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Control of Gonadotropin Release in the Atlantic Croaker (*Micropogonias undulatus*): Evidence for Lack of Dopaminergic Inhibition

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Gonadotropin (GTH) secretion is known to be under inhibitory dopaminergic control in several species of fish. To investigate whether this is also the case in the Atlantic croaker (*Micropogonias undulatus*), juvenile and adult croaker were treated with a gonadotropin-releasing hormone analog (des-Gly^{10} D-Ala^{6} Pro^{9} n ethylamide luteinizing hormone-releasing hormone (LHRHa), 1–100 ng/g body wt) in combination with various dopaminergic drugs (1–20 mg/kg body wt). None of the dopamine antagonists tested, metoclopramide, pimozide, haloperidol, and domperidone, were able to increase plasma GTH levels above those induced by treatment with LHRHa alone and in some cases the gonadotropin response to LHRHa was reduced. The dopamine agonists bromocryptine and apomorphine either had no effect on the normal response to LHRHa or increased it. None of the drugs tested had any detectable effect on GTH levels in the absence of LHRHa. These results provide evidence for a lack of dopaminergic inhibition in the control of GTH secretion in the Atlantic croaker.

There is now considerable evidence that gonadotropin (GTH) release from the pituitary gland is under dual control in some species of teleost fish. GTH release is stimulated by one or more gonadotropin-releasing hormones (GnRH) (Sherwood, 1987) and is inhibited by dopamine (DA) (Chang and Peter, 1983; de Leeuw *et al.*, 1985). Most of the evidence concerning the dopaminergic inhibition of gonadotropin release comes from studies in goldfish (*Carassius auratus*) and other cyprinid species (reviewed by Peter *et al.*, 1986) and in African catfish (*Clarias gariepinus;* reviewed by van Oordt and Goos, 1987). In the goldfish, dopamine or dopamine agonists can abolish the pituitary's response to luteinizing hormone-releasing hormone analogs (LHRHa) or to lesioning of dopaminergic tracts in the pituitary stalk and preoptic nucleus (Chang and Peter, 1983). Conversely, dopamine antagonists such as pimozide (PIM) and domperidone (DOM) can increase plasma GTH levels or, if administered in conjunction with LHRHa, can markedly potentiate the response to LHRHa. The dopaminergic inhibition of GTH release appears to be due to a direct effect on the pituitary (Chang *et al.*, 1984a, b) and to be mediated via D2 type receptors (Omeljaniuk *et al.*, 1987). The inhibition has been shown to be seasonal, being much more pronounced in mature fish than in immature or sexually regressed animals (Sokolowska *et al.*, 1985), and may vary diurnally (Billard *et al.*, 1987).

In the African catfish (order siluriformes), dopamine agonists have also been shown to decrease, and antagonists to increase the action of LHRHa. However, the drugs had no effect on basal or nonstimulated release of GTH from pituitary fragments *in vitro* (de Leeuw *et al.*, 1986, 1987). Pimozide, in conjunction with LHRHa, has also been shown to elevate plasma gonadotropin levels in European eel (*Anguilla anguilla*) (Dufour *et al.*, 1988), coho salmon (*Oncorhynchus kisutch*) (Van der Kraak *et*

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al., 1986), rainbow trout (*Salmo gairdneri*) (Billard *et al.*, 1984), and Chinese loach (*Paramisgurnus dabryanus*) (Lin *et al.*, 1986). The effects in salmonids, however, are much less pronounced than in the goldfish, and salmonids are capable of oocyte maturation and ovulation in response to LHRHa alone. It was suggested that dopaminergic inhibition of GTH release, while demonstrable, was of relatively minor physiological importance in these fish (Van der Kraak *et al.*, 1986). Thus it appears that there can be variable degrees of dopaminergic control of GTH release in teleost fish, the effect being dominant in cyprinids, and subordinate in salmonids.

By comparison, virtually no information is available concerning the neuroendocrine control of gonadotropin secretion in marine teleosts or teleosts belonging to other orders. In one study, Groves and Batten (1986) found histological evidence for an inhibitory effect of dopamine on GTH secretion in the Molly (*Poecilia latipinna*). The possibility that the release of GTH in fish of the family sciaenidae might not be under a strong dopaminergic control, however, was suggested by the fact that several sciaenids; spotted seatrout (*Cynoscion nebulosus*), orangemouth corvina (*Cy. xanthulus*), red drum (*Sciaenops ocellatus*), and Atlantic croaker (*Micropogonias undulatus*) have been induced to spawn in captivity using LHRHa alone (Thomas and Boyd, 1988). The recent development of a radioimmunoassay for the measurement of GTH in the Atlantic croaker (Copeland and Thomas, 1989) now permits several aspects of the reproductive physiology of this euryhaline perciform species to be investigated for the first time.

The purpose of this study was to investigate whether GTH release in the Atlantic croaker is under inhibitory dopaminergic control as has been demonstrated in the goldfish and several other teleost species. Plasma GTH levels were monitored following administration of LHRHa and dopaminergic drugs since this method has clearly demonstrated the effects of such drugs in previous studies (reviewed by Peter *et al.*, 1986).

MATERIALS AND METHODS

Experimental animals. Atlantic croaker were captured by gill-netting or by shrimp-trawl in the vicinity of Port Aransas, Texas. Immature fish, less than 1 year old and weighing 50 to 100 g, were captured throughout the summer months and were maintained in captivity for up to 1 year. Mature adult fish of unknown age and weighing 100 to 500 g were captured in October and November during the annual offshore spawning migration. Adult male croaker remained spermiating throughout the entire year in captivity. Female croaker contained ripe (postvitellogenic) ovaries until January after which the ovaries regressed. The fish were maintained indoors in a recirculating seawater system under constant conditions of photoperiod (14 hr light:10 hr dark), temperature (25°), and salinity (20 ppt). They were fed daily with chopped fish and beef liver.

Chemicals. All drugs utilized in this study were purchased from Sigma Chemical Co. (St. Louis, MO), with the exception of the LHRH analog (des-Gly^{10} D-Ala^{6} Pro^{9} n ethylamide LHRH) which was supplied by Bachem Ltd. (CA) and the dopamine antagonists pimozide and domperidone which were gifts from Janssen Pharmaceutical Co., Beerse, Belgium and Dr. R. E. Peter, University of Alberta, Edmonton.

Experimental protocols. Experiments with mature fish were conducted in a 17,000-liter holding tank which was divided into two or four compartments using a 1-cm mesh net. Experiments with immature (<100 g) fish were conducted in 350-liter tanks (5–10 fish/tank).

All drugs were injected intraperitoneally in acidified 0.7% NaCl (pH 6.3) containing 0.1% sodium metabisulfite or in a solvent of 1:9 dimethyl sulfoxide (DMSO)/propylene glycol. Pimozide was administered as a suspension, but all other drugs were injected as solutions. LHRHa was administered by intramuscular or intraperitoneal injection using a 0.1-ml syringe. Fish were anesthetized with a 5 ppm solution of quinaldine sulfate (Argent Ltd.). Blood samples were taken from the caudal sinus using heparinized syringes, plasma was prepared by centrifugation of the blood at 1500*g* for 10 min, and plasma samples were stored at −20° until assayed for GTH content. Details of individual experiments are given in the figure legends.

Gonadotropin assay. Gonadotropin content was measured in 25–100 μl plasma by a homologous radioimmunoassay, details of which are described elsewhere (Copeland and Thomas, 1989). This assay mea-

sures a maturational/steroidogenic gonadotropin which is capable of binding to the lectin concanavalin A. The assay sensitivity is 5 pg/tube.

Drugs tested. The following drugs were tested for their effects on gonadotropin release: apomorphine-HCl, bromocryptine mesylate (dopamine agonists); metoclopramide, pimozide, haloperidol, domperidone (dopamine antagonists); and reserpine (catecholamine depletor). The effects of dopamine itself were also investigated.

Statistical analyses. Results were compared by analysis of variance and Duncan's multiple range test following log transformation of the data. Significance was accepted at the 0.05 level. For statistical purposes, nondetectable hormone levels were assumed to be at the detection limit of the assay (50 pg/ml). All results are shown as means ± standard errors for groups of 5–10 fish/treatment.

RESULTS

1. Dose–response to LHRHa

Figure 1 shows the plasma GTH levels in immature croaker at 6 and 25 hr after a single injection of 0, 1, 10, or 100 ng/g body wt of LHRHa. Plasma GTH levels were increased significantly by all the doses of LHRHa tested at 6 hr postinjection. A dose–response relationship was shown with 1 and 10 ng LHRHa/g body wt. Injection with 100 ng LHRHa caused a submaximal GTH response. By 25 hr, plasma GTH levels remained elevated only in the high dose group (100 ng/g body wt).

2. Effects of Dopaminergic Drugs in Immature Atlantic Croaker

A single injection of pimozide (10 μg/g body wt) alone did not alter plasma GTH levels in immature croaker (measured 3 hr postinjection). However, concomitant administration of pimozide with LHRHa (100 ng/g body wt) significantly lowered the response to LHRHa (Fig. 2).

The results of two experiments with the dopamine antagonist domperidone are shown in Fig. 3. In the first experiment (Fig. 3A), the plasma GTH rise induced by a low dose of LHRHa (1 ng/g body wt) was significantly reduced by concomitant injection of domperidone (10 μg/g body wt). In the second experiment (Fig. 3B), the response to a higher dose of LHRHa (20 ng/g body wt) was reduced by a high dose of domperidone (100 μg/g body wt), but was unaffected by a lower dose of the drug (20 μg/g body wt).

The effects of dopamine and the dopamine agonist apomorphine on the plasma

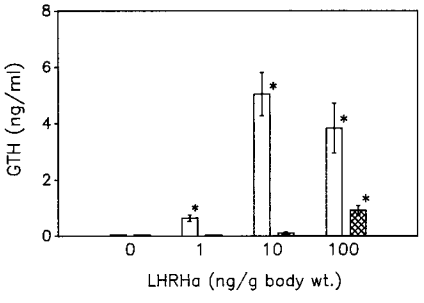

FIG. 1. Dose–response of plasma GTH levels in immature Atlantic croaker injected im with 0, 1, 10, or 100 ng LHRHa/g body wt. Blood samples were taken 6 hr (open bars) and 25 hr (hatched bars) after the injection. Each bar represents the mean ± SEM of 6–10 determinations. Asterisks denote mean values significantly different from controls (0 ng LHRHa) ($P < 0.05$).

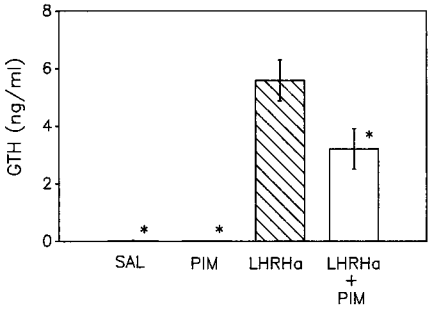

FIG. 2. Effects of LHRHa and pimozide on plasma GTH levels in immature Atlantic croaker. Groups of 10 fish were given a single ip injection of acidified saline (SAL), pimozide (PIM; 10 μg/g body wt), LHRHa (100 ng/g body wt, hatched bar), or LHRHa plus PIM at the same doses. Blood samples were taken 3 hr later. Results are expressed as means ± SEM. Asterisks denote mean values significantly different from the LHRHa group ($P < 0.05$).

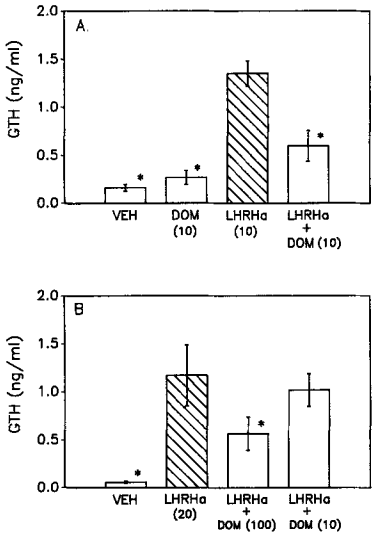

FIG. 3. Effects of LHRHa and domperidone on plasma GTH levels in immature Atlantic croaker. (A) Fish were given im injections of LHRHa (1 ng/g body wt, hatched bar) or acidified saline, and ip injections of domperidone (DOM, 10 μg/g body wt) or DMSO/propylene glycol (vehicle, VEH). Blood samples were taken 4 hr later. (B) Fish were given ip injections of LHRHa (20 ng/g body wt, hatched bar) or acidified saline, and domperidone (100 or 10 μg/g body wt) in vehicle. Blood samples were taken 3 hr later. For both experiments, results are expressed as means ± SEM (N = 11–15 and 7, respectively). Asterisks denote mean values significantly different from the LHRHa group ($P < 0.05$).

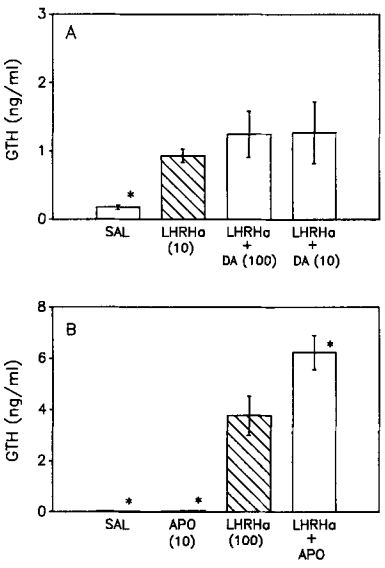

FIG. 4. Effects of LHRHa, dopamine, and apomorphine on plasma GTH levels in immature Atlantic croaker. (A) LHRHa (10 ng/g body wt) and dopamine (DA; 0, 10, and 100 μg/g body wt) were injected ip in acidified saline. Blood samples were taken 2 hr later. (B) LHRHa (100 ng/g body wt), apomorphine (APO; 10 μg/g body wt), or LHRHa plus APO were injected ip in acidified saline. Blood samples were taken 3 hr later. Results are expressed as means ± SEM (N = 7 and 10, respectively). Asterisks denote mean values significantly different from LHRHa groups (hatched bars) ($P < 0.05$).

GTH response to LHRHa are shown in Fig. 4. Dopamine at a dose of 10 or 100 μg/g body wt did not significantly alter the response to 10 ng/g body wt of LHRHa (Fig. 4A), though three out of seven fish in the high dose dopamine group had plasma GTH levels greater than any of the fish in the LHRHa group. Apomorphine (10 μg/g body wt) significantly increased the response to 100 ng/g body wt of LHRHa, but had no detectable effect on its own (Fig. 4B).

The effects of the dopamine agonist bromocryptine (BROMO) and the dopamine antagonists metoclopramide (MET) and haloperidol (HAL) on the response to LHRHa were also investigated in immature fish. Neither MET (10 μg/g body wt) nor BROMO (10 μg/g body wt) significantly altered the response to 100 ng/g body wt LHRHa (GTH values 3 hr postinjection: LHRHa, 4.67 ± 0.65; LHRHa + MET, 4.92 ± 0.50; LHRHa + BROMO, 3.67 ± 0.37; $\bar{x}$ ± SEM in ng/ml, N = 10, N.S.). Bromocryptine (50 μg/g body wt) also failed to alter the response to a low dose of LHRHa (20 ng/g body wt) (GTH values 6 hr postinjection: LHRHa, 1.84 ± 0.43; LHRHa + BROMO, 1.22 ± 0.53; $\bar{x}$ ± SEM in ng/ml, N = 9, N.S.). Concomitant administration of the dopamine antagonist haloperidol (10 μg/g body wt) with the low dose of LHRHa (20 ng/g body wt) caused a slight, but nonsignificant, decrease in the response to LHRHa (GTH values 4 hr postinjection: LHRHa, 1.48 ± 0.52;

LHRHa + HAL, 1.07 ± 0.33; $\bar{x}$ ± SEM in ng/ml, $N = 8$, N.S.).

3. *Effects of Dopaminergic Drugs in Mature Male Atlantic Croaker*

The results of four experiments in mature (spermiating) male croaker are shown in Fig. 5. In these experiments, single injections of the dopamine antagonists pimozide, domperidone, or metoclopramide or of the dopamine agonist apomorphine or dopamine itself all failed to significantly affect the response to LHRHa. None of the drugs increased plasma GTH levels when injected alone. While the differences were not found to be significant, the results of these experiments showed a consistent trend toward a decrease in the GTH response to LHRHa when the LHRHa was coadministered with pimozide (Fig. 5A) or apomorphine (Fig. 5C) and an increase when it was administered with dopamine (Fig. 5D).

4. *Effects of Dopaminergic Drugs in Regressed Adult Female Atlantic Croaker*

Figure 6 shows the plasma GTH levels in regressed female croaker injected with LHRHa (20 ng/g body wt) and the dopamine antagonists pimozide and domperidone (both 20 μg/g body wt). Pimozide effectively abolished the response to LHRHa

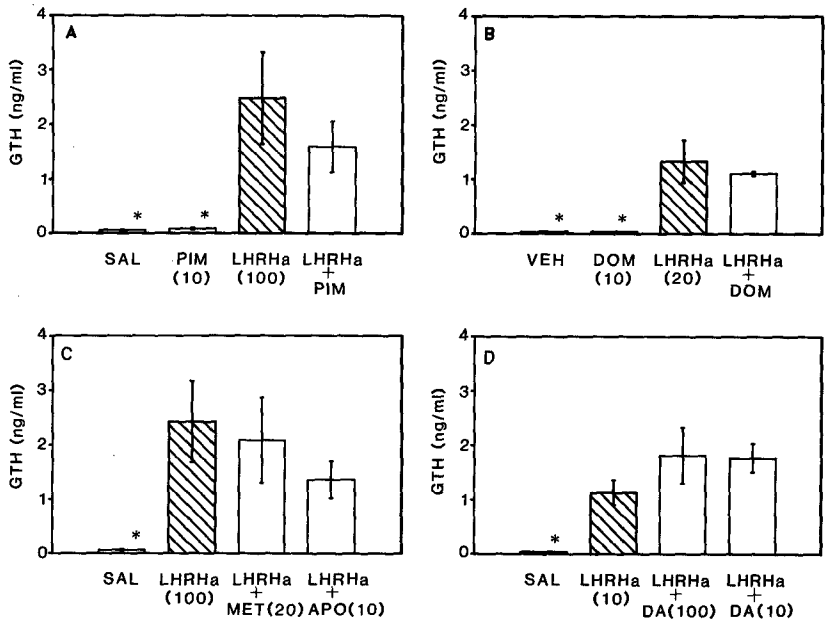

FIG. 5. Effects of LHRHa, dopamine, and dopaminergic drugs on plasma GTH levels in mature male Atlantic croaker. (A) Fish were given ip injections of pimozide (PIM; 10 μg/g body wt) or acidified saline (SAL), followed 3 hr later by injections of LHRHa (100 ng/g body wt). Blood samples were taken 3 hr after the second injection ($N = 7$). (B) Fish were given im injections of LHRHa (20 ng/g body wt) or acidified saline, and ip injections of domperidone (DOM; 10 μg/g body wt) or DMSO/propylene glycol solvent (VEH). Blood samples were taken 4 hr postinjection ($N = 4$). (C) Fish were given ip injections of LHRHa (100 ng/g body wt) and metoclopramide (MET; 20 μg/g body wt) or apomorphine (APO; 10 μg/g body wt), and blood samples were taken 4 hr later ($N = 7$). (D) LHRHa (10 ng/g body wt) and dopamine (DA; 0, 10, or 100 μg/g body wt) were injected ip in acidified saline. Blood samples were taken 2 hr later ($N = 5$). All results are shown as means ± SEM. Asterisks denote mean values significantly different from LHRHa group ($P < 0.05$).

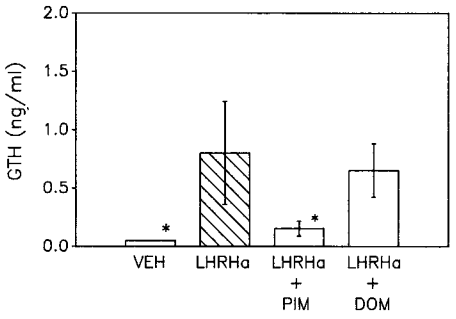

FIG. 6. Effects of LHRHa, pimozide, and domperidone on plasma GTH levels in sexually regressed adult female Atlantic croaker. LHRHa (20 ng/g body wt) was injected im in acidified saline, and pimozide (PIM) or domperidone (DOM; both 20 μg/g body wt) were injected ip in DMSO/propylene glycol vehicle (VEH). Blood samples were taken 4 hr later. Results are expressed as means ± SEM (N = 5). Asterisks denote mean values significantly different from the LHRHa group (hatched bar) ($P < 0.05$).

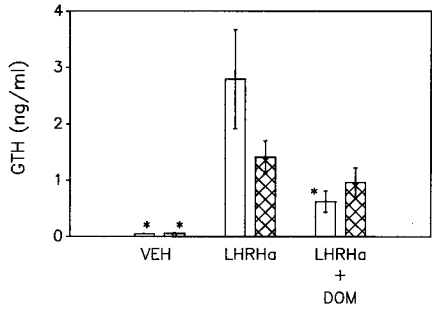

FIG. 7. Effects of LHRHa and domperidone on plasma GTH levels in mature female Atlantic croaker. LHRHa (20 ng/g body wt) was injected im in acidified saline and domperidone (DOM; 10 μg/g body wt) was injected ip in DMSO/propylene glycol solvent (VEH). Blood samples were taken 5 hr (open bars) and 29 hr (hatched bars) postinjection. Results are expressed as means ± SEM (N = 6). Asterisks denote mean values significantly different from the LHRHa group ($P < 0.05$).

($P < 0.05$) while domperidone had no effect.

In another experiment with regressed fish the dopamine antagonist haloperidol failed to alter the GTH response to 100 ng/g body wt of LHRHa at a concentration of 10 μg/g body wt (GTH values 24 hr postinjection: LHRHa, 3.11 ± 0.50; LHRHa + HAL, 3.42 ± 0.55; $\bar{x}$ ± SEM in ng/ml, N = 10, N.S.). The catecholamine depletor reserpine (RES) at a concentration of 10 μg/g body wt slightly augmented the response to 100 ng/g body wt of LHRHa, but this increase was not significant (GTH values 6 hr postinjection: LHRHa, 2.54 ± 1.07; LHRHa + RES, 4.21 ± 0.98; $\bar{x}$ ± SEM in ng/ml, N = 5, N.S.).

5. *Effects of Dopaminergic Drugs in Mature Female Atlantic Croaker*

Results of an experiment with mature female fish capable of spawning are shown in Fig. 7. The response to LHRHa (20 ng/g body wt) was greatly diminished at 5 hr postinjection by simultaneous administration of domperidone (10 μg/g body wt). After 29 hr the plasma GTH levels in LHRHa-treated fish remained elevated and the effect of domperidone had diminished. All of the fish treated with domperidone and three out of four fish treated with LHRHa alone proceeded to undergo oocyte maturation, hydration, and ovulation and viable eggs were collected.

In a second experiment, fish were treated twice in 24 hr with pimozide or reserpine (10 μg/g body wt) and once with LHRHa (100 ng/g body wt) administered with the second injection of drugs. Plasma GTH levels were raised in all fish receiving LHRHa alone at 6 and 24 hr after the second injection (6 hr, 1.16 ± 0.18; 24 hr, 8.22 ± 2.23; $\bar{x}$ ± SEM in ng/ml, N = 9). Reserpine transiently increased the response to LHRHa at 6 hr postinjection ($P < 0.05$) whereas pimozide did not (GTH values: LHRHa + PIM, 6 hr 1.23 ± 0.19, 24 hr 10.36 ± 2.03; LHRHa + RES, 6 hr 2.85 ± 0.66, 24 hr 13.68 ± 3.18; $\bar{x}$ ± SEM in ng/ml, N = 6). All the fish treated with pimozide plus LHRHa, five out of six treated with reserpine plus LHRHa, and six out of nine treated with LHRHa alone began the process of oocyte maturation and hydration. However, the hydration process was exaggerated compared to normal and most of the fish died prior to ovulating. Further, it

was not determined prior to this experiment whether all the fish were capable of spawning.

DISCUSSION

The results of the experiments described here provide no evidence for a dopaminergic inhibition of GTH release in the Atlantic croaker. On the contrary, in most experiments plasma gonadotropin levels were significantly increased by dopamine agonists (Fig. 4) and decreased by dopamine antagonists (Figs. 2, 3, 6, and 7). A wide variety of compounds having agonistic or antagonistic dopaminergic effects was tested. Experiments utilized fish in differing reproductive states with doses of drugs which have been found to be effective in other species of fish (Peter *et al.*, 1986). The dose of LHRHa used in many of the experiments (20 ng/g body wt) was selected to give a submaximal GTH response. The levels of GTH reported here are low compared to those found in other species of fish; however, the response to LHRHa was extremely reproducible. The levels of GTH recorded were capable of inducing ovulation in mature female fish and were 10-fold greater than the detection limit of the assay. Blood samples were taken at varying times following the administration of drugs during the period when the LHRHa-induced plasma GTH increase was maximal (Copeland and Thomas, 1989). In no case, however, was there any indication of a dopaminergic inhibition of GTH release.

Experiments with goldfish (*C. auratus*), performed similarly to those described here, can result in serum GTH levels at least 100-fold higher than those found in Atlantic croaker, the concentrations rising from basal levels of less than 5 ng/ml to hundreds of ng/ml following injections of LHRHa plus pimozide or LHRHa plus domperidone (Omeljaniuk *et al.*, 1987). In juvenile African catfish (*C. gariepinus*), basal GTH levels around 3 ng/ml were increased to 11 ng/ml 2 hr after a single injection of LHRHa and to 50 ng/ml following LHRHa plus pimozide, whereas in mature fish maximum plasma GTH levels were similar to those found in goldfish (de Leeuw *et al.*, 1985). The effects of pimozide were much less noticeable in coho salmon. LHRHa-induced plasma GTH levels were increased only 2-fold by pimozide (from 20 to 40 ng/ml) and pimozide had no effect on spawning success (Van der Kraak *et al.*, 1986). In all of these species, however, the effects of dopamine antagonists were clearly stimulatory, while the effects of dopamine or dopamine agonists were inhibitory on LHRHa-induced GTH release. It is clear that the response of Atlantic croaker to such treatments does not follow the pattern seen in any of these fish species. Zohar *et al.* (1987 and unpublished results) similarly found no effect of pimozide and only a very minor effect of domperidone in the gilthead seabream (*Sparus aurata*). It appears, therefore, that dopaminergic inhibition of GTH release may not be a universal phenomenon in teleost fish.

The most interesting finding of the present study was that in most instances dopamine agonists increased and dopamine antagonists decreased the GTH response to LHRHa. To our knowledge this is the first evidence for a stimulatory effect of dopamine on GTH release in a teleost fish. Injection of a dopamine agonist (apomorphine) alone failed to increase circulating GTH levels which suggests that DA does not act directly on the gonadotrope to stimulate GTH secretion. It is possible, however, that the effect of DA is mediated at the pituitary level and not centrally, since the dopamine antagonist domperidone, which does not cross the blood–brain barrier in mammals or in goldfish (Omeljaniuk *et al.*, 1987), was shown to affect the LHRHa-induced increase in plasma GTH levels. More comprehensive *in vivo* and *in vitro* studies will be required to confirm the stimulatory effects of DA in Atlantic croaker and to determine the mechanisms of DA action. The results of studies using

pharmacological agents must be interpreted with caution because drugs can have a variety of actions and their effects have not been thoroughly investigated in fish. A wide variety of dopaminergic drugs was tested in the present study in an attempt to minimize this problem.

One experiment with reserpine provided evidence that there is some catecholamine involvement in the process of final oocyte maturation. Reserpine plus LHRHa resulted in higher plasma GTH levels than LHRHa alone. Reserpine, however, causes the release of stored catecholamines as well as an inhibition of catecholamine synthesis, and can also have indirect effects on dopamine metabolism. The mechanism of this stimulation of GTH release, therefore, is not known. In the only other experiment with mature female fish, domperidone was shown to significantly reduce the LHRHa-induced rise in GTH levels.

The role of dopamine in the control of gonadotropin release in mammals has been reviewed by Steger and Morgan (1985). Dopamine, L-dopa, bromocryptine, and domperidone are all reported to be capable of having both stimulatory and inhibitory effects on gonadotropin release. Dopamine antagonists such as haloperidol and pimozide as well as dopamine agonists such as apomorphine have been shown to inhibit gonadotropin release. Barraclough *et al.* (1984) have reviewed their own extensive studies on the neuroendocrine control of reproduction in the rat and have clearly demonstrated an inhibitory role for dopamine and a stimulatory role for norepinephrine in the release of LHRH from the hypothalamus. However, the existence of other dopaminergic nerve tracts which have stimulatory effects on GTH release and ovulation in rats have also been demonstrated (MacKenzie *et al.*, 1984). Steger and Morgan (1985) concluded that the widely variable effects of dopamine and dopaminergic drugs on GTH release reported in the literature were largely due to differences in the endocrine status of the test subjects. In particular, the estrogen levels in female subjects appear to play an important, but as yet poorly understood, role in modulating the effects of dopamine. De Leeuw *et al.* (1987) have proposed a model to explain the relationship between steroid feedback and dopaminergic inhibition of GTH in the African catfish, whereby aromatizable androgens and estrogens are converted to catecholestrogens which are metabolized preferentially by the enzyme COMT (catechol *o*-methyl transferase), thus allowing levels of dopamine to increase. In this way increased circulating steroid levels indirectly inhibit gonadotropin release. How this model would relate to species where the dopaminergic control of GTH is less pronounced or where steroids can have both positive and negative feedback effects on GTH secretion (such as certain salmonid fish, reviewed by Goos, 1987) remains to be investigated.

The croaker GTH RIA was developed using antiserum raised against gonadotropin purified from pituitaries of mature fish captured just prior to spawning. The pituitary content of the GTH measured by this RIA increases more than 100-fold during the period of gonadal recrudescence. It is therefore probable that the hormone measured in this study is primarily a maturational GTH, similar to the GTH2 of Kawauchi *et al.* (1986). However, there may be more than one gonadotropin or form of gonadotropin in the Atlantic croaker, as in salmonids (Kawauchi *et al.*, 1986; Suzuki *et al.*, 1988a, b). The croaker GTH antiserum is likely to cross-react to some extent with any gonadotropin containing a common subunit, since the antiserum was raised against the entire GTH molecule, not its subunits. Indeed, preliminary results indicate that several isoelectric forms of croaker GTH are present in the pituitary and plasma of this species and can be detected by the croaker GTH RIA (Copeland and Thomas, 1989).

In the present investigation we have found no evidence for an inhibitory effect

of dopamine or dopamine agonist drugs on GTH release from the pituitary. Conversely, we have found some evidence that dopamine antagonist drugs can suppress the actions of exogenous LHRHa on GTH release, while dopamine agonists can potentiate them. The mechanisms of these effects are unknown as yet. It would appear that the role of dopamine in the control of GTH release in Atlantic croaker is different to that described in the other orders of teleost fishes investigated to date.

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