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The Regulation of Gonadotropin Release by Neurohormones and Gonadal Steroids in the African Catfish, *Clarias gariepinus*

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ABSTRACT

De Leeuw, R., Goos, H.J.Th. and Van Oordt, P.G.W.J., 1987. The regulation of gonadotropin release by neurohormones and gonadal steroids in the African catfish, *Clarias gariepinus*. *Aquaculture*, 63: 43–58.

The secretion of gonadotropic hormone (GTH) from the pituitary of teleosts is considered to be regulated by neuropeptides and neuroamines of cerebral origin and steroid hormones from the gonads. This paper reviews our studies concerning the control of GTH release in the African catfish, *Clarias gariepinus*. It was demonstrated that luteinizing hormone releasing hormone (LHRH) stimulates GTH release and that the gonadotropin release-inhibiting activity of dopamine is restricted to the LHRH-induced GTH release. With regard to the inhibitory action of steroid hormones on GTH release, a hypothesis was postulated which links together the inhibitory actions of dopamines and gonadal steroids. According to this hypothesis, only aromatizable androgens should feed back on the release of GTH and the effects of catecholestrogens and dopamine on GTH release should be comparable.

INTRODUCTION

In all teleost species, as in other vertebrates, the gonadotropic hormone (GTH) from the pituitary gland plays a central role in the regulation of the reproduction. Therefore, studies on the regulation of GTH synthesis and release provide information concerning the hormonal interactions during reproductive cycles. The secretion of GTH is considered to be regulated by neurohormones, i.e., neuropeptides and neuroamines, and by gonadal hormones. This paper reviews studies concerning the control of GTH release in the African catfish, *Clarias gariepinus* (Burchell) and compares the data with those obtained in other teleost species. Studies about the gonadotropic activity of the pituitary of the African catfish were only possible after the development of a

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specific and sensitive radioimmunoassay (RIA) for catfish GTH (Goos et al., 1986).

NEUROPEPTIDES

In mammals, the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) is stimulated by a luteinizing hormone-releasing hormone (LHRH). This neuropeptide of hypothalamic origin has been identified as a decapeptide with the linear sequence pGlu^1-His^2-Trp^3-Ser^4-Tyr^5-Gly^6-Leu^7-Arg^8-Pro^9-Gly^{10} NH_2.

In teleost fishes, the release of GTH is also stimulated by a releasing hormone referred to as gonadotropin-releasing hormone (GnRH). In several earlier studies experimental evidence has been provided for the presence of GnRH activity in the hypothalamus of teleosts (for review see Peter, 1983).

From brains of chum salmon, *Oncorhynchus keta*, Sherwood et al. (1983) purified GnRH and identified its primary structure as pGlu^1-His^2-Trp^3-Ser^4-Tyr^5-Gly^6-Trp^7-Leu^8-Pro^9-Gly^{10} NH_2. This salmon GnRH differs from mammalian LHRH in two amino acids at the seventh and eighth positions. In many teleost species synthetic LHRH or its superactive analogues stimulate GTH release, indicating an overlap in the biological activity of LHRH and GnRH (for review see Peter, 1986).

Immunocytochemical techniques have been used to localize material(s) reactive with antisera against LHRH in the brain and pituitary of teleosts. Summing up the results, a rather heterogeneous distributional pattern of LHRH immunoreactivity is obtained. However, from studies in platyfish, *Xiphophorus maculatus* (Schreibman et al., 1979, 1983), stickleback, *Gasterosteus aculeatus* (Borg et al., 1982), and goldfish, *Carassius auratus* (Kah et al., 1984) it appeared that the anteroventral preoptic region and the posterior part of the nucleus lateralis tuberis (NLT) are possible sites of GnRH origin. In the African catfish, Goos et al. (1985) found LHRH-immunoreactive material in perikarya of the nucleus preopticus and in neurosecretory fibres reaching the GTH-producing cells in the proximal pars distalis (PPD). At the ultrastructural level LHRH immunoreactivity was observed inside secretary granules of fibres bordering the GTH cells (Schild and Peute, 1985).

In the African catfish the superactive LHRH analogue Des Gly^{10}[D-Ala^6]-LHRH ethylamide (LHRHa) stimulates the release of GTH. An intraperitoneal (i.p.) injection of 0.1 mg LHRHa per kg body weight causes an increase of plasma GTH levels starting within 30 min after injection (Figs. 3 and 4).

In vitro studies on the African catfish show a stimulatory effect of LHRHa on the release of GTH from (i) pituitary fragments in a perifusion culture (Fig. 1a) (De Leeuw et al., 1986a), (ii) pituitary cell suspensions in a perifusion culture (Fig. 1b) (De Leeuw et al., 1986a), and (iii) isolated gonadotropic cells in a static culture (De Leeuw et al., 1984). Comparable results are

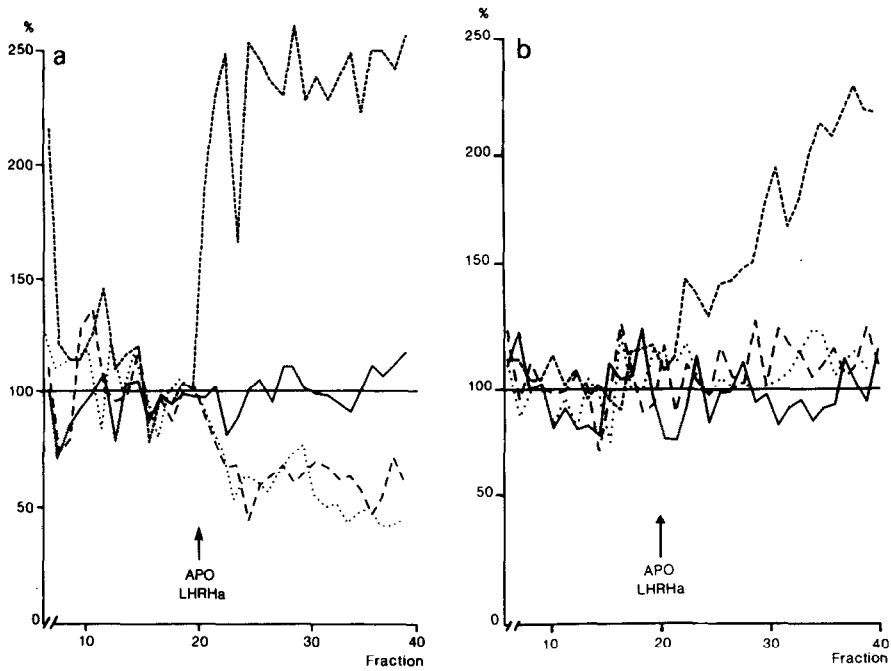

Fig. 1. (a) Release of GTH from perifused catfish pituitary fragments indicating percentage of basal release (%) versus 10-min interval fractions.
—— GTH release of untreated fragments; - - - GTH release in response to 10^{-7} *M* LHRHa; . . . . . GTH release in response to 10^{-5} *M* APO; — — — GTH release in response to 10^{-5} *M* APO followed by 10^{-7} *M* LHRHa.
(b) Release of GTH from perifused catfish pituitary cell suspensions indicating percentage of basal release (%) versus 10-min interval fractions.
—— GTH release in untreated cell suspensions; - - - GTH release in response to 10^{-10} *M* LHRHa; . . . . . GTH release in response to 10^{-5} *M* APO; — — — GTH release in response to 10^{-5} *M* APO followed by 10^{-10} *M* LHRHa.

reported for rainbow trout, *Salmo gairdneri*, pituitaries in a static culture (Crim and Evans, 1980; Crim et al., 1981; Fåhraeus-Van Ree et al., 1983), goldfish pituitary fragments in a perifusion culture (Mackenzie et al., 1984), and rainbow trout dispersed pituitary cells in a static culture (Weil et al., 1986). Most of these studies have been carried out with LHRH and its analogues. Exceptions are the studies by Mackenzie et al. (1984) and Weil et al. (1986) on the effects of synthetic salmon GnRH in comparison to that of LHRH or LHRHa on GTH secretion.

NEUROAMINES

In goldfish, lesions of the NLT-pituitary stalk region as well as destruction of the pituitary stalk cause a dramatic rise in GTH release (Peter et al., 1978).

This points towards the existence of a gonadotropin release-inhibitory factor (GRIF). On the basis of experiments in which lesions were placed in a variety of locations in the diencephalon, Peter and Paulencu (1980) concluded that GRIF originates from the anteroventral preoptic region. Since the observation that dopamine (DA) inhibits the increased GTH release caused by preoptic lesions (Chang and Peter, 1983), attention was focussed on DA as the possible GRIF. Indeed, blocking of DA synthesis resulted in an increase in serum GTH levels (Chang et al., 1983). In addition, the dopamine agonist apomorphine (APO) caused a decrease and its antagonist pimozide (PIM) an increase in GTH release (Chang and Peter, 1983). By using specific DA agonists and antagonists Chang et al. (1984c) suggested that the dopaminergic inhibition of GTH release might be mediated by receptors similar to the mammalian D-2 type. In vitro studies with transplanted goldfish pituitaries (Chang et al., 1984a) and dispersed goldfish pituitary cells (Chang et al., 1984b) led to the conclusion that dopamine acts directly on the gonadotropes; it inhibits spontaneous GTH release and suppresses the stimulatory effect of GnRH. A direct effect of DA on the gonadotropes in goldfish is supported by the immunocytochemical observations of Kah et al. (1986). They found DA-immunoreactive material in fibres in contact with the gonadotropes, suggesting a dopaminergic innervation of these cells.

In the African catfish APO inhibits the spontaneous GTH release from pituitary fragments but has no effect on the spontaneous GTH release from dispersed pituitary cells in a perifusion culture. In both systems APO inhibits the LHRHa-induced GTH release (Fig. 1) (De Leeuw et al., 1986a); for dose dependency, see Fig. 2. From these observations De Leeuw et al. (1986a) concluded that DA inhibits the GnRH-induced GTH release and that an interaction between DA and GnRH occurs at the level of the gonadotropes. In contrast with goldfish, spontaneous GTH release in the catfish is not inhibited by DA. The difference in the inhibitory response of APO on pituitary fragments and dispersed pituitary cells, respectively, was explained by assuming that the spontaneous release of GTH from fragments, containing nerve endings and neural elements, is, in fact, an endogenous GnRH-induced GTH release.

Also in vivo studies on the African catfish and other teleost species indicate a dopaminergic inhibition of only the GnRH-induced GTH release. PIM had slight effects on GTH release in juvenile catfish, and no effect at all in adults (De Leeuw et al., 1985b). Likewise, PIM alone could not increase GTH release in estradiol-treated female silver eel, *Anguilla anguilla* (Dufour et al., 1984) and female common carp, *Cyprinus carpio* (Billard et al., 1983). However, PIM greatly potentiates the effect of LHRHa in all the in vivo studies just mentioned.

The effect of DA and APO on GTH release was also tested in vivo. It was demonstrated that both drugs inhibit the LHRHa-induced GTH release, and that the spontaneous GTH release is not affected (Figs. 3 and 4). This indicates once more that DA acts as a GRIF by blocking the effect of GnRH.

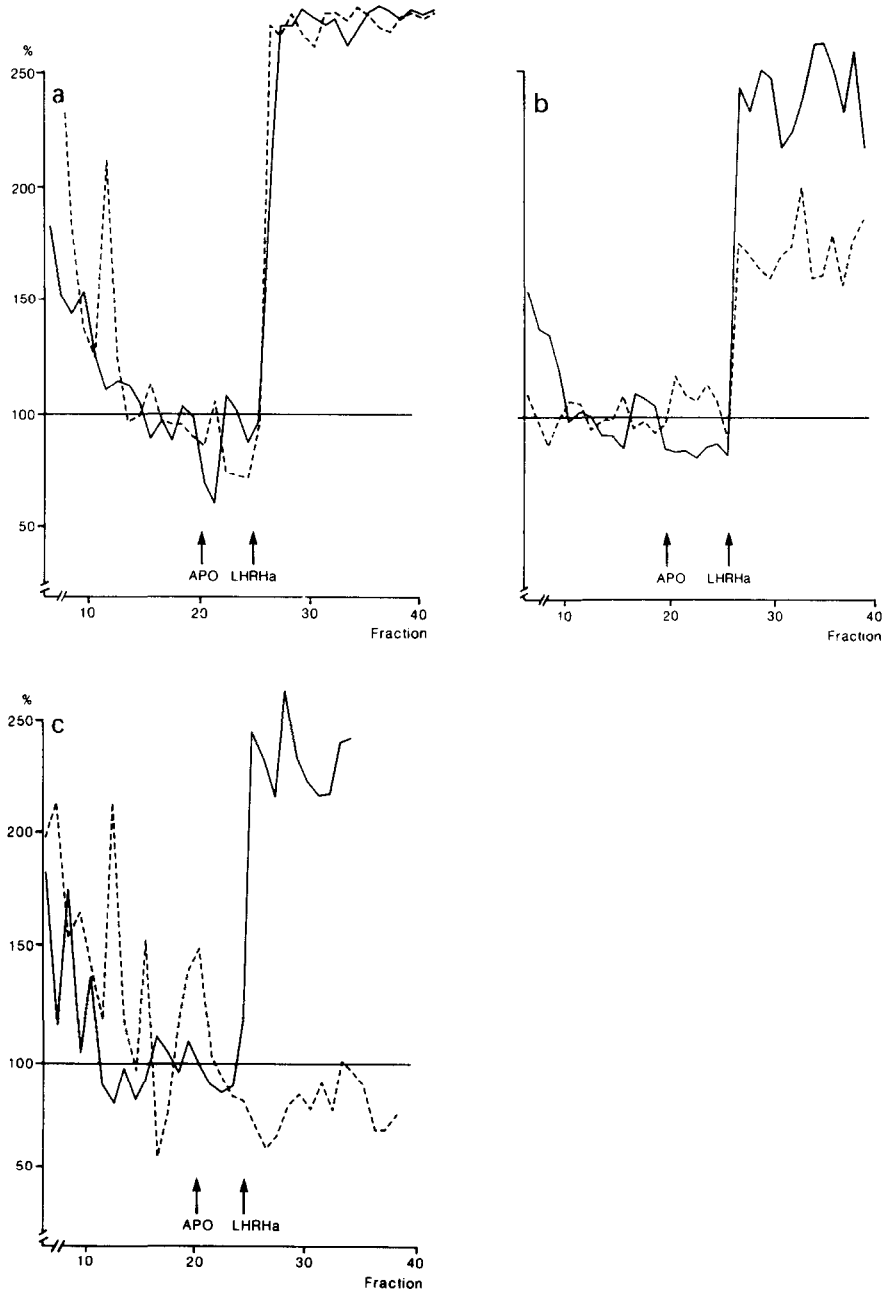

Fig. 2. Release of GTH from perifused catfish pituitary fragments indicating percentage of basal release (%) versus 10-min interval fractions.
—— GTH release in response to 10^{-7} *M* LHRHa (a, b, and c); - - - GTH release in response to different concentrations of APO followed by 10^{-7} *M* LHRHa; (a) 10^{-9} *M* APO + 10^{-7} *M* LHRHa, (b) 10^{-7} *M* APO + 10^{-7} *M* LHRHa, and (c) 10^{-5} *M* APO + 10^{-7} *M* LHRHa.

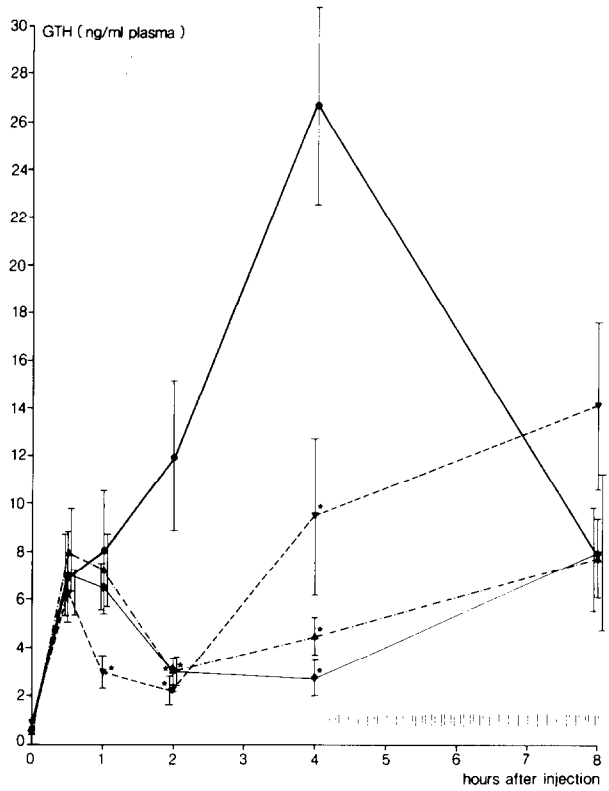

Fig. 3. Effect of intraperitoneal injections of LHRHa (0.1 mg/kg body weight dissolved in a vehicle consisting of 0.8% NaCl with 0.25% bovine serum albumin) and/or DA (0.01, 0.1, and 1.0 mg/kg body weight dissolved in a vehicle consisting of 0.7% NaCl with 0.1% sodium metabisulphite) on plasma GTH concentration in adult male African catfish.
——— LHRHa; —— LHRHa + 0.01 mg DA per kg body weight; -·-·- LHRHa + 0.1 mg DA per kg body weight; - - - LHRHa + 1.0 mg DA per kg body weight. The shaded bar represents plasma GTH values after a treatment with (i) LHRHa-vehicle, (ii) DA-vehicle, and (iii) DA 0.01, 0.1, and 1.0 mg/kg body weight. Values are $\bar{x} \pm$ SEM ($n = 10$). ★ significantly different from LHRHa group, $P < 0.05$.

The dopamine antagonist PIM also has anti-serotonin properties; however, experiments with LHRHa and drugs with anti-dopamine and/or anti-serotonin activities suggest that the effect of PIM on the LHRHa-induced GTH release in the African catfish is caused by the anti-dopaminergic activity of PIM (Goos et al., 1987).

In a variety of teleost species, including the African catfish, oocyte maturation and ovulation do not occur spontaneously under fish-farm conditions. In the African catfish, a single injection of LHRHa (0.1 mg/kg body weight) was observed to cause an increase of plasma GTH levels and in 80% of the treated females this increase was followed by ovulation (De Leeuw et al., 1985b).

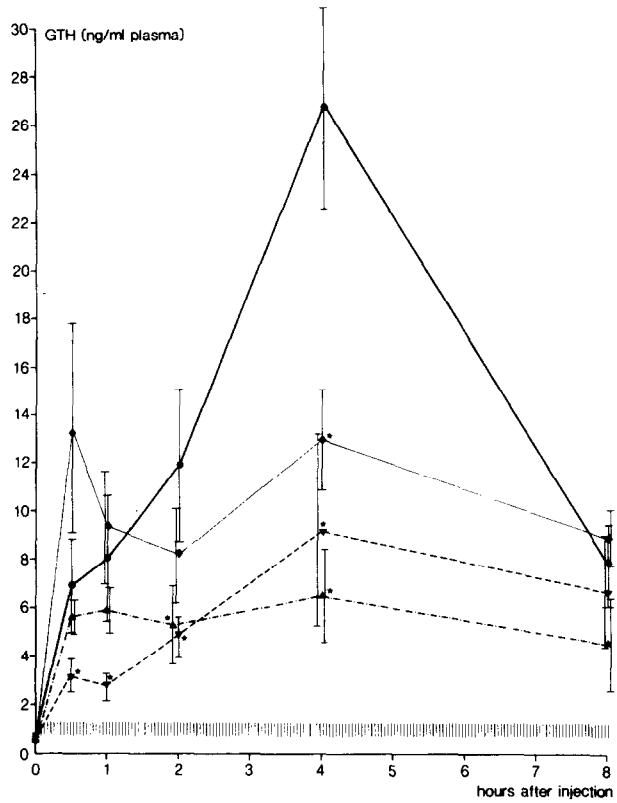

Fig. 4. Effect of intraperitoneal injections of LHRHa (0.1 mg/kg body weight dissolved in a vehicle consisting of 0.8% NaCl with 0.25% bovine serum albumin) and/or APO (0.01, 0.1, and 1.0 mg/kg body weight dissolved in a vehicle consisting of 0.7% NaCl with 0.1% sodium metabisulphite) on plasma GTH concentration in adult male African catfish.
——— LHRHa; —— LHRHa + 0.01 mg APO per kg body weight; - - - LHRHa + 0.1 mg APO per kg body weight; -·-·- LHRHa + 1.0 mg APO per kg body weight. The shaded bar represents plasma GTH values after a treatment with (i) LHRHa-vehicle, (ii) DA-vehicle, and (iii) APO 0.01, 0.1, and 1.0 mg/kg body weight. Values are $\bar{x} \pm$ SEM ($n = 10$). ★ significantly different from LHRHa group, $P < 0.05$.

However, later experiments have shown that this high rate of ovulation induced by LHRHa only is not a common phenomenon in the African catfish. Although postvitellogenic eggs are constantly present in mature females, monthly ovulation-inducing experiments showed that the successful induction of ovulation with LHRHa is restricted to the spring period, close to the natural breeding period. Indeed, although in many teleosts LHRH and its analogues can induce release of GTH, not seldom very high doses or multiple injections are needed for the induction of oocyte maturation and ovulation (for review see Donaldson and Hunter, 1983). In contrast, a combined treatment with PIM and LHRH analogues is very effective in inducing ovulation in goldfish (Sokolowska et

al., 1984), carp (Billard et al., 1983), loach, *Paramisgurnus dabryanus* (Lin et al., 1985), and African catfish (De Leeuw et al., 1985a; Richter et al., 1987). In the catfish a single injection of 5 mg PIM and 0.05 mg LHRHa per kg body weight led to oocyte maturation and ovulation; 12–14 h after injection the fish could be stripped and the eggs fertilized. This procedure yielded 30 000–60 000 eggs per female of 400–500 g, and over 80% normal, healthy larvae. Unfortunately, this simple method cannot be used for all species of cultured teleosts that fail to ovulate spontaneously under farm and hatchery conditions. In goldfish, for example, simultaneous injection of PIM and LHRHa led to low rates of ovulation, and for better results the dopamine antagonist and GnRH agonist had to be injected separately with some time interval (Sokolowska et al., 1984).

GONADAL HORMONES

In adult teleosts with a functionally active brain-pituitary-gonadal axis, gonadal hormones exert a negative feedback effect on GTH release. This follows from several physiological and morphological studies. In the Indian catfish, *Heteropneustes fossilis* (Bloch), androgens and estrogens suppress the gonadotropic activity of the pituitary (Sundararaj and Goswami, 1968). In rainbow trout, plasma GTH levels increase after gonadectomy (Billard et al., 1977; Van Putten et al., 1981); however, the intensity and duration of this response depends on the stage of the reproductive cycle (Billard, 1978; Bommelaer et al., 1981). Van Putten et al. (1981) demonstrated that the rise in plasma GTH in ovariectomized rainbow trout is accompanied with a disappearance of secretory granules and globules from the cytoplasm of the gonadotropes. Similar results were obtained for the African catfish. In this teleost species, castration of adult males leads to an increase of plasma GTH levels (Fig. 5), a decrease of pituitary GTH content (Fig. 6), and a degranulation of the gonadotropes (De Leeuw et al., 1986b). Apart from rainbow trout and African catfish, a degranulation following gonadectomy was also described by Borg et al. (1985) for the stickleback. A treatment of castrated African catfish with silastic capsules containing testosterone or androstenedione, restored the plasma steroid levels. Concomitantly, the plasma GTH level and the pituitary GTH content returned to normal (Figs. 5 and 6); in the gonadotropic cells a well developed granular endoplasmic reticulum and Golgi complex as well as numerous secretory granules and globules reappeared. These results indicate an inhibition of GTH release and a stimulation of GTH synthesis. In contrast, the non-aromatizable androgens 5α-dihydrotestosterone and 11β-hydroxyandrostenedione failed to abolish the castration effects on the gonadotropes (Figs. 5 and 6) (De Leeuw et al., 1986b). This confirms similar observations of Billard (1978). He observed a decrease in plasma GTH of castrated rainbow trout after an intraperitoneal administration of testosterone and estradiol, whereas the non-aromatizable

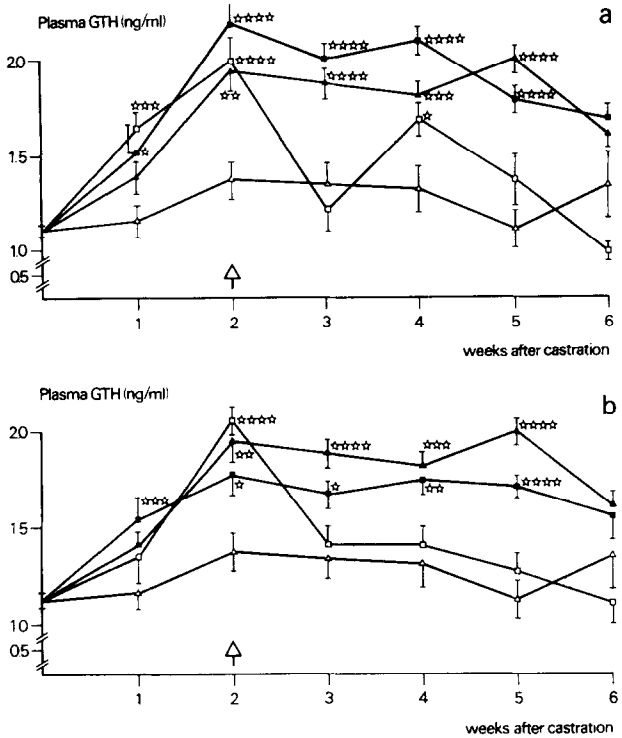

Fig. 5. The effect of castration and steroid implantation (arrow) on plasma GTH levels in the African catfish. (a): △ sham-operated, ▲ castrated without an implant, □ castrated followed by testosterone implantation, ■ castrated followed by 5α-dihydrotestosterone implantation. (b): △ sham-operated, ▲ castrated without an implant, □ castrated followed by androstenedione implantation, and ■ castrated followed by 11β-hydroxyandrostenedione implantation. Values are $\bar{x} \pm$ SEM ($n = 10$). Plasma GTH levels were compared with the plasma GTH levels of sham-operated catfish. ☆ $P < 0.05$, ☆☆ $0.05 > P > 0.02$, ☆☆☆ $0.02 > P > 0.01$, ☆☆☆☆ $0.01 > P > 0.001$. (From De Leeuw et al., 1986b. Reproduced from Cell Tissue Research, by permission.)

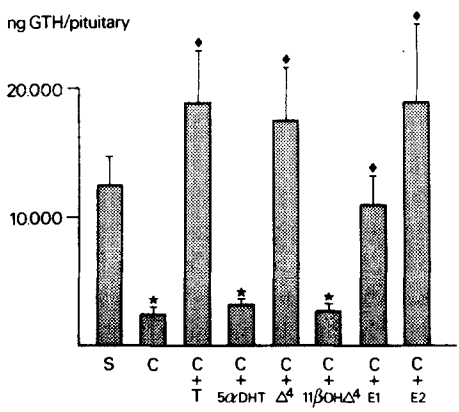

Fig. 6. The effect of castration and steroid implantation on pituitary GTH content in the African catfish at 6 weeks after castration. S sham; C castrated; C+T castrated+testosterone; C+5αDHT castrated+5α-dihydrotestosterone; C+Δ^4 castrated+androstenedione; C+11βOHΔ^4 castrated+11β-hydroxyandrostenedione; C+E1 castrated+estrone; C+E2 castrated+estradiol. Values are $\bar{x} \pm$ SEM ($n = 10$). ★ significantly ($P < 0.05$) different from sham-operated catfish; ♦ significantly ($P < 0.05$) different from castrated catfish. (From De Leeuw et al., 1986b. Reproduced from Cell Tissue Research, by permission.)

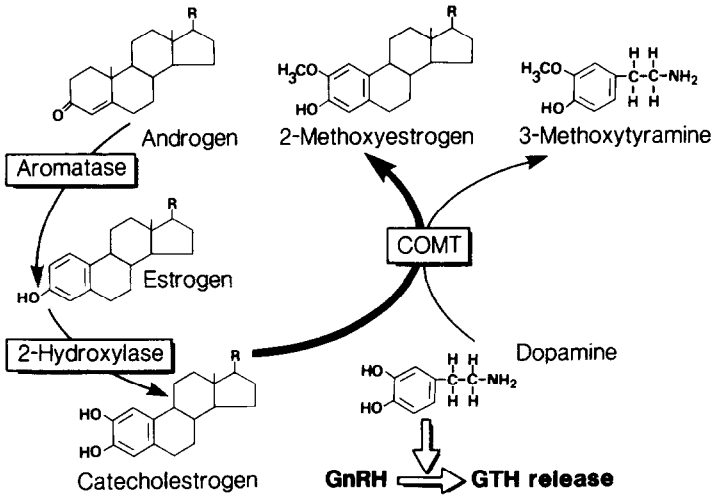

Fig. 7. Schematic representation of the interaction between gonadal steroids and dopamine in the regulation of GTH release in the African catfish, *Clarias gariepinus* (Burchell).

11-ketotestosterone is less effective. These observations led to the hypothesis that a conversion of androgens into estrogens (aromatization) is essential in the feedback regulation of GTH release.

In mammals, it has long been established that estrogens can induce a negative feedback on gonadotropin release. Knuppen (1981) and Breuer et al. (1981) speculated that estrogens affect gonadotropin release via catecholamines. They suggested the following model: estrogens are hydroxylated to catecholestrogens, which subsequently are methylated by the enzyme catechol-O-methyltransferase (COMT), resulting in the formation of inactive products. The same enzyme is also responsible for the methylation of catecholamines. However, since catecholestrogens form a better substrate for COMT (Ball and Knuppen, 1980), in their presence the methylation of catecholamines might be reduced. Consequently, catecholestrogens indirectly stimulate the catecholaminergic effect on gonadotropin release. As dopamine inhibits the GnRH-induced GTH release in teleosts such as goldfish, carp, eel and African catfish (see above), De Leeuw et al. (1985c) postulated for the African catfish that gonadal hormones inhibit gonadotropin release via dopamine. In teleosts, the main steroids produced by the testes are androgens (for review see Fostier et al., 1983). Therefore, it is proposed that these androgens first need to be aromatized to estrogens before they can stimulate the dopaminergic effect on GTH release according to the mechanism suggested above. Fig. 7 summarizes the hypothetical mechanism by which androgens and/or estrogens inhibit gonadotropin release in the African catfish.

According to this hypothesis, estrogens as well as catecholestrogens inhibit the release of GTH. Indeed, the negative effect of estrogens on GTH release has been demonstrated in several teleost species such as goldfish (Billard and

Peter, 1977), carp (Breton et al., 1975), and Indian catfish (Singh and Singh, 1979), in which the anti-estrogen clomiphene citrate causes an increase of circulating GTH. In addition, Billard (1978) and Bommelaer et al. (1981) could suppress the elevated plasma GTH levels in gonadectomized rainbow trout with estradiol. In castrated African catfish, the elevated levels of circulating GTH were not suppressed after the implantation of silastic capsules containing estradiol or estrone (De Leeuw et al., 1986b); however, both estrogens did restore the pituitary GTH content to levels observed in sham-operated catfish (Fig. 6). This elevation was accompanied by the ultrastructural appearance of GTH cells as present in castrated catfish treated with the aromatizable androgens testosterone and androstenedione. Steroid analysis after estradiol or estrone implantation revealed that both estrogens remained at a very low concentration (estradiol 240–280 pg/ml; estrone 14–26 pg/ml) in comparison with the plasma androgen levels after implantation (testosterone 7.6–18.5 ng/ml; androstenedione 2.2–4.9 ng/ml). De Leeuw et al. (1986b) suggested that these relatively low estrogen concentrations stimulate GTH synthesis, but higher levels are needed to inhibit GTH release.

Data about the effect of catecholestrogens on GTH release in castrated or intact teleosts are very limited. The only information available comes from an in vivo study in the African catfish. In this study, female catfish were injected with 0.1 mg 2-hydroxyestrone per kg body weight (intravenous) and/or 0.1 mg LHRHa per kg body weight (intraperitoneal). In the case of a combined treatment, 2-hydroxyestrone was injected 30 min before LHRHa. The following results were observed (Fig., 8): at 1 h after injection, i.e., 30 min after the LHRHa injection, the LHRHa-induced GTH release was somewhat potentiated, and at 4 and 8 h after injection the effect of LHRHa was inhibited by 2-hydroxyestrone; spontaneous GTH release was not affected by 2-hydroxyestrone. The observed stimulatory effect is difficult to explain. The inhibitory effect of 2-hydroxyestrone on the LHRHa-induced GTH release is in agreement with the hypothesis stated above. If catecholestrogens inhibit the release of GTH via catecholamines, a treatment with catecholestrogens should have the same effect as a treatment with catecholamines. Indeed, both catecholamines and catecholestrogens inhibit the GnRH-induced GTH release (Figs. 3, 4, and 8), whereas spontaneous GTH release is not affected.

In the African catfish the gonadotropic cells are innervated by both peptidergic and aminergic fibres (Van Oordt and Peute, 1983; Peute et al., 1984). Granules of peptidergic fibres were identified as LHRH immunoreactive (Schild and Peute, 1985), whereas granules of aminergic fibres were DA immunoreactive (Peute et al., 1987). In addition, DA can inhibit the GnRH-induced GTH release at the level of the gonadotropic cells (De Leeuw et al., 1986a). These observations indicate that an interaction between gonadal hormones, DA and GnRH according to the above-mentioned hypothesis, may occur at the level of the gonadotropes. If so, the enzymes involved in this mechanism

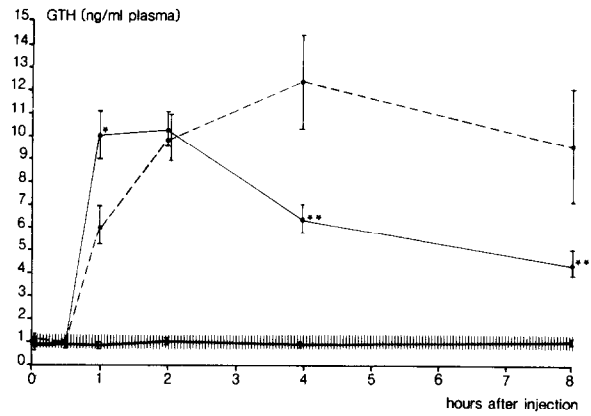

Fig. 8. The effect of 2-hydroxyestrone (2 OHE1; 0.1 mg/kg body weight dissolved in a vehicle consisting of 0.9% NaCl with 10% propyleneglycol and 0.2% ascorbic acid) on basal and LHRHa (0.1 mg/kg body weight dissolved in a vehicle consisting of 0.8% NaCl with 0.25% bovine serum albumin)-induced plasma GTH levels of male African catfish.
— — — LHRHa, injected at $t=\frac{1}{2}$; ▬▬ 2 OHE1, injected at $t=0$; —— 2 OHE1, injected at $t=0$, plus LHRHa, injected at $t=\frac{1}{2}$. The shaded bar represents plasma GTH levels after a treatment with (i) LHRHa-vehicle and (ii) 2 OHE1-vehicle. Values are $\bar{x}\pm$SEM ($n=9$). ★ significantly ($P<0.05$) higher than LHRHa group. ★★ significantly ($P<0.05$) lower than LHRHa group.

of interaction must be present in the gonadotropic cells. Indeed, De Leeuw et al. (1985c) succeeded in demonstrating aromatase, estrogen 2-hydroxylase and COMT activity in the gonadotropes of the African catfish. The isolated GTH cells used in this study were obtained by Percoll density centrifugation (De Leeuw et al., 1984). Evidence for a direct effect of gonadal hormones on gonadotropic cells influencing GTH release has been provided by Sage and Bromage (1970) and Groves and Batten (1986).

Other data suggest that the hypothalamus could also be the site of action for negative feedback regulation. Zambrano (1971) found an activation of neurons of the NLT of the goby, *Gillichthys mirabilis*, after gonadectomy. This activation could be suppressed by androgens. A similar activation of neurons in the NLT and nucleus preopticus was observed in castrated *Clarias batrachus* (Dixit, 1970). In this respect Timmers et al. (1987) localized aromatase activity in African catfish brain areas by means of microdissection and biochemical identification. The highest aromatase activity in this species was found in the preoptic area. In several teleost species aromatase activity has been found in brain areas that accumulate steroids (for review see Van Oordt, 1987). This might indicate a role of these brain areas in the control of the gonadotropic activity of the pituitary. Lambert and Van Oordt (1982) found estrogen 2-hydroxylase and COMT activity in brains of rainbow trout; unpublished results (1986) of Timmers and Lambert are indicative of co-localization of these enzymes in certain brain areas of the African catfish. This means that aromatizable androgens or estrogens might inhibit GTH release after being con-

verted into catecholestrogens both at the level of the gonadotropes in the pituitary, and at the cerebral level.

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