboratory has shown that salmon prolactin does not bind to trout GH receptors [40], and *Tilapia* prolactin receptors do not recognize *Tilapia* GH (P. Prunet, personal communication). It is therefore likely that in fish tissue prolactin and GH have totally distinct binding sites. Further experiments will be necessary to complete the study of GH binding to testicular sites in terms of specificity and of association and dissociation kinetics. However, as far as we can conclude from this study, testicular GH binding sites have characteristics similar to the liver binding sites that we previously described as specific GH receptors [40]. To our knowledge, this is the first time that specific GH binding to putative testicular GH receptors has been described. That only a low concentration of these binding sites is found in testicular tissue probably reflects the fact that mature trout testis is mainly composed of germ cells (spermatozoa) and contains only very few somatic cells per gram of tissue.

Our binding studies are in agreement with and complementary to recent data published by Lobie et al. [37] describing immunological localization of GH receptors in several cell types of the rat testis. Our data are also supported

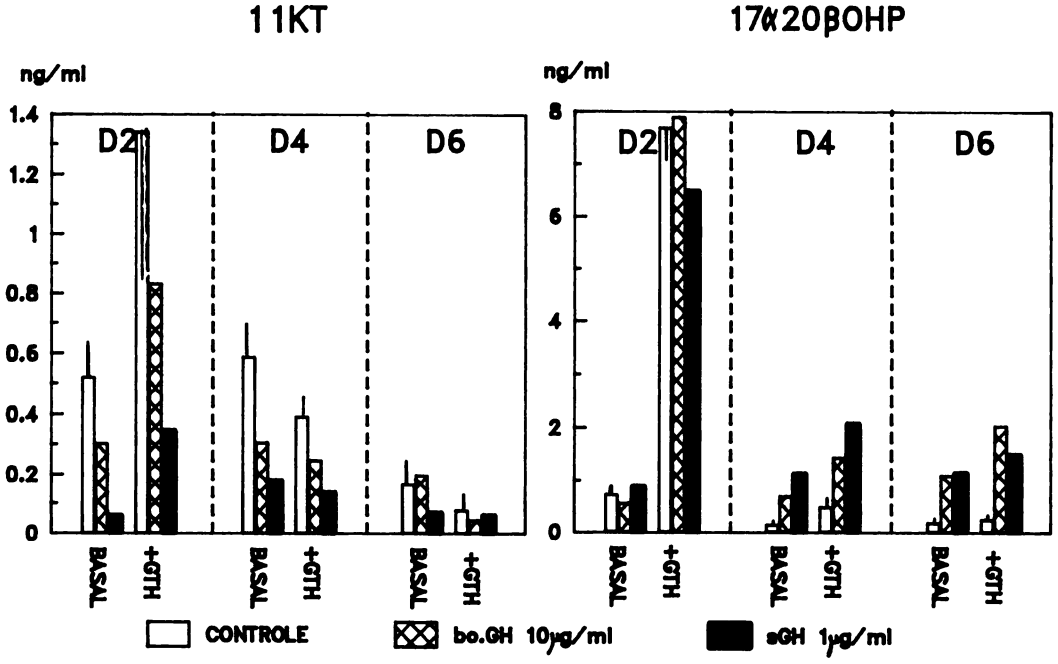

FIG. 4. Effect of sGH (1 μg/ml) or bGH (10 μg/ml) on 17α20βOHP and 11KT accumulation in incubation medium of testicular cells (isolated from the gonad of a fish in the spermiation stage). Cells were cultured for 6 days in the absence (BASAL) or presence (+GTH) of GtH (100 ng/ml). Media and hormones were replenished every 2 days. The control values (no GH) are means ± SD of triplicate culture dishes; for sGH- and bGH-treated cultures, steroids were assayed in pools of media from triplicate culture dishes.

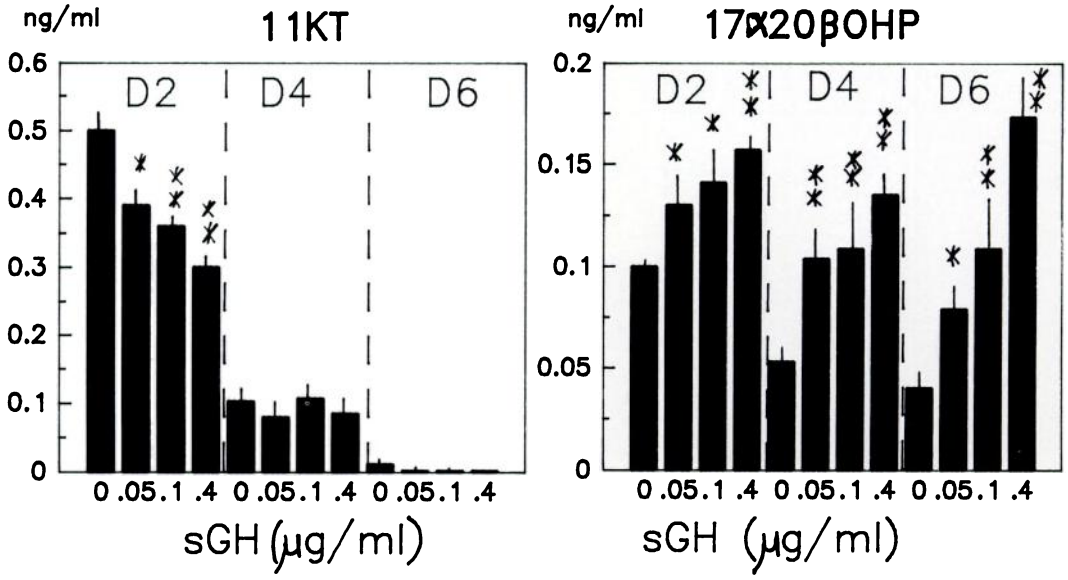

FIG. 5. Effect of increasing concentration of sGH on basal accumulation of 17α20βOHP and 11KT in culture media. Cells were isolated from a fish in the spermiation stage. Media and hormone were replenished every 2 days. Values are means ± SEM of 4 dishes. ANOVA: overall GH effect on 17α20βOHP is significant on Days 2, 4, and 6 ($p < 0.001$); GH effect on 11KT is significant on Day 2 ($p < 0.001$). *$p < 0.05$ and **$p < 0.01$: significantly different from control sGH = 0; Kruskal-Wallis test.

by the previous finding of Mathews et al. [36] that GH receptor mRNA is detectable in the rat testis.

Our in vitro data also suggested that sGH is able to induce changes in apparent steroid production by trout testicular cells in culture. Similar effects were obtained with a high concentration of bGH, which is consistent with the fact that this mammalian hormone is able to compete with sGH for binding to testis membranes (this study). Furthermore, bGH is known to affect trout growth when used at doses 10–100 times higher than sGH effective doses [46].

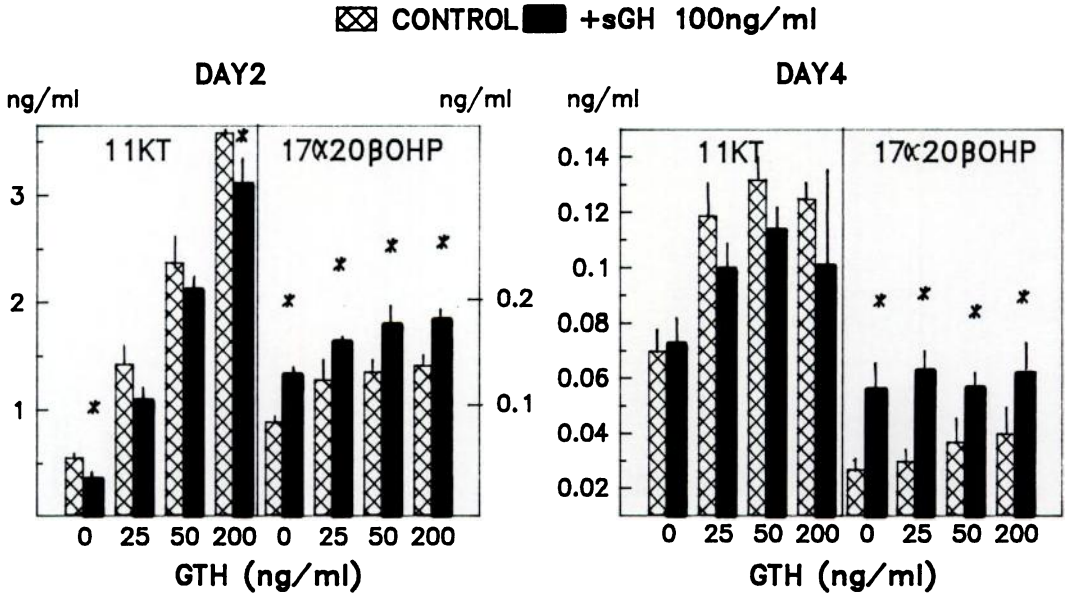

FIG. 6. Effect of sGH (100 ng/ml), added from Day 0 of culture, on steroid secretion on Day 2 and Day 4 of culture, in the absence (GTH, 0 ng/ml) or presence of increasing concentrations of GtH (25–200 ng/ml). Cells were isolated from a fish in the spermiation stage. Values are means ± SEM of triplicate culture dishes. ANOVA: overall GH effect on 11KT is significant on Day 2 ($p < 0.001$); GH effect on 17α20βOHP is significant on Days 2, 4, and 6 (Day 6 not shown) ($p < 0.001$). *$p < 0.05$: significantly different from the value obtained at the same GtH concentration but in the absence of GH; Kruskal-Wallis test.

TABLE 1. Response of 17α20βOHP to GH in five different culture experiments in relation to the physiological stage of the gonad used.

Experiment	Physiological stage[a]		17α20βOHP response to GH[c]
	11KT response to GtH[b]	Proposed stage	
1	14	before spermiation	2.2
2	6	beginning of spermiation	3.6
3	2.6	full spermiation	7.6
4	1.3	advanced spermiation	1.97
5	1	end of spermiation	1

[a]The physiological stage of the gonad is tentatively defined by the testicular cell responsiveness to gonadotropic stimulation and by sperm production.

[b]11KT response to GtH is the ratio of GtH-stimulated 11KT production (mean of 3 dishes) to basal 11KT production (mean of 3 dishes), during the first 48 h of culture (Days 0–2).

[c]17α20βOHP response to GH is the ratio of GH-stimulated 17α20βOHP (mean of 3 dishes) to basal 17α20βOHP (mean of 3 dishes), during Days 2–4 of culture.

The magnitude of responses to GH was highly variable between experiments; however, when an effect was present, GH always increased 17α20βOHP measured in the medium and tended to decrease 11KT accumulation during early stimulation. This last inhibitory effect is not consistent with the stimulation of testosterone obtained by Singh et al. [25] with a recombinant sGH preparation. We cannot exclude the possibility that this apparent contradiction is due to differences in the GH preparations; however, we believe that it is most probably the result of important differences in experimental conditions: Singh et al. used short-term (3 h) in vitro incubation of testicular explants. Whether this difference is due to a biphasic effect of GH on 11KT accumulation or to some dedifferentiation of the isolated cells in culture remains to be established. However, biphasic effects of GH action have been described in other tissues [47].

In our different experiments, GtH was found to increase androgen production at the beginning of culture (Day 2), but not necessarily after 4 or 6 days of stimulation. We previously showed [48] that this stimulatory effect of GtH decreases with duration of culture and/or stimulation and suggested that it may be due to a reduction of the steroidogenesis substrates or to some desensitization phenomenon after several days of GtH stimulation. The kinetics of 11KT response to GtH also depend on the physiological stage of the gonad [31].

The GH effects that we observed are unlikely to be due to GtH contamination since purified sGH contained less than 0.3% of this hormone and since, inversely to GH, GtH tends to stimulate 11KT strongly at the beginning of culture. Furthermore, GH effects on both 11KT and 17α20βOHP accumulation were observed even in the presence of a large concentration of GtH.

Testicular cell responsiveness was found to vary largely from culture to culture. The relative proportions of different cell types were not systematically compared in the different experiments presented here, nor did we monitor changes in the proportion of different cell types that may have occurred during culture. Therefore, we cannot exclude that GH action is mediated through differential proliferation of one or several cell types and that a variation in original cell composition contributes to the different amplitudes observed in response to GH. However, we believe from previous work [48, 49] that the cell populations obtained from fully mature testes (which exclude the regressing gonad) are unlikely to vary considerably in composition.

Variation in testicular cell responsiveness to GH seems, at least in part, to reflect differences in the physiological stage of the gonads used, with a maximum response of 17α20βOHP occurring during active spermiation, when 11KT response to GtH declines.

Results obtained with cells in culture do not necessarily reflect the physiological role of GH in steroid regulation. For example, GH effects might result from a nonspecific acceleration of a differentiation process that tends to affect trout steroidogenic cells in culture [48, 49]. However, in vivo, we found that large increases of both GH and 17α20βOHP occurred during spermiation. GH is known to vary greatly between individuals and during the day, and to be secreted in a pulsatile manner [43, 50]; it is therefore difficult to describe physiological variations of this hormone precisely. However, study of the individual hormonal profiles of ten fish during spermiation has shown that, in most cases, GH and 17α20βOHP increase simultaneously [51]. These in vivo data support the idea of a physiological link between the two hormones at this stage. It should be noted that GH rise is probably attributable to the decreased food intake accompanying reproduction in male fish [34].

Regulation of 17α20βOHP secretion in these species is of particular interest since this progestin is the most effective steroid to stimulate spermiation [52]; it is also known as the final oocyte maturation-inducing factor in the salmonid ovary [53] and is studied as a pheromone in the coordination of fish male and female sexual behavior during spawning [54]. In previous studies, we showed that the increase of 11KT during spermatogenesis is linked to an elevation of testicular receptivity to GtH [45]; however, the shift of the steroidogenic pathway towards 17α20βOHP production occurring during spermiation could not be accounted for [31]. Our present results allow us to suggest a role for GH during the late differentiation of trout testicular cells involved in steroidogenesis or steroid metabolism.

Because whole testis membrane preparations and cultures of mixed cell types were used in this study, we have no information concerning the type of cell involved in the testicular response to GH. In salmonids, somatic cells [48, 49] and also spermatozoa [55] are involved in 17α20βOHP production; therefore, several cell types are potential targets for direct or indirect GH action. In the rat, Lobie and collaborators [37] have detected immunoreactive GH receptor-like material in a large variety of testicular cells, including

Leydig cells, Sertoli cells, germ cells, and endothelial cells. It is known that GH action may directly affect the cells regulated or may be mediated through the modification of IGF-I and/or IGF-I binding protein(s) production in the target tissue. Tres et al. [8] suggested a role for GH on IGF-I secretion by mammalian Sertoli cells, and Closset et al. [21] clearly demonstrated an in vivo action of GH on IGF-I gene expression in the rat testis. Whether GH acts, in the testis, directly on the steroidogenic cells or through a testicular paracrine factor of the somatomedin family remains to be established and is currently under investigation.

In conclusion, our binding experiments directly show the possibility of GH acting at the testis level. GH is able to modify the testicular cells functioning in vitro; the changes that are seen depend upon duration of cell culture and/or exposure to GH and previous history of the testicular tissue. Our in vitro and in vivo data suggest a role for GH in the final stage of the salmonid testicular cycle (or possibly in the early stages of the following cycle).

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