Serotonin, GnRH-A, and dopamine interaction in the control of in vivo luteinizing hormone release in Prussian carp (Carassius gibelio Bloch) at the time of gonad recrudescence


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ABSTRACT: The effects of serotonin (5-HT), GnRH analogue (D-Ala$^6$ LHRH, GnRH-A) and dopamine antagonist – pimozide (PIM), on luteinizing hormone (LH) release in mature Prussian carp female (Carassius gibelio Bloch) were examined at the time of gonad recrudescence. Fish were intraperitoneally injected with 5-HT (10 mg/kg), GnRH-A (20 μg/kg) or PIM (5 mg/kg) or the combinations: 5-HT+GnRH-A, 5-HT+PIM, 5-HT+GnRH-A+PIM. Before the injection and 3, 6, 12 or 24 h after treatment blood samples were collected for LH levels determination by ELISA method. The analysis of LH concentrations, expressed as the percentage of pre-treatment, showed that serotonin alone had no influence on the spontaneous LH release, however the additive effects of serotonin and GnRH-A was observed. Serotonin potentiated the GnRH-A-stimulated LH release and potentiated also the effect of PIM. Extremely strong response to PIM and also to the combination with GnRH-A masked the participation of serotonin in the process of LH release in fish with recrudescing gonads. The interaction of serotonin GnRH-A and PIM in the control of LH release is discussed.

Keywords: gonadotropin-releasing hormone analogue; LH; pimozide – dopamine antagonist; serotonin

INTRODUCTION

The role of the serotonergic system in the control of fish maturation and reproduction is not definitively explained but the number of data showing its involvement in these processes is growing, also in the context of selective serotonin reuptake inhibitor (fluoxetine – an antidepressant drug) presence in the aquatic systems in significant concentrations (Kolpin et al. 2002; Brooks et al. 2003; Metcalfe et al. 2010). This potential disruptor of serotonin-mediated physiological processes concentrates also in the tissues of wild-caught fish (Brooks et al. 2005). The serious ecotoxicological effect of fluoxetine concentrations in aquatic systems is not only in the case of fish reproduction but also on feeding (Mennigen et al. 2009) and behaviour (Semsar et al. 2004; Mennigen et al. 2011).

To assess the potential role of serotonin reuptake inhibitor-induced reproductive impairment in fish, the role of serotonin in the control of hypothalamic-pituitary-gonadal axis function has to be definitively established. Serotonin immunoreactive pericaria are located in goldfish (Carassius auratus L.) hypothalamus (nucleus recessus lateralis and nucleus recessus posterioris) (Kah and Chambolle 1983). Also in the pituitary gland immunoreactive serotonergic fibres, cells, and nerve terminals were found: in goldfish (Kah and Chambolle 1983), three-spined stickleback – Gasterosteus aculeatus L. (Ekstrom and Van Veen 1984), rainbow trout – Oncorhynchus mykiss (Frankenhuis-van den Heuvel and Nieuvenhuys 1984), platyfish – Xiphophorus maculatus (Margolis-Kazan et al. 1985), molly – Poecilia latipinna (Groves and Batten 1985), and Atlantic croaker – Micropogonias undulatus (Khan...
and Thomas 1993). These morphological observations confirm serotonin involvement in the regulation of pituitary hormone secretion in fish, gonadotropic hormones among others. There is also a direct evidence on the effects of serotonin on gonadotropin secretion in fish: 5HT₂-like receptors mediate the in vivo and in vitro stimulatory effects of serotonin on gonadotropin release in goldfish (Somoza et al. 1988; Somoza and Peter 1991; Wong et al. 1998), in Atlantic croaker (Khan and Thomas 1992), and in red seabream (Senthilkumaran et al. 2001) at the level of GnRH cell body or nerve terminals (Yu et al. 1991).

In mammals, serotonin involvement in the regulation of gonadotropin LH and FSH release is not so well defined and data are still controversial: according to Pinilla et al. (1994) and Dow et al. (1994) serotonin stimulates gonadotropin release and according to others (Le Corre and Chemineau 1999a, b; Rodriguez et al. 1994; Chemineau et al. 2003) its action is inhibitory.

Gonadotropin (FSH and LH) secretion in fish is a resultant of the interaction of many external and internal factors: several neurohormones and neuropeptides affect their synthesis and/or release, directly at the pituitary level or indirectly at the level of hypothalamus. Some of them, like GnRH, dopamine, γ-amino butyric acid (GABA), pituitary adenylate cyclase activating peptide (PACAP), norepinephrine, neuropeptide Y (NPY), ghrelin, leptin, kisspeptin have been reviewed extensively (Lethimonier et al. 2004; Levavi-Sivan et al. 2010; Zohar et al. 2010), however, potential interactions between them affecting the synthesis and release of gonadotropins have not been definitively elucidated yet. Of so many at least two are considered as the most important: GnRH and dopamine (Peter and Yu 1997). It would be interesting to find out whether serotonin stimulates luteinizing hormone (LH) release acting on GnRH system or on dopamine release which in turn affects GnRH secretion. Khan and Thomas (1993) showed that in Atlantic croaker serotonin and GnRH systems are in close proximity what suggests that they may interact in the process of gonadotropin release control. Data on serotonin and dopamine concentrations in the brain and pituitary of mummichog (Fundulus heteroclitus), showed that dopamine content, high during gonadal recrudescence, lowers significantly during ovulation and spawning (Subhedar et al. 1997). Wong et al. (1998) have already demonstrated that 5-HT exerts its stimulatory action on gonadotropin release directly at the pituitary level, probably by interactions with other regulators, such as dopamine.

As there is no clear information on the interaction of these hormones in the process of gonadotropin secretion, the experiments on Prussian carp were undertaken in order to determine LH secretion under the influence of exogenous serotonin, GnRH analogue in fish with dopamine receptors blocked by the specific D2 receptor antagonist – pimozide (PIM).

### MATERIAL AND METHODS

Sexually mature female Prussian carp (Carassius gibelio Bloch) bred in the Fisheries Experimental Station of the Department of Ichthyobiology and Fisheries, University of Agriculture in Krakow, were used for the experiment conducted at the end of November.

Ninety-six fish netted from the wintering pond were transferred to eight 300-l glass aquaria (12 fish per aquarium) with aerated water. Water temperature was slowly raised and after two days it reached 18–19°C. Then fish were anaesthetized by means of immersion in Propiscin (IRS, Zabieniec, Poland) solution (0.3 ml/l of water), weighed (mean body weight (BW) of 82.2 ± 2.2 g), and individually marked by a gentle fin clip. Fish were at the stage of gonadal recrudescence: their gonadosomatic index (GSI) was of 5.4 ± 0.9%.

After 10-day lasting acclimatization to water temperature of 18–19°C and to the simulated photoperiod typical for November (ratio of light to dark phase of a day L : D was 8:16) fish were anaesthetized, blood samples (150 μl) were taken with heparinized 1-ml syringes, and then fish were subjected to intraperitoneal injections of the tested substances as follows: group 1 (Control – 0.6% saline solution), group 2 (5-HT – 10 mg/kg BW), group 3 (GnRH-A – 20 μg/kg BW), group 4 (PIM – 5 mg/kg BW), group 5 (GnRH-A + PIM), group 6 (5-HT + GnRH-A), group 7 (5-HT + PIM), group 8 (5-HT + GnRH-A + PIM).

5-HT – serotonin (creatinine sulfate monohydrate), GnRH-A (D-Ala⁷ LHRH), PIM – dopamine D₂-type receptor blocker were purchased from Sigma-Aldrich, St. Louis, USA. Serotonin and GnRH-A were dissolled in saline acidified with 0.1M HCl and PIM in propylene glycol acidified with 0.1M acetic acid. Each drug dose calculated for 1 kg of fish BW was dissolved in 1 ml.
At time 0 (before injection) and at 3, 6, 12, and 24 h post injection plasma blood samples were collected from all fish and then they were frozen and kept at –20°C until gonadotropin (LH) determination by ELISA method (Kah et al. 1989). Statistical analysis was performed using GraphPad Prism 5 software (Version 5.0, 2007) for MS Windows. The effects of serotonin, PIM, and GnRH-A on LH levels were expressed as percentage of pre-treatment. Kruskal-Wallis non-parametric ANOVA followed by Mann-Whitney U test (one tailed) with Bonferroni correction were applied to find the statistically significant differences between the groups (P < 0.05).

Data on the graphs are presented as the mean ± SEM percentage of pre-treatment values of gonadotropin LH.

The experiment was conducted according to the guidelines given by the Institutional Ethical Committee.

RESULTS

Effects of serotonin (5-HT) on the spontaneous or GnRH-A-stimulated LH release. In the case of fish treated with GnRH-A or serotonin alone at all times of blood sampling there were no statistically significant differences between control, serotonin or GnRH-A receiving fish. In 5-HT+GnRH-A treated fish LH levels were significantly higher at 6, 12, and 24 h post-injection in comparison to the control group at 12 and 24 h after injection respectively.

In fish receiving 5-HT+PIM statistically significant increase of LH levels was found at 6, 12, and 24 h sampling times after treatment in relation to control (by 162.6, 371.9, and 448.7%, respectively) as well as to serotonin group (by 125.3, 199.42, 386.2, and 438.1%, respectively) (Figure 1).

Effects of serotonin on GnRH-A-stimulated LH release in fish with dopaminergic system blocked by PIM. As shown in Figure 3, PIM given together with GnRH-A evoked a significantly higher LH levels increase compared to control at 12 and 24 h post-injection (by 1572.3 and 297.3%, respectively). The combination: 5-HT+GnRH-A+PIM at 6, 12, and 24 h after injections caused significant increase of LH concentrations in comparison to control group (by 464.6, 1204.9, and 572.2%, respectively) as well as to serotonin receiving group (by 501.42, 1219.2, and 507.6%, respectively). The comparison with GnRH-A injected fish at 6, 12, and 24 h after injections demonstrated LH increase (by 431.9, 1211.0, and 491.6% respectively – data not shown). Also in comparison to PIM alone injected fish the combination of all three compounds caused a significant increase in LH levels at 6 and 12 h sampling time by 395.2 and 968.0%, respectively (data not shown).

DISCUSSION

In teleost fish, gonadotropin LH concentration in the blood circulation depends on interaction of many hormonal factors acting at the level of the brain and/or pituitary, with two most studied: GnRH and dopamine, having opposite effect on this process. From the in vivo and in vitro study...
it is known that in fish serotonin stimulates LH secretion at the level of hypothalamus (GnRH cell body or nerve terminal – Yu et al. 1991; Schulz and Goos 1999; Senthilkumar et al. 2001) or pituitary gland (Somoza and Peter 1991; Khan and Thomas 1992; Wong et al. 1998). This influence at the level of hypothalamus may be direct or indirect (through interaction with other hormonal systems, like dopaminergic one), but this latter possibility has not been intensively studied so far. However, there is immunohistochemical data showing that interaction between serotonin and dopamine has the morphological base: Ekstrom and Van Veen (1984) in Gasterosteus aculeatus L. and Batten et al. (1993) in sea bass (Dicentrarchus labrax) brain found that all region of the brain is densely innervated by 5-HT fibres and terminals. In catfish (Clarias gariepinus) brain Corio et al. (1991) demonstrated that diencephalon contained a high number of serotonin- or dopamine-immunoreactive cell bodies confined to the same brain nuclei. On the other hand, there is an evidence (Kah et al. 1987) that dopamine and GnRH neurons are also co-localized in goldfish brain. Based on these observations we can conclude that there is morphological and anatomical base for the interaction between serotonin, dopamine, and GnRH systems in teleost fish.

Our in vivo experiments on the effects of serotonin on LH release in Prussian carp were conducted at the time of early gonadal recrudescence – at that time of the season fish response to GnRH stimulation is usually weaker (Sokolowska et al. 1985) probably because of stronger dopamine inhibition on LH secretion. Depending on the phase of fish gonad maturity the sensitivity of hypothalamo-pituitary-gonadal axis to hormonal stimulation differs, what has already been demonstrated for GnRH and dopamine (Sokolowska et al. 1985) or for endogenous opioid peptides (Sokolowska-Mikolajczyk et al. 2002).

The analysis of LH concentration in serotonin or GnRH-A receiving fish showed no significant changes during all times of blood sampling (3, 6, 12, and 24 h after treatment). Such lack of responsiveness may reflect the influence of the season (winter, early gonad recrudescence) on reproductive axis activity. However, the combination of both: serotonin and GnRH-A stimulated LH secretion (Figure 1) not only in comparison to control (at 6, 12, and 24 h post-injection) but also to serotonin alone (at 6, 12, and 24 h) or GnRH-A alone injected fish (at 6 and 12 h after treatment), which represents the potentiation of GnRH-A effect by serotonin. Also Khan and Thomas (1994) demonstrated similar potentiation in Atlantic croaker, in agreement with their earlier findings (Khan and Thomas 1993), that serotonin and GnRH systems are in close proximity. Our data confirm the connection between serotonin and GnRH system, as far as LH release from fish pituitary is concerned, in fish with dopamine inhibition on this gonadotropin secretion not changed by the treatment with PIM. This interaction gives stronger effect than inhibition exerted by dopamine at that stage of gonad maturity. How strong this inhibition is we demonstrated in PIM injected fish (Figure 2) – over 236% increase of LH level in relation to control values at 12 h post-injection were observed. Removed dopamine inhibition by PIM strongly potentiated LH releasing effects of GnRH-A – 1573.9 and 314.7% stimulation in relation to control group at 12 and 24 h post-injection respectively, were found (Figure 3) while the influence of GnRH-A alone was not detected – no differences from control values at any sampling time (Figure 1). Such stimulation was
surprising, whereas experiments were performed in November on fish with recrudescing gonads (gonad-osomatic index GSI = 5.4%). Usually the response to the stimulation at that time of the season is weak – this observation is confirmed by the lack of effects of GnRH-A alone in the present data. It shows also that dopamine inhibition on LH release is very strong at that time of the year. It is confirmed by the pronounced response to PIM (Figure 2), which is stronger than can be found in mature carp or goldfish, having GSI exceeding 20% (Mikolajczyk et al. 2004). The seasonality of maturation and reproduction in fish and accompanying them different activity and sensitivity of many systems controlling production and secretion of hormones, responsible for gonad maturation, is well known (Sokolowska et al. 1985, Saligaut et al. 1992; Sokolowska-Mikolajczyk et al. 2002). This seasonality was also demonstrated by Khan and Thomas (1992) in Atlantic croaker serotonin stimulated gonadotropin release in sexually mature females and in juvenile fish potentiated gonadotropin release by GnRH, but in fish undergoing sexual recrudescence gonadotropin releasing action of salmon GnRH analogue was not affected by the treatment with 5-HT. The authors suggest that the post-receptor mechanisms mediating gonadotropin releasing effect of these hormones are independent to each other and they may act additively and that 5-HT may be synthesized in the pituitary acting as an autocrine/paracrine factor. It may be as well taken up by the 5HT-positive cells from the blood entering the anterior pituitary (Wong et al. 1998).

Serotonin action in fish with dopamine system blocked with PIM was found at 6, 12, and 24 h post-injection (Figure 2) in comparison to control group. Later (at 12 and 24 h) the action of PIM was so strong (341% of LH increase in comparison to control) that it masked the participation of serotonin in this process. Significantly higher LH levels observed in 5-HT+PIM group at other sampling times, when compared to control group, were caused by PIM action only (no differences between 5-HT+PIM and PIM groups). The presence of the relationship between serotonin and dopamine may be more pronounced in fish with mature gonads but to prove that future studies will be required. It is known that dopamine and serotonin take part in endogenous rhythms related to reproduction – changes in dopamine and serotonin turnover were observed in the brain of teleost fish under different reproductive conditions (Dulka et al. 1992; Saligaut et al. 1992; Senthilkumaran and Joy 1993; Trudeau et al. 1993). Subhedar et al. (1997) found significant changes of dopamine and serotonin concentrations in the brain of killifish (Fundulus heteroclitus) during its reproductive cycle.

In the case of joint action of serotonin and GnRH-A in fish treated with PIM we observed tremendous increase of LH secretion (Figure 3): at 12 h post-injection it was 1204.9% in relation to control group. Such high stimulation was due to the combination of GnRH-A+PIM, and serotonin did not change in any way this process (no differences between 5-HT+GnRH-A+PIM and GnRH-A+PIM were found).

In summary, our experiment performed on Prussian carp at the stage of gonad recrudescence showed that:

– serotonin alone had no influence on the spontaneous LH release
– additive effects of serotonin and GnRH-A on LH release were observed

Figure 3. Effects of serotonin (5-HT) on luteinizing hormone (LH) secretion at 3, 6, 12, and 24 h post-injection in GnRH-A and pimozide (PIM; dopamine antagonist) treated fish. Bars represent the mean ± SEM percentage of pre-treatment values of gonadotropin LH. Different superscripts indicate significant differences within the same sampling time.
– serotonin potentiated the GnRH-A-stimulated LH release
– extremely high response to PIM alone and also to the combination with GnRH-A masked the potential participation of serotonin in the process of LH release in fish with recrudescing gonads.

REFERENCES


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