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GABAergic MODULATION OF GONADOTROPIN (GtH2) SECRETION FROM CARP (*Cyprinus carpio* L.) HYPOPHYSIS

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A B S T R A C T. Experiments *in vivo* and *in vitro* concerned the influence of GABA, agonists and antagonists of $GABA_A$ and $GABA_B$ receptor subtypes (isonipecotic acid - INIP, bicuculline - BIC, baclofen - BAC, 5-aminovaleric acid- AVA, muscimol - MUS) on gonadotropin (GtH2) secretion stimulated by GnRH-A analog in sexually mature carp around the spawning period. Influence of dopaminergic system on GtH2 release in fish was taken into account and that is why some experiments were carried out with the fish injected with pimozide - a dopamine antagonist.

These experiments demonstrated that GABA affected the GtH2 secretion from carp pituitary gland through the type A as well as type B receptors. It inhibited GtH2 secretion through the type A receptors at the level of hypothalamus (*via* inhibition of GnRH release). It also stimulated GtH2 release (at the hypothalamus level) by means of B type of GABA receptors due to impeding dopamine release, whereas at the level of hypophysis it inhibited GtH2 release through inhibition of GnRH secretion from the nerve endings.

Key words: GABA, COMMON CARP, SUBTYPES OF GABA RECEPTORS, GtH2

INTRODUCTION

The gamma-aminobutyric acid (GABA) is recognised as an inhibiting mediator of the central nervous system. Research in the recent years have demonstrated that GABA participates in regulating the secretion of hypothalamic hormones and some pituitary hormones in mammals (McCann and Rettori 1988), including gonadotropic hormones. However, opinions on an inhibiting or stimulating effect of GABA in these processes are controversial and not clear. According to Scott and Clarke (1993) GABA inhibited LH release by means of type A of GABA receptors, whereas studies of Wilson et al. (1990) proved that GABA inhibited this hormone secretion with the participation of type B of GABA receptors. However, results by Moguilevsky et al. (1991) indicated a stimulating role of GABA in gonadotropin secretion in mammals, although it depended on sexual maturity of the animals.

The problem of GABA contribution to the control of reproductive processes in fish has been even less studied, although there are data indicating the presence of GABAergic neurones in the brain (Martinoli et al. 1990) and in the hypophysis of goldfish. Moreover, occurrence of GABA containing neurones in close contact with the gonadotropic cells in hypophysis was established (Kah et al. 1992). The recent investigations have shown that GABA participates in regulating gonadotropin (GtH2) release in goldfish (Kah et al. 1992, Sloley et al. 1992, Trudeau et al. 1993) and in carp (Roelants et al. 1990, Popek et al. 1994). Although the above mentioned authors agree that GABA is the factor modulating GtH2 secretion, opinions on the character of the effect of this neuro-transmitter on gonadotropin secretion differ considerably. As claimed by Kah et al. (1991) and Trudeau et al. (1993), GABA had a stimulating effect in sexually immature goldfish only, whereas Roelants et al. (1990) and Popek et al. (1994) demonstrated an inhibitive effect of GABA on GtH2 secretion in mature carp during the period around spawning .

In view of the above summarised controversy with respect to the role of GABAergic system in regulating the pituitary gonadotropic function in such a closely related species as goldfish and carp, investigations of carp *in vivo* and *in vitro* were undertaken in order to evaluate the stimulating or inhibiting role of GABA, and to find out the types of receptors through which and at what level (hypothalamus or hypophysis) it acted. Influence of GnRH analog and a possible contribution of the dopaminergic system in the modulating function of GABA was also taken into consideration as it was found (Peter et al. 1986, Chang et al. 1990, Yu and Peter 1990, Yu et al. 1991) that dopamine participated in the processes of releasing both GnRH as well as GtH2 in fish.

MATERIAL AND METHODS

Experiments were carried out on five years old sexually mature female and four years old male common carp. Fish were caught from a pond, weighed and tagged. During three days before the commencement of every experiment, the fish were kept in 2 m^3 running water aerated tanks, having a temperature of 18°C and a simulated photoperiod.

Experiments *in vivo*

The fish were anaesthetised before every experiment in the ethylene glycol solution. In every experiment they were injected intraperitoneally twice during 3 hours: I - first injection at time „-3", II - second injection at time "0".

The following media were applied:

*physiological solution - 0.6 % NaCl aquatic solution

*g-aminobutyric acid (GABA) - Sigma Chemical Co., USA

*isonipecotic acid (INIP) - Interchim, France

*bicuculline (BIC) - Sigma Chemical Co., USA

*baclofen (BAC) - Sigma Chemical Co., USA

*5-aminovaleric acid (AVA) - Sigma Chemical Co., USA

*muscimol (MUS) - Sigma Chemical Co., USA

*pimozide (PIM) - Janssen Pharmaceutica N.V., Belgium

*(Des-Gly^{10}, D-Ala^{6})LHRH (GnRH-A) - Sigma Chemical Co., USA

Blood samples were taken from the caudal vein using a heparinized syringe, the blood was centrifuged, blood plasma collected and stored in - 20°C until gonadotropin GtH2 level was estimated using ELISA method (Kah et al. 1989).

The design of particular experiments is shown in the following tables:

EXPERIMENT 1

Influence of GABA on the *in vivo* GtH2 release from pituitary gland of carp females, stimulated by GnRH analog

GROUP	Number of fish	I injection (- 3 h)	II injection (0 h)
RF	6	RF	RF
GnRH-A	6	RF	GnRH-A (20μg/kg)
PIM	6	PIM (5 mg/kg)	RF
GABA 300/GnRH-A	7	GABA (300 mg/kg)	GnRH-A (20μg/kg)
GABA 30/GnRH-A	7	GABA (30 mg/kg)	GnRH-A (20μg/kg)
GABA 3/GnRH-A	7	GABA (3 mg/kg)	GnRH-A (20μg/kg)
GABA 0.3/GnRH-A	7	GABA (0.3 mg/kg)	GnRH-A (20μg/kg)
GABA 300/PIM	7	GABA (300 mg/kg) + PIM (5 mg/kg)	RF

EXPERIMENT 2

Influence of isonipecotic acid - INIP (an agonist of GABA type A receptors) on *in vivo* GtH2 release from pituitary gland of carp males, stimulated by GnRH analog

a)

GROUP	Number of fish	I injection (- 3 h)	II injection (0 h)
RF	6	RF	RF
GnRH-A	6	RF	GnRH-A (20μg/kg)
PIM	6	PIM (5 mg/kg)	RF
BIC 1/GnRH-A	8	INIP (10 mg/kg)	GnRH-A (20μg/kg)
BIC 1/PIM	8	INIP (10 mg/kg) + PIM (5 mg/kg)	RF

b)

GROUP	Number of fish	I injection (- 3 h)	II injection (0 h)
RF	6	RF	RF
GnRH-A	6	RF	GnRH-A (20μg/kg)
INIP 1/GnRH-A	7	INIP (1 mg/kg)	GnRH-A (20μg/kg)

EXPERIMENT 3

Influence of bicuculline -BIC (an antagonist of GABA type A receptors) on *in vivo* GtH2 release from carp male pituitary gland, stimulated by GnRH analog

GROUP	Number of fish	I injection (- 3 h)	II injection (0 h)
RF	6	RF	RF
GnRH-A	6	RF	GnRH-A (20μg/kg)
PIM	6	PIM (5 mg/kg)	RF
BIC 1/GnRH-A	7	INIP (1 mg/kg)	GnRH-A (20μg/kg)
BIC 1/PIM	7	BIC (1 mg/kg) + PIM (5 mg/kg)	RF

EXPERIMENT 4

Influence of baclofen -BAC (an agonist of GABA receptors of type B) on *in vivo* GtH2 release from carp female pituitary gland, stimulated by GnRH analog

GROUP	Number of fish	I injection (- 3 h)	II injection (0 h)
RF	8	RF	RF
GnRH-A	8	RF	GnRH-A (20μg/kg)
PIM	8	PIM (5 mg/kg)	RF
BAC 10/GnRH-A	8	BAC (10 mg/kg)	GnRH-A (20μg/kg)
BAC 10/PIM	8	BAC (10 mg/kg) + PIM (5 mg/kg)	RF

EXPERIMENT 5

Influence of 5-aminovaleric acid - AVA (an antagonist of GABA receptors of type B) on *in vivo* GtH2 release from carp female pituitary gland, stimulated by GnRH analog

GROUP	Number of fish	I injection (- 3 h)	II injection (0 h)
RF	8	RF	RF
GnRH-A	8	RF	GnRH-A (20μg/kg)
PIM	8	PIM (5 mg/kg)	RF
AVA 10/GnRH-A	8	AVA (10 mg/kg)	GnRH-A (20μg/kg)
AVA 10/PIM	8	AVA (10 mg/kg) + PIM (5 mg/kg)	RF

Experiments *in vitro*

Perifusions of whole pituitary glands were performed using GABA, AVA, muscimol - MUS (an agonist of GABA receptors of type B), BAC, and BIC. The designs of particular experiments are shown in the following tables:

EXPERIMENT 6

Influence of GABA on spontaneous GtH2 release from carp male pituitary gland *in vitro*

Number of fish	Experimental group	Control group
6	GABA 10^{-10} M	perifusion medium
6	GABA 10^{-9} M	perifusion medium
6	GABA 10^{-8} M	perifusion medium
6	GABA 10^{-7} M	perifusion medium
6	GABA 10^{-6} M	perifusion medium
6	GABA 10^{-5} M	perifusion medium
6	GABA 10^{-4} M	perifusion medium
6	GABA 10^{-3} M	perifusion medium

EXPERIMENT 7

Influence of GABA on GnRH analog stimulated GtH2 release from carp male pituitary gland *in vitro*

Number of fish	Experimental group	Control group
6	GABA 10^{-8} M + GnRH-A 10^{-7}M	GnRH-A 10^{-7}M
6	GABA 10^{-6} M + GnRH-A 10^{-7}M	GnRH-A 10^{-7}M
6	GABA 10^{-4} M + GnRH-A 10^{-7}M	GnRH-A 10^{-7}M

EXPERIMENT 8

Influence of 5-aminovaleric acid (AVA) on spontaneous GtH2 release from male carp pituitary gland *in vitro*

Number of fish	Experimental group	Control group
6	AVA 10^{-8} M	perifusion medium
6	AVA 10^{-6} M	perifusion medium
6	AVA 10^{-4} M	perifusion medium

Results of the *in vivo* experiments were analysed by means of single factor ANOVA. Duncan test was used to establish the differences between the groups. Results of the *in vitro* experiments were compared using two tailed t Student's test.

RESULTS

EXPERIMENTS *IN VIVO*

EXPERIMENT 1

GABA injection at time „-3" at a dose of 300 mg/kg, together with the GnRH analog (20µg/kg, at time "0") resulted in a statistically significantly greater GtH2 secretion level than in the fish injected GnRH analog only (Fig. 1).

After intraperitoneal injection of either 30 or 3 mg/kg of GABA together with the GnRH analog, there was no difference in GtH2 concentration in comparison with the control group - GnRH-A (Fig. 1).

The dose of 0.3 mg/kg of GABA with the GnRH analog resulted in significant changes of GtH2 secretion. At hour 3 as well as hour 9 after the second injection, this hormone secretion in the experimental group was significantly lower than in the control group - GnRH-A (Fig. 1).

Application of 300 mg/kg GABA with pimozide (both media injected at time „-3") resulted in a significantly higher GtH2 secretion than in the control group - PIM (Fig. 1).

EXPERIMENT 2

The intraperitoneal application of 10 mg/kg INIP (injected at time „-3") with GnRH analog (20µg/kg, injected at time "0") resulted in a significant increase of gonadotropin GtH2 secretion in relation to the control group GnRH-A (Fig. 2a).

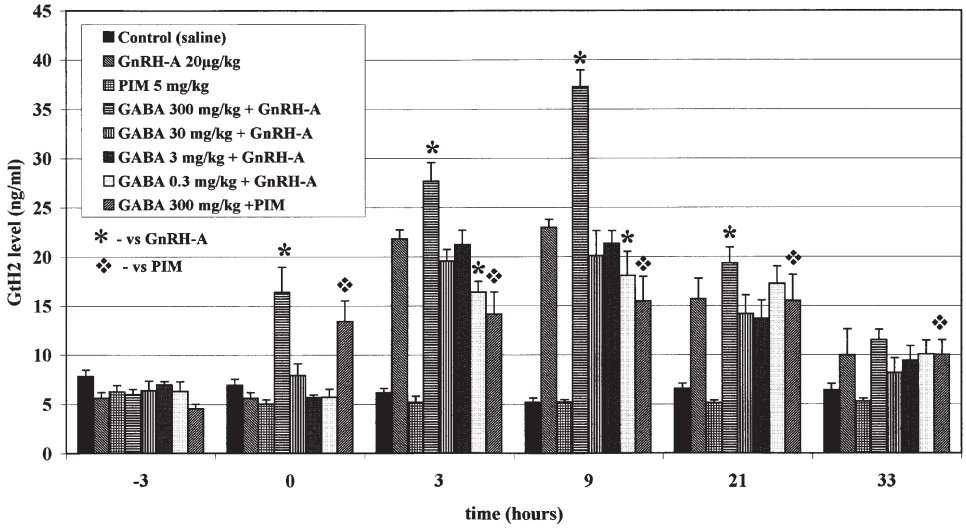

Fig. 1. Influence of various GABA concentrations on GtH2 secretion from carp pituitary gland *in vivo* ($p<0.05$)

A similar application of 1 mg/kg of this agonist together with GnRH analog did not result in any significant changes of GtH2 secretion (Fig. 2b).

After 3 hours since the application of 10 mg/kg of this medium with 5 mg/kg of pimozide (group BIC 1 mg/kg + PIM, injection time of both media „-3"), a significant increase of GtH2 was found in relation to the control - PIM (Fig. 2a).

EXPERIMENT 3

The intraperitoneal injection of 1 mg/kg of bicuculline (at time „-3") combined with GnRH-A (20µg/kg injected at time "0") brought about an increase of GtH2 secretion in relation to the control group - GnRH-A (Fig. 3).

Administration of 1 mg/kg bicuculline combined with 5 mg/kg of pimozide, both media injected at time „-3", resulted in a significant increase of gonadotropin concentration at hour 45 of the experiment in relation to the control - PIM (Fig. 3).

EXPERIMENT 4

The 10 mg/kg baclofen injection at time „-3" combined with 20µg/kg GnRH-A injection at time "0" resulted in an increase of GtH2 release (compared to the control - GnRH-A, Fig. 4).

The 10 mg/kg baclofen injection combined with 10 mg/kg of pimozide at time „-3" brought about a significant stimulation of GtH2 secretion at times O, 3, 9, and 21 hours as compared with the control (PIM) (Fig. 4).

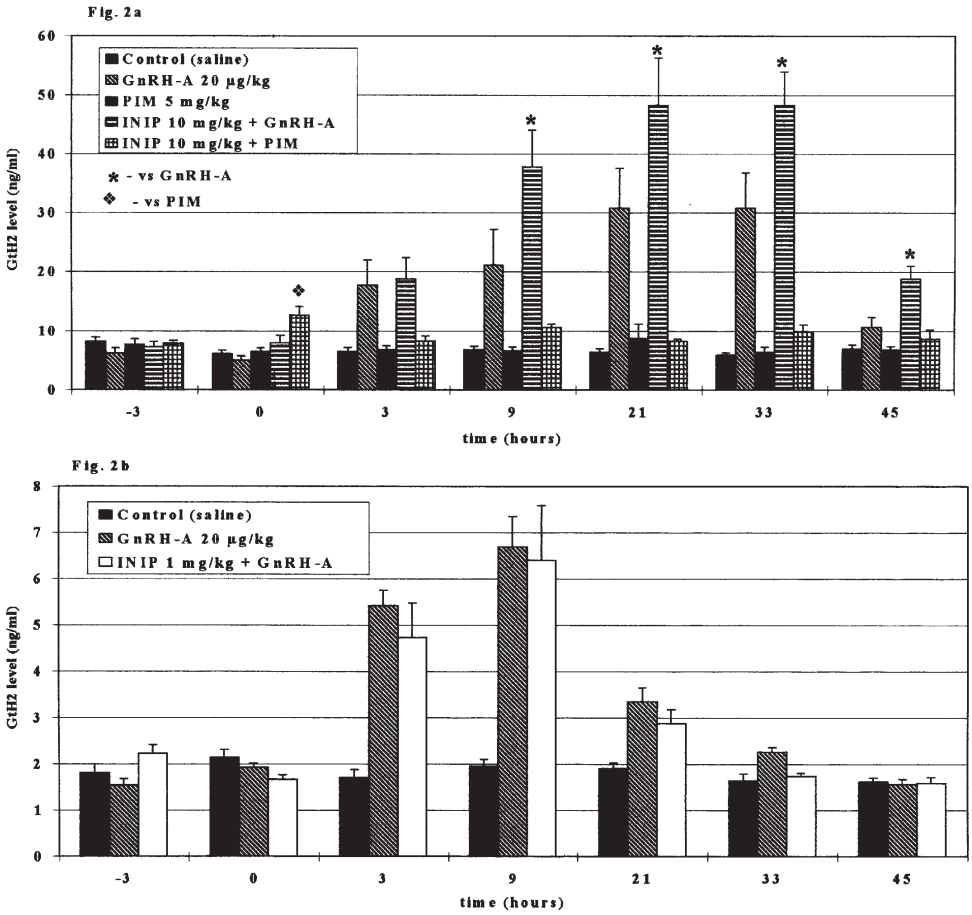

Fig. 2. a) Influence of isonipecotic acid (INIP) at the dose of 10 mg/kg on GtH2 secretion from the pituitary gland of male carp *in vivo* ($p<0.05$). b) Influence of isonipecotic acid (INIP) at the dose of 1 mg/kg on GtH2 secretion from the pituitary gland of male carp *in vivo* ($p<0.05$)

EXPERIMENT 5

The intraperitoneal injection of 10 mg/kg 5-aminovaleric acid (at time „-3") together with GnRH-A (20µg/kg, incjection time "0") resulted in a significant decrease of GtH2 secretion after 3 hours of its administration (Fig. 5).

AVA (dose 10 mg/kg) administered with pimozide (10 mg/kg, both media injected at time „-3") did not result in any significant changes of GtH2 secretion (compared to the control - PIM, Fig. 5).

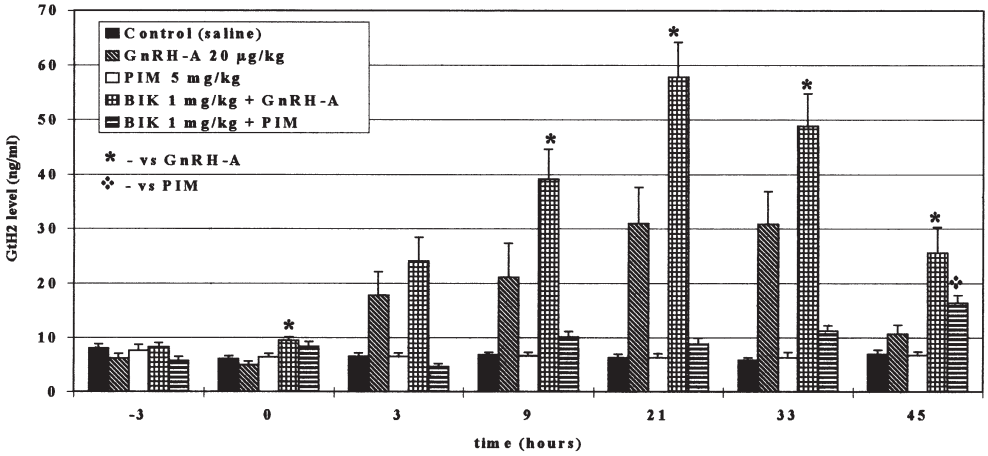

Fig. 3. Influence of bicuculline (BIC) at the dose of 1 mg/kg on GtH2 secretion from the pituitary gland of male carp *in vivo* ($p<0.05$)

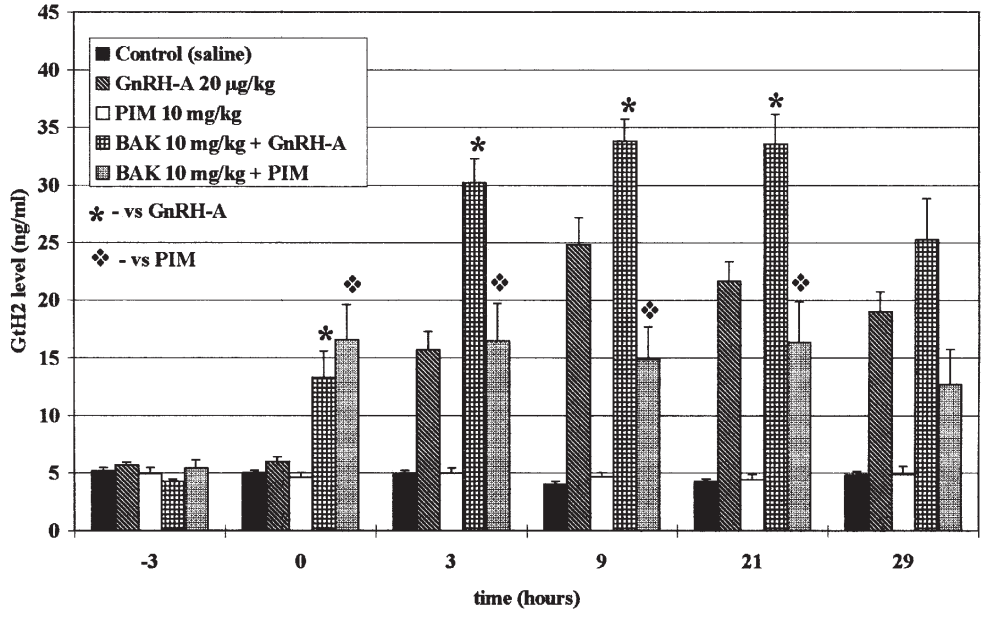

Fig. 4. Influence of baclofen (BAC) at the dose of 10 mg/kg on GtH2 secretion from the pituitary gland of female carp *in vivo* ($p<0.05$)

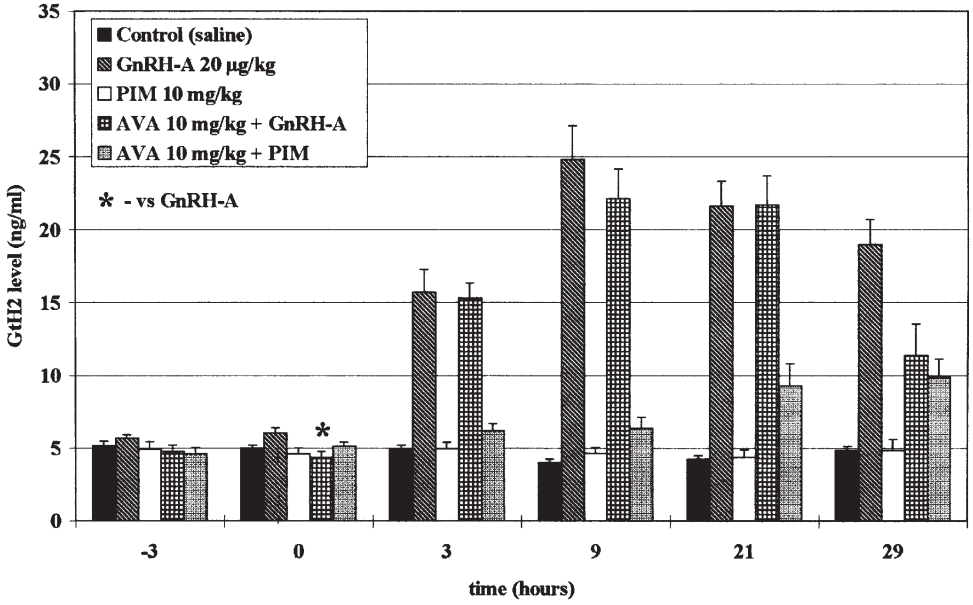

Fig. 5. Influence of 5-aminovaleric acid (AVA) at the dose of 10 mg/kg on GtH2 secretion from the pituitary gland of female carp *in vivo* ($p<0.05$)

EXPERIMENTS *IN VITRO*

EXPERIMENT 6

The perifusion of the whole pituitary glands with a medium containing gamma-aminobutyric acid (GABA) at concentrations of from 10^{-10} M to 10^{-3} M resulted in a decrease of GtH2 release in relation to the control (perifusion medium) at the concentration of GABA of 10^{-6} M. At this concentration, gonadotropin secretion level was significantly lower in 2 experimental fractions compared to the control (Fig. 6).

EXPERIMENT 7

The perifusion of pituitary glands with a medium containing GABA (10^{-8} M) and GnRH-A (10^{-7} M) resulted in retarding an increase of gonadotropin release stimulated by GnRH-A in one fraction. Application a higher GABA (10^{-6} M) concentration in perifusion did not significantly affect GtH2 release in relation to the control. Finally, a 10^{-4} M GABA concentration combined with GnRH-A brought about a significant increase of gonadotropin level in relation to the control in the case of two fractions (Fig. 7).

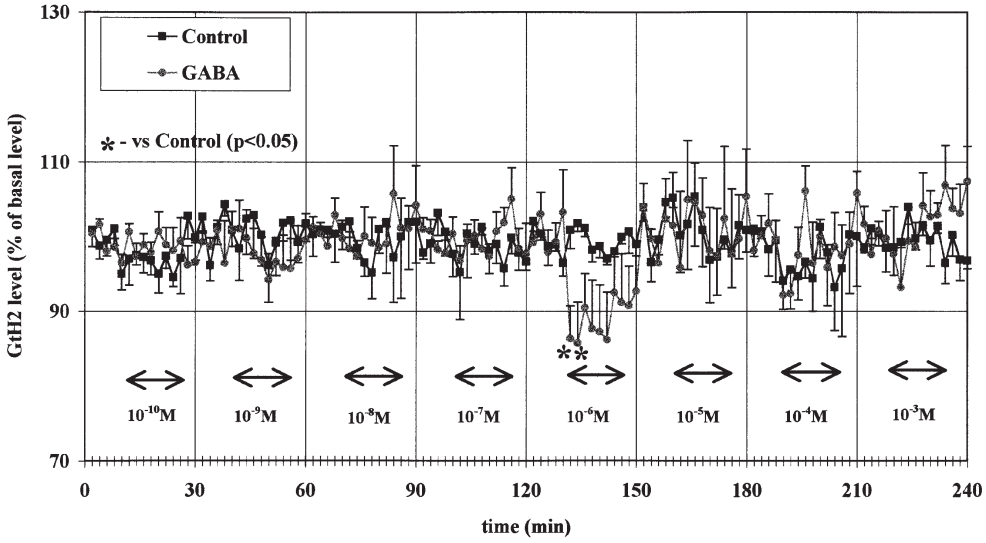

Fig. 6. Influence of various GABA concentrations on GtH2 secretion from the pituitary gland of male carp *in vitro* ($p<0.05$)

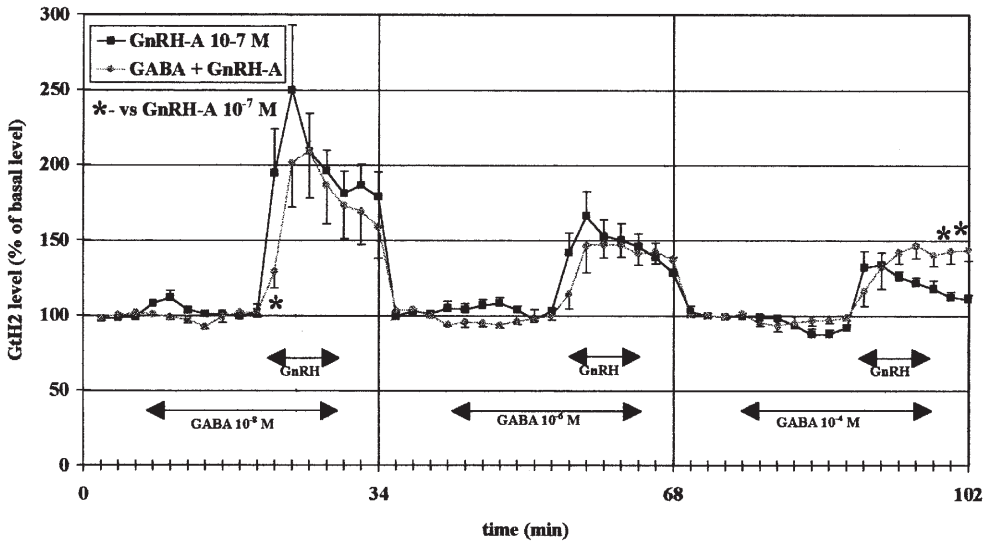

Fig. 7. Influence of various GABA concentrations on GtH2 secretion stimulated by GnRH-A from the pituitary gland of male carp *in vitro* ($p<0.05$)

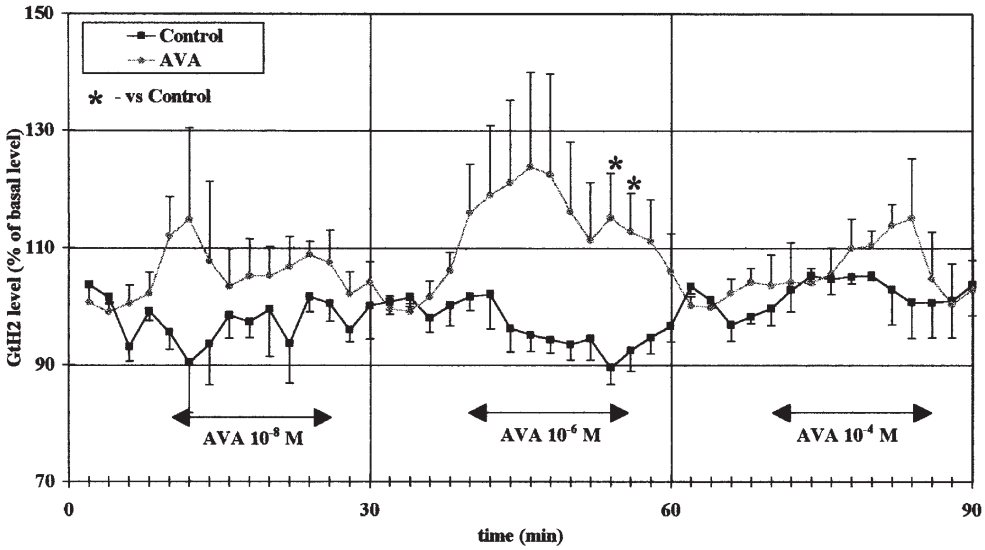

Fig. 8. Influence of various 5-aminovaleric acid (AVA) concentrations on GtH2 secretion from the pituitary gland of male carp *in vitro* ($p<0.05$)

EXPERIMENT 8

It was found that pituitary gland perifusion with a medium containing 5-aminovaleric acid at a concentration of 10^{-6} M resulted in a significant stimulation of gonadotropin GtH2 secretion (2 fractions). At the remaining concentrations, i.e. 10^{-8} M and 10^{-4} M, no significant differences in gonadotropin secretion were found between the control group (perifusion medium) and the experimental groups (Fig. 8).

DISCUSSION

Controversies as to the inhibiting or stimulating role of GABA in releasing gonadotropic hormones in mammals and in fish might result from the heterogenity of the GABAergic receptors. This is because it is accepted that at least two out of the three receptor subtypes participate in controlling the gonadotropin secretion. There are reports that GABA receptors of type A as well as of type B take part in regulating LH secretion in mammals (Wilson et al. 1990, Moguilevksy et al, 1991, Scott and Clarke 1993) or GtH2 secretion in fish (Roelants et al. 1990, Kah et al. 1991, Sloley et al. 1992, Trudeau et al. 1993b, Popek et al. 1994).

Studies on the effects of GABA as well as of the agonist and antagonists (of both subtypes) on the spontaneous gonadotropin release *in vivo* (unpublished data) demonstrated that GABA dose of the order of 300 mg/kg only and baclofen (agonist of type B of GABA receptors) produced a significant increase of GtH2 secretion in sexually mature carp. These results concur with those of Trudeau et al. (1993) pertaining to goldfish, on condition that the stimulating effect was detected in immature fish only.

There are many data in the available literature on the GABAergic modulation of GtH2 secretion as affected by GnRH. It is suggested that the GABA receptors are located in the neurones releasing GnRH from the preoptic region of hypothalamus in mammals and in fish (Nikolarakis et al. 1988, Jarry et al. 1991, Kah et al. 1992). In the present experiments, the dose of 300 mg/kg GABA resulted in a significant increase of GtH2 release when stimulated by GnRH analog (Fig. 1). However, smaller GABA doses did not change significantly GtH2 secretion, and the lowest of the applied doses - 0.3 mg/kg - resulted even in a decrease of GtH2 release (Fig.1). It seems that GABA dose of about 300 mg/kg could produce a pharmacological effect in carp, the more so that lower doses of this medium remained ineffective as regards the gonadotropin secretion, and the lowest one produced an opposite effect to that found at the 300 mg/kg dose.

In order to determine through which of its receptors GABA modulates the GtH2 release when stimulated by GnRH analog, agonists and antagonists of both types of GABA receptors were applied. The isonipecotic acid (type A receptor's agonist) at the dose of 10 mg/kg stimulated GtH2 secretion (Fig. 2a), whereas a ten times lower dose of this medium remained ineffective. Bicuculline (type B receptor's antagonist), similarly as INIP at the dose of 10 mg/kg, stimulated GtH2 release (Fig. 3). These results are surprising in that a similar effect was detected in the case of agonist and antagonist of the same receptor. Findings of Popek et al. (1994) confirm the results of these investigations on bicuculline. On the other hand, lack of data on the effect of INIP does not explain the results of this investigation. One can only conjecture that the applied INIP dose (10 times higher than the BIC dose), likewise the highest GABA dose, exerted a pharmacological action.

The published data on mammals and fish evidence that GABA may stimulate as well as an inhibit GnRH secretion trough type A receptors (Jarry et al. 1991, Leranth et al. 1985, Masotto and Negro-Vilar 1986, Hales et al. 1994, Martinez de la Escalera et al. 1994). It is possible that such a diverse action results from the fact that type A recep-

tors affect not only the GnRH releasing neurones, but also the factors modulating GnRH release.

The problem of the contribution of type B receptors in regulating GnRH-A stimulated GtH2 secretion in carp was also an interesting issue. The agonist of this receptor, BAC, applied at the dose of 10 mg/kg, enhanced the GnRH-A effect (Fig. 4). The antagonistic medium (AVA) resulted in an opposite effect, i.e. a statistically significant decrease of GtH2 secretion (Fig. 5). Although there are no published accounts on fish, data related to mammals indicate that baclofen does not affect LH release when stimulated by LHRH.

Experiments conducted with the participation of GABA (300 mg/kg), INIP (10 mg/kg), BIC (1 mg/kg), BAC (10 mg/kg) and of AVA (10 mg/kg) applied to fish together with pimozide (dopamine receptor antagonist) in order to block the inhibiting effect of dopamine on GtH2 secretion, showed that in such conditions all media, except AVA, brought about a significant increase of GtH2 secretion. The effect of GABA and of INIP can be regarded as pharmacological, similarly to the case of GtH2 secretion stimulated by GnRH-A. The stimulating influence of bicuculline and baclofen was confirmed. These results are an indirect evidence that - apart from type A receptors - also type B receptors can take part in regulating the GtH2 secretion, possibly through modulation of the dopaminergic system at the hypothalamus level.

While studying the modulating effect of GABA on gonadotropin secretion in fish, it was necessary to take into consideration the anatomic specificity of the bond between hypothalamus and pituitary gland, which involved an indirect innervation of cells of the hypophyseal glandular part by hypothalamic neurones. The perifusions of the whole hypophyses by media containing various GABA concentrations (10^{-10} - 10^{-3} M) demonstrated that a significant decrease of GtH2 secretion occurred at the concentration of 10^{-6} M (Fig. 6). These findings contradict those of Kah et al. (1992) who did not notice any GABA effect on GtH2 secretion from dispersed cells of goldfish hypophysis. In such a system there were no bonds between the endings of dopaminergic neurones, the endings of GnRH releasing neurones, and the gonadotropic cells. The inhibiting GABA effect obtained in the case of the whole hypophysis perifusion might have resulted from the effect of GABA on neurones with GnRH or on dopaminergic neurones. There are some data which confirm the above findings, viz. the GtH2 secretion stimulation in the static incubation of hypophysis fragments of goldfish in the presence of GABA (Kah et al. 1992).

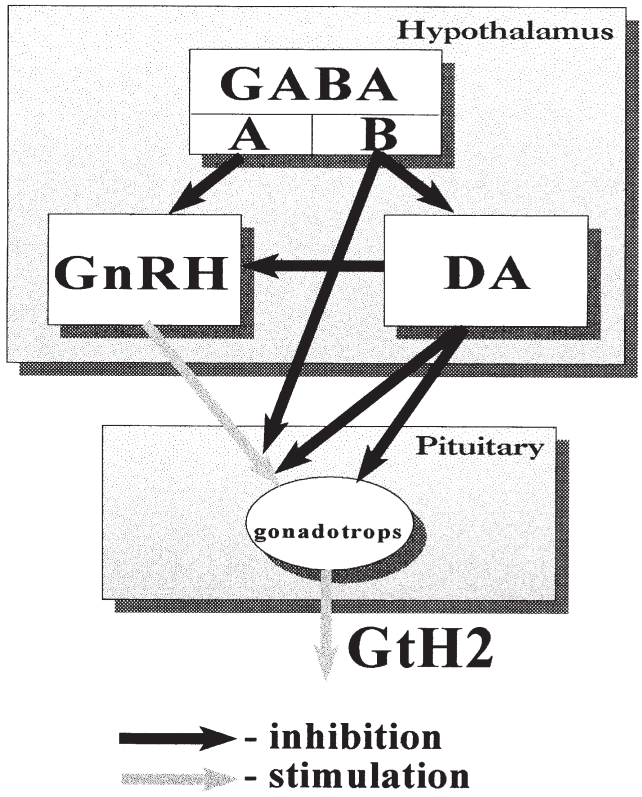

Fig. 9. Suggested layout of influence of GABAergic system on GtH2 secretion

Investigations in perifusion system of the influence of muscimol, bicuculline, and baclofen did not reveal any significant effects on GtH2 secretion (data not shown). 5-aminovaleric acid produced a significant increase of GtH2 secretion at the concentration of 10^{-6} M (Fig. 6). Therefore, the stimulatory effect of AVA on GtH2 secretion, and the fact that GABA inhibits spontaneous and stimulated by GnRH-A release of GtH2 *in vitro*, imply that type B of GABA receptors can probably be responsible for inhibition of GnRH receptors from the nerve ending at the pituitary level. It should be noted that baclofen, having an activity opposite to AVA, did not produce any changes in GnRH secretion from perifused pituitary glands. Perhaps the GnRH releasing neurones are in a state of tonic inhibition with the participation of type B GABA receptors (Lux-Lantos et al. 1993). This problem, however, requires more detailed study with respect to the GtH2 release stimulated by GnRH-A *in vitro*.

Considering the above discussed results one can make several generalisations as regards the effects of GABAergic system on GtH2 secretion :

1. GABA affects GtH2 secretion from the carp hypophysis through both GABA-A as well as GABA-B receptors, exerting its influence mainly through the first type of receptor.
2. By the intermediary action of GABA-A receptors, g-aminobutyric acid inhibits GtH2 secretion at the hypothalamus level trough inhibiting GnRH release.
3. By the intermediary action of GABA-B receptors, g-aminobutyric acid affects GtH2 secretion in two ways:

   a) at the hypothalamus level it stimulates GtH2 secretion through inhibiting the dopamine (DA) release

   b) at the level of hypophysis it inhibits GtH2 release inhibiting GnRH secretion from neurone endings.

These interactions are illustrated in Fig. 9.

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STRESZCZENIE

GABAergiczna MODULACJA SEKRECJI GONADOTROPINY (GtH2) Z PRZYSADKI MÓZGOWEJ KARPIA (*Cyprinus carpio* L.)

Doświadczenie *in vivo* i *in vitro* obejmowały badanie wpływu GABA, agonistów i antagonistów GABA-A i GABA-B receptorów (kwas izonipekotynowy - INIP, bikukulina - BIC, baklofen - BAC, kwas 5-aminowalerianowy - AVA, muscimol - MUS) na sekrecję gonadotropiny GtH2 stymulowaną analogiem GnRH u dojrzałych płciowo karpi w okresie okołotarłowym. Uwzględniając wpływ systemu dopaminergicznego na sekrecję GtH2 niektóre doświadczenia przeprowadzono na karpiach iniekowanych piozydem - antagonistą dopaminy.

Wyniki doświadczeń wykazały, że GABA oddziaływuje na sekrecję GtH2 z przysadki mózgowej poprzez obydwa podtypy receptorów GABA. Poprzez podtyp A hamuje sekrecję GtH2 na poziomie podwzgórza (poprzez hamowanie uwalniania GnRH). Poprzez podtyp B działa w dwojaki sposób na poziomie podwzgórza stymuluje sekrecję GtH2 poprzez eliminowanie hamującego wpływu dopaminy, natomiast na poziomie przysadki mózgowej hamują uwalnianie GtH2 poprzez hamowanie sekrecji GnRH z zakończeń nerwowych.

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