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STUDIES ON MECHANISMS OF RELEASING PITUITARY GONADOTROPIN (GtH2) FROM CARP (*Cyprinus carpio*) HYPOPHYSIS

Piotr Epler, Krzysztof Bieniarz, Włodzimierz Popek, Mirosława Sokołowska-Mikołajczyk, Tomasz Mikołajczyk

Department of Ichthyobiology and Fisheries Academy of Agriculture, Kraków

A B S T R A C T. Levels of GtH2 and of GnRH in carp females and males were investigated during a 4 year period in relation to the gonadal maturation. GtH in males remained at the level of 10-20 ng ml^{-1} and maximum occurred at the commencement of spermination. The level of GtH in the pituitary gland increased with the fish age. The GtH2 level in females changed slightly (9.97 - 33.25 ng ml^{-1}) during the investigation except at the oocyte vacuolisation stage (161.4 - 214.51 ng ml^{-1}). GtH2 accumulation in the pituitary gland increased since the 13th month, from 2.64 to 617.35 ng/ml of the homogenate, and of GnRH since the 33rd month, from 0.6 to 9.55 ng/ml. The *in vivo* experiments on gonadotropin (GtH2) release from carp hypophysis proved that NPY alone raised gonadotropin secretion and intensified GnRH action, but GABA affected GtH2 secretion through the dopaminergic system decreasing the hormone secretion. Stress induced by capture and injections during artificial spawning did not influence the level and rhythm of GtH2 release from pituitary glands, thus allowing to disregard this factor in assessing artificial spawning success.

Key words: GtH2, COMMON CARP, PITUITARY GLAND, NPY, GABA, STRESS

INTRODUCTION

Sexual maturation in fish is controlled by internal factors (hypothalamus, pineal gland, pituitary gland, gonads), the activities of which are regulated by environmental parameters (Bieniarz, Epler 1992 - a review). It has been finally established that there are two gonadotropins in fish: GtHI (mainly controlling vitellogenesis), and GtHII which regulates the last stages of oocyte maturation and ovulation (Suzuki et al. 1988). In the hypothalamus of fish several releasing hormones (GnRHs) were found: chicken II (cGnRH), salmon (sGnRH), catfish I and II (cfGnRH), which release gonadotropins (Conn et al. 1991, Peter et al. 1990). However, sexual maturation processes in fish are subjected to a more complicated regulation involving such factors as neuropeptide (Breton et al. 1989; Breton et al. 1990), GABA-ergic system (Roelants et al. 1990), and cholinergic system (Mikołajczyk et al. 1993). It was also shown that calcium was an intracellular mediator at stimulated GnRH and spontaneous release of GtH2 from gonadotrops (Mikołajczyk et al. 1990 a and b).

The aim of this paper is a presentation of further investigations on the regulation of gonadotropin (GtH2) releasing processes in carp in the biological cycles as well as under the influence of selected internal and external factors.

RESEARCH DESCRIPTION

Five investigations are reported in the sections below.

I. Studies on GtH2 level in hypophysis, GnRH in hypothalamus and hypophysis during growth and maturation of females (Bieniarz et al. 1992) and males (Billard et al. 1992) of carp (*Cyprinus carpio*)

The investigations were carried out on 500 females and 500 males of carp. Blood samples from the caudal vein and the pituitary gland and hypothalamus of carp aged 5 to 46 months were taken once in 3 months. GtH2 level in blood and hypophysis was determined using RIA method according to Breton et al. (1971), and the GnRH content in hypothalamus and hypophysis according to Breton et al (1986). Gonad samples were collected and their maturity stages were assessed in histological preparations according to the scale of Sakun and Bucka (1968).

It was discovered that GtH2 level in all the examined fish females varied from 9.79 to 33.25 ng ml^{-1} irrespective of maturity stage. The only increase of the gonadotropin level was observed between 38 and 39 month, at the period of oocyte vacuolisation (138 - 198 ng ml^{-1}). The GtH2 level in the pituitary gland began to increase from 2.64 in the 13th month of life to 617 ng ml^{-1} in the 52 month. The content observed in the hypophysis increased between 33rd and 52nd month of life from 1 to 3.55 μg ml^{-1}. GnRH level in hypothalamus did not show any synchronisation with age but varied considerably, from 0.06 to 412 μg per hypothalamus. Statistical analysis revealed that there was correlation only between age and GnRH content in the pituitary gland.

A relatively high GtH2 level was discovered in the blood of males (ca. 10 ng ml^{-1}) between the 5th and 10th month of life, at the time of no testicular development and no hormone accumulation in the hypophysis. Until month 19, GtH2 level in fish remained at an average level of 13.2 ng ml^{-1} of plasma. GtH level in hypophysis was from 2.03 to 4.8 μg per gland. From the month 19 to 37, gonad development and a stable GtH level, varying from 10 to 20 ng ml^{-1} of blood plasma, were observed. As from the month 38 in males, a consistently high gonadotropin level was observed, of the order of 130 - 200 ng ml^{-1}, which returned to the pre-spawning quantities from the

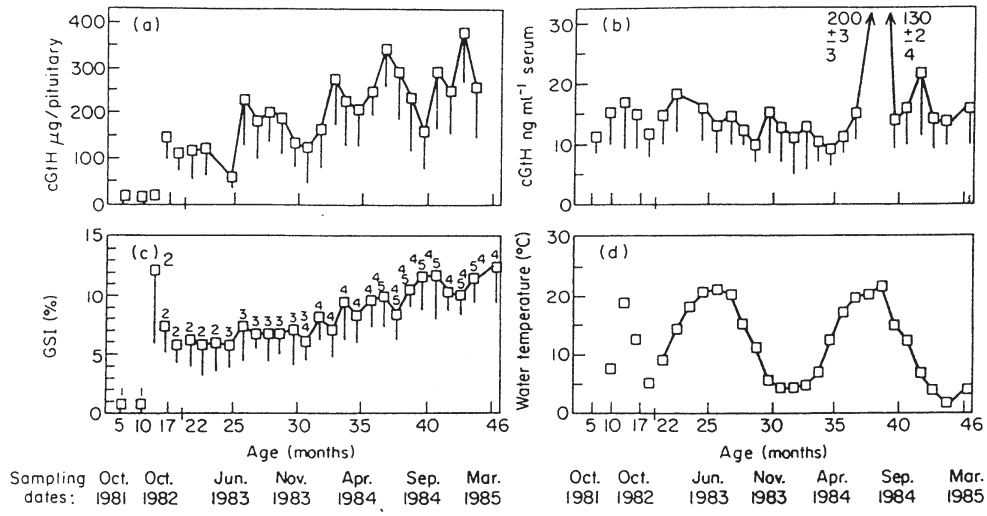

Fig. 1. Changes in some parameters measured in male carp for a period of 4 years post-hatching (fish were immature in October 1981 and March 1982 at 5 and 10 months of age, and males were mature for the rest of the time). (a) Total c-GtH content in the pituitary, (b) c-GtH concentration in bloodstream, (c) GSI, (d) Water temperature: daily average temperature from the previous sampling. From 5–22 months sampling was at 3–6 month intervals; from 22–44 months sampling was every month. Average values are given with S.D.

month 40-41 of life (September - October). Afterwards, low GtH2 level remained until March when the investigation terminated (Fig. 1). Mean values of GtH2 in the hypophysis fluctuated and varied greatly among individuals, although there was an upward trend in gonadotropin concentration with age of the fish.

Determinations of the sGnRH began in the month 35 and lasted till 46th month. They revealed a high concentration in hypophysis (6 µg per gland) in the period 35th - 42nd month, and relatively low in other months (Fig. 2). GnRH level in hypothalamus remained low from 38th to 43rd month, reaching a maximum (above 2 µg/gland) in the month 44 (February) when the GnRH level in hypophysis was the lowest (Fig. 3).

II. Influence of neuropeptide Y on gonadotropin release in teleost fishes (Breton et al. 1991)

Carp females aged 6 years, and mature rainbow trout females were treated during the period around spawning. The females were administered either 3 µg NPY or physiological solution to the IIIrd brain ventricle, using the stereotactic method described by Sokołowska (1982). Physiological solution or LHRH-A was injected intrape-

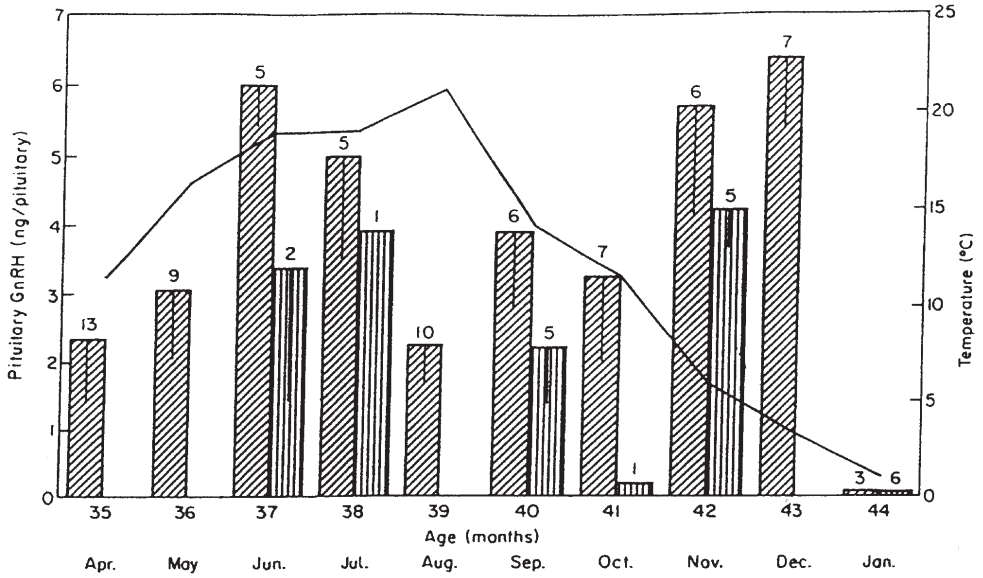

Fig. 2. Changes in blood 11-ketotestosterone during growth and seasonal maturation of male carp. Values a-re mean and S.D. Numbers above bars are the numbers of specimes. Maturity stages trace average daily temperature for the previous month.

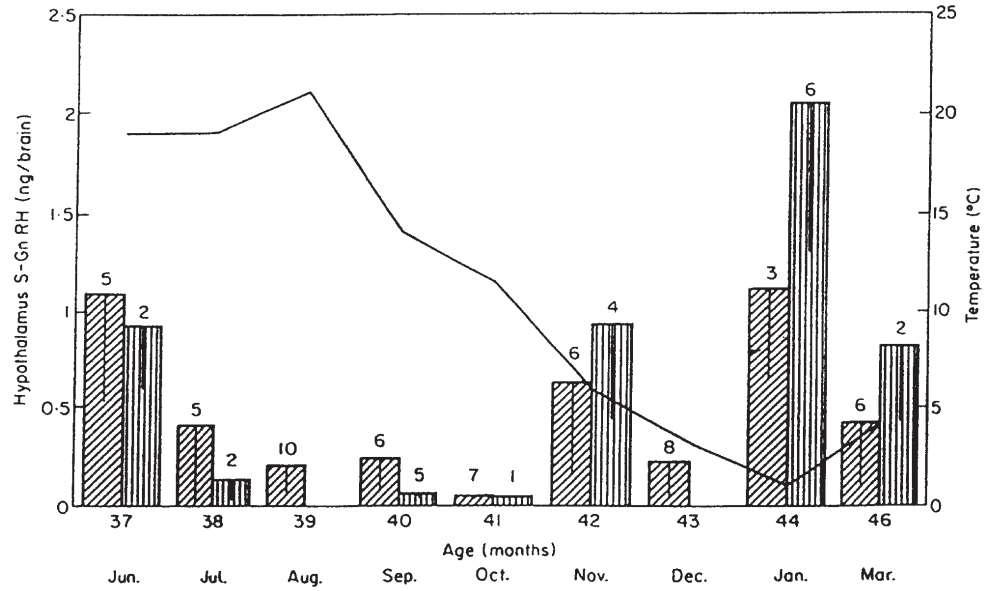

Fig. 3. Changes in blood 17α hydroxy 20β dihydroprogesterone during growth and sexual maturation of male carp. Values are mean S.D.

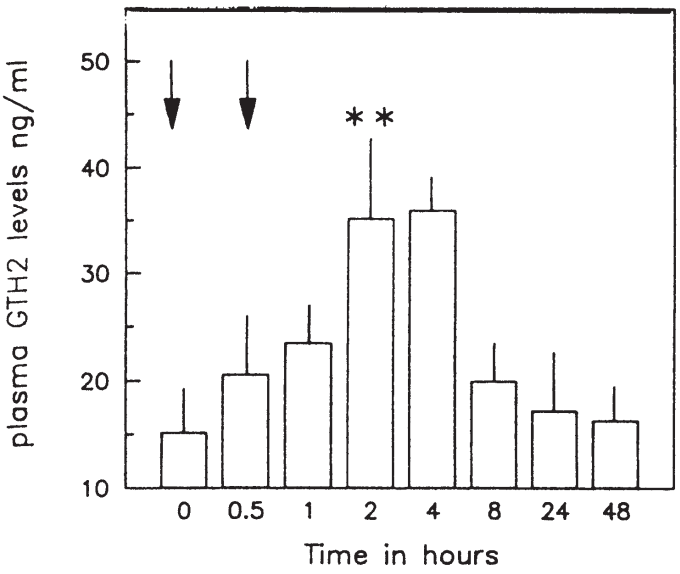

Fig. 4. Effects of icv infusion of neuropeptide Y (NPY) on the GtH2 secretion in the common carp *Cyprinus carpio*. Arrows indicate the time of injection; first, icv; second, saline ip.
**Statistically higher than time O; P<0.01.

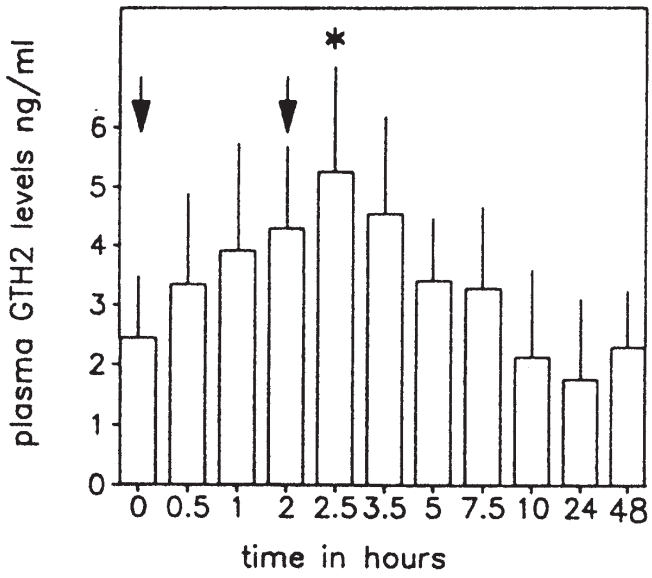

Fig. 5. Effect of intraperitoneal injection of neuropeptide Y (NPY) on the GtH2 secretion in the rainbow trout. Arrows indicate the time of injection; first, NPY injection 20 μg/kg body wt, second, saline injection,
*Statistically higher than time O; P<0.05.

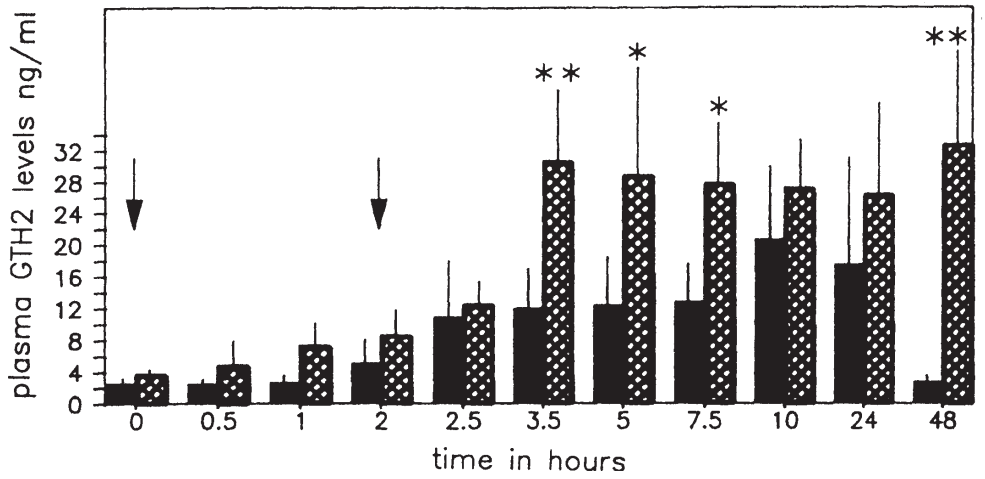

Fig. 6. Effects of intraperitoneal injection of neuropeptide Y (NPY) on the response to intraperitoneally injected LHRHa in the rainbow trout. The arrows indicate the time of treatments. Hatched bars correspond to Group IV, receiving first NPY then LHRHa alone at time 2 hr. Statistically higher in Group IVt than in Group IIIt; **P<0.01, *P<0.05.

ritoneally during three minutes after neuropeptide Y administration. Four groups of rainbow trout females were given intraperitoneally at time 0 and 2 h later the following: group I - physiological solution, group II - 20 μg kg^{-1} NPY and physiological solution, group III - 20 μg kg^{-1} LHRH-A and physiological solution, group IV - 20 μg kg^{-1} NPY and 20 μg LHRH-A. Blood from the caudal vein was sampled up to 48 h and GtH2 level was determined by means of RIA method according to Breton et al. (1978).

Results of these experiments showed that in both species NPY brought about a twofold increase of GtH2 level, with a maximum 2 - 4 h after injections (Fig. 4 and 5). After 8 h the gonadotropin level dropped to the basic secretion value. When NPY was injected first, followed by LHRH-A, GtH2 level increased much more than in the cases of separate NPY or LHRH-A administration (Fig. 6).

III. The effect of bicuculline (a $GABA_A$ receptor antagonist) on GtH2 level in fish stimulated *in vivo*

The aim of this study was an assessment of GABA-ergic contribution to the stimulation of gonadotropin release from carp hypophysis, in the case of its individual action as well as in connection with LHRH-A, using bicuculline as a blockage of receptors through which the GABA (*g* - aminobuthyric acid) operates. The research was conducted on 65 mature carp females of the mean weight of 3.6 kg, transferred from

TABLE 1

Desing of experiments

Group	Number of fish		First injection (-2 hr)	Second injection (0 hr)
	Exp. 1	Exp. 2		
1	8	8	SAL + VEH	SAL
2	8	8	PIM + VEH	SAL
3	8	8	SAL + BIC	SAL
4	8	8	PIM + BIC	SAL
5	8	8	SAL + VEH	LHRH-A
6	8	8	PIM + VEH	LHRH-A
7	8	8	SAL + BIC	LHRH-A
8	8	8	PIM + BIC	LHRH-A

ponds to artificially aerated (6 mg l^{-1}) 1.6 m^3 tanks with electronically controlled temperature (±20°C). Photoperiod corresponded to the natural one: L:D=16:8.

The fish were divided into 8 groups (Table 1). Blood was sampled from the caudal vein, 1 and 2 h before the injection, at the time of injection (0 h), and after 12, 24 and 48 h. Gonadotropin (GtH2) level was determined using RIA method (Breton et al. 1971).

Results of this experiment (Fig. 7) demonstrated the highest GtH2 level in group 8 at 12 and 24 h after the injection. Owing to the application of bicuculline, the influence of GABA on gonadotropic cells was eliminated by blocking the receptors. It can be concluded that the GABA-ergic system hindered GtH2 release from the carp pituitary gland during the spawning period.

IV. Influence of stress on the level of GtH2 release and rhythm in mature carp females (Roelants et al. 1993)

Investigations on the impact of stress (measured by the cortisol level in blood) on the GtH2 release rhythm and level in mature carp females during the spawning period were carried out in order to determine whether such a condition can alter the achieved results in a significant degree.

The investigations were carried out on fish captured from tanks (group 1) and on fish with cannulae implanted to dorsal aorta, freely swimming in the tank during blood sampling (group 2). Blood cortisol level was estimated by the RIA method (Khun et al. 1986) and GtH2 level - according to Breton et al. (1971).

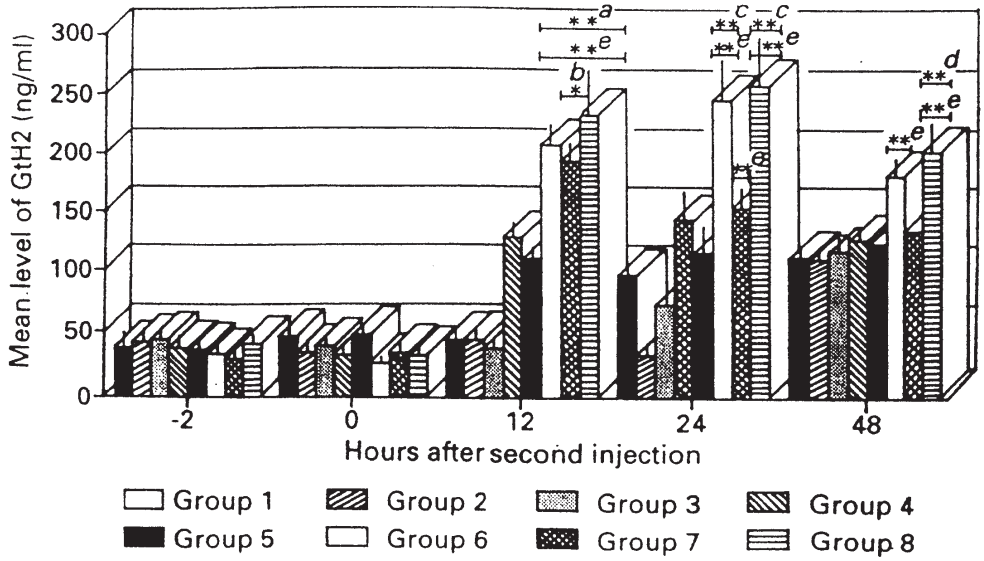

Fig. 7. Changes in blood GtH2 levels in mature female carp after BIC, PIM and LHRH-A treatment. Group 1: (SAL + VEH + SAL); Group 2: (PIM + VEH + SAL); Group 3: (SAL + BIC + SAL); Group 4: (PIM + BIC + SAL); Group 5: (SAL + VEH + LHRH-A); Group 6: (PIM + VEH + LHRH-A); Group 7: (SAL + BIC + LHRH-A); Group 8: (PIM + BIC + LHRH-A). Significance: a, compared with the value in groups 1, 2 and 3; b, compared with the value in group 5; c, compared with the value in groups 1, 2, 3, 4 and 5; d, compared with the value in group 2; e, compared with the value in this group before second injection. Levels of significance; one symbol (*) P<0.05; two symbols (**) P<0.01.

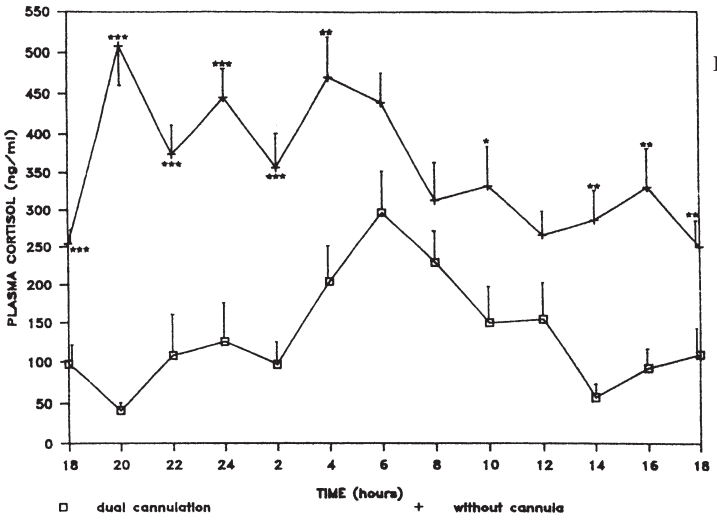

Fig. 8. The comparison of the mean plasma cortisol concentrations between not cannulated (n=10) and cannulated (n=8) carps. Vertical lines represent standard error. *, ** and *** - significant differences between the not cannulated and the cannulated groups sampled at the same time ($p<0.05$, $p<0.01$ and $p<0.001$, respectively).

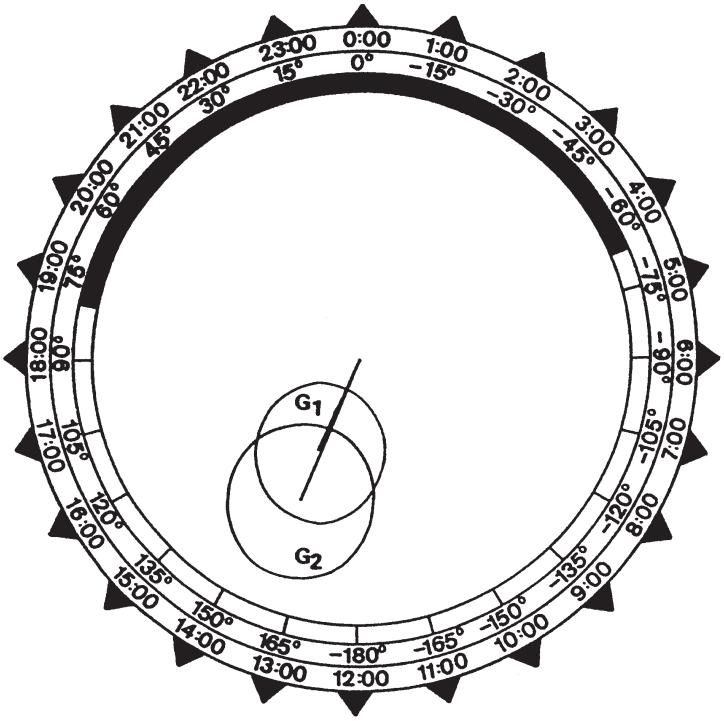

Fig. 9. The circadian changes of plasma gonadotropin, expressed by cosinor and ellipse analysis, G1 = samples taken via cannulae (start at 18:00 h); G2 = samples taken from intact fish by puncturing the caudal vasculature (start at 18:00* h). Key to ellipses:

	n	P	n	Mesor	Amplitude	Acrophase
Females			Samples	ng/cm	ng/cm	Hours
				M±SE	A±SE	t_0±SE
G1	8	$6x10^{-6}$	100	52±5	34±1	13.9±0.05
G2	10	$2x10^{-6}$	128	45±5	52.2±0.7	13.5±0.1
C1 vs	G2			NS	p<0.01	p<0.01

amplitude (A) is the maximal deviation of the theoretical curve $y = M+A \cos w (t+t_0)$ from the mean level or the mesor (M). The acrophase (t_0) is the hour of maximal deviation from this curve. The P value was used to test the null hypothesis that the amplitude is equal to zero.

During the experiments, fish were under stress estimated by cortisol levels (Fig. 8) At the same time the steroid level was significantly lower in group 2 (cannulated fish). The GtH level analysis by means of the cosinor circle and error ellipse method showed that a regular rhythm of GtH2 release to blood (Fig. 9) took place in both groups, and the hormone level in these groups did not differ (Fig. 10).

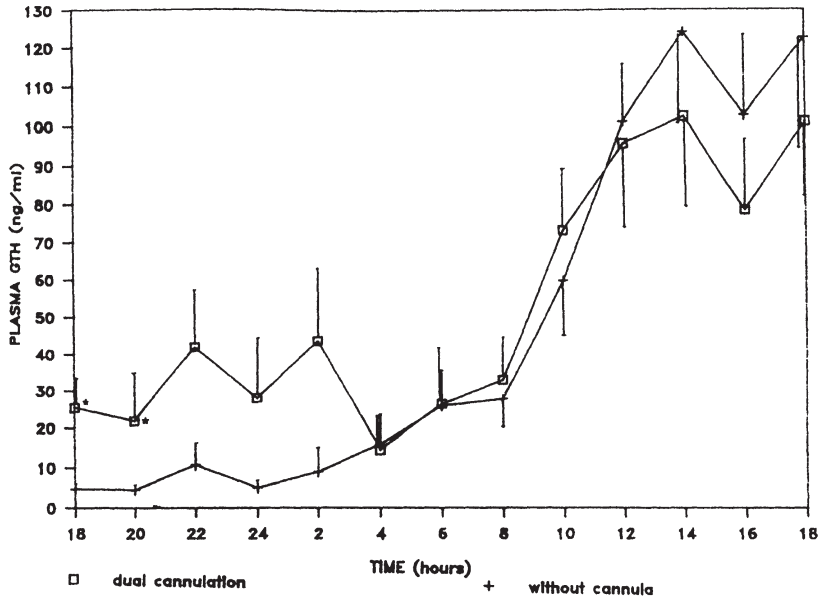

Fig. 10. The comparison of the mean plasma GtH2 concentrations between not cannulated (n=10) and cannulated (n=8) carps. Vertical lines represent standard error. *, ** and *** - significant differences between the not cannulated and the cannulated groups sampled at the same time ($p<0.05$, $p<0.01$ and $p<0.001$, respectively).

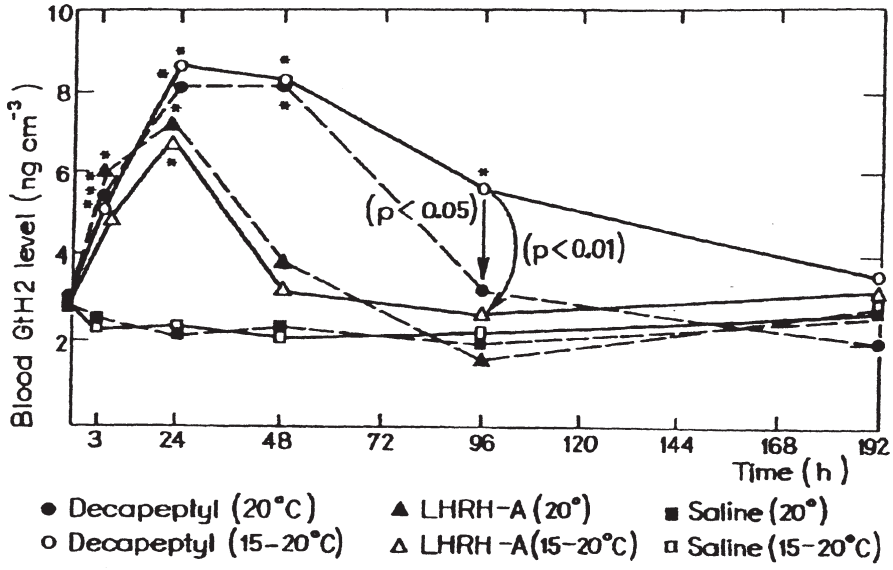

Fig. 11. Changes in GtH2 level in carp females stimulated by injections of the LHRH analogue in pure from and as DECAPEPTYL, in the presence of two temperature regimes. *Significant differences ($p<0.01$).

V. Effects of temperature on GtH2 release stimulated by LHRH analogue (D-Trp$_6$ LHRH) (Popek et al. 1993)

The aim of this research was to determine the impact of two thermal conditions, a constant spawning temperature (20^o C) and rising temperature, from 15 to 20^o at the rate 1 degree daily, on the GtH2 level stimulated by the analogue and its form of prolonged action (Decapeptyl). During the spawning season, 64 mature carp females of the average weight of 2.7 kg were transferred from ponds to tanks (1.6 m^3, oxygenation 6.0 mg O_2 l^{-1}, electronically controlled temperature, photoperiod 16L : 8D). The fish were divided into 6 groups (Fig. 11). The experimental groups were administered 25 μg kg^{-1} of LHRH-A or Decapeptyl. Blood was sampled from the caudal vasculature, 3, 24, 48, 96, 120, 144, 168, and 199 h after injections. GtH2 level was determined according to Breton et al. (1971).

The experiment showed that the increase of GtH2 in both groups, i.e. LHRH-A or Decapeptyl injected, was very close to each other after 72 h (Fig. 11)irrespective of the temperature, but after 96 h the GtH level was significantly higher in the Decapeptyl injected group at rising temperatures (15-20^{0}C) than in the Decapeptyl injected fish but exposed the constant temperature of 20^{o}C.

RECAPITULATION

Changes of gonadotropin concentrations in the blood and pituitary gland as well as GnRH level in hypothalamus and in the pituitary were determined for the first time in carp during its growth and maturation (Bieniarz et al. 1982). It was demonstrated that GtH2 level remained practically invariable during the growth and first maturation phases, whereas a definite increase of gonadotropin in blood was observed during the last stages of oocyte maturation and somewhere around the spawning period.

Important data were discerned also in the research on GtH2 and GnRH concentrations in hypophysis and hypothalamus. These data showed that GtH accumulation in the pituitary gland began at the age of 13 month of the fish life, but GnRH accumulation - at the age of 33 month. Later than 35th month also GnRH concentration in hypothalamus increased. Similar results were observed in males (Billard et al. 1992). Some quantities of GtH2 were also discovered in blood yet before the commencement of testes development and the hormone accumulation in hypophysis. From among the investigated internal factors in carp, the active ones were: 1.- neuropeptide Y (Breton

et al. 1991) which raised GtH2 level when acting alone, and also intensified the action of GnRH factor releasing gonadotropin from hypophysis, and 2.- *g*-aminobuthyric acid (GABA) which hindered GtH2 release in mature carp females (Popek et al. 1994). The application of a blockage of $GABA_A$ receptors, through which the system restrained GtH2 secretion, indicated that this process, as in the case of pimozide, occurred at the level of hypophysis through an endogenic dopamine.

Essential data were also obtained while determining stress impact on the level and rhythm of GtH2 release from carp hypophysis *in vivo*. It was proved that irrespective of the handling of fish during blood sampling (blood taken by means of a cannula from freely swimming fish or using a syringe and needle on captured fish), the animals were under stress (assessed by the cortisol level). Notwithstanding this, a much higher hormone concentration was found in non-cannulated fish (Roelants et al. 1993).

The presented investigations have, apart from their cognitive values which allow further optimisation of artificial spawning stimulation, a practical aspect. It was demonstrated (Popek et al. 1993) that the thermal regime resembling the natural course of temperature, i.e. consisting of a gradual increase of water temperature to the "spawning" level, was more advantageous in stimulating artificial spawning , in particular in applying GnRH-A form of prolonged activity. In such a case, the GtH2 level in blood remained longer and was higher than at constant temperature, provided that the way of stimulation was similar.

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REFERENCES

Bieniarz K. et al. 1982 - Maturational gonadotropin hormone (GtH) and gonadotropin releasing hormone (GnRH) changes during growth and sexual maturation on female carp (*Cyprinus carpio* L.) - Proc.Fourth Int.Symp.Reprod.Physiol.Fish., Norwich 81

Bieniarz K., Epler P. 1992 - Advances in reproductive endocrinology of fish - J.Physiol.Pharmacol. 43, 4(1): 215–222

Billard R. et al. 1992 - Testicular and some hormonal changes during the first four years of life in the mirror carp, *Cyprinus carpio* L. - J.Fish Biol. 41: 473–487

Breton B. et al. 1971 - Dosage radioimmunologique d'une hormone gonadotrope de carp, *Cyprinus carpio* L. - C.R.Acad Sci. Paris 272: 1515–1517

Breton B., Prunet P., Reynaud P. 1978 - Sexual differences in salmon gonadotropin - Ann.Biol.Anim.Biochem.Biophys. 18: 759–765

Breton B. et al. 1986 - Immunoreactive gonadotropin releasing hormone-like material in the brain and the pituitary gland during the periovulatory period in the brown trout *Salmo trutta* L., relationship with the plasma and pituitary gonadotropin - Gen.Comp.Endocrinol. 61: 109–119

Breton B. et al. 1989 - Neuropeptide Y (NPY) on maturational gonadotropin release from rainbow trout pituitary glands - Fish.Physiol.Biochem. 7: 77–83

Breton B. et al. 1990 - Studies on the mode of action of neuropeptide Y (NPY) on maturational gonadotropin (GtH) secretion from perifused rainbow trout pituitary glands - Fish.Physiol.Biochem. 8: 339–346

Breton B. et al. 1991 - Neuropeptide Y stimulates in vivo gonadotropin secretion in teleost fish - Gen.Comp.Endocrinol. 84: 277–283

Conn P.M. et al. 1991 - Gonadotropin releasing hormones in the African catfish, *Clarias gariepinus* - Proc.Fourth Int.Symp.Reprod.Physiol.Fish., Norwich: 48–50

Kuhn E.R., Corneillie S., Ollevier F. 1986 - Circadian variations in plasma osmolarity, electrolytes, glucose and cortisol in carp, *Cyprinus carpio* L. - Gen.Comp.Endocrinol. 61: 459–468

Mikołajczyk T. et al. 1990a - Calcium ions as a mediator in GnRH action on gonadotropin release in the common carp (*Cyprinus carpio* L.) - Reprod.Nutr.Dev. 30: 483–492

Mikołajczyk T. et al. 1990b - Involvement of voltage dependent calcium channels (VDCC) in the action of GnRH on GtH release in common carp (*Cyprinus carpio* L.): Comparison with K action - Reprod.Nutr.Dev. 30: 619–628

Mikołajczyk T., Weil C., Breton B. 1993 - Nicotine stimulates maturational gonadotropin (GtH2) release from carp (*Cyprinus carpio* L.) pituitary cells - Comp.Biochem.Physiol. 105C: 83–88

Peter R.E., Habibi H.R., Chang J.P. 1990 - Actions of gonadotropin releasing hormone (GnRH) in the goldfish - Progr.Comp.Endocrinol.34: 393–398

Popek W. et al. 1993 - The effect of carp female exposure to different thermal conditions followed by stimulation, with LHRH analogue (D-Trp^6 LHRH), on the level of gonadotropin (GtH2) and oocyte maturity - Pol.Arch.Hydrobiol. 40,3–4: 339–345

Popek W. 1994 - The effect of bicuculline (a $GABA_a$ receptor antagonist) on LHRH-A and pimozide stimulated gonadotropin (GtH2) release in female carp (*Cyprinus carpio* L.) - Comp.Biochem.Physiol. 108C,1: 129–135

Roelants I. et al. 1990 - A presumptive role for GABA in the stimulatory effects of Des-Gly^{10} (D-Ala^6) LHRH-ethylamide and pimozide on the gonadotropin release in carp - Life Sci. 47: 1801–1812

Roelants I. et al. 1993 - The effect of handling stress on the circadian release pattern of cortisol and gonadotropin (GtH2) in female carp (*Cyprinus carpio* L.) - Pol.Arch.Hydrobiol. 40, 3–4: 347–353

Sakun O.F., Buckaya I.A. 1968 - Determination of maturational stages and investigation of sexual cycles in fishes - Zh.Min.Ryb.Hoz. 1–14, Murmansk

Sokołowska M. 1982 - The influende of luteinizing-releasing hormone (LHRH) on serum gonadotropin levels and oocyte maturation in female carp (*Cyprinus carpio* L.) - Comp.Biochem.Physiol. A 73: 273–278

Suzuki K., Kawauchi H., Nagahama Y. 1988 - Isolation and characterization of two distinct gonadotropins from chum salmon pituitary glands - Gen.Comp.Endocrinol. 71: 292–301

STRESZCZENIE

BADANIA NAD MECHANIZMAMI UWALNIAJĄCYMI GONADOTROPINĘ PRZYSADKOWĄ (GtH2) Z PRZYSADKI MÓZGOWEJ KARPIA (*Cyprinus carpio*)

Wciągu 4 lat badano poziom GtH2 i GnRH u samców i samic karpia w odniesieniu do stadium dojrzałości gonad. GtH2 u samców utrzymuje się we krwi na poziomie 10-20 ng/ml osiągając maksimum w okresie spermacji. Poziom GtH2 w przysadce mózgowej wzrastał wraz z wiekiem ryby. U samic GtH2 we krwi zmieniało się nieznacznie w zakresie 9,97-33,25 ng/ml w czasie całego okresu badawczego, za wyjątkiem stadium wakuolizacji oocytów (161,4-214,51 ng/ml). Akumulacja GtH2 w przysadce mózgowej wrastała w czasie ostatnich 13 miesięcy z 2,64 do 617,35 μg/ml.

W doświadczeniach *in vivo* wykazano, że uwalnianie się GtH2 następuje pod wpływem neuropeptydu Y. NPY intensyfikuje również działanie GnRH na komórki gonadotropowe.

Stres, który wywoływany jest łapaniem i injekowaniem ryb w celu wywołania sztucznego tarła nie wpływa na poziom oraz rytm uwalniania GtH2 z przysadki mózgowej co pozwala wyeliminować ten czynnik przy określaniu efektywności owulacji i tarła.

ADRES AUTORÓW:

Prof. dr hab. Krzysztof Bieniarz
Prof. dr hab. Piotr Epler
Dr hab. Mirosława Sokołowska-Mikołajczyk
Dr hab. inż. Włodzimierz Popek
Dr inż. Tomasz Mikołajczyk

Katedra Ichtiobiologii i Rybactwa
Akademii Rolniczej w Krakowie
30-149 Kraków
ul. T. Spiczakowa 6