Binding and action of salmon growth-hormone (sGH) in the mature trout testis

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BINDING AND ACTION OF SALMON GROWTH-HORMONE (sGH) IN THE MATURE TROUT TESTIS

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Abstract :

The ability of salmon growth hormone (sGH) to bind specific testicular receptors and its potential aptitude to affect testicular steroidogenesis was examined in salmonids at the end of the reproductive cycle. We found that GH and 17α-hydroxy, 20ß-dihydroprogesterone (17α20ßOHP) both increased in male blood plasma during spermiation. *In vitro*, sGH modulates steroidogenesis : in particular it increases 17α20ßOHP accumulation in the culture medium of testicular cells isolated from spermiating trout. Purified sGH is able to interact with specific binding sites in mature testis membranes.

Introduction :

While the GH relationship with reproductive physiology in male vertebrates has been suggested by several indirect *in vivo* studies, few direct effects of GH on the testicular tissue have yet been demonstrated. Injections of bovine GH increase the expression of testicular IGF1 mRNA in hypophysectomized immature rats (Closset *et al.* 1990). In fish, Singh *et al.* (1988) have reported that recombinant salmon GH could increase androgen secretion by gonadal tissue from hypophysectomized *Fundulus heteroclitus* and intact trout.

The action of GH on gonadal steroidogenesis has been documented in rat ovary. In *Carassius auratus*, Van der Kraak *et al.* (1990) have shown that Carp GH could directly affect *in vitro* ovarian secretion of testosterone and estradiol by potentiating the action of GtH.

The demonstration of high GH circulating levels at the end of the reproductive cycle in goldfish, a catastomid and male trout make it particularly interesting to study potential GH binding to testicular cells and to investigate the potential role of GH in the steroidogenic changes observed during spermiation.

Results :

GH and steroid levels during the end of the reproductive cycle : The changes in GH and steroid hormone concentrations in male trout blood were followed in individuals, before, during and after their spermiation period and results are shown in Figure 1. Plasma levels of 17α20ßOHP are known to be low during the early stages of the gametogenetic cycle ; as shown in figure 1, they remained low until the end of spermatogenesis (0.5 ± 0.15 ng/ml - M ± SD - in February) then rose abruptly during early spermiation (maximum values : 4 to 25 ng/ml)

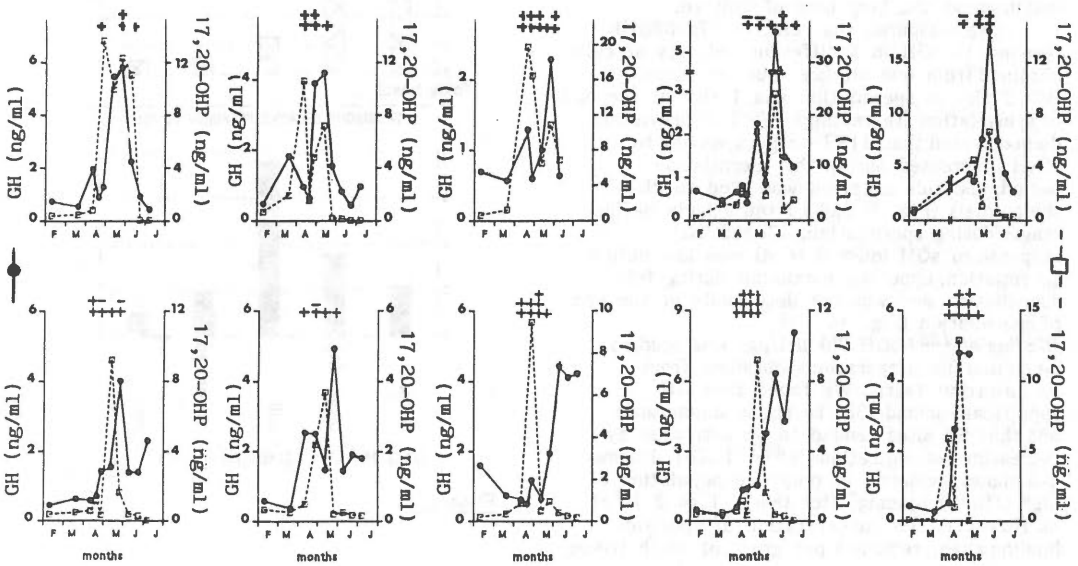

Figure 1 : Plasma levels of GH ____ and 17,20-OHP ---- in 10 male trouts; +/-, +, +++ indicate increasing sperm productions.

and returned to low values after spermiation (0.1 ± 0.04 ng/ml). During this same period of time plasma 11KT concentrations decreased progressively from April (56.6 ± 16.3 ng/ml) to July (0.57 ± 0.21 ng/ml) (not shown in Fig. 1). GH levels have been shown to be low during gonadal development and in this experiment they were still low at the end of spermatogenesis (0.62 ± 0.06 ng/ml). GH levels were greatly increased during the spermiation period (maximum values : 5 to 18 ng/ml) ; afterwards concentrations of this hormone returned to low values in 7 of 10 fish (one died during the experiment). GH is known to vary largely between individuals and during the day and to be secreted in a pulsatile way. It is therefore difficult to describe physiological variation of this hormone precisely. However, individual profiles show that, in most cases, GH and 17α20ßOHP tend to be elevated simultaneously.

GH action in vitro : Crude testicular cells (a mixture of interstitial cells, peritubular cells, Sertoli cells and contaminating spermatozoa) were prepared from testes before, during, or at the end of the spermiation period. The cells were dispersed by perfusion of the testis with collagenase and pronase, then cultured for 2, 4 or 6 days in the absence or presence of purified sGH. Media and hormones were renewed every 2 days. We found that sGH increased the accumulation of 17α20ßOHP in testicular cell culture medium (Fig. 2). This effect developed over the culture duration and was maximum after 6 days ; it was dose dependent and occurred even in the presence of an optimum gonadotropin concentration. Accumulation of 11KT tended to be reduced in the presence of sGH ; this effect was maximum at the beginning of culture.

We compared the relative 17α20ßOHP response to sGH in 5 different cultures of cells obtained from one mature -but not running- fish, 3 fish in spermiation and 1 fish at the end of spermiation (regressing). We had previously demonstrated that 11KT responsiveness to $sGtH_2$ decreased during the spermiation period, and this criterion was used to classify the animals (Fig. 3) and to tentatively define stages during spermiation. 17α20ßOHP response to sGH (days 2 to 4) was low before spermiation, appeared maximum during full spermiation, and was not detectable at the end of spermiation (Fig. 3).

Binding of ^{125}I-sGH (60 μci/μg) was studied on membrane preparations obtained from mature trout testis. We found that GH specifically bound (SB) to testis membranes and that SB sites tended to be saturated by increasing concentrations of ^{125}I-sGH. Figure 4-A shows evidence of only one population of high affinity binding sites (Ka = 1 to 2 10^9 M^{-1}). However, the concentration of specific binding sites, reported per gram of fresh tissue, was about 125 fold less in testicular tissue than in the liver. Figure 4-B shows that unlabelled sGH and bovine GH were able to compete with ^{125}I-sGH for interaction with the specific binding sites, while salmon gonadotropin ($sGtH_2$) had no effect.

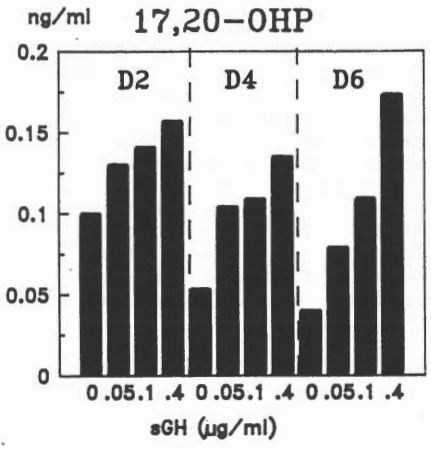

Figure 2:

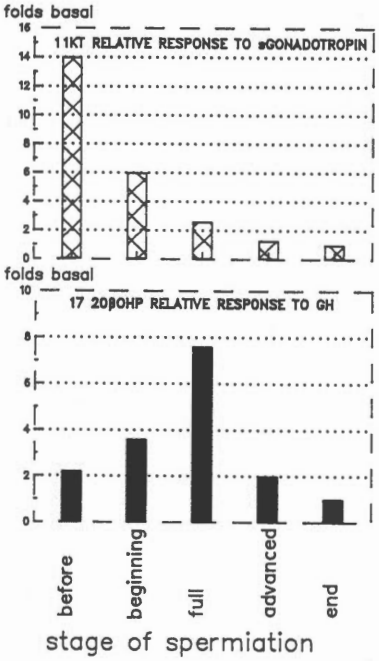

Figure 3:

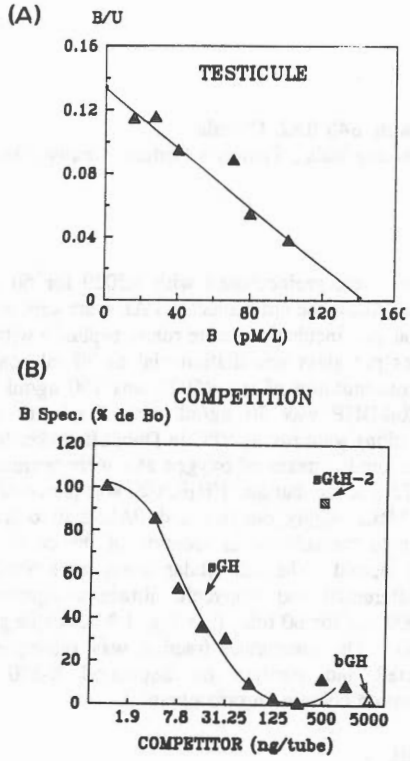

Figure 4

Discussion :

In vivo endocrine profiles show that GH and 17α20ßOHP both increase during spermiation. Increase of GH has been attributed to a diminution or arrest of food intake around spawning. In a previous study we had found that increased 17α20ßOHP production during spermiation could not be explained by an increased receptivity to GtH or by the circulating levels of the gonadotropin. On the other hand, in this study purified sGH is able to increase apparent 17α20ßOHP production by trout testicular cells. Furthermore, this effect seems to depend on the precise stage of the gonad and to be maximum during spermiation. We do not yet know the mechanism by which GH acts. Its effect might result from the acceleration of a differentiation process occuring in culture, or from a specific action (direct or indirect) on steroid-metabolising cells. Also we cannot exclude the possibility that the variation of relative GH response is due to specific multiplication of one (or several) particular cell types. Finally, we have shown that salmon GH is able to interact with specific binding sites in testicular tissue. These sites are saturable, and present a high affinity and a low capacity for salmon GH and are different from maturational gonadotropin receptors. This is the first time that GH binding to putative testicular GH receptors is described. It is in accordance with the recent detection of low levels of GH receptor/binding protein mRNA (Mathews *et al*, 1989) and of GH receptor/binding-protein immunological activity (Lobie *et al.* 1990) in the rat testis. The doses of unlabelled sGH effective in competion binding studies (4 to 250 ng/ml) are compatible with the lower GH concentration tested in culture (50 ng/ml) or with the high GH circulating levels in male trout during spermiation. These data support the biological relevance of testicular GH receptors.

Taken together, these data show that GH could act on testicular tissue (at least at certain stages of spermatogenesis). Our *in vivo* observations and *in vitro* results suggest that GH is physiologically associated with 17α20ßOHP production during spermiation although demonstrative *in vivo* experimental data are as yet unavailable.

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Proceedings

of the

Fourth International Symposium

on the

Reproductive Physiology of Fish

University of East Anglia, Norwich, U. K., 7-12 July 1991

Edited by: A. P. Scott, J. P. Sumpter, D. E. Kime, and M. S. Rolfe

Published by FishSymp 91, Sheffield.