Changes in Endocrine Profiles and Spermiation Response in Carp after LHRH Analogue Injection

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LHRH analogue [(D-Ala₆-desGly₁₀)LHRH ethylamide] injection at a dose of 30 µg/kg body weight accelerated spermiation in carp. The amount of collectable milt increased rapidly at 12 h after injection and was maintained at high levels for 24 h. The spermatocrit gradually increased throughout the experiment, and motility of the spermatozoa did not appear to be affected.

Plasma gonadotropin (GtH) level showed a significant rise at 5 min after the LHRH analogue injection. The level decreased at 40 min but increased again gradually and reached a peak at 24 h. The control group had a lower plasma GtH level than the injected group throughout the experiment. Plasma testosterone increased significantly from 1 h and was maintained at a high level for 24 h. Plasma 11-ketotestosterone also showed a similar pattern of changes. No significant changes were recognized for both androgens in the control fish.

From these results, it is possible to postulate that exogenous LHRH analogue stimulated an early and rapid discharge of gonadotropin from the pituitary gland, and that spermiation was enhanced by the action of the successive secretion of gonadotropin and androgens.

Many investigations carried out on mammals, birds and reptiles have shown that some of the synthetic LHRH and their analogues (LHRH-a) have the same biological properties as the natural hypothalamic releasing factors, as these substances will induce oocyte maturation and ovulation in female and spermiation in males.¹⁻⁷⁾ Similar results have been obtained in female fish such as carp,⁸⁾ rainbow trout,⁹⁾ Atlantic salmon,¹⁰⁾ goldfish,¹¹⁻¹³⁾ medaka,¹⁴⁾ ayu,¹⁵⁾ plaice and goby,¹⁶⁾ *Tinca*,¹⁷⁾ *Hypthalmichthys*,¹⁸⁾ and Chinese carp.^{19,20)} In these fishes, oocyte maturation and ovulation were accelerated after injection with LHRH or its analogue. Spermiation was also accelerated after administration of LHRH-a in pike,²¹⁾ carp²²⁾ and Atlantic salmon²³⁾ also stimulated spermiation. The results of a recent experiment by one of the authors (FT) indicated that LHRH-a effectively stimulated spermiation in carp.²⁴⁾

The purpose of this study was to investigate the early responses of the endocrine system, especially the plasma GtH and androgen changes and the spermiation response, of carp within 24 h after

treatment with LHRH-a. In addition, the histological changes of the gonadotrophs were also investigated by electron microscopy.

Materials and Methods

The experiments were carried out at the laboratory of Fish-Culture, Tokyo University of Fisheries, from March to April 1985. Three-year-old male carp with an average weight of 1.2-2.0 kg were used. Before handling, the fish were anesthetized with 350 ppm 2-phenoxyethanol. The males that gave a slight amount of milt after hand stripping were selected for this experiment.

Five to eight males were either injected intraperitoneally with 0.9% NaCl as a control group or with LHRH analogue [(D-Ala₆-desGly₁₀)-LHRH ethylamide] at a dose of 30 µg/kg body weight.

Milt was obtained by hand stripping just before injection and at 5 and 30 min, and at 1, 3, 6, 12, 24, and 48 h after injection. About 0.1 ml of blood was also obtained using heparinized syringes from the caudal vasculature at each

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sampling time. After centrifugation at 3600 rpm the plasma was separated and stored at -70°C until analysis. Spermatocrit was measured with a hemacytometer using microhematocrit tubes sealed at one end and centrifuged at 12,000 rpm for 5 min. Sperm motility was classified under the light microscope using $1\ \mu\text{l}$ of stripped milt diluted with $100\ \mu\text{l}$ of distilled water. The types of spermatozoa movement was recorded as following: forward movement (+++), rotatory movement (++), vibration movement (+), and no movement (-). The duration of movement was determined from the time of activation by distilled water until the end of movement.

Plasma GtH was measured using a double antibody radio-immunoassay (RIA) with a purified preparation of silver carp gonadotropin described by Kobayashi *et al.*²⁵⁾

Plasma testosterone, 11-ketotestosterone and 17α , 20β dihydroxyprogesterone were measured by RIA according to the method developed by Aida *et al.*¹⁶⁾ and Kobayashi *et al.*,²⁵⁾ respectively.

During the experiment, fish were kept individually in 36 l tanks with dechlorinated slow-running water, and the water temperature averaged 19°C .

Duncan's multiple-range test was used to determine differences among experimental groups.

The pituitaries were removed from 2 or 3 fishes sacrificed before injection or 5 min and 24 h after injection with LHRH-a, and were fixed in glutaldehyde-osmium solution. Ultrathin sections were made after embedding in Quetol 812, and stained with Pb-nitrate.

Results

Volume of Milt

At 5 and 30 min, and 1 and 3 h following LHRH-a injection, the amount of collectable milt per body weight was less than 0.02% and not significantly different from the control group. However, the milt volume rapidly increased 12 h after the injection and remained elevated for 24 h; then, slightly decreased after 48 h (Fig. 1). The amount of milt from control fish was low at 6 h and slightly increased at 12 h, after which time it decreased.

Spermatocrit

The spermatocrit value before injection was $16.3 \pm 10.69\%$ and increased gradually from 18.3 ± 10.14 to $47.8 \pm 5.72\%$ after injection. In the control group the spermatocrit was measured on

the males which gave small amount of the milt at 24 h after 0.9% NaCl injection.

Sperm Motility

It was found that the spermatozoa of carp were usually motionless in undiluted sperm, but became active after dilution with distilled water. In the control fish, which scarcely gave milt at 24 h, the rate of spermatozoa movement began from forward movement (+++) and stopped within about 1 min (45–67 sec). In the LHRH-a injected fish, energetic forward movement (+++) of most spermatozoa in the milt obtained in each time began at the time of activation and then stopped within 1 min.

Plasma GtH

Plasma GtH before injection was 8.8 ± 0.38 ng/ml. In the LHRH-a injected group, plasma GtH level increased at 5 min to 40.5 ± 11.0 ng/ml. The levels, however, decreased rapidly at 30 min reaching a level of 14.1 ng/ml. This level was not statistically different from that of the control group (9.0 ± 0.20 ng/ml). At 6 h, plasma GtH gradually increased again reaching a peak at 24 h (51.2 ± 12.35 ng/ml). The control group had a plasma GtH range from 7.8 ± 0.34 ng/ml to 9.2 ± 0.5 ng/ml throughout the experiment (Fig. 1).

Plasma Androgens

Before LHRH-a injection, plasma testosterone and 11-ketotestosterone were 6.5 ± 2.5 ng/ml and 3.4 ± 1.0 ng/ml, respectively. At 1 h, plasma testosterone had increased significantly to 14.6 ± 4.7 ng/ml, and also at 3 h to a level of 22.6 ± 3.0 ng/ml (Fig. 1). It was maintained at a high level for 12 h but it decreased after 24 h.

Plasma 11-ketotestosterone increased significantly at 1 h to a level of 5.8 ± 1.58 ng/ml and reached 13.6 ± 7.98 ng/ml at 3 h. The level was still high at 24 h with 25.9 ± 10.0 ng/ml.

In the control group, both testosterone and 11-ketotestosterone showed a slight increase from 3 h to 12 h, but this increase was not statistically significant. Plasma 17α , 20β -dihydroxyprogesterone levels were varied among individuals and showed no statistical significance between injected and control groups.

Gonadotrophs

The gonadotrophs of carp were thought to be the larger basophilic cells in the meso-adenohypophysis which possessed the granules of different size. Some of them contained large

Fig. 1. Changes in the amount of collectable milt (expressed as percent to the body weight) and the levels of plasma gonadotropin (GtH) as well as androgens (testosterone and 11-ketotestosterone) in male carp after LHRH-analogue injection.

vacuoles within the cytoplasm (Fig. 2A). Larger granules were usually round in shape, while middle-sized and small granules were round or ellipsoidal.

Five min after LHRH-a injection, most of the gonadotrophs reduced in size and almost all large and middle-sized granules as well as the intracellular vacuoles had disappeared (Fig. 2B). Small and somewhat ellipsoidal granules were condensed in the periphery of cytoplasm. Most parts of intercellular spaces expanded and contained some materials probably discharged from the gonadotrophs.

The gonadotrophs at 24 h after injection contained both large and middle-sized granules. While the small granules were less in number. Plentiful intercellular spaces were also observed.

Discussion

The purpose of this study was to examine the changes in plasma GtH and androgen levels and the spermiation responses of carp after LHRH-a injection over a period of 24 h. It was found that LHRH-a at a dose of 30 $\mu\text{g/kg}$ body weight could cause an elevation in plasma GtH level and also accelerate spermiation. The amount of milt increased rapidly at 12 h after injection and was maintained high for 24 h, after which time it decreased. Almost all the spermatozoa in the milt from LHRH-a injected animals showed

immediate active forward movement. The duration of movement after activation was quite similar to that reported by Ginzburg,²⁸⁾ and indicated that the spermatozoa possessed a good quality of motility.

The present results suggested that LHRH-a stimulated the secretion of GtH from the pituitary gland within a short time since plasma GtH was already elevated at 5 min after injection. Plasma GtH, in turn, stimulated production of the androgens such as testosterone and 11-ketotestosterone, which may eventually control the spermiation process.

Several previous studies have tried to identify the trigger of spermiation. Takashima *et al.*²⁴⁾ found that the amount of milt and 11-ketotestosterone in serum were both increased following an elevation in plasma GtH, artificially evoked with LHRH-a injection in carp. However, Yamazaki and Donaldson²⁷⁾ suggested that the spermiation in goldfish was regulated by 17-methyltestosterone and dehydroepiandrosterone. In rainbow trout, it was found that the concentration of 11-ketotestosterone was high in spermiating male²⁹⁾ but very low in females.²⁹⁾ Fostier *et al.*³⁰⁾ reported that the quantities of collectable milt in rainbow trout were positively correlated with 11-ketotestosterone levels, but not with GtH levels. Hunt *et al.*³¹⁾ analysed serum testosterone and 11-ketotestosterone in rainbow trout after GtH injection and observed early rise in the former

Fig. 2. Histological changes in the gonadotrophs of male carp after LHRH-analogue injection.

A: Before injection, Large (gl) and middle-sized as well as small granules of various sizes (gm) are distributed in the cell, A part of endoplasmic reticuli (e) is dilated. N; nucleus, Large vacuole (v) can be seen.

B: Five min after LHRH-analogue injection, Large and middle-sized granules

followed by an increase in the later. These results taken together seem to indicate that the testosterone and 11-ketotestosterone play key roles in the process of spermiation.

On the other hand, Kobayashi *et al.*³²⁾ reported the effectiveness of HCG on induction of collectable milt in goldfish, and postulated involving role of testosterone and 11-ketotestosterone in spermatogenesis and $17\alpha,20\beta$ -dihydroxyprogesterone in spermiation. Similar explanations for the action of testicular steroid were presented in salmonid fish.³³⁻³⁵⁾

In the present study, plasma testosterone and 11-ketotestosterone were both increased at 1 h after LHRH-a injection. 11-ketotestosterone continued to increase for 6 h, while testosterone reached maximum levels after 3 h and had decreased at 24 h. The role of $17\alpha,20\beta$ -dihydroxyprogesterone was not clear in this study because of individual variations in plasma concentration. A possible explanation for these observations is that testosterone produced in the testis in response to GtH may be gradually transformed to 11-ketotestosterone and accelerate the spermiation.

The depletion of secretory granules from the gonadotrophs and dilatation of intercellular spaces among the gonadotrophs at 5 min after LHRH-a injection indicate active discharge of the secretory substances. This phenomenon may be causally related to the rapid increase in plasma GtH in the males receiving LHRH-a. On the other hand, within the gonadotrophs of the male at 24 h after the injection, both large granules and vacuoles were observed. Thus, it is possible that the increase in plasma GtH observed after 6 h of injection may be the result of *de novo* synthesis and subsequent discharge of GtH. However, the mechanism for this bimodal discharge of GtH from the pituitary gland after LHRH-a injection is unclear because of insufficient informations concerning biological half-time of exogeneous LHRH or its analogues, we can not conclude that the exogeneous LHRH-a is enough to stimulate GtH secretion from the pituitary gland after several hours. The possibility that the concentration of LHRH-a injected is enough to stimulate GtH secretion from the pituitary gland after several hours should be checked experimentally.

The possibility of involvement of positive feedback system should be also examined.

In conclusion, the present results clearly showed that exogenous LHRH-a can stimulate rapid discharge of stored GtH from the pituitary gland, followed later by androgen production and ultimately, spermiation. From an aquacultural point of view, the technique used in this study seems appropriate to obtain milt from male carps during the spawning season.

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Thave disappeared, and smaller granules (gs) become compact. Numerous intercellular spaces (is) are formed.

C: Twenty-four h after injection, Large and middle-sized granules have increased in number, Endoplasmic reticuli appear to be dilated.

Bars indicate 1 μ m.

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