Gonadotrophs but not somatotrophs carry gonadotrophin-releasing hormone receptors: receptor localisation, intracellular calcium, and gonadotrophin and GH release

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Abstract

Gonadotrophs are the primary target cells for GnRH in the pituitary. However, during a limited period of neonatal life in the rat, lactotrophs and somatotrophs respond to GnRH as well. Also, in the adults of a number of teleost fishes (e.g. carp, goldfish, and tilapia but not trout), GnRH is a potent GH secretagogue. In studying hypophysiotrophic actions of the two forms of GnRH present in the African catfish (Clarias gariepinus), chicken GnRH-II ([His5,Trp7,Tyr8]GnRH; cGnRH-II) and catfish GnRH ([His5,Asn8]GnRH; cfGnRH), we have investigated the effects of GnRH on catfish gonadotrophs and somatotrophs. GnRH binding was examined by incubating dispersed pituitary cells attached to coverslips with 125I-labelled sGnRHa, a salmon GnRH analogue with high affinity for the GnRH receptor. Following fixation and immunohistochemistry using antisera against catfish LH and GH, 125I-labelled sGnRHa was localised autoradiographically and silver grains were quantified on gonadotrophs and somatotrophs. Specific binding of 125I-labelled sGnRHa was restricted to gonadotrophs. Both cfGnRH and cGnRH-II dose-dependently inhibited 125I-labelled sGnRHa binding to gonadotrophs. To substantiate the localisation of functional GnRH receptors, the effects of cfGnRH and cGnRH-II on the cytosolic free calcium concentration ([Ca2+]i) were examined in Fura-2-loaded somatotrophs and gonadotrophs. GnRH-induced increases in [Ca2+]i, appeared to be confined to gonadotrophs, in which both endogenous GnRHs caused a single and transient increase in [Ca2+]i. The amplitude of this [Ca2+]i transient depended on the GnRH dose and correlated well with the GnRHs’ effect on LH release. In vivo experiments demonstrated that GnRH treatments which markedly elevated plasma LH levels had no effect on plasma GH levels, while a dopamine agonist (apomorphine) significantly elevated plasma GH levels. We conclude that the two endogenous forms of GnRH in the African catfish are not directly involved in the regulation of the release of GH, suggesting that GnRHs cannot be considered as GH secretagogues in teleosts in general.

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Introduction

Gonadotrophin-releasing hormones (GnRHs) form a family of— to date — nine decapeptides (Powell et al. 1994). In the brain of most placental mammals one form of GnRH, which can be present in two molecular variants, is synthesised (Gautron et al. 1992). In metatherian species (King et al. 1990, 1994) and in submammalian vertebrates, more than one form of GnRH is found in the brain (Powell et al. 1994, Sherwood et al. 1994). Teleost fish lack a functional hypothalmo-hypophysial portal system; instead the pituitary is directly innervated by GnRH nerve fibres (Peter et al. 1990, Kah et al. 1993). Hence, catfish GnRH ([His5,Asn8]GnRH; cfGnRH) and chicken GnRH-II ([His5,Trp7,Tyr8]GnRH; cGnRH-II), the two endogenous GnRH peptides in the African catfish (Bogerd et al. 1992), are not only present in the brain but also in the pituitary (Schulz et al. 1993, Zandbergen et al. 1995). A well-established function of GnRH is to stimulate the release of gonadotrophin. Two types of gonadotrophins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), are found in all tetrapods except for the squamate reptiles (Licht 1983). Many fish species also show two gonadotrophins (Kawauchi et al. 1989) and it has recently been proposed to adopt the general vertebrate nomenclature and to call these...
hormones fish FSH and LH respectively (Prat et al. 1996). However, in some fishes only the LH-like form has been identified (reviewed by Schulz et al. 1995), including the African catfish (Koide et al. 1992). Both endogenous GnRHs stimulate the release of LH in catfish, cGnRH-II being approximately 150-fold more potent than cfGnRH; this difference in potency correlates with the relative receptor affinities of the GnRHs, but may be compensated by the large excess of cfGnRH over cGnRH-II in the catfish pituitary (Schulz et al. 1993). In goldfish, Carassius auratus (Marchant et al. 1989), common carp, Cyprinus carpio (Lin et al. 1993) and tilapia hybrids, Oreochromis niloticus × O. aureus (Melamed et al. 1995), GnRHs also stimulate the release of growth hormone (GH), and GnRH receptors are present on goldfish gonadotrophs and somatotrophs (Cook et al. 1991). Conversely, there is conflicting evidence regarding the effect of GnRH on the release of GH in the rainbow trout, Oncorhynchus mykiss, A. j. oregoni (Stojilkovic & Catt 1992, Stojilkovic et al. 1994). The role of [Ca\(^{2+}\)], in the mechanism of action of GnRH has been established for teleost fish as well, regarding the GnRH-stimulated release of GH in goldfish (Jobin & Chang 1992, Wong et al. 1994). Also, in the rat, GnRH stimulates pituitary GH (and prolactin (PRL)) secretion, at least during a limited period of neonatal life (Andries & Deneufville 1995).

The first step in GnRH action is the binding to its specific plasma membrane receptor. A single class of high-affinity GnRH-binding sites was characterised in the pituitary of the African catfish (de Leeuw et al. 1988). GnRH induces a rapid increase in the gonadotroph's cytosolic free calcium concentration ([Ca\(^{2+}\)]), in mummus (Stojilkovic & Catt 1992, Stojilkovic et al. 1994). The role of [Ca\(^{2+}\)], in the mechanism of action of GnRH has been established for teleost fish as well, regarding the gonadotrophs (van Asselt et al. 1989, Chang et al. 1993, Levavi-Sivan & Yaron 1993, Mollard & Kah 1996), but also regarding the GnRH-stimulated release of GH in goldfish (Jobin & Chang 1992, Wong et al. 1994). The present study on hypophysiotrophic actions of cfGnRH and cGnRH-II was conducted to investigate if GnRHs function as GH secretagogues in the African catfish.

A preliminary report (Schulz et al. 1995) was presented at the Fifth International Symposium on the Reproductive Physiology of Fish (2–8 July 1995) in Austin, TX, USA.

Materials and Methods

Experimental animals and GnRH peptides

African catfish were bred and raised in the laboratory as described previously (de Leeuw et al. 1985), except that catfish pituitary extract instead of human chorionic gonadotrophin was used to induce ovulation. Mature male catfish (8–15 months old) were used for the experiments. Synthetic cfGnRH (American Peptide Co., Sunnyvale, CA, USA) and cGnRH-II (Peninsula Laboratories, Belmont, CA, USA) were used in all experiments. An analogue of salmon GnRH ([D-Arg\(^{6}\),Trp\(^{2}\),Leu\(^{8}\), Pro\(^{2}\)-NEt]GnRH (sGnRHa); Syndel Laboratories Ltd, Vancouver, British Columbia, Canada), showing a high affinity in binding studies conducted with a catfish pituitary membrane preparation (Schulz et al. 1993), was used for the receptor localisation studies.

Pituitary cell dispersion and GnRH receptor localisation

Fish were killed by decapitation and the pituitaries were removed for enzymatic dispersion as described previously (Lescroart et al. 1996). The cell suspension was diluted to 250 000 cells/ml with L-15 medium (Leibovitz 1963). Life Technologies, Paisley, Strathclyde, UK) containing 26 mm sodium bicarbonate, and 200 μl aliquots were placed on glass coverslips (24 x 24 mm) in six-well tissue culture plates (Costar, Cambridge, MA, USA). Cells were cultured for 90 min at 25 °C, 5% CO\(_2\) to allow attachment of the cells to the coverslip, after which 2 μl L-15 medium containing 26 mM sodium bicarbonate and horse serum (Gibco, Paisley, Strathclyde, UK; 20% v/v) was added. After overnight incubation the cells were rinsed with 1 ml of a balanced salt solution (BSS: 1 mM CaCl\(_2\), 5.4 mM KCl, 0.4 mM KH\(_2\)PO\(_4\), 1 mM MgCl\(_2\), 0.8 mM MgSO\(_4\), 0.14 mM NaCl, 1.3 mM Na\(_2\)HPO\(_4\), 5 mM D(+)-galactose, 5 mM Na-pyruvate, 15 mM Hepes; pH 7.4) for 5 min, followed by 1 ml BSS for 20 min. BSS was then removed and 80 μl aliquots of BSS containing \(^{125}\)I-labelled sGnRHa (75 000 c.p.m.) were placed on the coverslips for 20 min at room temperature. sGnRHa was iodinated and purified according to Habibi et al. (1987). For displacement studies, radioinert sGnRHa (10 μM; non-specific binding), cfGnRH (1 nm–100 μM), or cGnRH-II (0.1 nm–10 μM) were added to BSS for the incubation with \(^{125}\)I-labelled sGnRHa.

Following incubation with \(^{125}\)I-labelled sGnRHa, the culture plates were put on ice, and 1.5 ml chilled fixative (0.1 M phosphate buffer, pH 7.4, containing 2% (w/v) paraformaldehyde and 2% (v/v) glutaraldehyde) was added for 20 min. The culture plates were removed from the ice and the cells were washed with respectively 1 ml 0.1 M phosphate buffer, pH 7.4, and 1 ml 10 mM PBS (0.15 % NaCl), pH 7.4, for 5 min each. The cells were then incubated with 1 ml PBS containing 0.05% (w/v) BSA for 30 min, and washed three times for 5 min with 1 ml PBS containing 0.25% (v/v) Triton X-100 (PBS/Triton). The cells were subsequently incubated for 1 h with 1 ml antiserum against catfish LHβ subunit (diluted 1:8000) or against catfish GH (diluted 1:4000) in PBS/Triton containing 0.05% (w/v) BSA at room temperature. Visualisation of the primary antibodies was carried out as described previously (Peute et al. 1984) using the peroxidase-antiperoxidase–3,3'-diaminobenzidine procedure. The purification of the pituitary hormones and the generation of polyclonal antisera have been described previously (Koide et al. 1992, Lescroart et al. 1996). For control purposes, the primary antisera were replaced with preimmune serum, resulting in the complete loss of specific staining.
Following the immunohistochemical procedure, the coverslips were air-dried and mounted on slides (26 x 76 mm) with the cells on top. They were coated in a dark-room with nuclear emulsion K-2 (Ilford, Mobberley, Cheshire, UK) diluted 1:1 with distilled water, and air-dried horizontally overnight. The autoradiograms were exposed in lightproof boxes for 2 weeks at 4 °C; controls for both positive and negative chemography were included. The slides were developed in freshly prepared Kodak D-19 diluted 1:1 with distilled water for 4 °C; controls for both positive and negative chemography were included. The slides were developed in freshly prepared Kodak D-19 diluted 1:1 with distilled water for 4 min, rinsed in distilled water containing 1% (v/v) acetic acid for 1 min, fixed in 20% (w/v) sodium thiosulphate for 7 min, and rinsed in distilled water for 15 min (Kopriwa 1975). Finally, the autoradiograms were air-dried, shielded with a coverslip using a 1:1 mixture of glycerine/gelatine, and photographed with transmitted light- and dark-field illumination.

**Analysis of autoradiograms**

Silver grains above most cells fused to clusters, so we measured the area occupied by the grains using the IBAS image analysis system (Kontron/Zeiss, Eching, Germany). Autoradiograms were microscopically examined (objective 100 x oil immersion, projective 2 x 0 x) and scanned with a Panasonic b/w CCD camera type WC-CD50, digitised four times and averaged to improve the signal to noise ratio (frame size 640 x 512 pixels; 256 grey levels; pixel size 0.051 x 0.054 mm). To improve the edges of the silver grains, their contours were enhanced. Subsequently, the boundary of a cell of interest was indicated on-screen with a pointing device. The boundary was extended for 90 nm to take into account the influence of 125I as radiation source (Nadler 1979). Since the grey level of the background in each image can vary, semiautomatic selection of the silver grains demanded the application of a so-called dynamic discrimination. This method operates with a threshold that is dependent on the grey level of the local neighbourhood and was carried out in two steps for separate and clustered grains respectively. The resulting selections were combined and measured. The net concentration of label on each cell was calculated as grain area per cell area after subtraction of the background from the total labelling.

Two independent experiments were carried out for the displacement studies. In each experiment, 20 immunostained cells were selected at random and analysed for each GnRH concentration. The results of both experiments were combined as no significant differences were observed between the two experiments.

**Enrichment of gonadotrophs and somatotrophs**

Dispersed pituitary cells were separated into five fractions on a discontinuous Percoll density gradient as described previously (de Leuw et al. 1984). After fractionation, the cells were washed four times with PBS, pH 7.4, and cultured overnight. One aliquot was cultured overnight on glass coverslips as described above (30 000 cells/coverslip) to determine immunohistochemically the distribution of gonadotrophs and somatotrophs over the fractions.

**Intracellular calcium response of single cells**

After overnight incubation, enriched somatotrophs and gonadotrophs (fractions 3 and 4 respectively; see Fig. 3) were washed four times with 4 ml L-15 medium containing 5% (v/v) horse serum, leaving 1 ml medium in the wells to cover the cells in between wash steps. The cells were loaded with 10 μM Fura-2/AM (Molecular Probes, Eugene, OR, USA) in 1 ml L-15 medium containing 0.02% (w/v) Pluronic (Molecular Probes; Poenie et al. 1986) for 1 h at room temperature. The cells were then washed four times with 5 ml PBS containing 1 mM CaCl₂, pH 7.4, placed in a Leiden perifusion chamber (volume 400 μl; Ince et al. 1985), and superfused with the same buffer for 5 min at a flow rate of 1 ml/min. [Ca²⁺]ᵢ measurements on single cells were performed by dynamic video imaging using the MagiCal hardware and TARDIS software provided by Joyce Loebl (Dukesway, Team Valley, Gateshead, Tyne & Wear, UK) as described previously (Willems et al. 1993). The relative changes in [Ca²⁺]ᵢ were measured as the fluorescence emission ratio at 492 nm after excitation at 340 and 380 nm. Upon challenging (1, 2, or 11 min), GnRHs were added to the superfusion buffer. On average, 35 ± 4 (s.e.m., n=9) single cells were monitored simultaneously and responding and non-responding cells were counted during an accelerated replay of the consecutive emission frames. In each reacting cell the [Ca²⁺]ᵢ response to GnRH was quantified by taking the four ratio frames with the highest fluorescence intensity (peak [Ca²⁺]ᵢ response), and calculating the mean of these four frames.

**Static culture**

After enzymatic dispersion, the cell suspension was diluted with L-15 medium containing 26 mM sodium bicarbonate and 400 μl aliquots were plated at a density of 125 000 cells/well in 48-well tissue culture plates (Costar). Cells were cultured for 90-120 min at 25 °C, 5% CO₂ to allow attachment of the cells, after which 100 μl L-15 medium containing 26 mM sodium bicarbonate and 25% (v/v) horse serum (Gibco; final concentration 5%) was added. The incubation was continued for 2 days. Before GnRH challenge experiments, the cells were washed three times with L-15 medium, exchanging 350 μl each time, thus leaving 150 μl medium in the wells to protect the cells from exposure to air. Cells were allowed to rest for 30 min at 25 °C. Another 350 μl incubation medium was replaced by L-15 medium containing freshly diluted GnRHs at different concentrations. After incubation for 30 min at 25 °C in the presence of GnRH, the medium was collected, centrifuged (10 min, 200 g, 4 °C), and
250 µl supernatant was stored at –20 °C until assayed for catfish LH by RIA (Goos et al. 1986), using highly purified LH (Koide et al. 1992) as standard and label.

**LH and GH plasma levels**

Fish (n=8/group) were injected i.p. with a dopamine agonist (apomorphine), cGnRH, or cGnRH-II (10 mg/kg, 250 µg/kg, and 5 µg/kg body weight respectively). Shortly before and at 0.5, 1, 2, 4, 8, and 24 h after injection blood samples were taken and LH and GH plasma levels were measured. GH was quantified by RIA as described by Lescroart et al. (1996). LH plasma levels were quantified by RIA as described above.

**Data analysis**

Data on GnRH receptor labelling, [Ca\(^{2+}\)], responses, and in vitro hormone release were processed statistically by ANOVA, followed by Fisher’s least significant difference test (α=0.05). Differences between pre- and post-injection hormone levels were analysed by a two-tailed Student’s t-test.

**Results**

**GnRH receptor localisation**

Following autoradiography, silver grains were almost exclusively associated with the anti-LHβ-positive, relatively large gonadotrophs (Fig. 1a and b), indicating the presence of sGnRHa binding sites on these cells. After GH immunostaining, only a small number of silver grains were observed on somatotrophs, whereas larger, immunonegative cells (probably gonadotrophs) were strongly labelled (Fig. 1c and d). This indicated that sGnRHa-binding sites are not present on somatotrophs.
GnRH and LH and GH secretion in catfish

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GnRH and LH and GH secretion in catfish

Displacement studies were carried out with radioinert sGnRHa, cfGnRH and cGnRH-II, and the silver grain distribution on single gonadotrophs and somatotrophs was quantified. The addition of an excess of radioinert sGnRHa (10 μM) caused an almost complete loss of silver grains on gonadotrophs (Fig. 2). Both cfGnRH and cGnRH-II reduced the silver grain area in a dose-dependent manner. To achieve a half-maximal reduction, 100-fold higher concentrations of cfGnRH than of cGnRH-II were needed (approximately 1 μM cfGnRH vs 10 μM cGnRH-II; Fig. 2). The small area occupied by silver grains found on somatotrophs could not be reduced by radioinert peptides.

In vitro [Ca^{2+}]_i response and release of LH

To test if GnRH-binding sites were functional receptors, the effects of cfGnRH and cGnRH-II on the [Ca^{2+}]_i of single cells were studied using pituitary cell suspensions enriched in gonadotrophs or somatotrophs. Immunohistochemistry revealed that, after density gradient fractionation, fraction 3 contained the highest proportion of somatotrophs, whereas fractions 4 and 5 mostly contained gonadotrophs (Fig. 3). Fractions 3 and 4 were used to study effects of GnRHs on the [Ca^{2+}]_i. An elevation of [Ca^{2+}]_i in response to GnRH was observed in 16 and 20% of the cells in fraction 3, and in 76 ± 3% (n=6) of the cells in fraction 4. These data closely correlate with the percentage of gonadotrophs found after immunohistochemistry in the respective fractions (Fig. 3). This observation is in agreement with the idea that only gonadotrophs possess functional GnRH receptors coupled to [Ca^{2+}]_i mobilisation.

GnRH-responsive cells did not show spontaneous [Ca^{2+}]_i oscillations (Fig. 4a). Baseline ratio values were similar between individual cells (0.655 ± 0.014; n=99 cells). A transient rise in [Ca^{2+}]_i in response to GnRH was observed in all gonadotrophs which responded with the same sensitivity to a particular GnRH concentration. The amplitude of the [Ca^{2+}]_i response depended on the concentration of a particular GnRH (Figs 4c and 5). The [Ca^{2+}]_i response in single gonadotrophs (n=10) to a prolonged challenge with 1 nM cGnRH-II (11 min; Fig. 4b) shows a rapid, initial rise followed by a slower decrease to pre-challenge levels. No GnRH-induced oscillatory changes in [Ca^{2+}]_i were observed. Discontinuation of cGnRH-II superfusion caused [Ca^{2+}]_i to further

Figure 2 Displacement of 125I-labelled sGnRHa bound to immunohistochemically identified, single gonadotrophs (top) or somatotrophs (bottom) by increasing concentrations of cfGnRH (10^{-8} to 10^{-4} M) and cGnRH-II (10^{-10} to 10^{-6} M). Total and non-specific binding was determined in the absence (T) and presence of 10^{-9} M radioinert sGnRHa. Receptor labelling was visualised using autoradiography, and quantified by computerised image analysis of the silver grain distribution above immunoreactive cells. Bars represent the mean ± S.E.M. (n=40 cells per GnRH concentration). Groups sharing the same letter are not significantly different (ANOVA, followed by Fisher's least significant difference test, α=0.05).

Figure 3 Percent distribution of cells stained by anti-GH (hatched bars) or by anti-LHβ (solid bars) in the crude pituitary cell suspension and after Percoll gradient separation. Bars represent the mean ± S.E.M. (n=3-6).

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The release of LH was dose-dependent for both GnRHs, but 100- to 1000-fold higher concentrations of cfGnRH than of cGnRH-II were needed to induce similar responses. The GnRH effects on the release of LH correlated with the \([\text{Ca}^{2+}]_c\) response of the gonadotrophs.

**LH and GH plasma levels**

Apomorphine had no effect on the LH plasma levels, whereas cfGnRH and cGnRH-II resulted in significantly elevated LH plasma levels 1 h after injection (Fig. 6a).

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Figure 4 The effect of cGnRH-II challenges on \([\text{Ca}^{2+}]_c\) in single gonadotrophs of African catfish. The duration of the GnRH challenges are represented by horizontal bars. Note the absence of spontaneous and GnRH-induced oscillatory changes in \([\text{Ca}^{2+}]_c\). (a) Single 1-min challenge with 10 nM cGnRH-II, (b) single, long-term challenge (11 min) with 1 nM cGnRH-II and (c) consecutive 2-min challenges with 0.5 and 10 nM cGnRH-II.

Figure 5 Peak \([\text{Ca}^{2+}]_c\) responses (% of basal Fura-2 ratio) of single gonadotrophs (1-min GnRH challenge), and LH levels (% of basal LH release) in the medium of a primary culture of dispersed pituitary cells (30-min GnRH challenge) after stimulation with cfGnRH (top) and cGnRH-II (bottom). Bars represent the mean ± S.E.M. \([\text{Ca}^{2+}]_c\) measurements: n=54, 16, and 38 for 0, 10⁻⁷, and 10⁻⁵ M cfGnRH respectively; n=45, 16, and 29 for 0, 10⁻¹⁰, and 10⁻⁸ M cGnRH-II respectively; in vitro LH release: n=6 per GnRH concentration). Different upper case letters indicate significant differences of the \([\text{Ca}^{2+}]_c\) responses to GnRH; different lower case letters indicate significant differences of the LH release in response to GnRH (ANOVA, followed by Fisher’s least significant difference test, α=0.05).
associated with the gonadotrophs, while all other cell types remained unlabelled. The labelling could be inhibited by an excess of radioinert sGnRHa. The two native GnRHs of the African catfish competed with $^{125}$I-labelled sGnRHa for the binding sites on gonadotrophs in a dose-dependent fashion. Moreover, the concentrations of cGnRH-II (10 nM) and of cfGnRH (1 µM) inducing a half-maximal displacement of $^{125}$I-labelled sGnRHa correspond with the relative receptor binding affinities of these two GnRHs (Schulz et al. 1993), with their effects on the $[Ca^{2+}]_i$ in catfish gonadotrophs in vitro (this study), and the GnRHs' potency to induce the release of LH (Schulz et al. 1993, this study). We therefore conclude that the present autoradiographic technique detects GnRH receptors on gonadotrophs.

Some silver grains were found on pituitary cells identified as somatotrophs, but they were not displaceable by the GnRHs tested, indicating that they do not indicate specific sGnRHa-binding sites. As we have not studied the binding of radiolabelled cGnRH-II or cfGnRH, it cannot be ruled out that catfish somatotrophs possess GnRH-binding sites that show affinity to one of the two endogenous catfish GnRHs, but that do not bind sGnRHa. However, the present study shows that somatotrophs are not likely to share the GnRH-induced increases in $[Ca^{2+}]_i$ with the gonadotrophs, and doses of cfGnRH and cGnRH-II that resulted in a high LH response failed to evoke increases in the plasma levels of GH. We therefore conclude that, in the African catfish, GnRHs are not directly involved in the regulation of GH secretion.

These data are not in agreement with the GnRH-stimulated release of GH in goldfish (Marchant et al. 1989), common carp (Lin et al. 1993), and tilapia hybrids (Melamed et al. 1995), or with the localisation of GnRH receptors on goldfish somatotrophs (Cook et al. 1991). Although the GnRH receptors on goldfish gonadotrophs and somatotrophs appear to represent different receptor subtypes, the GnRHs native to the goldfish, cGnRH-II and salmon GnRH ([Trp$^7$,Leu$^8$]GnRH; sGnRH), act on both receptor subtypes (Habibi et al. 1992, Murthy & Peter 1994), and their action involves increases in $[Ca^{2+}]_i$ in both cell types (Jobin & Chang 1992, Wong et al. 1994). The likely absence of GnRH-mediated changes in $[Ca^{2+}]_i$ in catfish somatotrophs lends further support to the notion that this cell type is devoid of functional GnRH receptors. The present results are in line with the absence of GnRH effects on GH release in rainbow trout (Blaise et al. 1995).

Thus, the involvement of GnRH in the release of GH is not a feature common to teleost fish in general: representatives of the orders Cypriniformes and Perciformes show GnRH effects on GH release, in contrast to representatives of the orders Salmoniformes and Siluriformes. In the rat, GnRH stimulates the release of PRL and GH during a limited period in neonatal life (Andries & Denef 1995). Moreover, a subgroup of the gonadotrophs

Conversely, apomorphine augmented the plasma levels of GH, while the two GnRHs were not effective (Fig. 6b). Also at other times after injection (0:5, 2, 4, 8, and 24 h) these GnRHs did not lead to changes in GH plasma levels (results not shown).

**Discussion**

Gonadotrophs and somatotrophs are intermingled in the proximal pars distalis of the pituitary in teleost fish (van Oordt & Peute 1983, Peute et al. 1984), rendering it difficult to study differential characteristics of these cell types using tissue sections. However, the combination of autoradiography and immunohistochemistry on dispersed pituitary cells greatly facilitated the study of GnRH binding and of the effects of GnRH on individually identified gonadotrophs and somatotrophs and provided the possibility for quantifying the binding.

After incubation of catfish pituitary cells with $^{125}$I-labelled sGnRHa, a significant amount of silver grains was
respond to GRF as well (Kasahara et al. 1994) and some of the gonadotrophs that respond to GnRH by displaying an increase in [Ca\(^{2+}\)]\(_i\) respond to GRF as well (GnRH by displaying an increase [Ca\(^{2+}\)]\(_i\)-positive cells). Thus, the African catfish seems to share the presence in other species, including a catfish (MeRory et al. 1989, Tomic et al. 1994, Stojilkovic & Catt 1992). At the same time, the reason for this discrepancy is not clear.

In conclusion, GnRH receptors were shown to be confined to gonadotrophs in the African catfish pituitary. The GnRHs' relative receptor affinities correlated with their respective amplitude-modulated [Ca\(^{2+}\)]\(_i\) responses in gonadotrophs, and with their LH release activities. Catfish gonadotrophs do not show spontaneous or GnRH-induced oscillations of [Ca\(^{2+}\)]\(_i\). The lack of GnRH receptors on somatotrophs and the inability of GnRHs to influence [Ca\(^{2+}\)]\(_i\) in somatotrophs and to increase GH plasma levels, supports the notion that GnRHs are not functioning as GH-releasing factors in the African catfish.

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