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Short Communication

STIMULATION OF SPERMATION IN CARP IN RESPONSE TO LH-RH AND D-Ala⁶-LH-RH ETHYLAMIDE

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ABSTRACT

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The present experiment shows that spermiation was stimulated in the common carp following the injection of LH-RH and its alanine⁶ LH-RH ethylamide analog (LRH-A). The response was maximal at doses of 50 µg/kg body weight of LH-RH and 10 µg/kg body weight of LRH-A. Giving a higher dose of LRH-A (50 µg/kg) did not further increase the volume of milt collected.

INTRODUCTION

The number of spermatozoa harvested from the common carp (*Cyprinus carpio*) is usually low compared to the number produced during spermatogenesis and remaining in the testis. In induced reproduction and artificial insemination, it is desirable to increase the number of available spermatozoa by stimulating spermiation. Carp pituitary extracts are usually used for this purpose (Clemens and Grant, 1964, 1965; Rothbard and Rothbard, 1982), but other hormones should be tested. Among these, HCG has proved to be ineffective (F. Takashima, unpublished data, 1981), and LH-RH has been widely used in Chinese carp (Chinese National Bureau of Aquatic Products, 1980).

The present paper compares the effectiveness of synthetic LH-RH's on the production of spermatozoa in carp.

MATERIAL AND METHODS

We used adult male carp raised on the fish-farm at Jouy-en-Josas in recycled, filtered water (Petit, 1974). The fish were about 7 years old and weighed between 1 and 2 kg. They were visually separated from the females and kept under natural photoperiod at 10–13°C throughout the experiment.

The fish, usually fed commercial pellets at a rate of 1% of body weight, were starved during the present experiment which took place between April and May. They were divided into 5 groups each of 10 fish. Two groups were given a daily intraperitoneal injection of LH-RH (Sigma) at a dose of 10 or 50 $\mu\text{g/kg}$ of body weight for 4 days. Two other groups received a similar treatment of LH-RH analog (LRH-A) (D-Ala⁶-Des Gly¹⁰ LH-RH ethylamide, Sigma). Group 5 was given only the solvent (500 $\mu\text{l/kg}$ body weight of a NaCl solution at 7 g/l). The fish were injected at about 10 a.m. On days 4 and 5 we measured the volume of sperm collected by squeezing the abdomen. On day 5 an aliquot of the sperm of each male was diluted by 1/3000 in saline solution at 7 g/l in order to measure the optical density (OD) (spectrophotometer Jobin and Yvon 10 L at 410 nm) and estimate the concentration of spermatozoa. Before any injection or sampling the males were anesthetized with phenoxy-ethanol (0.5 ml/l) and put in a bath of malachite green (15 mg/l for 30 s to 1 min).

The doses were compared statistically by analysis of variance and covariance (body weight and sperm volume). The results on the two neurohormones were compared by a non-parametric method (Kruskal-Wallis test) because the variances were not homogeneous.

Fig. 1. Volume of milt collected in common carp 4 (D4) and 5 (D5) days after injection of 10 and 50 $\mu\text{g/kg}$ body weight of LH-RH and LRH-A. C: control group receiving only the solvent.

RESULTS

The results obtained on the volume of milt harvested, presented in Fig. 1, are expressed in percent of body weight. The volume of collected milt was at D4 lower than at D5. The responses were very similar on both days, i.e. significantly higher production of spermatozoa with 50 μg than with 10 μg of LH-RH, whereas LRH-A responses were not increased at 50 μg in comparison with 10 μg . In all cases, strong individual variations were observed, e.g. at D5 the volume of collected sperm varied between 2.2 and 13.7 ml after treatment with 10 μg of LRH-A. The controls which received only the solvent produced a smaller number of spermatozoa. Spermatozoal concentrations did not vary significantly between treatments, the OD varying between 0.19 ± 0.01 and 0.21 ± 0.03 , i.e., between 20.9 and 23.1 billions of spermatozoa per ml of milt.

DISCUSSION

This study showed that spermiogenesis in the carp was stimulated by administering a gonadotropin releasing hormone. This stimulation resulted in an increase in milt volume and in the number of spermatozoa. The response increased significantly with a dose of mammalian decapeptide (LH-RH). Maximal response to LRH-A was obtained with a dose of 10 $\mu\text{g}/\text{kg}$; no further increase occurred at a dose of 50 $\mu\text{g}/\text{kg}$. These doses were relatively high compared with those which are effective in mammals, but they are lower than that needed to induce ovulation in the common carp (at least 50 μg — Kung pers. commun., 1982; L.W. Crim, K. Bieniarz, R.E. Peter and R. Billard, unpublished data, 1981). At a lower dose (e.g. 1 $\mu\text{g}/\text{kg}$ of LRH-A) the response is very low (F. Takashima, unpubl. data, 1981) in carp, whereas it is greater in other species such as grass carp (Kung, pers. commun., 1982). It should be noted that the water temperature was relatively low in our experiment. LRH-A was also successfully used to advance the onset of spermiogenesis in landlocked salmon (Weil and Crim, 1982, 1983). This method of stimulating spermiogenesis may be used as a test for roughly assessing the biological activity of GnRH, which is easy to use in fish farms, as suggested for carp pituitary extracts (Rothbard and Rothbard, 1982). In fact, the biological activity of various GnRH preparations presently on the market is not indicated, and the fish-farmer and scientist can only refer to the weight of the products. Unfortunately, while this test can be used for LH-RH, it is not entirely valid for LRH-A because the response to the highest dose used here (50 $\mu\text{g}/\text{kg}$) was not increased and, in fact even decreased. Such a suppression of response to a high dose has already been reported for goldfish in which several LRH-A injections at high doses, compared to lower doses, caused a decrease in plasma GtH secretion (Peter, 1980).

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