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The Effect of Luteinizing Hormone-Releasing Hormone Analogue (LHRHa) in Combination with Different Drugs with Anti-Dopamine and Anti-Serotonin Properties on Gonadotropin Release and Ovulation in the African Catfish, *Clarias gariepinus*

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ABSTRACT

Goos, H. J. Th., Joy, K. P., De Leeuw, R., Van Oordt, P. G. W. J., Van Delft, A. M. L. and Gielen, J. Th., 1987. The effect of luteinizing hormone-releasing hormone analogue (LHRHa) in combination with different drugs with anti-dopamine and anti-serotonin properties on gonadotropin release and ovulation in the African catfish, *Clarias gariepinus*. *Aquaculture*, 63: 143-156.

Under hatchery conditions the reproduction of the African catfish depends on artificial induction of egg maturation and ovulation. In this study the effect of a number of potential psychotropic drugs with variable anti-dopamine and/or anti-serotonin properties in combination with LHRHa on gonadotropin release and ovulation was investigated.

Drugs with a potent anti-dopaminergic character caused a preovulatory gonadotropin surge, which seems to be independent of their anti-serotonergic properties. One drug, however, did not follow this rule. Drugs exhibiting low interaction with dopamine and high interaction with serotonin receptors had no effect on the LHRHa-induced gonadotropin release.

INTRODUCTION

The preovulatory surge of gonadotropin (GTH) which precedes oocyte maturation and ovulation does not occur in most cultured fishes kept in con-

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fined waters in the laboratory or in ponds. In artificial breeding of fishes, therefore, a surge of GTH is usually induced by injecting pituitary extracts and/or human chorionic gonadotropin (HCG) (Eding et al., 1982; see review, Lam 1982; Mollah and Tan, 1983; Rowland, 1983; Juario et al., 1984; Richter and Cattel, 1985). The GTH secretion in teleosts is regulated by two neuroendocrine pathways, viz, a peptidergic (gonadotropin-releasing hormone, GnRH) and an aminergic (gonadotropin release-inhibitory factor, GnRIF) that project on or near the GTH cells (Ball, 1981; Van Oordt and Peute, 1983; Peter, 1983; Van Oordt, 1987). Synthetic mammalian LHRH and its superactive analogues have been used to stimulate GTH release in several teleost species, but they have varying effects on oocyte maturation and ovulation (Donaldson and Hunter, 1983). Ever since the demonstration of the presence of a GnRIF in goldfish, *Carassius auratus* (Peter and Crim, 1978; Peter et al., 1978; Peter and Paulencu, 1980), investigations, both in vivo and in vitro, have shown that dopamine (DA) acts as a GnRIF and inhibits at the pituitary level the spontaneous (direct effect) and/or the LHRH-induced (indirect effect) release of GTH (Chang and Peter, 1983; Chang et al., 1983, 1984a, b, c; De Leeuw et al., 1985b, 1986). This has considerably enhanced the prospect of inducing ovulation at the pituitary level, by stimulating the endogenous production of GTH to the preovulatory level. Thus, pimozide, a DA antagonist, which potentiates the effect of LHRHa to stimulate GTH release, has proved to be an efficient ovulation-inducing agent and the combination has been tried successfully in common carp, *Cyprinus carpio* (Billard et al., 1983), goldfish (Chang and Peter, 1984; Sokolowska et al., 1984) and the African catfish, *Clarias gariepinus* (De Leeuw et al., 1985a, b).

In the present study, we have evaluated the effects of seven potential psychotropic drugs with variable antagonistic actions towards dopamine and/or serotonin (5HT) on the GTH release and ovulation. Further we have attempted to correlate their effects on LHRH-induced GTH release with their specific anti-dopamine or anti-serotonin characteristics. The purpose of this study was a search for other LHRHa potentiating drugs since such agents are not commercially available for fish farming, and to investigate the importance of serotonin as stimulating or inhibiting substance for GnRH action.

MATERIALS AND METHODS

Pharmacological characterization of the drugs

Interaction with dopamine receptors

To characterize the interaction of the drugs with dopamine receptors two assays were used: (i) the in vitro test for inhibition of spiperone binding to dopaminergic (D2) receptors in rat brain striatal membrane homogenates (SPIPS test), and (ii) the apomorphine climbing test in mice (ACT).

(i) SPIPS test. Spiperone binds reversibly to dopaminergic receptors present in rat brain striatal membranes. Using labelled spiperone, the binding can be assessed by separation of bound and non-bound spiperone. Drugs with dopaminergic blocking properties, like the neuroleptic haloperidol, can inhibit the binding of spiperone.

The method used was identical to that described by Seeman et al. (1976) and Titeler et al. (1976). For routine measurements the ^{3}H-spiperone concentration was 0.2 n*M*, the tissue concentration was 5 mg original tissue per ml, and the incubation time was 45 min. Non-specific binding was defined as the amount of ^{3}H-spiperone binding in the presence of an excess of haloperidol (10^{-6} *M*) and appeared to be about 10% of total binding. Displacement curves were obtained for the various compounds by measuring specific binding in the presence of at least five different concentrations and IC_{50} and p*K*i values were obtained using a computer program. Every observation was obtained at least in triplicate. Binding is expressed in a p*K*i value, which is the negative logarithm of K_i, the affinity constant for specific binding to the receptor.

Interpretation of the results. For the interpretation of the results of the SPIPS assay, as for those of the other tests (see below), the figures have to be considered in connection with those from other assays. Sometimes clinical characteristics of the test drugs also have to be taken into account. The SPIPS figures may be interpreted as follows: p*K*i > 8, strong interaction with dopamine receptors; p*K*i 6–8, moderate interaction; p*K*i > 6, little interaction with dopamine receptors. The SPIPS values as such do not give an indication about the DA antagonistic effect of the drugs. However, they usually correlate well with the results from in vivo assays more directed to DA antagonistic activity (ACT test, below; see Table 2).

(ii) ACT. Mice treated with apomorphine-HCl, 0.25–1.5 mg/kg subcutaneously (s.c.), tend to adopt a vertical position along the wall of a wire mesh cylinder, standing or climbing. This "climbing behaviour" is supposed to be elicited by apomorphine-mediated stimulation of dopamine receptors, presumably in the striatum.

Many drugs affect the climbing behaviour, but dopamine antagonists generally inhibit it in doses too low to interfere with spontaneous motor activity and/or motor coordination in mice.

Experiments were performed according to Protais et al. (1976). Male HaM/KR Swiss mice (Broekman Institute, Stiphout, The Netherlands) of 24–26 g were injected s.c. with test compound or vehicle and 30 min later with 1 mg/kg of apomorphine-HCl. Climbing behaviour of mice is visually scored at 30 and 60 min after apomorphine administration. Inhibition of climbing is calculated as a percentage of control values. Two-tailed Yates test provides statistical evaluation of the data. The results are expressed as ED50s.

Interpretation of the results. See also remarks concerning interpretation under the SPIPS assay. ED50 values < 1 indicate strong to moderate DA-receptor antagonistic activity. Higher values have to be considered as low or no activity.

Interaction with serotonin receptors

For characterization of the serotonergic blocking properties of the drugs two test systems were used: (i) interaction with tryptamine-D-receptors in rat fundus strips (TDRF test) (peripheral receptors) and, (ii) mianserin binding in cortex membranes (MIA test) (central nervous system receptors).

(i) TDRF test. Serotonin induces contractions in a rat fundus strip placed in an organ bath by interaction with specific receptors on the smooth muscle cells. Drugs with affinity for these receptors can induce similar contractions and/or inhibit serotonin-induced contraction.

Log dose responses with serotonin were made on rat fundus strips in vitro according to Van Rossum (1963) and De Graaf et al. (1983). Male rats (Centraal Proefdier Bedrijf: WU, TNO, Zeist, The Netherlands) of 200–400 g were used for the experiments. Antagonistic properties of the test compounds against serotonin were established and expressed in a pA2 value, which is a quantitative measure of competitive antagonism of the compounds. pA2 is the negative logarithm of the concentration of antagonist needed to double the amount of serotonin to obtain 50% contraction of the organ.

(ii) MIA test. Mianserin binds reversibly to rat brain serotonin receptors. Drugs, like pyrilamine, with affinity for serotonergic receptors, inhibit the binding of mianserin.

The rapid filtration assay as described by Dumbrille-Rosse et al. (1980) was used to measure the interaction of the test compounds with rat brain serotonin receptors. For routine measurements the ^{3}H-mianserin concentration was 0.5 nM, the tissue concentration was 3.8 mg fresh tissue per ml, and the incubation time was 40 min. Non-specific binding was defined as the amount of ^{3}H-mianserin binding in the presence of 10^{-6} M pyrilamine and 10^{-6} M mianserin, and appeared to be about 40% of total binding. For calculation procedure, see under SPIPS test.

Interpretation of the results of the TDRF and MIA tests. pA2 > 8, strong interaction with serotonin receptors; pA2 7–8, moderate affinity; pA2 < 7, little interaction with serotonin receptors.

The TDRF and MIA assays are both tests for interaction with serotonin receptors. While the MIA-assay only gives an indication of binding to serotonin receptors, the TDRF assay specifically provides information about possible agonistic or antagonistic activity. Usually the results from the two assays

correlate well, in particular for antagonists. Differences in results may also be considered as differences in interaction of drugs with receptors in peripheral tissue and central nervous tissue, respectively.

Experimental animals

The stock of catfish used in the present investigation was raised in the laboratory by artificial breeding using HCG (Chorulon®, Intervet, Boxmeer, The Netherlands) as a maturation- and ovulation-inducing agent. The fish were 13–14 months old at the time of the experiments. A fortnight before starting the experiments, about 150 mature female catfish were transferred to experimental tanks with a continuous flow of copper-free recirculating water at $23 \pm 1\,^{\circ}C$ and under a photoperiod normal for the time of the year in The Netherlands. They were fed with 'Trouvit' trout pellets (Trouw, Putten, The Netherlands) twice daily. On the day previous to each experiment, the fish were weighed (about 500 g) and kept individually. They were not fed during the experiments. The investigations were conducted in the months of December and January 1985/86. Each experiment consisted of three groups: LHRHa, drugs, and combinations of LHRHa and drugs, with five fish in each group. The experiments were run concurrently. All treatments were carried out in the morning, between 8.30–9.30 h.

LHRHa groups. Five groups of fish were injected intraperitoneally with 0.05 mg of the LHRH analogue Des-Gly^{10}[D-Ala^{6}]LHRH ethylamide (Intervet) per kg body weight. LHRHa was dissolved in 0.7% NaCl containing 0.25% bovine serum albumin (fraction V, Sigma).

Drug groups. Seven groups of fish were used to test the effect of different drugs alone on GTH release. All drugs were prepared by Organon International B.V., Oss, The Netherlands. The drugs, referred to as Org 5222, Org 30067, Org 10490, Org 8282, Org 4716, Org 30207 and Org GB94 (mianserin), (for their composition, see Table 1) were dissolved in 1 ml of absolute ethanol to which was added 9 ml of LHRHa-vehicle (1:10). Wherever the drugs formed suspensions or recrystallised after the addition of LHRHa-vehicle, the solutions were ultrasonicated before injection. They were injected intraperitoneally at a dose of 5 mg per kg body weight. This dose was chosen after trial experiments; however, this does not indicate the threshold dose for each drug.

LHRHa+drug groups. In another seven groups of fish, LHRHa and each of the drugs together were administered intraperitoneally at the doses mentioned above. The injections were made one immediately after the other.

Control group. Three groups of animals were injected intraperitoneally with

TABLE 1

The tested drugs, indicated by their code numbers and their chemical composition

Code	Chemical composition
Org 5222:	trans-5-chloro-2,3,3a,12b-tetrahydro-2-methyl-1H-dibenz[2,3:6,7]oxepino[4,5-c]pyrrole (Z)-2-butenedioate (1:1)
Org 30067:	6,7,8,9-tetrahydro-3,7-dimethyl-5H-dibenz[b,i][1,6]oxazecine (Z)-2-butenedioate (1:1)
Org 10490:	6,7,8,9-tetrahydro-7-methyl-5H-dibenz[b,i][1,6]oxazecine (Z)-2-butenedioate (1:1)
Org 8282:	2,3,4,9-tetrahydro-2-methyl-1H-dibenzo[3,4:6,7]cyclohepta[1,2-c]pyridine (Z)-2-butenedioate (1:1)
Org 4716:	trans-1,2,3,4,4a,13b-hexahydro-2-methyldibenzo[2,3:6,7]thiepino[4,5-c]pyridine (Z)-2-butenedioate (1:1)
Org 30207:	3-chloro-6,7,8,9-tetrahydro-7-methyl-5H-dibenzo[b,i][1,6]thiazecine (Z)-2-butenedioate (1:1)
Org GB94:	1,2,3,4,10,14b-hexahydro-2-methyldibenzo[c,f]pyrazino[1,2-a]azepine monohydrochloride

equivalent volumes of (1) LHRHa vehicle, (2) the drug vehicle and (3) LHRHa vehicle and drug vehicle. These groups served as controls for the above experiments and were carried out only once.

Blood sampling and radioimmunoassay. In all the experiments, blood samples were taken by caudal puncture before treatments ($t=0$). After the injections, blood samples were drawn at 0.5, 1, 2, 4, 8 and 24 h after injection. The samples were centrifuged at 3000*g* for 10 min and plasma was stored at $-40\,^{\circ}C$ until assayed. Plasma GTH measurements were made by the homologous radioimmunoassay, as described by Goos et al. (1986).

Checking ovulation. After the last sampling, the fish were checked for ovulation by gently pressing the abdomen. Fish that yielded a copious stream of mature (green-brown) eggs were rated as ovulated and scored individually.

Statistical analysis. The level of significance of differences in plasma GTH levels between different groups was tested with Student's 't' test.

RESULTS

Pharmacological properties of the drugs

The results of the SPIPS, ACT, TDRF and MIA assays, are shown in Table 2. For comparison, the results for pimozide (obtained from Janssen Pharmaceutica, Beerse, Belgium) are also presented. Test drugs Org 5222, Org 30067 and Org 30207 exhibit a high antidopaminergic effect. In the SPIPS test they

TABLE 2

Pharmacological profiles of the tested drugs and pimozide as measured in the in vitro test for inhibition of spiperone binding to dopaminergic receptor in rat brain striatal membranes (SPIPS test), the apomorphine climbing test (ACT), the test for interaction with tryptamine-D-receptors in rat fundus strips (TDRF test) and the mianserin binding test in cortex membranes (MIA test)

	SPIPS p*Ki*	ACT ED50	TDRF pA2	MIA pA2
Org 5222	8.6	0.04	9.3	9.1
Org 30067	9.7	0.009	8.7	9.5
Org 10490	7.5	0.4	6.7	8.2
Org 8282	6.3	7.0	8.1	8.6
Org 4716	6.4	6.0	8.0	8.5
Org 30207	11.0	0.06	8.9	> 10
Org GB94	4.4	6.5	7.1	9.3
Pimozide	7.2	0.3	6.4	7.4

Interaction with DA receptors: SPIPS: > 8, strong; 6–8, moderate; < 6, little interaction; ACT; < 1, strong to moderate DA receptor antagonistic activity.
Interaction with 5-HT-receptors: TDRF and MIA: > 8, strong; 7–8, moderate; < 7, little interaction with 5-HT receptors.

reach p*Ki* values > 8, in the ACT test the ED50s are < 1. The drugs Org 10490, Org 8282 and Org 4716 have only moderate effects (SPIPS test 7.5, 6.3 and 6.4, ACT test 0.4, 7.0 and 6.0, respectively), while Org GB94 has very little interaction with dopamine receptors (p*Ki* = 4.4 in the SPIPS test and ED50 = 6.5 in the ACT test). Pimozide shows a moderate interaction with DA receptors.

With the exception of Org 10490 (pA2 = 6.7 in the TDRF test), all test drugs showed a strong interaction with serotonin receptors. Pimozide had only moderate affinity for serotonergic receptors (pA2 = 6.4 and 7.4 in the TDRF test and MIA test, respectively).

Effect of LHRHa (Fig. 1)

Initial levels of plasma GTH in blood samples taken immediately before treatment were 1.39 ± 0.21 ng/ml in the LHRHa group and 1.22 ± 0.2 ng/ml in the vehicle-injected control group; 0.5, 1, 2, 4 and 8 h after the LHRHa injection, a significant rise in plasma GTH level was noticed. Only one fish had ovulated in this group. After 24 h, the plasma GTH level was low and did not show any significant difference from the control.

Effect of drugs (Fig. 1)

In all groups that had received the various drugs alone, the plasma GTH level was not different from that of the initial level before treatment or was not detectable (< 0.8 ng/ml). No fish had ovulated in any of the groups.

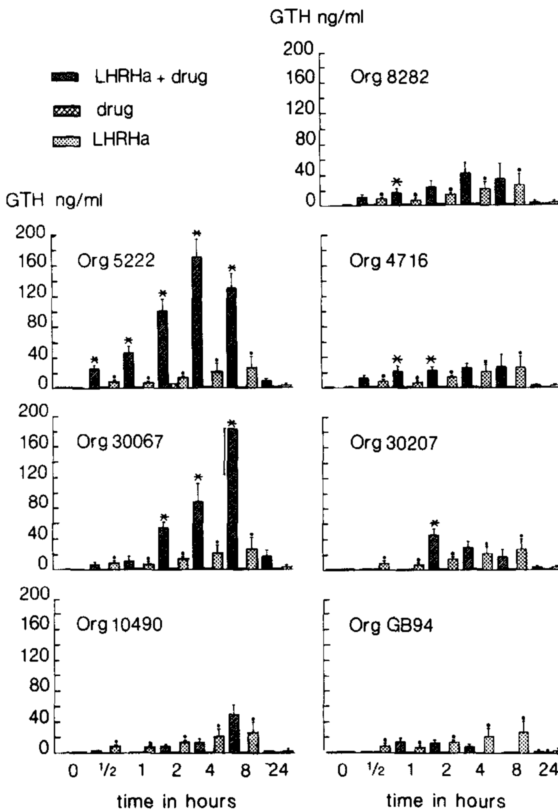

Fig. 1. The effect of LHRHa, in combination with seven potential psychotropic drugs with variable anti-dopaminergic and anti-serotonergic properties, on gonadotropic hormone plasma levels in female catfish, *Clarias gariepinus*.
•: significantly different compared to $t=0$ values (indicated for LHRHa-injected animals); $P<0.05$.
*: significantly different from LHRHa-injected animals at the same sampling time (indicated for the LHRHa-drug-injected animals); $P<0.05$.
None of the drugs or vehicle treatments (the latter are not indicated) caused a significant change in plasma GTH levels.

Effect of combination of drugs and LHRHa (Fig. 1)

In groups injected with Org 30067 and Org 5222, each in combination with LHRHa, the plasma GTH level showed a significant and steady increase during 8 h of treatment, when compared to the initial GTH level before injections or to that of the vehicle-injected control group. In the Org 30067+LHRHa combination, there was no significant change in GTH level at 0.5 and 1 h, compared to the LHRHa-treated group; however, the GTH level showed a significant difference at 2 ($P<0.01$), 4 and 8 h ($P<0.02$ and <0.05, respec-

tively). At 24 h, the GTH level did not show any significant difference from that of the LHRHa-treated group. In the Org 5222 + LHRHa group, there was a highly significant ($P < 0.001$) increase in the GTH level at all sampling times during 8 h in comparison to the LHRHa-treated group. Although the GTH level was noticed to be maximum at 4 h in the combination Org 5222 + LHRHa, and at 8 h in the combination Org 30067 + LHRHa, there was no significant difference in the GTH level between 4 and 8 h. All fish treated with the combination Org 30067 + LHRHa and Org 5222 + LHRHa had ovulated.

The combinations, Org 8282 + LHRHa, Org 4716 + LHRHa and Org 10490 + LHRHa caused the plasma GTH level to increase in comparison to the initial GTH level or to that of the vehicle-injected control group. The GTH concentration was significantly higher compared to the LHRHa-treated group at 1 h ($P < 0.05$) in the Org 8282 + LHRHa group, and at 1 and 2 h ($P < 0.05$) in the Org 4716 + LHRHa group. It was significantly lower at 1 h ($P < 0.001$) in the Org 10490 + LHRHa group. In all of the three last groups, only one fish of each had ovulated. After 24 h the GTH level had decreased to the control level.

In the Org 30207 + LHRHa group, the plasma GTH level had significantly increased and was maximal at 2 h ($P < 0.001$) and declined thereafter. In the Org GB94 + LHRHa group, the GTH response was low, although it was significantly higher than the control values at 1 ($P < 0.01$), and 2 ($P < 0.02$) h. No fish in these groups had ovulated.

DISCUSSION

Although mammalian synthetic LHRH or its analogues have consistently been shown to stimulate gonadotropin release in teleosts (for reviews, see Donaldson and Hunter, 1983; Peter, 1983), their ability to cause oocyte maturation and ovulation has not been unequivocally established in all species investigated. Induction of oocyte maturation and/or ovulation has been reported in the carp (Sokolowska et al., 1978; Weil et al., 1980); goldfish (Lam et al., 1975); plaice, *Pleuronectes platessa*, and goby, *Acanthogobius flavimanus* (Aida et al., 1978; ayu, *Plecoglossus altivelis* (Hirose and Ishida, 1974); rainbow trout, *Salmo gairdneri* (Crim et al., 1983); and coho salmon, *Oncorhynchus kisutch* (Donaldson et al., 1981; Van Der Kraak et al., 1983). On the other hand, in goldfish, single or multiple injections of LHRH or its analogues in different doses and regimes could not induce a very high rate of ovulation (Chang and Peter, 1984; Sokolowska et al., 1984). Similarly, in the African catfish, in both previous (De Leeuw et al., 1985b) and present investigations, a single intraperitoneal injection of LHRHa could not induce a high rate of ovulation. In the present study, only one out of the five fish had ovulated, and GTH measurements at different sampling times show that both the magnitude and time-course of the response varied among the animals. In this species, the LHRHa

treatment has been found to be more effective (to induce ovulation) in spring, close to breeding season in nature (De Leeuw et al., 1985b), possibly due to the high sensitivity of GTH cells to LHRHa or low level of DA inhibition during that period.

The low frequency of ovulation resulting from LHRHa treatment alone has been fully understood following the results of lesion and pars distalis transplantation studies in goldfish (Peter and Crim, 1978; Peter and Paulencu, 1980; Peter et al., 1978, 1984). The surge of GTH that precedes oocyte maturation and ovulation is the result of a stimulation by GnRH in combination with a decreased inhibition by GnRIF. The GnRIF is identified as dopamine (DA) on the basis of both in vivo and in vitro studies using catecholamine agonists and antagonists. Intraperitoneal injections of 6-OHDA, α-methylparatyrosine and carbidopa (inhibitors of DA synthesis) resulted in an increase in GTH release in goldfish (Chang et al., 1983). Administration of dopamine, and apomorphine and bromocriptine (DA agonists), decreased the spontaneous and the LHRHa-induced GTH release in goldfish (Chang and Peter, 1983; Chang et al., 1984a,b). In the African catfish, apomorphine depressed the release of GTH from perifused pituitary fragments and abolished the LHRHa-induced GTH release from both pituitary fragments and cell suspensions (De Leeuw et al., 1986). Administration of pimozide (a DA antagonist) in goldfish (Chang and Peter, 1984; Sokolowska et al., 1984), common carp (Billard et al., 1983), estradiol-treated eel (Dufour et al., 1984) and the African catfish (De Leeuw et al., 1985b) greatly potentiated the LHRHa-induced GTH release. Similarly, metoclopramide (another DA antagonist) also potentiated the LHRHa-induced release of GTH in goldfish (Chang et al, 1984b). Injections of phentolamine (α-adrenergic antagonist), and the sympathomimetic agent octopamine did not have any effect on GTH release (Chang et al., 1983, 1984b), suggesting that the effects of DA on GTH release are specific.

The potentiating effect of pimozide on LHRH-induced GTH release has led to the use of the combination as an ovulation-inducing agent to produce the preovulatory surge of GTH and has been successfully tried in the common carp (Billard et al., 1983), goldfish (Chang and Peter, 1984; Sokolowska et al., 1984) and the African catfish (De Leeuw et al., 1985a). Similarly, the combination of metoclopramide and LHRHa has also increased the frequency of ovulation in goldfish, but was less potent than the pimozide+LHRHa combination. In the present study, out of the seven drugs tested, the combinations of LHRHa with Org 30067 and Org 5222 have produced the preovulatory surge of GTH between 4 and 8 h after the treatment. The two combinations have yielded 100% ovulation. These drugs have been tested for the first time in any species and were administered, like pimozide+LHRHa, in a single intraperitoneal injection of 5 mg/kg body weight and 0.05 mg/kg body weight, respectively (De Leeuw et al., 1985a). Although a statistically significant difference in GTH level was not observed at 4 h and 8 h due to large individual variations, the

peak GTH level was found at 8 h (or beyond) in the Org 30067+LHRHa combination, like pimozide+LHRHa, and at 4 h in the Org 5222+LHRHa combination. The latter combination appears to have a more rapid effect on GTH release than the former.

On the other hand, the combinations Org 8282+LHRHa, Org 4716+LHRHa and Org 10490+LHRHa did not produce the preovulatory surge of GTH between 4 and 8 h. Only one fish of each group had ovulated and it is likely that the effect was due to LHRHa, the drugs having only a moderate potentiating effect on LHRHa-induced GTH release. The combinations Org 30207+LHRHa and Org GB94+LHRHa produced a very poor GTH release response. There was no difference between these groups and the LHRHa-treated group. No fish in these combinations had ovulated.

With the exception of the drug Org 30207, the dopamine antagonistic properties of the other drugs correlate well with their effects on the LHRHa-induced GTH secretion; Org 5222 and Org 30067 are strong dopamine antagonists and both drugs caused a preovulatory GTH surge when administered in combination with LHRHa.

The moderate dopamine antagonists Org 10490, Org 8282 and Org 4716 together with LHRHa caused an increase in GTH release but no sufficient for ovulation while Org GB94 had no effect. Although Org 30207 has strong anti-dopaminergic properties, it was ineffective in combination with LHRHa. At present, there is no real explanation for this. The pharmacokinetic properties in the catfish of all drugs used in the present study are unknown. It might be that the drug Org 30207 is ineffective because of unfavourable pharmacodynamics. Another explanation might be its strong antiserotonergic properties. Since serotonin has been found to stimulate GTH synthesis and release (Groves, 1984), a strong anti-serotonergic drug could prevent GTH release in spite of its anti-dopaminergic character. This is supported by comparing the results of Org 10490 and pimozide. Both drugs are almost equal in their anti-dopaminergic properties. However, pimozide in combination with LHRHa causes a preovulatory GTH surge (De Leeuw et al., 1985b) while Org 10490 does not; Org 10490 shows stronger interaction with serotonin receptors than does pimozide (see Table 2).

From the present experiments it seems likely that serotonin has no gonadotropin release-inhibiting activity. The drugs Org 4716 and Org GB94 (mianserin) do not enhance the GTH release caused by LHRHa; both drugs are strong serotonin antagonists but weak dopamine antagonists.

The fact that plasma GTH concentration was not affected in response to treatment with the drugs alone is in good agreement with observations on the effect of pimozide on GTH release. It has been reported that pimozide, when injected alone, had no effect on GTH release in estradiol-treated eel (Dufour et al., 1984) and mature catfish (De Leeuw et al., 1985a). In goldfish, mild but variable effects were reported (Chang and Peter, 1984; Sokolowska et al., 1984).

Similarly, the various drugs used in the present study did not have any effect on spontaneous GTH release on their own, but the successful combinations have potentiated the LHRHa-induced GTH release, suggesting interactions of the two regulatory systems at the pituitary level. The exact mechanism(s) of their modulating role in GTH release needs further investigations at the receptor and postreceptor level.

From the aquacultural point of view, the combinations of Org 30067, and Org 5222, each with LHRH analogue, are promising and may be practised with ease to induce oocyte maturation and ovulation in difficult-to-spawn species in captivity. The method is simple to adopt, and efficient, and may overcome many of the difficulties and uncertainties encountered with other available methods of inducing ovulation (Donaldson and Hunter, 1983). However, before they can be used commercially, these combinations must be tested in other economically important culture species, and standard procedures must be developed.

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