Rainbow trout gonadotropin receptors are preferentially activated by their cognate ligand

by

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ABSTRACT. - To better improve understanding of the molecular mechanisms involved in the actions of gonadotropins in fish, a structural and functional characterization of trout gonadotropin receptors has been undertaken. The present study describes two distinct gonadotropin receptors in trout showing similarities with those of other vertebrates but also differences in structural determinants of the FSHr. As observed in their mammalian counterparts, trout LH receptor (LHR) harbours a large N-terminal hormone binding domain with 9 leucine repeat repeats (LRR) flanked by a proximal and distal cysteine rich region (CRR). A similar sequential organization of 9 clustered LRR and 2 CRR motifs is also observed on mammalian FSH receptors however this is absent in the trout with only one out of four conserved cysteine residues being present upstream of the first LRR motif. The functional specificity of trout gonadotropin receptors was investigated in the heterologous mammalian COS-7 cells using homologous (trout) and heterologous (chinook salmon) purified gonadotropins. Contrary to published data in catfish and zebrafish, trout gonadotropin receptors show a high selectivity for their respective cognate ligand.

Key words. - rainbow trout - Oncorhynchus mykiss - Gonadotropin receptors - Functional specificity.

Introduction

In fish, two heterodimeric glycoproteins are released into the plasma from the gonadotroph cells of the anterior pituitary. These gonadotropins, now referred to as FSH and LH, regulate gonadal functions including gametogenesis and steroidogenesis.

Gonadotropins bind to membrane bound receptors mainly expressed on the somatic cells of the gonads. In fish, the presence of two distinct gonadotropin receptors was evidenced by the molecular cloning of two different cDNA in several fish species including salmon, catfish, and zebrafish. The fish gonadotropin receptors belong to the G protein-coupled receptor (GPCR) superfamily, and specifically to the subfamily of glycoprotein hormone receptors.

Although the overall structure is similar to that described in tetrapods, marked differences exist with regards to the hormonal specificity of teleostean fish receptors to gonadotropins. Depending on the species, a promiscuous activation of one or the other fish gonadotropin receptors was reported in functional studies using mammalian cell lines to express fish receptors.

In this report, the functional characterization of rainbow trout FSH and LH receptors is presented using homologous and heterologous purified gonadotropins.

Materials and Methods

Animal

Male and female trout (Oncorhynchus mykiss) held at the INRA/PEIMA fish farm (Sizun, France) under natural photoperiod and temperature were anaesthetised in 2-phenoxyethanol and killed in order to collect the gonads.

Cloning of gonadotropin receptors cDNA

Amino acid sequences of known glycoprotein hormone receptors were aligned and one specific primer was designed from each of the two conserved regions spanning the third extracellular loop and the seventh transmembrane alpha helix. Total RNA extracted from a recrudescent (stage II) testis was reverse transcribed using oligo-dT15 and the Moloney Murine Leukaemia Virus reverse transcriptase (MMLV) (Promega, Madison, WI) according to manufacturer’s instructions. A partial cDNA was obtained for each gonadotropin receptor and used as probes to screen a stage III-IV testicular cDNA library. For LHR, a full length cDNA (clone 2b-1) was isolated. For FSHR, a single partial cDNA named FSHR B8 (1337 bp) was first obtained and a 5’ RACE-PCR was performed using the high fidelity elongase (Gibco BRL Life Technologies, Cergy Pontoise, France) to determine the 5’end of the corresponding transcript. The nucleotide sequences of the full length cDNA encoding either FSHR (2783 bp) or LHR (2756 bp) have been submitted to Genbank (AF439405 and AF439404).

Functional characterization of rainbow trout FSHR and LHR

The putative open reading frame, including the stop codon of each cDNA, was PCR amplified and cloned into the pcDNA 3.1/V5-TOPO expression vector (Invitrogen). Vectors expressing gonadotropin receptors were cotransfected independently in COS-7 cells together with a cAMP-responsive reporter construct pCRE-Luc (Stratagene, La Jolla, CA) and pcMV beta-galactosidase (Clontech, Mountain View, CA) using the FuGENE6 reagent (Roche Applied Science, Meylan, France). Cells were stimulated...
with purified gonadotropins for 6 h and the luciferase activity was measured using the luciferase assay kit (Promega). The β-galactosidase activity was determined according to manufacturer’s instructions (Promega). Each stimulation was performed in triplicate and each experiment repeated at least twice. Data were analyzed using the non parametric Mann-Whitney U test of the Statistica software (Statsoft, France) based on the ranking method. Rainbow trout and chinook salmon (Oncorhynchus tshawytscha) gonadotropins were purified using metal ion affinity chromatography and dye-ligand chromatography. The homogeneity and specificity of the gonadotropins preparations were checked by Reverse-Phase HPLC and radio immunoassay.

Results and discussion

Cloning of transcripts encoding trout gonadotropin receptors

Two full-length cDNA encoding proteins related to the glycoprotein hormone receptors were isolated from trout testis. The encoded proteins show the highest similarity (99%) with Atlantic salmon (Salmo salar) and amago salmon (Oncorhynchus rhodurus) gonadotropin receptors. Lower homologies were observed with other teleostean (70 to 80%) and mammalian (60%) counterparts. The phylogenetic analysis confirms that the amino acid sequences of trout glycoprotein receptors segregate into two different clades, the FSHr and LHr clades, and originate from a common ancestral gene (not shown). The presence of two distinct genes encoding gonadotropin receptors in trout is in agreement with previous reports in other fish species including amago salmon, Atlantic salmon, African catfish, channel catfish, and zebrafish. Therefore, there is strong evidence that two distinct gonadotropin receptors are well conserved among Euteleost as observed in tetrapods.

The overall structure of the putative rainbow trout FSHr (rtFSHR) and LHr (rtLHR) is similar to that of the members of the other G protein-coupled receptor superfamily with the presence of three main functional regions: extracellular, transmembrane and intracellular domains. The large extracellular domain includes a cluster of 9 repeated sequences related to imperfect leucine rich repeats (LRR) as observed in other vertebrate counterparts. However, a proximal cysteine rich flanking region that normally links the N-terminal end of the protein to the LRR cluster in mammalian FSHr and LHr, is not found in rtFSHR. This structural change is observed in salmoniform and perciform FSHR. In zebrafish and catfish, only two out of the four cysteine residues liable to form a disulfide bond are present.

Pharmacological characterization of rainbow trout gonadotropin receptors

The functionality of the trout putative gonadotropin receptors was studied by transient transfection assays in COS-7 cells expressing one or the other receptor (Fig. 1).

No reporter gene expression was measured when cells expressing no receptor were incubated in presence of gonadotropins (not shown). In addition, no basal activity of the gonadotropin receptors was detected (not shown).

The stimulation of the rtFSHR expressing cells with
rtFSH (25 to 800 ng/ml) resulted in a dose-dependent response curve with a limited but significant (p < 0.05) maximal 2.5 fold induction of the reporter gene expression and an ED50 around 40 ng/ml. Such a weak FSH-induced response was previously reported in amago salmon, catfish, and zebrafish, regardless of the direct and indirect intracellular cAMP quantification systems used. No activation of rtFSHR was observed in presence of rtLH up to 800 ng/ml. Similar results were obtained with chinook gonadotropins (cFSH and cLH). The FSH receptor selectivity appears to be much higher in trout than in African catfish (Siluriforms) and zebrafish (Cypriniforms), two phylogenetically closely related species in which FSHR is efficiently activated by both hormones. Interestingly, in Siluriforms and Cypriniforms, but not in Salmoniforms, the N-terminal end of the FSHR exhibits two out of four conserved cysteine residues of a structural determinant required for efficient LH binding in mammals. Whether the missing cysteine residues in the salmonid FSH receptors are involved in the functional selectivity of the FSHR remains to be investigated.

Regarding rtLHR, rtLH induced luciferase reporter gene expression in a dose-dependent manner from 50 to 1000 ng/ml (ED50 = 120 ng/ml). The rtLHR was highly responsive since a significant (p < 0.05) 8.9 fold maximal induction was obtained at 1000 ng/ml. cLH treatment resulted in a similar dose-dependent responsiveness to that determined in presence of rtLH with an 11-fold maximal induction obtained at 400 ng/ml. The highest concentrations of rtFSH (1000 ng/ml) were also able to induce a poor but significant (p < 0.05) two fold induction of the reporter gene. Contrary to rtFSH, cFSH had no significant effect on rtLHR transactivation at the tested doses (25 to 400 ng/ml). Although a contamination of the purified rtFSH fraction with residual rtLH cannot be totally excluded, such contamination was not detected using a specific and sensitive LH radio immunoassay. In addition, our data are consistent with those reported in amago salmon where homologous LH did not activate FSHR, whereas a unique high concentration (5µg/ml) of FSH transactivated FSHR and also LHR, albeit to a much lesser extent (Oba et al., 1999a; 1999b). However, the amago gonadotropin receptors were recently found to be highly specific for their cognate ligand using recombinant hormones originating from another salmonid species (Ko et al., 2007).

Conclusion

In contrast to catfish and zebrafish, trout gonadotropin receptors have a marked hormone selectivity for their cognate ligand.

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References

