Characterization of the receptor for gonadotropin-releasing hormone in the pituitary of the African catfish, *Clarias gariepinus*


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Abstract

Receptors for gonadotropin-releasing hormone (GnRH) were characterized using a radioligand prepared from a superactive analog of salmon GnRH (sGnRH), D-Arg^6^-Pro^9^-sGnRH-NEt (sGnRHa). Binding of $^{125}$I-sGnRHa to catfish pituitary membrane fractions reached equilibrium after 2 h incubation at 4°C. Displacement experiments with several GnRH analogs as well as other peptides, demonstrated the specificity of $^{125}$I-sGnRHa binding. Specific binding was enhanced in the presence of the cation chelator ethylene bis(oxethylenenitrilo) tetra-acetic acid (EGTA), indicating an inhibitory effect of cations on GnRH-receptor binding. The binding of $^{125}$I-sGnRHa to pituitary membranes was found to be saturable at radioligand concentrations of 5 nM and above. A Scatchard analysis of the saturation data suggested the presence of a single class of high-affinity binding sites ($K_a = 0.901 \pm 0.06 \times 10^9$ M$^{-1}$, $B_{max} = 1678 \pm 150$ fmol/mg protein). A comparative study on $^{125}$I-sGnRHa binding to pituitary membrane fractions of male and female catfish, indicated that there were no differences in binding affinity and binding capacity between both sexes. The results demonstrate the presence of specific, saturable GnRH receptors in the African catfish pituitary.

Introduction

In mammals luteinizing hormone-releasing hormone (LHRH) stimulates the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH). Binding of LHRH to specific plasma membrane receptors on the pituitary gonadotropes is the first step leading to gonadotropin secretion (Conn et al. 1981). Radioligand receptor assays with pituitary homogenates (Pedroza et al. 1977; Clayton et al. 1980), membrane fractions (Spons 1973; Marian et al. 1981), and cells in culture (Naor et al. 1980; Meidan et al. 1982; Loumaye et al. 1982; Andries and Denef 1986; Koiter et al. 1986), have been used extensively to characterize the LHRH-receptor interaction. Most of these studies were carried out with superactive analogs of LHRH modified at position 6 and 10. Substitution of D-amino acids of position 6 stabilizes the peptide against biological degradation (Koch et al. 1977) and substitution of ethylamide (NEt) for gly10 amide enhances the binding affinity (Loumaye et al. 1982). The combination of both substitutions attribute to the enhanced potency of superactive analogs such as D-Ser (t-Bu)$^6$-Pro$^9$-LHRH-NEt (Buserelin) and D-Ala$^6$-Pro$^2$-LHRH-NEt (LHRHa) (Gorospe and Conn 1987).

In teleost fishes also, the release of gonadotrophic hormone (GTH) is stimulated by a gonadotropin-releasing hormone (GnRH) (Peter 1983; De Leeuw et al. 1987a). From brains of chum salmon, Sherwood et al. (1983) purified GnRH and identified its
primary structure as Trp⁷-Leu⁸-LHRH (sGnRH). This sGnRH is known to be present in a number of other teleost species (Sherwood et al. 1984; King and Millar 1985; Powell et al. 1986; Wu et al. 1986), and several in vivo and in vitro studies demonstrated the bioactivity of sGnRH in goldfish (Peter et al. 1985; MacKenzie et al. 1984), rainbow trout (Weil et al. 1986), and catfish (De Leeuw et al. 1987). In contrast with other vertebrates, teleosts do not have a hypothalamo-hypophysial portal system and in many species, including the African catfish, the regulation of GTH by GnRH involves a direct innervation of the gonadotropes by GnRH-containing nerve fibres (Goos et al. 1985; Peute et al. 1987).

Recently, radioreceptor assays have been used to demonstrate specific, saturable GnRH receptors in pituitary membrane fractions of goldfish (Habibi et al. 1987) and pituitary homogenates of winter flounder (Crim et al. 1988). The aim of this study was to develop a method for demonstrating GnRH-binding sites on African catfish pituitary membranes, thereby allowing determination of receptor number, affinity, and specificity. Such a system is a prerequisite for investigations regarding the role of GnRH receptors in the physiological control of pituitary gonadotropin secretion. The sGnRH analog D-Arg⁶-Pro⁹-sGnRH-NEt (sGnRHa), which was found to be more potent compared to sGnRH in goldfish (Peter et al. 1985), was used as labeled and unlabeled ligand for binding to pituitary membranes of female catfish.

Materials and methods

Animals

African catfish, Clarias gariepinus, were bred in the laboratory. The breeding techniques have been reported in a previous paper (De Leeuw et al. 1985). Fish were kept in a copper-free recirculation system at 25 ± 2°C, exposed to a photoperiod normal for the time of year in the Netherlands, and fed with Trouvit trout pellets (Trouw, Putten, The Netherlands). Experiments were performed mainly between July and January and included sexually mature fish with a body weight between 500 and 700 g.

Hormones

Salmon GnRH (sGnRH: Trp⁷-Leu⁸-LHRH), and its superactive analog D-Arg⁶-Pro⁹-sGnRH-NEt (sGnRHa) were purchased from Peninsula laboratories Europe Ltd., Merseyside, England and Syn-del laboratories, Vancouver, Canada, respectively. sGnRHa was of 98.0% purity on analytical High Performance Liquid Chromatography. D-Ser(t-Bu)⁶-Pro⁹-LHRH-NEt (Buserelin) was a gift from Dr. M. Schorr, Hoechst AG, Frankfurt am Main, FRG. D-Ala⁶-Pro⁹-LHRH-NEt (LHRHa) was kindly provided by Dr. J.Th. Gielen, Intervet International B.V., Boxmeer, The Netherlands. Human Chorionic Gonadotropin (hCG) was obtained from Organon, Oss, The Netherlands. Somatostatin, Arg⁸-vasopressin and thyrotropin releasing hormone (TRH) were from Sigma, St. Louis, M.O., USA.

Radioiodination of sGnRHa

sGnRHa was iodinated using chloramine-T and purified by CM-cellulose chromatography, as described for Buserelin by Marian and Conn (1980). The reaction mixture of the iodination was composed of 5 μg sGnRHa in 10 μl phosphate buffer (0.1 M, pH 7.5), 1 mCi (10 μl) Na¹²⁵I (specific activity: 15–17 mCi/μg iodide; Amersham, Houten, The Netherlands), and 250 ng chloramine-T in 10 μl phosphate buffer. After 2 min at room temperature the reaction was terminated by dilution with 500 μl 2 mM ammonium acetate, pH 4.5. The mixture was applied to a carboxymethyl cellulose column (Sigma: 0.7 meq/g, fine mesh) equilibrated with 2 mM ammonium acetate, pH 4.5. The column was first eluted with equilibration buffer to remove unbound ¹²⁵I, and subsequently with 100 mM ammonium acetate, pH 4.5, to elute the labeled analog.

The purity of the iodinated peptide was further tested by electrophoresis. For this purpose samples were applied to strips (1.5 cm wide) of Whatman