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To cite this version:
Hao-Ren Lin, Chun Peng, Glen van Der Karaak, Richard E. Peter, Bernard Breton. Effects of [d-Ala6, Pro9-NEt]-LHRH and catecholaminergic drugs on gonadotropin secretion and ovulation in the Chinese loach (Paramisgurnus dabryanus). General and Comparative Endocrinology, 1986, 64 (3), pp.389-395. 10.1016/0016-6480(86)90073-0 . hal-01608551

HAL Id: hal-01608551
https://hal.science/hal-01608551
Submitted on 2 Jun 2020

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Effects of [D-Ala⁶, Pro⁹-NEt]-LHRH and Catecholaminergic Drugs on Gonadotropin Secretion and Ovulation in the Chinese Loach (Paramisgurnus dabryanus)

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Accepted July 6, 1986

The effects of [D-Ala⁶, Pro⁹-NEt]-LHRH (LHRH-A) alone and in combination with drugs which influence the actions of dopamine or the synthesis of catecholamines on gonadotropin (GtH) secretion and ovulation in the loach (Paramisgurnus dabryanus) were investigated. LHRH-A alone stimulated an increase in serum GtH levels in the loach, but was a relatively ineffective treatment for the induction of ovulation. Injection of the dopamine receptor antagonist pimozide caused a marked potentiation of the GtH-release response to LHRH-A, and combined injections of pimozide and LHRH-A were an effective treatment for the induction of ovulation. Reserpine, a drug which causes depletion of catecholamines from presynaptic terminals, also caused a marked potentiation of the GtH-release response to LHRH-A and combined treatment induced ovulation. Similarly, administration of α-methyl-para-tyrosine to block conversion of tyrosine to L-dopa, or carbidopa to block conversion of L-dopa to dopamine, potentiated the GtH-release response to LHRH-A and induced ovulation. In contrast, the use of diethyldithiocarbamate, to block conversion of dopamine to noradrenaline, failed to augment the action of LHRH-A on GtH release and ovulation. The present results provide further evidence to suggest that dopamine functions as a gonadotropin release-inhibitory factor in teleosts, and demonstrate that the use of drugs which block either the synthesis or the actions of dopamine potentiates the action of LHRH-A in teleosts. © 1986 Academic Press, Inc.

Recent studies have demonstrated that gonadotropin (GtH) secretion in teleosts is regulated by the stimulatory effects of a gonadotropin-releasing hormone (GnRH) and the inhibitory effects of a gonadotropin release-inhibitory factor (GRIF; for review see Peter, 1982, 1983; Peter et al., 1986). Dopamine functions as a GRIF in goldfish by acting directly at the level of the pituitary to modulate the actions of GnRHI as well as by modulating the spontaneous release of GtH (Chang and Peter, 1983a; Peter et al., 1985; Sokolowska et al., 1985a, b), common carp (Billard et al., 1983; Lin et al., 1985b, 1986), coho salmon (van der Kraak et al., 1986), and bream (Lin et al., 1985b). Furthermore, injection of PIM together with LHRH-A is highly effective in inducing ovulation in goldfish (Chang and Peter, 1983a; Sokolowska et al., 1984, 1985a) common carp (Billard et al., 1983; Lin et al., 1985b, 1986), Chinese loach (Lin et al., 1985a), African catfish (de Leeuw et al., 1985), and bream and silver carp (Lin et al., 1985b). If dopamine acts as a GRIF, then drugs which block the synthesis of dopamine or cause depletion of dopamine on GtH release in goldfish (Chang and Peter, 1983a; Peter et al., 1985; Sokolowska et al., 1985a, b), common carp (Billard et al., 1983; Lin et al., 1985b, 1986), coho salmon (van der Kraak et al., 1986), and bream (Lin et al., 1985b).
from presynaptic terminals should also influence the response to LHRH-A. To test this hypothesis, we have investigated the effects of LHRH-A in combination with drugs which block the synthesis of catecholamines or cause depletion of catecholamines from presynaptic terminals on GtH secretion and ovulation in the Chinese loach.

MATERIALS AND METHODS

Loach, 20 to 40 g body wt, were obtained from a local supplier (Guangdong Province, China) during the spawning season (November–April). Fish were held indoors in 250-liter aquariums at 20–22°C, or outdoors at ambient temperature without vegetation. Sexually mature (preovulatory) females were selected for experiments on the basis of a soft and distended abdomen. Individual fish were identified by means of a fin clip and weighed 1–2 days prior to hormone or drug treatment.

LHRH-A was purchased from the Ningbo Fish Hormone Factory, Zhejiang Province, China. PIM was a gift from Janssen Pharmaceutics Ltd., Beerse, Belgium. Reserpine (RES), α-methyl-para-tyrosine (α-MPT), carbidopa (CBD), and diethyldithiocarbamate (DDC) were purchased from Sigma, St. Louis, Missouri. LHRH-A was dissolved in freshwater teleost physiological saline (PS; Burnstock, 1958); drugs were suspended or dissolved in a vehicle (Veh) of 0.7% NaCl with 0.1% sodium metabisulfite. The dosages of LHRH-A and drugs utilized, in micrograms per gram body weight, and the injection schedules are provided under Results. Control groups received PS and/or Veh. All injections were intramuscular near the base of the dorsal fin; injection volumes were 5 μl/g body wt. Blood was sampled at 8 and 24 hr after injection by puncturing the caudal vasculature with a 25-gauge 0.5-in. needle attached to a 1.0-ml disposable syringe. Fish were checked for ovulation 24 hr after injection by abdominal massage, and then killed by spinal transection to determine gonadal condition by visual observation. Blood samples were allowed to clot on ice for several hours, and the serum was separated by centrifugation and stored at −25°C.

Serum GtH levels were determined by radioimmunoassay (RIA) using an antiserum directed against the β subunit of carp GtH and carp GtH for the assay standards and tracer. The assay protocol followed that previously described by Peter et al. (1984) except for the use of the carp GtH β-subunit antiserum at a final dilution of 1:1,000,000. Serial dilutions of loach serum and pituitary extract resulted in binding inhibition curves parallel to the carp GtH standard (data not shown). Interassay variation was determined by repeat measurement of two pools of loach serum with low and high GtH content. Intraassay variation in the measurement of the low serum GtH pool was 7.3% (N = 12) measured at about 80% of maximal binding and in the high serum GtH pool was 4.9% (N = 12) measured at about 20% of maximal binding. Interassay variation was 14.8% (N = 10) and 12.7% (N = 10) for the low and high GtH content serum pools, respectively.

GtH data were analyzed by one-way analysis of variance and Duncan’s multiple range test following log10 transformation. Fisher’s exact probability test was used to compare the number of ovulated fish between groups (P < 0.05).

RESULTS

Injection of LHRH-A alone at 0.01 or 0.05 μg/g body wt was effective in stimulating an increase in serum GtH levels in the loach (Figs. 1–6). However, the rate of ovulation induced by LHRH-A alone was generally low, usually at about 25% of the test animals but ranging up to 40%.

As shown in Fig. 1, combined injections of PIM + LHRH-A and RES + LHRH-A resulted in higher serum GtH levels than injections of LHRH-A + VEH at 8 hr post-
GONADOTROPIN SECRETION IN LOACH

injection; the magnitude of the increase was greatest in RES + LHRH-A injected fish. At 24 hr, serum GtH levels in PIM + LHRH-A injected fish were similar to LHRH-A + VEH injected fish; however, the serum GtH levels in RES + LHRH-A injected fish remained significantly elevated. Injections of PIM + PS or RES + PS failed to stimulate an increase in serum GtH levels at 8 or 24 hr after injection when compared to the VEH + PS injected group. The ovulatory response to injections of PIM alone, RES alone, or LHRH-A did not differ from control fish; the combination of PIM or RES + LHRH-A resulted in a significant ovulatory response (80%; 8/10).

The effects of graded amounts of RES administered together with LHRH-A (0.05 μg/g body wt) on serum GtH levels and ovulation are shown in Fig. 2. Injections of RES alone at dosages of 0.5 or 5 μg/g body wt stimulated a modest but significant increase in serum GtH levels at 8 and 24 hr postinjection when compared to VEH + PS injected fish; injection of a low dosage of RES alone (0.05 μg/g body wt) was ineffective. Administration of RES alone failed to induce ovulation. Injection of RES (0.5 or 5 μg/g body wt) together with LHRH-A stimulated a significant increase in serum GtH levels at 8 hr postinjection compared to VEH + LHRH-A injected fish. At 24 hr, only fish receiving the high dose of RES (5 μg/g body wt) + LHRH-A maintained elevated serum GtH levels compared to VEH + LHRH-A injected fish. Serum GtH levels in fish receiving a low dose of RES (0.05 μg/g body wt) + LHRH-A did not differ from the levels found in fish receiving LHRH-A alone at either 8 or 24 hr. Combined injections of RES (0.5 and 5 μg/g body wt) + LHRH-A resulted in a significant increase in the number of fish which ovulated compared to treatment with either LHRH-A alone or LHRH-A and a low dose of RES (0.05 μg/g body wt).

The effects of RES (5 μg/g body wt) and α-MPT (50 mg/g body wt) alone and in combination with LHRH-A (0.05 μg/g body wt) on serum GtH levels and ovulation in the loach are shown in Fig. 3. Although RES alone stimulated an increase in serum GtH levels at 8 and 24 hr postinjection and α-MPT alone increased serum GtH levels at 8 hr postinjection compared to VEH + PS injected fish, these treatments were ineffective in inducing a high rate of ovulation. Administration of RES + LHRH-A or α-MPT + LHRH-A resulted in significantly higher serum GtH levels at

Fig. 2. Effects of graded dosages of RES (0.05, 0.5, and 5 μg/g body wt) alone and in combination with LHRH-A (0.05 μg/g body wt) on serum GtH levels and ovulation in loach. See caption to Fig. 1 for details of the legend.

Fig. 3. Effects of RES (5 μg/g body wt) or α-MPT (50 mg/g body wt) alone and in combination with LHRH-A (0.05 μg/g body wt) on serum GtH levels and ovulation in loach. See caption to Fig. 1 for details of the legend.
The effects of RES (5 μg/g body wt) and CBD (10 μg/g body wt) alone and in combination with LHRH-A (0.05 μg/g body wt) on serum GtH levels and ovulation in the loach are shown in Fig. 4. Both RES and α-MPT in combination with LHRH-A resulted in a significant increase in the rate of ovulation compared to treatment with LHRH-A alone.

The effects of RES (5 μg/g body wt) and graded amounts of DDC alone and in combination with LHRH-A (0.01 μg/g body wt) on serum GtH levels and ovulation in the loach are shown in Fig. 5. RES alone stimulated a small but significant increase in serum GtH levels at 8 and 24 hr postinjection whereas a high dose of DDC (50 μg/g body wt) alone was ineffective. Fish receiving the high dose of DDC + LHRH-A had significantly lower serum GtH levels than fish receiving LHRH-A alone at 8 hr but were similar at 24 hr postinjection. Serum GtH levels in fish receiving a lower dose of DDC (5 μg/g body wt) + LHRH-A were similar to the levels in fish receiving LHRH-A alone at 8 and 24 hr postinjection. The administration of a high dose of DDC alone as well as low or high doses of DDC + LHRH-A were ineffective in inducing ovulation. In contrast, injection of RES + LHRH-A resulted in significantly higher serum GtH levels at 8 and 24 hr postinjection compared to fish receiving LHRH-A alone, and RES + LHRH-A was highly effective in inducing ovulation.

A final experiment was conducted to compare the effects of a combined injection of LHRH-A (0.01 μg/g body wt) with PIM, RES, α-MPT, or CBD at similar dosages (5 μg/g body wt) on serum GtH levels and...
ovulation in the loach (Fig. 6). Each of the drugs administered alone failed to stimulate an increase in serum GtH levels compared to VEH + PS treated fish and were ineffective in inducing ovulation. LHRH-A alone and in combination with CBD had similar effects on serum GtH levels; treatment with LHRH-A in combination with PIM, RES, or α-MPT resulted in higher serum GtH levels than injection of LHRH-A alone. At 8 and 24 hr, RES + LHRH-A injected fish had higher serum GtH levels than α-MPT + LHRH-A treated fish. The ovulatory response of fish injected with α-MPT + LHRH-A or CBD + LHRH-A was similar to fish receiving LHRH-A alone. In contrast, fish treated with PIM + LHRH-A or RES + LHRH-A had a significantly greater ovulatory response than fish receiving LHRH-A alone.

DISCUSSION

The present results demonstrated that LHRH-A stimulates GtH release in sexually mature (preovulatory) Chinese loach; however, treatment with LHRH-A alone was relatively ineffective in inducing ovulation. PIM potentiated the GtH-release response to LHRH-A; the combined treatment was highly effective in inducing ovulation. This suggests that dopamine has GRIF activity in the loach and confirms the results of our earlier study (Lin et al., 1985a) in which we demonstrated that treatment with PIM and LHRH-A was an effective technique for inducing ovulation in this species.

The actions of RES, α-MPT, CBD, and DDC have been well characterized (Gilman et al., 1980). RES causes depletion of catecholamine neurotransmitters by blocking the transport of neurotransmitters into intragranular stores in presynaptic terminals. The drug α-MPT blocks the formation of L-dopa from tyrosine; CBD blocks conversion of L-dopa to dopamine; DDC blocks conversion of dopamine to norepinephrine. Intraperitoneal injections of RES, α-MPT, and CBD caused a significant although relatively small increase in serum GtH levels in goldfish (Chang et al. 1983). In the present study on Chinese loach, RES, α-MPT, and CBD caused a small but significant increase in serum GtH levels, although a consistent response to these drugs could not be demonstrated in each of the experiments.

RES, α-MPT, and CBD each potentiated the action of LHRH-A (Figs. 1–6) resulting in significantly higher serum GtH levels.

![Graph](image-url)  
**Fig. 6.** Effects of PIM, RES, α-MPT, and CBD (all dosages 5 μg/g body wt) alone and in combination with LHRH-A (0.01 μg/g body wt) on serum GtH levels and ovulation in the loach. See Fig. 1 for details of the legend.
than in animals treated with LHRH-A alone. However, a high dosage of DDC failed to potentiate the action of LHRH-A in the Chinese loach with DDC (50 µg/g body wt) + LHRH-A injected fish having lower serum GtH levels at 8 hr postinjection than fish receiving LHRH-A alone (Fig. 5). Taken together, these results indicate that any drug that blocks the synthesis of dopamine, but not the synthesis of norepinephrine, can potentiate the action of injected LHRH-A, presumably by effectively reducing the level of dopamine acting as GRIF on the GtH cells in the pituitary. Based on the present results alone we cannot exclude the possibility that dopamine also influences the activity of GnRH neurons. However, it would seem that these effects would be of somewhat lesser importance than direct actions on the GtH cells as LHRH-A alone stimulates only a modest increase in GtH secretion. The present results also confirm a preliminary study showing that RES, 6-hydroxydopamine, α-MPT, and CBD, but not DDC, potentiate the GtH releasing activity of LHRH-A in goldfish (Peter et al., 1986). These studies together lend further support to the idea that GRIF activity is specific to dopamine in goldfish (Chang et al., 1984) and teleosts in general (Peter et al., 1986).

PIM and RES were more effective, on a weight-specific basis, than α-MPT or CBD in potentiating the actions of LHRH-A on GtH release and ovulation (Fig. 6); however, it is unclear, based on the present results, whether one of the former two (Pim or RES) is more effective than the other. PIM and RES were of equal effectiveness based on the results shown in Fig. 6, although this may reflect the relatively high doses of the two drugs used in this study. The results presented in Fig. 2 demonstrate that the effects of RES are dose dependent, with levels of RES a low as 0.5 µg/g body wt causing a marked potentiation of LHRH-A effects on GtH release and the induction of ovulation. In separate studies, PIM (0.5 µg/g body wt) injected together with LHRH-A (0.05 µg/g body wt) was shown to be effective in inducing ovulation in the loach (Lin et al., 1985a). The effects of RES may be more prolonged than PIM in that the combination of RES + LHRH-A maintained elevated serum GtH levels at 24 hr postinjection whereas the serum GtH levels in PIM + LHRH-A injected fish were similar to LHRH-A + VEH injected fish (Fig. 1). Other studies on common carp have also demonstrated a more prolonged elevation of serum GtH levels following injection of RES + LHRH-A compared to PIM + LHRH-A (Lin et al. 1986). However, interpretation of differences in the effectiveness of PIM and RES is further complicated by differences in their solubility in the injection vehicle; RES being highly soluble and PIM relatively insoluble in the vehicle used in the present work. Further tests are warranted using an injection vehicle which permits solubilization of both drugs.

In summary, the present results demonstrate that drugs which either cause depletion of catecholamines, or that block catecholamine synthesis at steps up to and including the production of dopamine, potentiate the GtH releasing action of LHRH-A in the loach; blocking the conversion of dopamine to norepinephrine has no apparent effect on the action of LHRH-A. These results confirm the specificity of dopamine as GRIF in the loach.

ACKNOWLEDGMENTS

This work was supported by Grant 3-P-83-1101 from the International Development Research Centre of Canada to H.-R. Lin and R. E. Peter. G.V.D.K. was supported by a Fellowship from the Alberta Heritage Foundation for Medical Research.

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