Temporal Responsiveness of the Ovary of Goldfish to Gonatropin

by

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ABSTRACT

Female goldfish were acclimated to an 8 h light photoperiod and 14-15°C, and, in separate experiments, injected daily for 18 and 14 days with low doses of a partially purified salmon gonadotropin (sGtH) or a highly purified carp gonadotropin (cGtH), respectively at either the onset of light, or 4 or 8 h after the onset of light. The response to GtH injection was measured by the gonadosomatic index (GSI) and by histological analyses of oocyte development. There was a response to sGtH injected at 8 h and to cGtH at 4 h and 8 h after the onset of light. Female goldfish acclimated to a 16 h light photoperiod and injected with low doses of cGtH at either the onset of light or at 4, 8, 12 or 16 h after the onset of light for 14 days had an increased GSI in response to the cGtH injected at 12 h after the onset of light. These results demonstrate that there is a daily variation in responsiveness of the ovary of goldfish to GtH.

INTRODUCTION

Daily cycles in the serum levels of gonadotropin (GtH) of female goldfish are influenced by sexual stage of development, temperature and photoperiod (Hontela and Peter, 1978, 1980). Based on the finding that significant daily cycles in serum levels of GtH are usually present in female goldfish with recrudescing or mature ovaries, whereas such cycles are absent in fish with regressed ovaries, it was hypothesized that daily cycles of GtH are of importance

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Manuscript received March 1981.

Abstracting Keywords: goldfish, gonadotropin, gonad responsiveness, gonad recrudescence.

in stimulating gonadal development (Hontela and Peter, 1978). This is supported by results from pinealectomy experiments. In goldfish and other teleosts undergoing gonadal recrudescence, pinealectomy under long photoperiod and warm conditions causes gonadal regression, whereas stimulation of gonadal development occurs if the fish are under short photoperiod and warm conditions (Fenwick, 1970; Urasaki, 1972, 1973, 1976; de Vlaming, 1975; de Vlaming and Vodcnik, 1978; Vodcnik et al., 1978; Hontela and Peter 1980); Hontela and Peter (1980) found that a daily cycle of serum GtH was absent following pinealectomy of mature female goldfish on a long photoperiod, whereas a cycles was induced in fish on a short photoperiod.

There are significant daily changes in the amount of cyclic 3'5'-adenosine monophosphate accumulated by oocytes of *Mugil cephalus* following treatment *in vitro* with salmon GtH (sGtH), luteinizing hormone (LH), and prostaglandins (Kuo and Watanabe, 1978). A temporal variation in the responsiveness of gonads of teleosts to GtH is further suggested by the finding that there was stimulation of development of gonads of *N. crysoleucas* when sGtH was injected 3 h after onset of light, but not at 10 h, on conditions of 15.5 h light daily photoperiod (15.5 L 8.5 D) and 15°C (de Vlaming and Vodcnik, 1977). However, a temporal variation in responsiveness to injection of LH was not found by these authors.

In the present study temporal variation in responsiveness of the gonads to injection of GtH was investigated in female goldfish.

MATERIALS AND METHODS

Goldfish, *Carassius auratus*, common or comet variety, of about 20 g body weight, were purchased from Grassfork Fisheries Company, Inc., Martinsville, Indiana.

Hormone Preparations

Control fish were injected intraperitoneally (ip) with physiological solution (PS; 25 μ l/5 g body weight). A partially purified salmon GtH SG-G100 (sGtH; Donaldson et al., 1972) was injected ip at a dosage of 1.25 μ g/5 g in experiment 1 (50 μ g sGtH/ml PS).

A highly purified carp GtH (cGtH) was injected ip at a dosage of 0.40 μ g cGtH/5 g body weight (5 μ g cGtH/ml PS) in experiments 2 and 3. The cGtH was prepared as follows: acetone dried carp pituitary powder was extracted by 0.05 M tris buffer in 0.5 m NaCl, pH-7.8, containing 0.1 mM dithiothreitol. Glycoproteins were separated from the extract by adsorption on Conconavale A Sepharose after adjusting the above buffer to contain 1 mM concentration of MgCl₂, CaCl₂ and MnCl₂. 0.15 M α -methyl-D-mannopyranoside was

added to the above buffer to elute the glycoproteins from the column. The glycoprotein fraction was submitted to a second run on the same column. After concentration, the total glycoproteins were chromatographed on a agarose acrylamide Ultrogel ACA₅₄ column, 100 x 5 cm, in 0.05 M tris buffer in 0.15 M NaCl and 1 mM ethylenediamine tetraacetic acid (EDTA), pH 7.8. The biologically active fraction, as determined by the *in vitro* trout oocyte maturation assay (Jalabert et al., 1974), was further purified by adsorption on a Biogel DEAE A cellulose column, 20 x 1.6 cm, in 0.01 M tris buffer, pH 7.8, and elution with a linear gradient of 0.01 M tris buffer, pH 7.8, and 0.4 M NaCl. The final purification of the biologically active fraction was by elution through an ACA₅₄ column, 100 x 1.6 cm, with 0.01 M (NH₄)₂CO₃, pH 8.0. Molecular uniformity of the biologically active fractions from the last two columns was confirmed by polyacrylamide gel electrophoresis.

Experiment 1: Seventy female goldfish were selected in February 1978, when the fish were undergoing ovarian recrudescence. Groups of 10 were held in 45 L aquaria at 14-15°C and 8 L/16 D photoperiod. The fish were fed daily at lights-on and again 4 h later. After 10 days of acclimation injections were started and continued for 18 days; one group of uninjected fish served as normal controls. Injections of PS or sGtH (1.25 µg sGtH/5 g) were given at lights-on, or 4 h or 8 h after lights-on. At the end of the experiment the total body weight and ovarian weight of each fish was determined for calculation of the gonado-

somatic index ($GSI = \frac{\text{gonad weight}}{\text{total body weight}} \times 100$). Differences in GSI between

groups were determined by analysis of variance and Duncan's multiple range test (Steele and Torrie, 1960). In addition, a small segment from the middle of one ovary of each fish was fixed in Bouin's solution, processed by routine paraffin wax histological techniques, and stained with hematoxylin and eosin. The number of oocytes in the primary yolk stage, and secondary and tertiary yolk stages combined (Yamazaki, 1965) in six 1.5-mm² sections of the ovary of each fish were counted. The final results were expressed as the number of oocytes per 1.0 mm², and the differences between groups determined by Duncan's multiple range test. These particular oocyte stages were characterised because primary yolk stage oocytes have just begun active uptake of vitellogenin, whereas tertiary yolk stage oocytes have completed vitellogenesis, and all three stages are dependent on GtH for maintenance and stimulation (Yamazaki, 1965; Upadhyay et al., 1978; Idler and Ng, 1979).

Experiment 2: Seventy female goldfish were selected in December 1978. The experimental conditions and analysis of results were the same as in experiment 1, except that the fish were injected with cGtH (0.40 µg/5 g)* instead of sGtH, and the injections were for 14 days. There was some mortality during the experiment and 10 males were included by error, which accounts for

the discrepancy between the number of fish at the start and end of the experiment.

Experiment 3: One hundred and ten female goldfish were selected in January 1979, and divided into groups of ten, as before. The environmental conditions were 14-15°C and 16L/8D photoperiod. After 12 days of acclimation, injections were started and continued for 14 days; one group served as normal controls. Injections of PS or cGtH (0.40 µg/5 g) were given at lights-on, or 4, 8, 12, or 16 h after lights-on. At the end of the experiment the ovarian GSI was determined and the results analyzed as described above. There was some mortality during the experiment and 7 males were included by error, which accounts for the discrepancy between the number of fish at the start and end of the experiment.

RESULTS

The ovarian GSI response to injection of sGtH at three different times during an 8-h daily light period (experiment 1) is shown in Fig. 1. There was a greater response to sGtH injection given at the end of the light period compared to when it was injected at the start of the light period. Also, the injection of sGtH at the end of the light period caused an increased GSI compared to controls injected with PS at the same time of day. The fish injected with sGtH at 4 h after the onset of light were intermediate and not significantly different from the respective PS-injected controls or from fish injected with sGtH at the onset or end of the light period. The normal control group was not significantly different from any of the experimental groups, perhaps reflecting a suppressive effect on gonadal growth of the stress of daily anesthesia and injection.

Table 1 summarizes the effects on oocyte development for the fish in experiment 1. There was a tendency for a decrease in the number of primary yolk oocytes and an increase in the number of secondary and tertiary yolk oocytes in the fish injected with sGtH at 8 h after the onset of light. This suggests that there was recruitment of primary yolk oocytes into the secondary and tertiary yolk stage oocytes, but the response was not clear in this experiment due to the overlap between groups.

Fig. 2 shows the ovarian GSI of the fish in experiment 2. Injection of cGtH at 8 h after the onset of light caused a significant increase in GSI compared to all other groups except for the group injected with cGtH at 4 h after the onset of

*The same dosage as used by Cook and Peter (1980), but mistakenly indicated by them as 0.125 µg/5 g.

Fig. 1. The gonadosomatic index (GSI) of female goldfish injected daily for 18 days with salmon gonadotropin (sGtH) or physiological solution (PS) at three different times during the light phase of an 8 h daily photoperiod. The number of fish in the group is given in brackets at the bottom of each column, along with the code designating the group. Significance at $P < 0.05$ was tested by Duncan's multiple range test; groups underlined by the same line are not significantly different.

Table 1. Summary of the effects on oocyte development of injecting female goldfish with salmon gonadotropin (sGtH) or physiological solution (PS) daily for 18 days. The injection schedule and code for designating each group is the same as in Fig. 1. Significance at $P < 0.05$ was tested by Duncan's multiple range test; groups underlined by the same line are not significantly different.

Group	N	Primary Yolk Stage Oocytes/mm ²	Secondary and Tertiary Yolk Stage Oocytes/mm ²
Normal (N)	10	3.70 $\pm$ 0.54	1.00 $\pm$ 0.33
PS 0 h (P0)	10	4.69 $\pm$ 0.55	0.49 $\pm$ 0.33
sGtH 0 h (G0)	10	3.04 $\pm$ 0.61	0.64 $\pm$ 0.32
PS 4 h (P4)	10	3.61 $\pm$ 0.39	0.39 $\pm$ 0.26
sGtH 4 h (G4)	10	3.13 $\pm$ 0.60	0.79 $\pm$ 0.32
PS 8 h (P8)	10	3.49 $\pm$ 0.42	0.93 $\pm$ 0.35
sGtH 8 h (G8)	10	2.69 $\pm$ 0.64	1.66 $\pm$ 0.38
		G8 G4 P8 P4 N G0 P0	P0 G0 G4 P4 P8 N G8

Fig. 2. The gonadosomatic index (GSI) of female goldfish injected daily for 14 days with carp gonadotropin (cGtH) or physiological solution (PS) at three different times during the light of an 8 h daily photoperiod. For further details see the caption to Fig. 2.

light. Injection of cGtH at 4 h after the onset of light caused a significant increase in GSI compared to all but one of the control groups.

Table 2 shows that the group injected with cGtH at 8 h after the onset of light had fewer primary yolk oocytes than all the other groups. This same group had a greater number of secondary and tertiary yolk oocytes than all other groups except for the group injected with cGtH at 4 h after the onset of light, which itself had a greater number of oocytes at these stages than all the control groups. This confirms that the groups with the increased GSI had advancement in oocyte development.

Fig. 3 shows the ovarian GSI response to injection of cGtH at five different times during the light phase of a 16L/8D photoperiod. The fish injected with cGtH at 12 h after the onset of light had a significantly greater GSI than all other groups.

Table 2. Summary of the effects on oocyte development of injecting female goldfish with carp gonadotropin (cGtH) or physiological solution (PS) daily for 14 days. The injection schedule and code designating each group is the same as in Fig. 2. Significance at $P < 0.05$ was tested by Duncan's multiple range test; groups underlined by the same line are not significantly different.

Group	N	Primary Yolk Stage Oocytes/mm ²	Secondary and Tertiary Yolk Stage Oocytes/mm ²
Normal (N)	6	5.20 ± 0.29*	0
PS 0 h (P0)	5	5.94 ± 0.31	0
cGtH 0 h (G0)	9	4.99 ± 0.59	0.80 ± 0.29*
PS 4 h (P4)	8	5.27 ± 0.52	0
cGtH 4 h (G4)	5	4.35 ± 0.56	1.41 ± 0.40
PS 8 h (P8)	4	5.25 ± 0.23	0
cGtH 8 h (G8)	7	2.67 ± 0.60	1.81 ± 0.40
		<u>G8 G4 G0 P8 P4 P0 N</u>	<u>N P0 P4 P8 G0 G4 G8</u>

* Values are mean ± standard error.

Fig. 3. The gonadosomatic index (GSI) of female goldfish injected daily for 14 days with carp gonadotropin (cGtH) or physiological solution (PS) at five different times during the light of a 16 h daily photoperiod. For further details see the caption to Fig. 2.

DISCUSSION

The results demonstrate that there is a temporal variation in responsiveness to GtH by the ovaries of goldfish. Female goldfish acclimated to a short photoperiod (LD = 8:16) and 14-15°C had a greater response to injection of low doses of sGtH at 8 h after the onset of light, and to cGtH at 4 h and 8 h after the onset of light, compared to injection at the onset of light. Goldfish acclimated to a long photoperiod (LD = 16:8) and 14-15°C had a marked temporal variation in responsiveness to cGtH, responding to cGtH injected at 12 h after the onset of light, and not at other times during the light phase. This confirms and extends the previous suggestion that a temporal variation in responsiveness to injection of GtH occurs in teleosts (de Vlaming and Vodicnik, 1977).

Female goldfish, undergoing ovarian recrudescence or with a mature ovary, exposed to a long photoperiod (LD = 16:8) and 12°C have a daily apogee in serum GtH levels at about 8 h or 12 h after the onset of light (Hontela and Peter, 1978). The timing of this peak in serum GtH levels corresponds well with time of day that female goldfish exposed to LD = 16:8 and 14-15°C were responsive to injection of low doses of cGtH. Cook and Peter (1980) have shown that injection of cGtH, at the same dosage as used here, in goldfish acclimated to 12°C causes high blood levels and a peak in blood levels of GtH at 1 and 3 hours, respectively, after the ip injection, and by 24 hours after injection the blood levels were decreased to near that in controls. Thus, there probably is coincidence in the timing of the daily cycle of serum GtH levels found by Hontela and Peter (1978) and the time of high blood levels of GtH following injection of cGtH at 12 hours after the onset of light in fish acclimated LD = 16:8 and 14-15°C in the present work. However, the data from experiments on goldfish acclimated to a short photoperiod do not correspond so closely. Goldfish, with a mature ovary, exposed to a short photoperiod (LD = 8:16) and 12°C had apogees in serum GtH levels at the onset of light and 4 h after the offset of light; fish with recrudescing ovaries had a similar cycle but the variations were not statistically significant (Hontela and Peter, 1978). In the present work, female goldfish exposed to LD = 8:16 and 14-15°C were responsive to injection of low doses of sGtH or cGtH at the end and middle of the light phase. Perhaps, in the present work, the stress of daily anesthesia and injection altered endogenous hormone cycles and thereby altered the gonadal cycle of responsiveness to GtH. On the other hand, perhaps the daily apogees in serum GtH in goldfish on LD = 8:16 and 12°C found by Hontela and Peter (1978) do not correspond well with the time of greatest ovarian responsiveness in fish under these conditions; gonadal recrudescence in goldfish in highly stimulated by long but not short photoperiods (for review see Hontela and Peter, 1978, 1980). Irrespective of the presence or absence of correlation between these two sets of data, the present findings provide support for the hypothesis

that daily cycles of secretion of GtH are of importance in stimulating gonadal activity in goldfish.

Teleost fishes have two GtH's, one, relatively high in carbohydrate content, which has ability to induce oocyte maturation, ovulation, spermiation, steroidogenesis and cyclic AMP activity, and a second, relatively low in carbohydrate content, that stimulates the uptake of vitellogenin by oocytes and, to a greater or lesser extent depending on the species, steroidogenesis (Idler et al., 1975; Idler and Ng, 1979; Ng and Idler, 1979). The GtH measured by radioimmunoassay by Hontela and Peter (1978, 1980) and the cGtH used in the present study (see Materials and Methods) are equivalent to the GtH that has a high carbohydrate content and the broad spectrum of biological activity. The sGtH used in the present study (SG-G100) can restore sexual function in hypophysectomized goldfish (Donaldson et al., 1972), and it is likely that this preparation contains two GtH's with division of actions similar to the two GtH's described above (Idler et al., 1975; Idler and Ng, 1979; Ng and Idler, 1979), as well as other contaminations (B. Breton, unpublished results). The similarity of the response by the goldfish to the different dosages of cGtH ($0.40 \mu\text{g}/5 \text{ g}$) and sGtH ($1.25 \mu\text{g}/5 \text{ g}$) in the present study can be accounted for by the contaminants in the sGtH and perhaps also to some difference in responsiveness of goldfish, a cyprinid fish, to salmonid versus cyprinid GtH. Nevertheless, the present study demonstrates that there is a daily variation in responsiveness of the ovary to the GtH high in carbohydrate content, but since we did not use the GtH low in carbohydrate content alone in any experiments we are not able to conclude whether there is a temporal variation in responsiveness to it as well.

The temporal variation in ovarian responsiveness to the high carbohydrate-content GtH in goldfish presumably reflects a daily cycle of receptors. What induces such a daily cycle of responsiveness is not known. It may be that the variation in responsiveness is inherent to the gonads. This would seem unlikely, however, since the gonads would then have cyclical responsiveness to this GtH independent of external factors that could synchronize or induce the cycle. A more probable basis for the temporal variation in responsiveness is that it is induced by variations in blood levels of this GtH that must phase in a certain way with some other physiological cycle to effect a response in the gonads. A likely candidate in this regard is the low carbohydrate-content GtH; unfortunately this "second" GtH is not readily available for study. Other hormones may also be involved. Thyroid hormone has a synergistic action with GtH during ovarian recrudescence in goldfish (Hurlburt, 1977), and it can synchronize the temporal fattening response to injections of prolactin in killifish (Meier, 1970). In addition, temporal synergism between cortisol and prolactin in Gulf killifish (Fivizzani and Meier, 1976), and corticosterone and prolactin in sparrows (Meier et al., 1971) occurs such that when daily injections of the hormones are phased in a certain way development of the testes is

stimulated. Also, corticosteroids have been demonstrated to sensitize oocytes of goldfish to the maturational effects of GtH (Jalabert, 1976). However, our experiments indicate that corticosteroids do not synchronize the temporal variation in responsiveness of the gonads of goldfish to GtH (R. Peter and C. Paulencu, unpublished results). Additional studies are needed to solve this problem.

ACKNOWLEDGEMENTS

This work was supported by NATO research grant 1305 and Natural Sciences and Engineering Research Council of Canada grant A6371 to R.E.P. We thank N. E. Stacey for reviewing the manuscript and C. Nahorniak for technical assistance.

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