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INDUCED MATURATIONAL GONADOTROPIN RELEASE AFTER INTESTINAL ABSORPTION ENHANCEMENT OF PIMOZIDE + S-GNRH-A IN COMMON CARP

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Introduction

It has been well established that D2 - type dopamine receptor blockers, as pimozide (Pim), potentiate the ability of salmonid gonadotropin hormone releasing hormone (s-GnRH), and its superactive analogues (s-GnRH-a) to induce the release of maturational gonadotropin (GtH_2) in cyprinids (Billard et al, 1987). The present study compares the oral and rectal route of delivery of s-GnRH-a and Pim, with the traditional way of parenteral administration. The effect of co-administration of absorption enhancers to lower the physical barrier and of peptidase inhibitors to reduce the metabolic barrier and evaluated, as well.

Methods

This study was performed at the Department of Ichthyobiology and Fisheries (DIF) of the Agricultural Academy in Cracow.

Animals: End April 1991, 80 sexually mature female carps (b.w. = 3.9 $\pm$ 0.8 kg, mean $\pm$ SD) were netted from outdoors mixed stock ponds and brought into the laboratory to be anesthetized, weighed and numbered. Per group of eight they were then placed in a 1000 L flow-through basin and exposed for four days to 20°C $\pm$ 0.5 under a simulated natural photoperiod ambient for the time of year (14 L : 10 D). The carps were not fed.

Materials: Pimozide and oleic acid were generous gifts from respectively Janssen Pharmaceuticals Ltd, Beerse, Belgium and Mosselman Ltd, Belgium. Polyoxyethylene sorbitan monooleate (T80) and chicken egg white trypsin inhibitor type II were respectively purchased from Serva Fine Biochemicals and Sigma Chemicals Co., Ltd., while EDTA disodium salt could be obtained from J.T. Baker bv. The salmonid gonadotropin

hormone releasing hormone used in this study was, (Des-Gly10-D-Arg6, Trp7, Leu8, Pro9)-LHRH ethyl amide from Bachem Inc., USA.

Table I: Treatment closure of various groups

Group	N	Body* weight	Solution	Route of delivery
1 (S)	8	4.3 ± 1.1	PBS	rectal
2 (R)	8	3.9 ± 0.4	GnRH-a + Pim PBS + enhancer	rectal
3 (PR)	8	4.1 ± 0.3	GnRH-a + Pim PBS	rectal
4 (RE)	8	3.6 ± 0.5	GnRH-a + Pim PBS + enhancer + EDTA	rectal
5 (PO)	8	4.2 ± 1.1	GnRH-a + Pim PBS	oral
6 (RT)	8	3.3 ± 0.4	GnRh-a + Pim PBS + enhancer + trypsin inhibitor	rectal
7 (IP)	8	3.5 ± 0.4	GnRH-a + Pim PBS	I.P.
8 (RETE)	8	4.2 ± 1.2	GnRH-a + Pim PBS + enhancer + EDTA + trypsin inhibitor	rectal
9 (OETE)	8	4.2 ± 1.2	GnRH-a + Pim PBS + enhancer + EDTA + trypsin inhibitor	oral
10 (IV)	8	4.1 ± 0.6	GnRH-a + Pim PBS	I.V.

* values are means ± SD

Just prior to injection all drugs were freshly made up. For rectal injection a flexible polyethylene tubing was introduced 2.3 cm up the descending intestine via the anal porus. For oral intubation a flexible polyethylene tubing was introduced in the intestine via a rigid guiding tubing, which was inserted between the tooth plates.

- GnRH = s-GnRH-a, 20 μg/ml or 10 μg/kg fish b.w.
- Pim = pimozide, 10 mg/ml or 5 mg/kg fish b.w.
- PBS = phosphate buffered saline, pH = 7.4; 0.058 M
- Enhancer = 4% w/v polyoxyethylene sorbitan monooleate + 0.6% w/v oleic acid
- EDTA = Na_2EDTA, 0.25% w/v
- Trypsin inhibitor = chicken egg white trypsin inhibitor, 5 mg/ml PBS.

All groups got the same vehicle volume, being 0.5 ml/kg. Blood samples (200-500 μl) were collected in preneparinized syringes from the caudal vein at the time of injection and 6, 12, 24 and 31 hours later. Plasma samples were kept frozen at -20°C until assayed for

GtH_2 by ELISA according an earlier described method (Kah et al, 89). Differences in hormone levels between treatment groups were analyzed using one-way ANOVA and considered significant if $p < 0.05$.

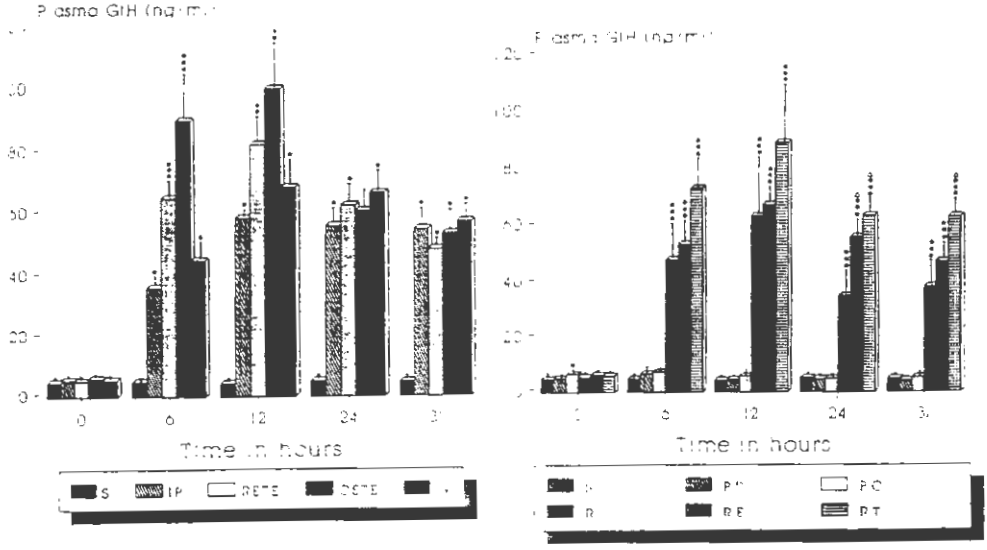

Fig. 1A/1B: Effects of 10 µg/kg fish b.w. s-GnRH-a and 5 mg/kg fish b.w. pimozide, being bolus delivered via various routes, on the release of maturational gonadotropin (GtH_2) as indicated under Table I. The mean plasma levels of GtH_2 are expressed in bars; lines represent the upper SEM range (n = 8).

1A ● vs to intrarectal bolus intubation of vehicle control (S)

* vs to Pim + GnRH intraperitoneal injected (IP)

○ vs to Pim + GnRH-a intravenous injected (IV)

1B ● vs to intrarectal bolus intubation of vehicle control (S)

* vs intrarectal bolus intubation of Pim + GnRH-a in vehicle control (PR)

○ vs oral bolus intubation of Pim + GnRH in vehicle control (PO)

Δ vs rectal bolus intubation of Pim + GnRH-a in vehicle + enhancer (R)

Results and discussion

PANEL A:

1. Intraperitoneal and intravenous injection of GnRH-a + Pim, in comparison to the control vehicle administration, gave significantly higher GtH_2 values at any post treatment sampling time.
2. At least at the sampling time six hours after delivery, both rectal and oral intubation of GnRH-a + Pim in vehicle + intestinal absorption enhancer + trypsin inhibitor + Na_2EDTA resulted in significantly higher GtH_2 values than intraperitoneal or intravenous injection of the same drugs in vehicle only.
3. Between the oral and rectal route of administration of these drugs no significant different GtH_2 release effect has been discovered at any sampling time.

PANEL B:

1. The so-called natural occurring ability of the fish gut to absorb intact bioactive peptides and polypeptides seems to be of a limited nature. The data of panel B, clearly demonstrate that oral or rectal intubation of both drugs, GnRH-a and pimozide in isotonic PBS but without co-administration of absorption enhancer or peptidase inhibitor does not evoke any GtH_2 release as compared to vehicle control.
2. At any post-treatment sampling time the intestinal absorption enhancement formulation induced a significant higher bioactivity of Pim + GnRh-a than the same compounds delivered orally or rectally without enhancement.
3. Addition of the chicken egg white trypsin inhibitor to an absorption enhancement formulation resulted also in an increased bioactivity of pimozide + GnRH-a, as twenty four hours and thirty one hours post-treatment significantly higher GtH_2 plasma values were recorded than in the group with the same route of delivery (oral or rectal) but with enhancement and without peptidase inhibition.
4. Addition of Na_2EDTA to an absorption enhancement formulation also resulted in a significant higher GnRH-a + Pim induced GtH_2 release thirty one hours post-treatment. But it is to discuss whether this is related to the peptidase inhibiting activity rather than to a physical absorption enhancement effect of this Ca-chelate.
5. A slight significant difference was recorded in the zero hour control GtH_2 values between carps that got GnRH-a + Pim in vehicle orally and these which got the same formulation rectally.

Conclusions

The present study demonstrates that rectally and orally intubated peptidergic drugs can be target to the blood circulation system without important loss of their bioactivity if intestinal absorption enhancement is used. It also underlines the fact that inhibition of digestive enzymes can increase preservation of bioactivity of intestinal absorbed peptidergic drugs. This results may possibly be extended to agastric teleosts other than carp and to gastric fish. But, a suitable feeding regime as yet to be found to monitor non vital functions in teleost fish by dietaryl uptake of such bioactive peptides.

References

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