DAILY CHANGES IN THE GONADOTROPIN LEVELS AND RESPONSE OF CARP OOCYTES TO HYPOPHYSIAL HOMOGENATE

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Abstract—Daily changes in carp gonadotropin levels in adult female carp and daily changes in carp oocyte sensitivity to carp hypophysial homogenate, *in vitro* and *in vivo*, were investigated.

A total of three series of experiments were carried out. Gonadotropin levels were radioimmunologically determined.

The results of series 1 and 2 experiments were subjected to statistical analysis with the use of cosinors circle and ellipse of errors. It has been found that in the mature female carp in the pre-spawning period with the light periods being long (L:D = 16:8) the apogee for gonadotropin occurs 10 hr after the onset of the light period.

The sensitivity of the oocytes, in terms of the percentage of mature oocytes (after GVBD) following a 24-hr incubation of ovarian fragments with the hypophysial homogenate, reached the highest value at 1300, i.e. 9 hr after the onset of the light period.

It was also found that the injections of carp hypophysial homogenate made at 0900 were much more efficient in inducing ovulation than those at 2100.

Key words—Carp, circadian, gonadotropin, oocytes, rhythm, sensitivity.

Introduction

The levels of many hormones in blood plasma, such as cortisol (1) or gonadotropin (GtH) in goldfish (*Carassius auratus*) undergo rhythmical changes during the day (2), and are connected with the stage of gonadal maturity since they occur only in specimens with mature gonads. A stage of gonadal maturity also affects daily fluctuations in catecholamin levels in the brain of eel (*Anguilla anguilla*) (3) (Popek; unpublished observations) and the feed-back between steroid hormone levels and gonadotropin release in rainbow trout (*Salmo gairdnerii*) (4, 5) and goldfish (6). Some other studies (Bieniarz *et al.*: unpublished observations) have revealed that the levels of steroid hormones, e.g. estradiol or 11-ketotestosterone in carp in the prespawning period, also undergo rhythmical changes during the day.

The above data suggest that daily variations in the gonadotropin levels may be an important stimulus in gonadal development. Those findings have been confirmed by De Vlaming and

Vodicnik (7) who stated that a stimulating effect of salmon gonadotropin (SG-G100) on the gonads of *Notemigonus crysoleucas* occurred only when injections were made 3 hr after the onset of the light period at water temperature of 15°C and L:D = 15.5:8.5. Similar injections made 10 hr after the onset of the light period produced no results.

All these experiments indicate that parallel with daily changes of GtH levels in blood plasma, similar daily changes in ovarian sensitivity, or even of individual oocytes to gonadotropin should be expected. The latest studies by Peter *et al.* (8) seem to confirm those suggestions since they have shown that the response of goldfish oocytes to GtH injections undergoes rhythmical variations during a 24-hr period.

The aim of the present study was to trace daily variations in the levels of gonadotropin in carp, a fish of high economic value in many countries, and to estimate how far the oocyte sensitivity corresponds to those changes both *in vitro* and *in vivo*.

Since hormonal stimulation of sexual maturation and ovulation in carp is becoming more and more popular, our present investigations may be of practical value.

Material and Methods

5- and 6-year-old female carp (*Cyprinus carpio* L.) weighing 4–5 kg were used. Studies were conducted in late May and early June by the Department of Ichthyobiology and Fisheries, Academy of Agriculture, Kraków-Mydlniki. The ratio of the light to dark period was 16:8 (the onset of the light period at 0400). A total of three series of experiments were performed.

The fish used in series 1 and 2 experiments were kept in concrete containers of 2 m³ in which the water was changed four times during 24 hr. Those used in series 3 were kept isolated by metal netting in part of a conventional carp pond (occupying an area of 6 m², 0.8 m deep). The temperature of the water in the containers and the pond was in the range 18–20°C, oxygen content was in the range 6–8 mg/l. Before starting the experiments the fish were acclimated to the above-mentioned conditions for a period of 10 days.

Series 1. Daily variations in gonadotropin levels in the blood of mature female carp

Three experiments were performed. A total of 130 female carp were divided into 13 groups (1–13) of 10 placed in various concrete containers. From the group 1 fish, 1 ml of blood was sampled during 24 hr every 4 hr, beginning at 1000, i.e. experiment I. From group 2 blood was taken during 24 hr also every 4 hr, but starting at 1200, i.e. experiment II.

From the fish of the remaining groups blood was sampled only once in each group, beginning at 1400 for group 3, at 1600 for group 4, at 1800 for group 5, etc. up to 1000 on the following day for group 13. An interval of 2 hr of blood sampling was applied, i.e. experiment III.

Thus, in experiments I and II, blood was sampled every 4 hours from the same fish, while in experiment III it was done every 2 hours but each time from a fish of a different group. For schematic presentation of series 1 see Table 1.

Blood (1 ml) was taken with heparinized syringe from the caudal veins. After centrifugation the plasma samples were frozen at –20°C and sent in dry ice to the Laboratoire de Physiologie des Poissons INRA in Rennes (France), where gonadotropin level was radio-immunologically determined (9). According to a specific method reported earlier.

Series 2. Daily variations in the oocyte response to carp hypophysial homogenate

Two experiments were performed, and a total of 12 female carp were used.

Experiment I. From six females every 4 hr during 24-hr ovarian fragments were taken *in vivo* (10) with a trocar (300–450 oocytes after a completed process of vitellogenesis) starting at 0900.

Experiment II. From the remaining six females, every 4 hr during 24-hr ovarian fragments were taken *in vivo* with a trocar, starting at 2100.

In both experiments the ovarian samples taken every 4 hr from each female were divided immediately into three parts (c. 100–150 oocytes each). One part was cleared in Serr's liquid to determine maturation stage based on the position of the nucleus in the oocyte immediately after sampling. The second part was placed in incubation dishes with 2 ml of culture medium Basic Salt Solution (BSS) Cortland at pH 7.6 with streptomycin and penicillin in the amount of 100 i.u./1 ml supplemented with carp hypophysial homogenate (chh) 100 µm/ml for a 24-hr period. The third part (control group) was placed in the same medium as mentioned above, also for a 24-hr period, however, instead of chh a salt solution (0.9% NaCl) was used. Following a 24-hr incubation, from the moment the ovarian fragments were placed in the medium, ovarian fragments were fixed and cleared in the Serr's liquid and the stage of oocytes maturity was estimated by calculating the percentage of oocytes after germinal vesicle break down (GVBD). For schematic presentation of series 2 see Figure 1.

Carp hypophysial homogenate was made of

Table 1. Design of series 1

Series 1	Times of sampling													
	1000	1200	1400	1600	1800	2000	2200	2400	0200	0400	0600	0800	1000	1200
Experiment I <i>n</i> = 10	Group 1		Group 1		Group 1		Group 1		Group 1		Group 1		Group 1	
Experiment II <i>n</i> = 10	↓	Group 2		Group 2		Group 2		Group 2		Group 2		Group 2		Group 2
Experiment III <i>n</i> = 130	↓	↓	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Group 10	Group 11	Group 12	Group 13

Figure 1. Design of experiment 1, series 2, start at 0900. Experiment 2 was made according to the same design except start of sampling which was at 2100. K¹, Ovarian fragments in which the percentage of oocytes after GVBD was stated immediately after sampling; P, Ovarian fragments in which the percentage of oocytes after GVBD was defined after 24 hr of incubation in BSS Cortland with carp hypophysial homogenate; K², Ovarian fragments in which the percentage of oocytes after GVBD was stated after 24 hr of incubation in BSS Cortland alone, without any hormone.

the hypophyses of 3-year-old carp, which had been killed 2 years before the start of the experiments. The collected hypophyses after being fixed in acetone, were dried, powdered and thoroughly mixed. The same chh was also used in series 3. Up to the moment of being used in the experiment it was kept in a hermetic vessel at -4°C.

Series 3. Sensitivity of carp oocytes in vivo to carp hypophysial homogenate

For the experiments a total of 24 female carp kept in an isolated part of the carp pond were used. They were divided into two groups of 12 fish and subjected twice to hypophysation. Group 1 fish were given a first intramuscular injection at 0900 in the amount of 0.5 mg of chh per 1 kg body weight, and 24-h later a second injection 4 mg of chh per 1 kg body weight.

Group 2 fish were injected in a similar way but the injections were made at 2100.

Twenty four hours after the second injection a check-up examination of females (through the massage of abdominal walls for a possible occurrence of ovulation) was made.

The results of the first and second series of experiments were analysed statistically using the cosinor analysis (11) whereas the results of series 3 were expressed as percentages of fish in which full or partial ovulation had occurred.

Results

Series 1

No occurrence of circadian changes in the blood gonadotropin levels of female carp was found in experiments I and II, in which blood samples had been taken from the same fish. The GtH level was in the range 3.38–15.34 ng/ml in experiment I and 2.36–4.82 ng/ml in experiment II at various times of a 24-hr period.

In experiment III an occurrence of the circadian changes in the blood gonadotropin levels of female carp was stated. The highest value of GtH level (15.51 ng/ml) was found at 1400 (acrophase 10.246°, amplitude 2.18), i.e. 10 hr after the onset of the light period (Figure 2). The lowest values (3.38 and 2.36 ng/ml) were found at 1000 and 1200.

Series 2

In experiment I an incidence of the circadian changes as the oocyte response to chh was found. The greatest increase in the percentage of mature oocytes (after GVBD) as a result of a 24-hr incubation with chh (29.0) was recorded in the ovarian fragments taken at 1300 (acrophase 8.528°, amplitude 4.9688), i.e. 9 hr after the onset of the light period (Figure 3), the lowest one (8.0) being recorded at 0500.

No statistically significant changes were found in the percentage of mature oocytes in the control groups. In group K₁: max. 7.6 at 2100, min. 5.0 at 0900; in group K₂: max. 22.3 at 1700, min. 5.0 at 0500.

In experiment II an incidence of the circadian changes at the oocyte response to chh was found. The greatest increase in the percentage of mature

oocytes (after GVBD) following a 24-hr incubation with chh (30.1) was recorded in the ovarian fragments sampled at 1300 (acrophase 10.627^h , amplitude 10.22), i.e. 9 hr after the onset of the light period. The lowest one (9.0) was recorded at 2100 (Figure 4). No statistically significant changes in the percentage of mature

oocytes were stated in the control groups. In group K_1 : max. 20.7 at 1300, min. 5.0 at 0500; in group K_2 : max. 24.5 at 1300, min. 9.5 at 0100.

Series 3

In the fish of group 1 (injection at 0900) complete ovulation was found in six females

Figure 2. Circadian changes in carp blood gonadotropin of mature carp females. G_1 , Samples taken during 24 hr, every 4 hr from the same fish, start at 1000; G_2 , Samples taken during 24 hr, every 4 hr from the same fish, start at 1200; G_3 , Samples taken during 24 hr, every 2 hr from different fish, start at 1000.

Key to ellipses Series I	<i>n</i>		Mesor	Amplitude C	Acrophase ϕ
	Females	Blood samples			
Experiment I	10	70	3.6251	1.0173	9.453 ^h
Experiment II	10	70	4.7476	1.6647	5.248 ^h
Experiment III	130	130	5.1826	2.1803	10.246 ^h

(50%) from which 180–350 cm³ per fish of the ovulated oocytes were obtained. In one female (9%) a partial ovulation was stated and 150 cm³ of ovulated oocytes obtained. In the remaining five females (41%) no ovulation was found.

In the fish of group 2 (injection at 2100) a

complete ovulation was stated in two females (12%) with 300 and 350 cm³ of ovulated oocytes. Partial ovulations were also ascertained in two females (12%) with 100 and 150 cm³ of ovulated oocytes. In eight females (66%) ovulations were not stated.

Figure 3. Circadian changes in carp oocyte sensitivity to carp hypophysial homogenate. Experiment I, series 2: K₁, Percentage of oocytes after GVBD defined before incubation *in vitro*; P, Percentage of oocytes after GVBD defined after 24 hr of incubation in BSS Cortland with hypophysial homogenate; K₂, Percentage of oocytes after GVBD defined after 24 hr of incubation without hypophysial homogenate.

Key to ellipses		n		Mesor	Amplitude	Acrophase
Series 2		Females	Ovarian samples		C	φ
1	2	3	4	5	6	7
Experiment I	K ₁	6	6	6.0206	1.0486	1.305 ^h
	P		42	12.7933	4.9688	8.528 ^h
	K ₂		42	11.5070	4.1154	8.651 ^h

Figure 4. Circadian changes in carp oocyte sensitivity to carp hypophysial homogenate. Experiment 2, series 2. For explanation see Fig. 3.

1	2	3	4	5	6	7
Experiment II	K ¹		6	9.3548	4.1015	8.170 ^h
	P	6	42	16.3765	10.2187	10.627 ^h
	K ²		42	16.0467	4.5346	9.974 ^h

Discussion

Our experiments have proved that in female carp during the pre-spawning period distinct circadian changes occur in the gonadotropin levels as well as the corresponding circadian changes in the response of the oocytes to gonadotropin.

This study has shown that in the mature female carp in the pre-spawning period with the

light days being long (L:D = 16:8) the apogee for gonadotropin occurs 10 hr after the onset of the light period. It is consistent with the studies by Hontela and Peter (12) who have found that in goldfish, a species closely related to the carp and spawning under similar conditions, namely during a long light day, with mature ovaries, the GtH apogee is reached 8–12 hr after the onset of the light period with L:D = 16:8.

Thus, it can be said, that in the two mentioned

fish species the circadian changes in the gonadotropin levels of mature females are very similar. The sensitivity of the oocytes to GtH in terms of percentage of mature oocytes (after GVBD) following a 24-hr incubation of ovarian fragments with the hypophysial homogenate, reached the highest value at 1300, i.e. 9 hr after the onset of the light period. Those results are consistent with the studies by Peter *et al.* (8) and confirm the conclusions obtained from the studies on goldfish that the sensitivity of the oocytes to carp gonadotropin undergoes changes during 24 hr. In spite of the fact that in this study a different species of fish was used and a new method of investigation was applied, the oocytes in both cases have shown the greatest sensitivity to the carp gonadotropin a few hours after the onset of the light period; in the goldfish it occurred after 4–8 hr with the light day being short (L:D = 8:16) and after 12 hr with the longer light period (L:D = 16:8) while in the case of carp it was 9 hr after the onset of the light day.

Similar variations in the oocyte sensitivity to salmon gonadotropin (SG-G100) were found by Kuo and Watanabe (13) in *Mugil cephalus*. In those studies the highest concentration of the cyclic AMP was stated in the oocytes incubated with salmon gonadotropin 3 and 12 h after the onset of the light period. Studies by Kuo and Watanabe, however, conducted on a species of fish placed in taxonomy far from the carp and goldfish, although based on a slightly different technique (oocyte sampling a few times daily: their incubation *in vitro* among others with

salmon gonadotropin in culture medium) suggest that this kind of circadian rhythm in the oocyte sensitivity is a characteristic feature of most teleosts.

The data obtained in series 1 and 2 experiments concerning circadian changes in the gonadotropin level and oocyte sensitivity to hypophysial homogenate, have found full support in the *in vivo* studies conducted in series 3 experiments. Those studies have demonstrated that the hypophysial homogenate injected to carp females in the period in which sensitivity of oocytes to GtH is at its highest (experiments *in vitro*) gives much better results in terms of percentage of the ovulated females than injections given to them during the day when the sensitivity of the oocytes to the hypophysial homogenate is at its lowest.

No circadian changes of gonadotropin were found when blood sampling during 24 hr concerned the same females (series I—experiments I and II). It may be explained only by a stress resulting from frequent captures of fish from the basin (seven times during 24 hr).

The fact of finding a low percentage of females, which ovulated as a result of an injection of the hypophysial homogenate at 2100, seems to support the view that it is the time during 24 hr in which the oocytes display the lowest sensitivity to the hypophysial homogenate.

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