Arch. Ryb. Pol.	Archives of Polish Fisheries	Vol. 8	Fasc. 1	129-143	2000

THE EFFECT OF ZINC ON MATURATIONAL GONADOTROPIN (GtH2) SECRETION MODULATED BY GABAergic SYSTEM IN COMMON CARP *(Cyprinus carpio* L)

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ABSTRACT: Studies were carried out on the effects of GABA and agonists or antagonists of its receptors on *in vivo* or *in vitro* spontaneous secretion as well as LHRH analog (D-Ala^6 LHRH) stimulated maturational gonadotropic hormone (GtH2) secretion in carp fed the diet containing zinc. A possible modulatory action of dopamine in these processes was examined by simultaneous administration of pimozide, a dopamine (D2) receptor antagonist. Injections of isonipecotic acid - an antagonist of $GABA_A$ receptors (INIP), or bicuculline, an antagonist of $GABA_A$ receptors (BIC), increased the GnRH stimulated GtH2 secretion only in the control fish, but not in fish fed the diet containing zinc, suggesting that zinc inhibited this stimulatory influence. By contrast baclofen, an agonist of $GABA_B$ receptors (BAC), increased the GtH2 response to GnRH-A only in zinc treated fish. *In vitro* experiments demonstrated that GABA did not modify the GnRH-A stimulated GtH2 secretion from the pituitary of normal or zinc treated fish. These results suggest that although zinc does not affect the *in vivo* GtH2 release modulated by GABAergic drugs, it can block the tonic GABAergic inhibition of GtH2 secretion *in vitro* Therefore, zinc may temporarily eliminate the $GABA_A$ type of receptors from the GABAergic regulation of GtH2 secretion.

Key words: GABA, AGONISTS AND ANTAGONISTS OF $GABA_A$ AND $GABA_B$ RECEPTOR TYPES, CARP, MATURATIONAL GONADOTROPIN (GtH2), ZINC ENRICHED DIET

INTRODUCTION

The toxicity of zinc to fish organism is well known (Alabaster and Lloyd 1980, Bengtsson 1974a, 1974b). Increased concentration of zinc compounds up to 1 mg dm^{-3} in inland waters of Poland (among others in commercial ponds and spawning grounds) drew attention to the problem of fish gonad maturation and spawning in waters polluted with zinc (Szulkowska-Wojaczek 1992). High doses of zinc, exceeding organism demands caused, depending on the concentration and time of exposure, a decrease of fecundity in *Pimephales promelas* (Brungs 1969), a delay of spawning and diminution of egg viability in *Brachydanio rerio* (Speranza et al. 1977), spermatogenesis inhibition and ovarian atresia in *Puntius conchonius* (Kumar and Pant 1984), decrease of egg size and larval deformation in *Catostomus commersoni* (Munkittrick and Dixon 1989). Zinc concentrations of 3 mg dm^{-3} decreased hatchability and increased the percentage of deformed larvae in guppy and goldfish (Bieniarz et al. 1994, 1996, 1997),

lowered oocyte sensitivity to pituitary homogenate stimulation, decreased the number of hatched larvae and increased the number of deformed larvae (Bieniarz et al. 1996).

There is some information that zinc can affect hypothalamic and pituitary hormone secretion in fish. Rearing of goldfish and guppies in the sublethal concentrations (in case of goldfish: 2.5 mg dm^{-3}) did not inhibit growth nor increased mortality, but it lowered gonadotropin GtH2 level in a statistically significant way causing a significant reduction of progeny numbers (Bieniarz et al. 1997).

It is well established that in teleost fish the secretion of maturational gonadotropin (GtH2) is stimulated by gonadotropin-releasing hormone (GnRH) and inhibited by dopamine (DA) (Chang et al. 1984, Peter et al. 1991), and that there are other factors involved in the control of GtH2 secretion. GABAergic system is one of these factors. However, the opinions on the character of the effect of this neurotransmitter on gonadotropin secretion differ considerably. (Roelants et al. 1990, Kah et al. 1991, Trudeau et al. 1993b, Chyb 1996, Sokołowska et al. 1997). Therefore, the aim of our work was to examine not only the effects of zinc on *in vivo* or *in vitro* spontaneous GtH2 secretion, but also to investigate the possible role of zinc in the modulation of the GtH2 response to LHRH analog and GABAergic drugs. A possible modulatory action of dopamine in these processes was examined by a simultaneous administration of pimozide a dopamine (D2) receptor antagonist.

MATERIAL AND METHODS

I. *IN VIVO* EXPERIMENTS

124 mature control male carp (fed a normal diet) and 140 males fed a zinc enriched diet ($ZnSO_4$ - 60 mg of zinc per g) were used for the experiments. Fish were fed an appropriate diet for one full season and 2 month of the next season. The fish were caught from conventional carp ponds at the beginning of the experiment, weighed, and labelled individually before transferring to concrete flow-through basins (volume 2 m^3). They were kept for 3 days in the basins in artificially aerated (8 mg O_2 l^{-1}) water of 18°C, in a simulated natural photoperiod. In all *in vivo* experiments the fish were injected intraperitoneally with:

Saline (SAL) - 0.6% Nad,

Isonipecotic acid - INIP (a $GABA_A$ receptor agonist) at a dose of 10 mg kg^{-1} (Interchim -France),

Bicuculline - BIC (a $GABA_A$ receptor antagonist) at a dose of l mg kg^{-1} (Sigma Chemical Co., USA),

Baclofen - BAC (a $GABA_B$ receptor agonist) at a dose of l mg kg^{-1} (Sigma Chemical Co., USA),

[Des Gly^{10}, D-Ala^6] LHRH - (GnRH-A) at a dose of 20 μg kg^{-1} (Sigma Chemical Co., SA)

Pimozide - PIM (a dopamine D2 receptor antagonist) at a dose of 10 mg kg^{-1} (Janssen Pharmaceutica N. V., Belgium).

The fish were anaesthetised before all the manipulations with ethylene glycol (3 ml for 10 L of water). All fish were injected twice, three hours apart: SAL or PIM or/and GABAergic drug were given at the time of the first injection (time «-3") and GnRH-A or SAL were always administered as the second injection three hours later (time »0"). The details of the experimental procedures are presented in Table l.

TABLE 1

The design of the *in vivo* experiment

FISH FED STANDARD DIET OR DIET CONTAINING ZINC			
Experimental group (n=8)		Control group (n=8)	
1^{st} injection	2^{nd} injection	1^{st} injection	2^{nd} injection
(-3 hours)	(0 hours)	(-3 hours)	(0 hours)
INIP	SAL	SAL	SAL
BIC	SAL	SAL	SAL
BAC	SAL	-	-
INIP	GnRH-A	SAL	GnRH-A
BIC	GnRH-A	SAL	GnRH-A
BAC	GnRH-A	SAL	GnRH-A
INIP+PIM	SAL	PIM	SAL
BIC+PIM	SAL	PIM	SAL
INIP+PIM	GnRH-A	PIM	GnRH-A
BIC+PIM	GnRH-A	PIM	GnRH-A

Blood samples (500 μl) were taken from fish caudal vasculature with the use of heparinized 1 ml syringes, just before the first (-3 hours) and before the second injection (0 hours), as well as 3, 9, 21, 33 and 45 hours after the second injection. Plasma

was collected and kept frozen until gonadotropin (GtH2) was measured using the ELISA method (Kah et al. 1989). All samples were analysed in duplicates.

The results of the experiments were analysed with the analysis of variance and the significance of differences between the groups was calculated using Duncan's multiple range test

II. *IN VITRO* EXPERIMENTS

24 five year old male carp were used for *in vitro* experiments. Twelve fish were fed a zinc-containing diet (similarly as in the *in vivo* treatment) and twelve fish a standard diet. Fish were anaesthetised and the pituitary glands were removed after decapitation. They were placed in ice-cold mineral medium prepared according to Jalabert (Jalabert et al. 1973), supplemented with glucose l g ml^{-1} and 0.3% of BSA (Sigma Chemical CO., USA, RIA grade). The glands were rinsed several times and transferred into the perifusion columns (l cm^3 of volume) with P-2 Bio-Rad Laboratories, USA. The flow rate was maintained at 15 ml $hour^{-1}$. For details concerning the perifusion system see Mikołajczyk et al. 1996. The fractions started to be collected after 90 minutes of rinsing using an automatic fraction collector (Neolab, Germany). Each perifusion consisted of two control columns (pituitaries of fish fed a standard diet) and two experimental columns (pituitaries of fish fed the diet containing zinc) and was repeated three times to have six replicates in the same group. The design of the experiment is presented in Table 2. In brief: in case of the perifusions testing the spontaneous secretion of GtH2 under the influence of GABA (10^{-8}, 10^{-6} or 10^{-4} M) fractions were collected as follows: four 2 minutes fractions before GABA application, eight 2-minute fractions during GABA pulse (one concentration), and three 5-minute fractions after the pulse. After each pulse of GABA

TABLE 2

The design of the *in vitro* experiment

FISH FED STANDARD DIET OR DIET CONTAINING ZINC		
	No of pituitary glands	Treatment
Control columns	6	GABA 10^{-8} M GABA 10^{-6} M GABA 10^{-4} M
Experimental columns	6	GABA 10^{-8} M + GnRH-A 10^{-7} M GABA 10^{-6} M + GnRH-A 10^{-7} M GABA 10^{-4} M + GnRH-A 10^{-7} M

pituitaries recovered during 90 minutes of rinsing and then the next concentration was tested in the same way as the first one.

In case of perifusions testing GnRH-A stimulated GtH2 secretion under the influence of GABA, fractions were collected as follows: four 2-minute fractions before GABA application, six 2-minute fractions during the pulse of the tested concentration of GABA, four 2-minute fractions during the pulse of GABA with GnRH-A (10^{-7} M) and three 5-minute fractions after the pulse. Each concentration of GABA was tested in the same way after 90 minutes of recovery (rinsing).

The fractions of perifusate were collected and kept frozen until gonadotropin (GtH2) measurement with the same method as plasma samples in *in vivo* experiments.

The perifusion profiles are presented as mean percentage of the basal GtH2 levels. The basal level (100%) was calculated as the mean GtH2 level of the first three fractions before each drug administration. The results were analysed using two tailed Student's t-test.

RESULTS

IN VIVO EXPERIMENTS

There were no significant differences in spontaneous GtH2 secretion under the influence of isonipecotic acid (INIP) at the dose of 10 mg kg^{-1}, bicuculline (BIC) at the dose of 1 mg kg^{-1}, baciofen (BAC) at the dose of l mg kg^{-1} in fish fed standard diet or in fish fed the diet containing zinc (data not shown).

In fish fed the standard diet, INIP (10 mg kg^{-1}) caused statistically significant increase of GnRH-A stimulated gonadotropin secretion at 9, 21, 33 and 45 hours after the second injection in comparison with GnRH-A only treated fish (Fig 1A). The same treatment did not changed GtH2 secretion in fish fed the diet containing zinc in comparison with GnRH-A only injected fish (Fig. 1B).

BIC (l mg kg^{-1}) in fish given the standard diet increased significantly GnRH-A stimulated gonadotropin secretion at 9, 21 33 and 45 hours after the second injection in comparison with GnRH-A only injected fish (Fig. 2A), whereas in the fish fed the diet containing zinc no significant changes in gonadotropin secretion were found in comparison with GnRH-A treated fish (Fig. 2B).

Injection of BAC (l mg kg^{-1}) in fish fed standard diet did not cause any significant changes in GnRH-A stimulated GtH2 secretion (Fig 3A). In carp fed the diet with zinc, a significant increase of GtH2 secretion was observed at 0, 3 and 45 hours after the second injection (Fig. 3B).

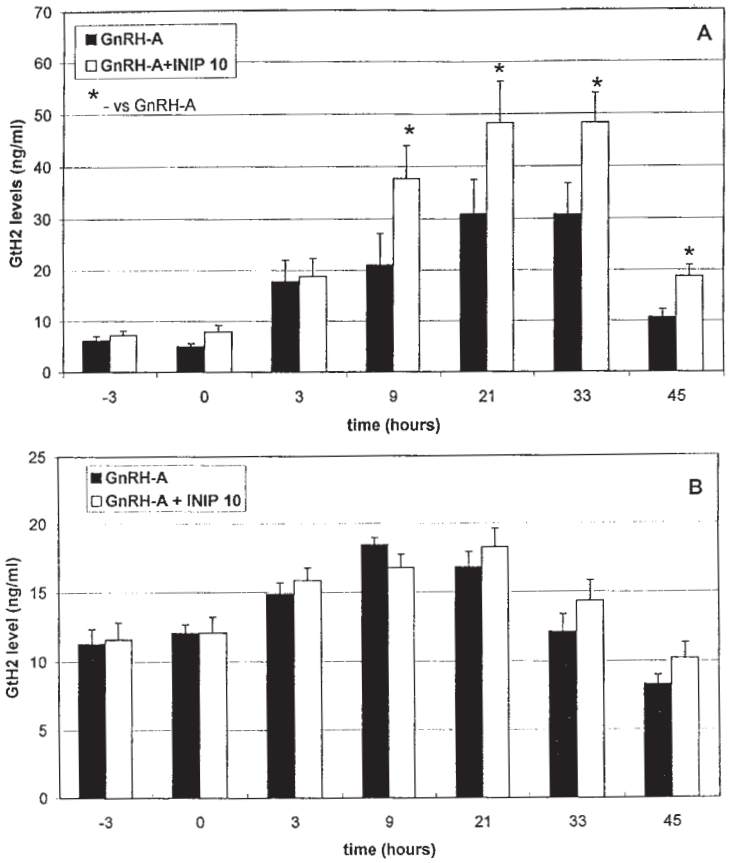

Fig. 1. The effects of isonipecotic acid (INIP) at the dose of 10 mg kg^{-1} on GnRH-A stimulated GtH2 release *in vivo* in male carp fed standard diet (A) or diet containing zinc (B).

In fish fed standard diet and treated with PIM and INIP (10 mg kg^{-1}) a significant increase of GtH2 release was found at 3 hours (Fig. 4A). No effects of INIP on GtH2 secretion were found in fish fed the diet with zinc (Fig. 4B).

Injections of PIM and BIC (l mg kg^{-1}) significantly increased gonadotropin level at 45 hours after the second injection in fish fed standard diet (Fig. 5A), whereas changes in GtH2 secretion were not observed in fish fed the diet with zinc (Fig. 5B).

There were no significant changes in gonadotropin release under the influence of INIP at a dose of 10 mg kg^{-1} in fish fed standard diet and treated with PIM and GnRH-A (Fig. 6A). INIP caused a significant decrease of GtH2 secretion in fish fed zinc-enriched diet at 33 hours after the second injection compared with PIM and GnRH-A treated fish (Fig. 6B).

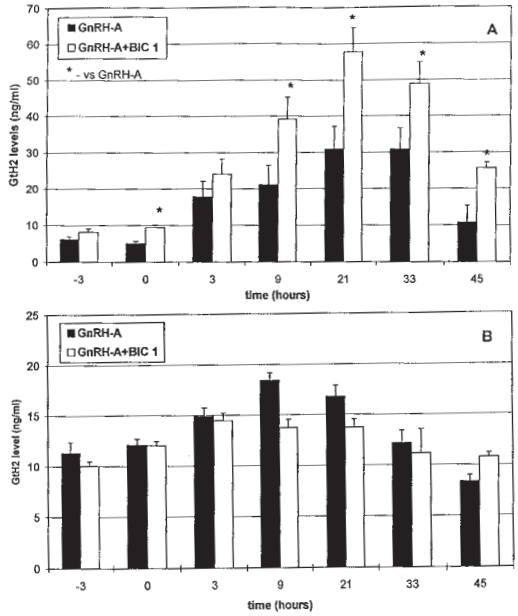

Fig. 2. The effects of bicuculline (BIC) at the dose of 1 mg kg^{-1} on GnRH-A stimulated GtH2 release *in vivo* in male carp fed standard diet (A) or diet containing zinc (B).

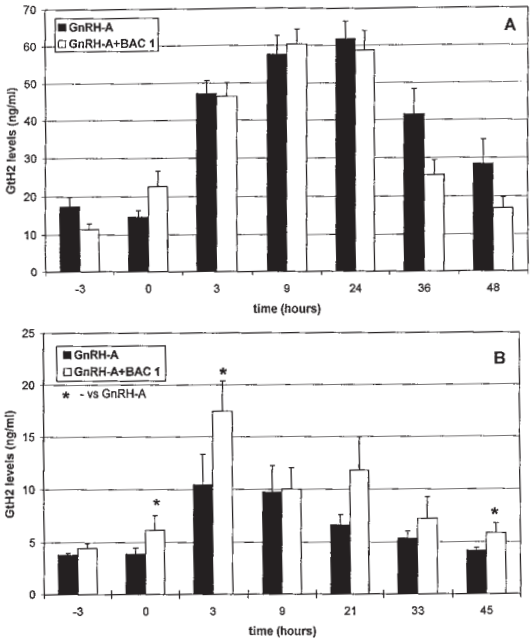

Fig. 3. The effects of baclofen (BAC) at the dose of 1 mg kg^{-1} on GnRH-A stimulated GtH2 release *in vivo* in male carp fed standard diet (A) or diet containing zinc (B).

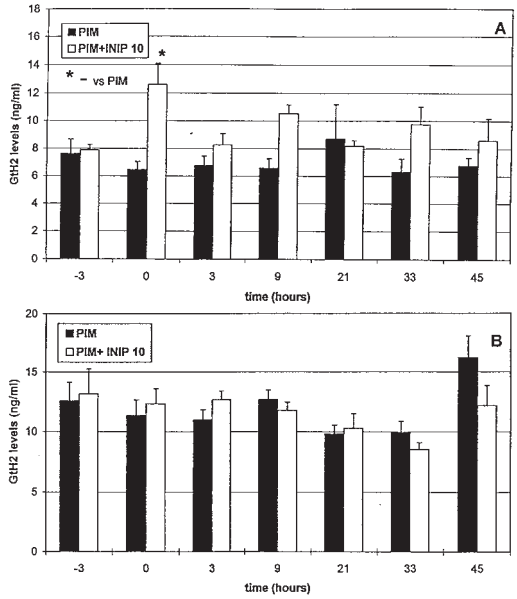

Fig. 4. The effects of isonipecotic acid (INIP) at the dose of 10 mg kg^{-1} on GtH2 release *in vivo* in male carp fed standard diet, injected with pimozide (A,) or males fed zinc-containing feed and injected with pimozide (B).

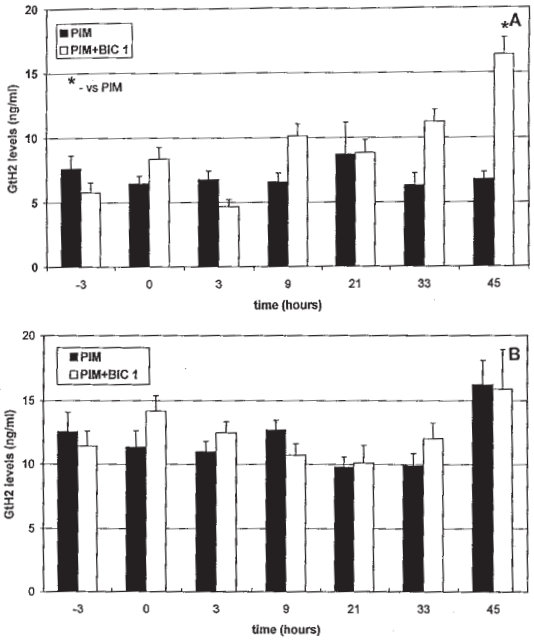

Fig. 5. The effects of bicuculline (BIC) at the dose of 1 mg kg^{-1} on GtH2 release *in vivo* in male carp fed standard diet, injected with pimozide (A), or males fed zinc-containing feed and injected with pimozide (B).

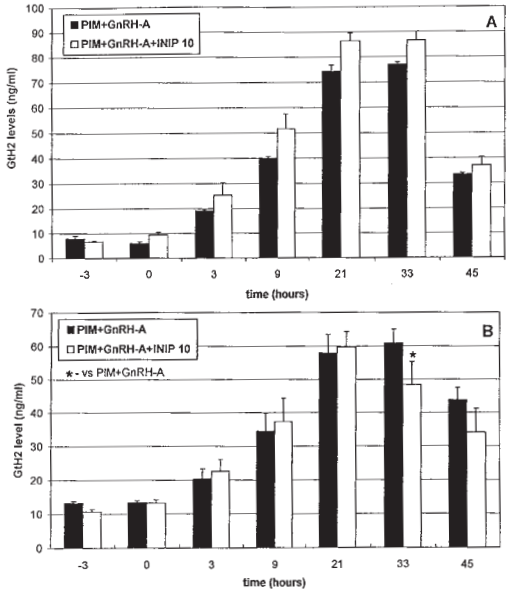

Fig. 6. The effects of isonipecotic acid (INIP) at the dose of 10 mg kg^{-1} on GnRH-A stimulated GtH2 release *in vivo* in male carp fed standard diet and injected with pimozide (A), or males fed zinc-containing feed and injected with pimozide (B).

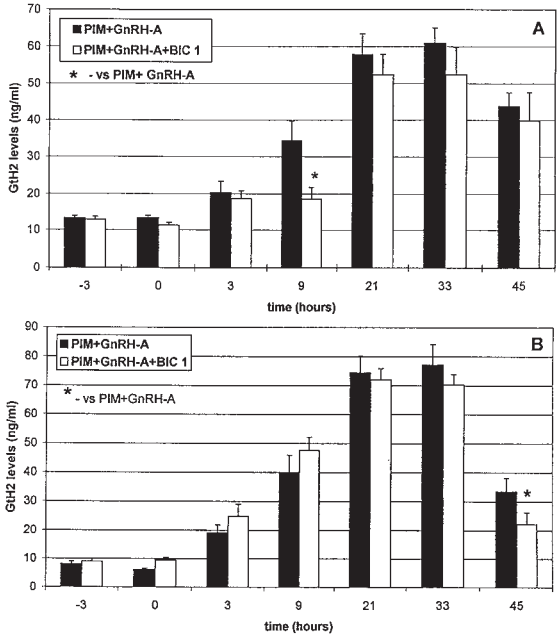

Fig. 7. The effects of bicuculline (BIC) at the dose of 1 mg kg^{-1} on GnRH-A stimulated GtH2 release *in vivo* in male carp fed standard diet and injected with pimozide (A), or males fed zinc-containing feed and injected with pimozide (B).

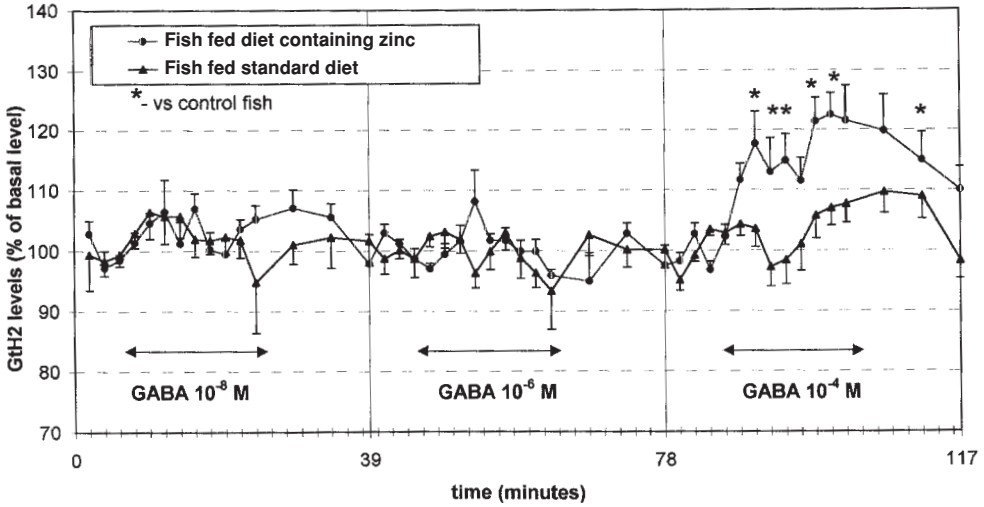

Fig. 8. The effects of GABA (10^{-8}, 10^{-6} or 10^{-4} M) on spontaneous GtH2 release *in vitro* from the pituitary of male carp fed standard diet (triangles) or diet containing zinc (circles).

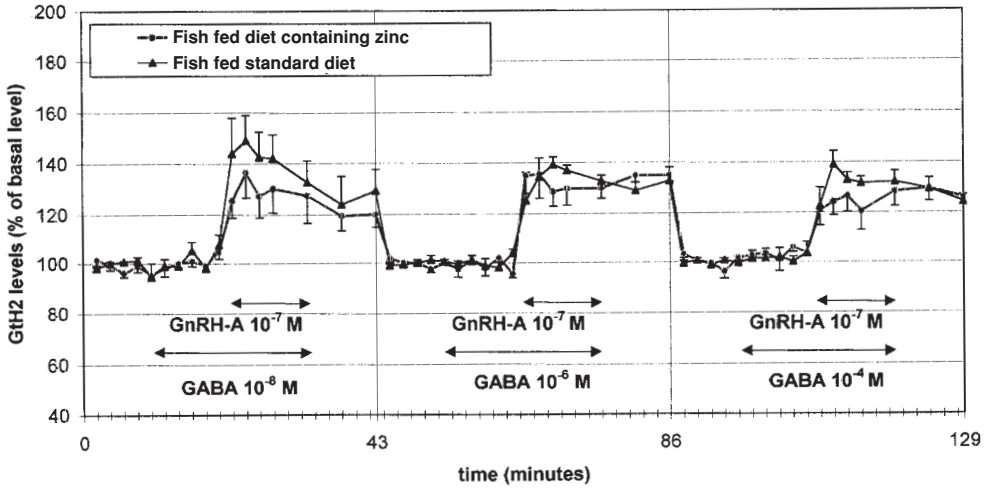

Fig. 9. The effects of GABA on GnRH-A stimulated GtH2 release *in vitro* from the pituitary of male carp fed standard diet (triangles) or diet containing zinc (circles).

BIC (l mg kg^{-1}) significantly decreased gonadotropin secretion at 9 hours after the second injection in fish fed standard diet and treated with PIM and GnRH-A (Fig. 7A), and in fish fed the diet with zinc - at 45 hours after the second injection compared with PIM and GnRH-A treatment (Fig. 7B).

IN VITRO EXPERIMENTS

a) The effects of GABA on a spontaneous GtH2 release from the pituitary of carp fed standard diet or a diet containing zinc.

GABA perifused at the concentration of 10^{-4} M significantly stimulated gonadotropin release from isolated pituitary glands of carps fed the diet containing zinc compared with pituitaries of fish fed standard diet. Other GABA concentrations (10^{-6} M or 10^{-8} M) were ineffective (Fig. 8).

b) The effects of GABA on GnRH-A (10^{-7} M) stimulated GtH2 release from the pituitary of carp fed standard diet or a diet containing zinc.

All three concentrations of GABA (10^{-8} M, 10^{-6} M or $l0^{-4}$ M) did not significantly change GnRH-A stimulated GtH2 secretion from the pituitary of experimental fish compared with the control fish (Fig. 9).

DISCUSSION

GABA stimulates gonadotropin release in immature goldfish acting through the A-type of receptors (Kah et al. 1991, Sloley et al. 1992, Trudeau et al. 1993a, b). In mature carp, however, GABA may inhibit GtH2 release (Popek et al. 1994, Roelants et al. 1990) also through the A receptors at the level of hypothalamus inhibiting of GnRH release from the neurone endings (Chyb 1996, Sokołowska et al. 1997). GABA may also stimulate GtH2 release in male carp through the B-receptors by inhibiting dopamine release at the level of hypothalamus (Chyb 1996, Sokołowska et al. 1997). Certain type of GABA receptors can be differentially modulated by zinc (Legendre and Westbrook 1991). GABAc receptors show diverse sensitivity and only a subset is inhibited at low concentrations (Kaila 1994). These differential effects suggest that zinc can modulate GABA-mediated synaptic neurotransmission, and this may represent a physiological role of Zn^{2+} in the brain (Xie et al. 1994). That zinc may affect pituitary function is shown by greater effectiveness of Zn-LHRH over LHRH alone in stimulating the release of LH and FSH in rats (Kochman et al. 1992).

In mature carp fed a diet containing 60 mg g^{-1} zinc for one full season and two months of the second season, the intraperitoneal injections of isonipecotic acid, bicuculline or baciofen did not cause any significant changes in plasma GtH2 between zinc-treated and control fish (data not shown). However, the results of the *in vitro* experiments showed that GABA at concentration of $l0^{-4}$ M significantly stimulated GtH2 secretion in zinc-treated fish (Fig. 8). This demonstrates that zinc did

not affect the spontaneous *in vivo* GtH2 release, but could block the tonic inhibition of GtH2 secretion by GABA. Akaike et al. (1987) and Aguayo and Alarcon (1993) found that zinc was a competitive type A agonist of GABA receptors, although Legendre and Westbrook (1991) indicated that it was a non-competitive agonist. The blocking of inhibitory action by GABA *(via* type A receptors) on GtH2 secretion in the zinc treated carp suggests that zinc exposure may result in increased pituitary GtH2 secretion in response to *in vitro* stimulation by GABA.

Experiments in which the injections were tested of INIP, BIC, or BAC on GnRH-A stimulated gonadotropin secretion demonstrated that INIP and BIC increased the GnRH stimulation of GtH2 only in the control fish (Figs. l and 2), suggesting that zinc exposure inhibited this stimulatory influence. By contrast, BAC increased the GnRH-A stimulation only in zinc-treated fish (Fig.3). *In vitro* experiments demonstrated that GABA did not modify the GnRH-A stimulated GtH2 secretion from the pituitary of the control or zinc treated fish (Fig. 9). The results of these experiments suggest that zinc may temporarily eliminate $GABA_A$ receptor from the GABAergic regulation of GtH2 secretion. This is in agreement with the data of Akaike et al. (1987) and Legendre and Westbrook (1991) showing that zinc may be a competitive or noncompetitive type A agonist of GABA receptors, and can remove the inhibitory effects of GABA on GtH2 secretion.

The role of dopaminergic system in the control of gonadotropin release in fish is well established. Chyb (1996) demonstrated that type B of GABA receptors were involved in stimulating GtH2 secretion in carp by the modulation of dopaminergic system at the level of hypothalamus. In rats, baclofen inhibited dopamine secretion *in vitro* and this effect was abolished by the specific $GABA_B$ receptor antagonist (Locatelli et al. 1979). $GABA_B$ receptors were also involved in the inhibition of dopamine secretion from TIDA neurons in rats (Casanueva et al. 1981, Kimura et al. 1993). In our experiments injections of PIM (a dopamine receptor antagonist) stimulated significantly increase of GtH2 secretion under the influence of INIP or BIC in the control but not in zinc treated carps (Figs. 4 and 5). In case of GnRH-A stimulated gonadotropin release under the influence of PIM and INIP or BIC, a significant inhibition of the secretion was observed in zinc treated fish (Figs. 6 and 7). In the control carps BIC but not INIP significantly decreased GnRH-A stimulated gonadotropin release. The results of the experiments in which fish were treated with PIM are not so conclusive, but they show that the response to the applied GABAergic drugs differs depending on the presence of zinc in the fish diet.

ACKNOWLEDGEMENTS

This work was supported by the Polish Committee of Scientific Research (KBN) Grant no. 5 S311 013 04. to MSM.

REFERENCES

Aguayo L. G., Alarcon J.M. 1993 - Modulation of the developing rat sympathetic GABA receptor by zinc, benzodiazepines, barbiturates and ethanol - J. Pharmacol. Exp. Therapeutics. 267: 414-1422

Akaike N.T., Yakaushiji N., Tokutomi M., Carpenter D.0.1987 - Multiple mechanisms of antagonism of y-aminobutyric acid (GABA) responses - Cell. Moll. Neurobiol. 7: 97-103

Alabaster J. S., Lloyd R. 1980 - Water quality criteria for freshwater fish - London, Butterworths. 1980.

Bengtsson B.E. 1974a - Vertebral damage to minnows (*Phoxinus phoxinus* L.) exposed to zinc-0ikos 25:134-139

Bengtsson B.E. 1974b - The effects of zinc on the ability of the minnow Phoxinus phoxinus L. to compensate for torque in a rotating water current - Bull. Environ. Contam. Toxicol. 12: 654-658

Bieniarz K., Epler P., Sokołowska-Mikołajczyk M. 1994 - Effect of zinc on *guppy (Poecilia reticulata*) reproduction - Pol. Arch. Hydrobiol. 41: 489-493

Bieniarz K., Epler P., Sokołowska-Mikołajczyk M. 1996 - Goldfish (*Carassius auratus gibelio* Bloch) breeding in different concentrations of zinc - Pol. Arch. Hydrobiol. 43: 365-371

Bieniarz K., Epler P., Sokołowska-Mikołajczyk M., Popek W. 1997 - Reproduction of fish in conditions disadvantageously altered with the salts of zinc and copper -Arch. Ryb. Pol. 5: 21-30

Brungs W. A. 1969 - Chronic toxicity of zinc to the fathead minnow (*Pimephales promelas* Rafinesque L.) - Trans. Am. Fish. Soc. 98: 272-279

Chang J.P., Peter R.E., Nahomiak C.S., Sokołowska M. 1984 - Effects of catecholaminergic agonists and antagonists on serum gonadotropin concentrations and ovulation in goldfish: Evidence for specificity of dopamine inhibition of gonadotropin secretion - Gen. Comp. Endocrinol. 55: 351-360

Casanueva F., Apud J., Locatelli V., Martinez-Campos A., Civati C., Racagni G., Cocchi D., Müller E.E. 1981 - Mechanisms subserving the stimulatory and inhibitory components of γ-aminobutyric acid-ergic control of prolactin secretion in rat - Endocrinology 109: 567-575

Chyb J. 1996 - Rola systemu GABAergicznego w kontroli uwalniania gonadotropiny (GtH2) z przysadki mózgowej karpia *Cyprinus carpio.* (The role of GABAergic system in the control of gonadotropin (GtH2) release secretion from carp *(Cyprinus carpio* L.) pituitary gland.) PhD Thesis. Agricultural Academy in Kraków 1-89

Jalabert B., Bry C., Szollosi D., Fostier A. 1973 - Etude comparee de 1'action des hormones hypophysaires et steroides sur la maturation in vitro des ovocytes de la Truite et du Carassin (poissons teleosteens) - Ann. Biol. Anim. Biochim. Biophys. 13: 59-72

Kaila K. 1994 - Ionic basis of $GABA_A$ receptor channel function in the nervous system -Progr. Neurobiol. 42: 489-537

Kah O., Trudeau V.L., Sloley B.D., Martinoli M.G, Chang J.P, Yu K.L., Peter R.E. 1991 - Implication of GABA in the neuroendocrine regulation of gonadotropin release in goldfish (*Carassius auratus*) - In: Proceedings of the Fourth International Symposium on Reproductive Physiology of Fish (Scott AP, Sumpter JP, Kime, DE, Rolfe MS, eds.). Norwich, U. K. 7-12 July 1991, Fish Symp. Sheffield

Kah **O.,** Pontet A.J., Rodriquez N., Calas A., Breton B. 1989 - Development of an enzyme-linked immunosorbent assay for goldfish gonadotropin - Biol. Reprod. 40: 68-73

Kimura F., Jinnai K., Funabashi T.A. 1993 - $GABA_B$ - receptor mechanism is involved in the prolactin release in both male and female rats - Neurosci. Letters. 155: 183-186

Kochman K., Gajewska A., Kozłowski H., Masiukiewicz E., Rzeszotarska B. 1992 -Increased LH and FSH release from the anterior pituitary of ovariectomized rat, in vivo, by copper-, nickel- and zinc-LHRH complexes. - J. Inorg. Biochem. 48: 41-46

Kumar A., Pant S. C. 1984 - Comparative effects of the sublethal poisoning of zinc, copper and lead on the gonads of the teleost *Puntius conchonius* Ham. -Toxicol.Lett.23:189-194

Legendre P., Westbrook. G.L. 1991 - Noncompetitive inhibition of γ-aminobutyric acid-A channels by zinc - Mol. Pharmacol. 39: 267-274

Locatelli P.D., Cocchi D., Frigerio C., Betti R., Krogsgaard-Larsen P., Racagni G., Muller E.E. 1979 - Dual γ-aminobutyric acid control of prolactin secretion in the rat. Endocrinology 105: 778-785

Mikołajczyk T., Epler P., Sokołowska-Mikołajczyk M., Breton B., Chyb J., Ćwioro E. 1996 - The effects of nicotine in combination with antidopaminergic drugs on the maturational gonadotropin (GtH2) secretion from perifused common carp (*Cyprinus carpio* L.) pituitary glands - Pol. Arch. Hydrobiol. 43: 373-378

Munkittrick K.R., Dixon D.G. 1989 - Effects of natural exposure to copper and zinc on egg size and larval copper tolerance in white sucker (*Catostomus commersoni*) -Ecotoxicol. Environ. Saf. 18: 15-26

Peter R.E., Trudeau V.L., Sloley B.D. 1991 - Brain regulation of reproduction in teleosts - Bull. Inst. Zool. Academia Sinica, Monograph. 16: 89-119

Popek W., Breton B., Sokołowska-Mikołajczyk M., Epler P. 1994 - The efFects of bicuculline (a GABA receptor antagonist) on LHRH-A and pimozide stimulated gonadotropin (GtH2) release in female carp (*Cyprinus carpio* L.). Comp. Biochem. Physiol. 108C: 129-135

Roelants I., Epler P., Mikołajczyk T., Breton B., Bieniarz K., Ollevier F. 1990 - A presumptive role for GABA in the stimulatory efFects of of Des-Gly^{10}, [D-Ala^{6}]-LHRH-ethylamide and pimozide on the gonadotropin release in carp - Life Sci. 47: 1801-1812

Sloley B.D., Kah O., Trudeau V.L., Dulka J.G., Peter R.E. 1992 - Amino acid neurotransmitters and dopamine in brain and pituitary of the goldfish involvement in the regulation of gonadotropin secretion - J Neurochem. 58: 2254-2262

Sokołowska-Mikołajczyk M., Chyb J., Mikołajczyk T., Epler P., Bieniarz K., Popek W. 1997 - GABAergic modulation of gonadotropin (GtH2) secretion from carp (*Cyprinus carpio* L.) hypophysis. Arch. Ryb. Pol. 5: 137-154

Speranza A.W., Seeley V.A., Perlmutter A. 1977 - The effect of sublethal concentrations of zinc on reproduction in zebrafish, *Brachydanio rerio* Hamilton-Buchanan - Environ. Pollut. 12: 217-222

Szulkowska-Wojaczek E., Marek J., Dobicki W., Polechoński R. 1992 - Metale ciężkie w środowisku stawowym (Heavy metals in pond environment) - Zesz. Nauk. AR Wrocław, Zootechnika 37, 218: 7-25

Trudeau V.L., Murthy C.K., Habibi H.R., Sloley B.D., Peter R.E. 1993a - Effects of sex steroid treatments on gonadotropin-releasing hormone-stimulated gonadotropin secretion from the goldfish pituitary - Biol. Reprod. 48: 300-307

Trudeau V.L., Sloley B.D., Peter R.E. 1993b - GABA stimulation of gonadotropin-II release in goldfish - involvement of GABA(A) receptors, dopamine and sex steroids - Am. J. Phys. 265: 348-355

Xie **X.,** Hider **C.C.,** Smart T.G. 1994 - Modulation of GABA-mediated synaptic transmition by endogenous zinc in mature rat hippocampus in vitro - J. Physiol. 3478: 75-86

STRESZCZENIE

WPŁYW CYNKU NA SEKRECJĘ GONADOTROPINY DOJRZEWANIA (GtH2), MODULOWANĄ PRZEZ SYSTEM GABAergiczny U KARPIA (*Cyprinus carpio* L.)

Badano wpływ GABA oraz jego agonistów i antagonistów na spontaniczną lub stymulowaną analogiem LHRH (D-Ala^{6} LHRH) sekrecję gonadotropiny dojrzewania (GtH2) u samców karpia żywionych

przez półtora sezonu wegetacyjnego paszą z dodatkiem siarczanu cynku. Badania prowadzono w systemie *in vivo* oraz *in vitro*.

Agonista receptorów GABA typu A - INIP (isonipecotic acid) oraz bikukulina (antagonista tego typu receptorów) nie spowodowały zmian w spontanicznej sekrecji GtH2 ani u ryb kontrolnych, ani żywionych paszą z dodatkiem cynku, natomiast powodowały wzrost stymulowanej analogiem LHRH sekrecji GtH2 tylko u ryb kontrolnych. Sugeruje to hamujący wpływ cynku na sekrecję GtH2 stymulowaną przez związki GABAergiczne. Doświadczenia nie wykazały wpływu GABA na stymulowaną analogiem LHRH sekrecję GtH2 z izolowanych przysadek mózgowych ryb kontrolnych i ryb karmionych paszą z dodatkiem cynku.

Uzyskane rezultaty sugerują, że cynk nie wpływa na modulowane przez środki GABAergiczne uwalnianie GtH2 natomiast może blokować toniczne hamujące działanie systemu GABAergicznego na sekrecję GtH2

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