General and Comparative Endocrinology 163 (2009) 329–339

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Pharmacological characterization, localization and quantification of expression of gonadotropin receptors in Atlantic salmon (*Salmo salar* L.) ovaries

Eva Andersson [a], Wouter Nijenhuis [b], Rune Male [c], Penny Swanson [d], Jan Bogerd [b], Geir Lasse Taranger [a], Rüdiger W. Schulz [a,b,*]

[a] *Institute of Marine Research, Research Group Reproduction and Growth in Fish, P.O. Box 1870, Nordnes, N-5817 Bergen, Norway*
[b] *Utrecht University, Science Faculty, Department Biology, Division Endocrinology & Metabolism, Kruyt Building, Padualaan 8, NL-3584 CH Utrecht, The Netherlands*
[c] *University of Bergen, Science Faculty, Department of Molecular Biology, P.O. Box 7803, N-5020 Bergen, Norway*
[d] *NOAA Fisheries, Northwest Fisheries Science Center, 2725 Montlake Blvd. East, Seattle, WA 98112, USA*

ARTICLE INFO

Article history:
Received 24 September 2008
Revised 17 April 2009
Accepted 1 May 2009
Available online 12 May 2009

Keywords:
Fsh receptor
Lh receptor
cDNA cloning
Pharmacology
Expression
Ovary
In situ hybridisation
Fish
Salmon

ABSTRACT

The gonadotropins Fsh and Lh interact with their receptors (Fshr and Lhr, respectively) in a highly specific manner in mammals with little overlap in biological activities. In fish, the biological activities seem less clearly separated considering, for example, the steroidogenic potency of both Fsh and Lh. Important determinants of the biological activity are the specificity of hormone–receptor interaction and the cellular site of receptor expression. Here, we report the pharmacological characterization of Atlantic salmon Fshr and Lhr, identify receptor-expressing cells in the ovary, and validate receptor mRNA quantification systems. For the pharmacological studies, we used highly purified coho salmon gonadotropins and found that the Fshr preferentially responded to Fsh, but was also activated by ~6-fold higher levels of Lh. The Lhr was specific for Lh and did not respond to Fsh. Photoperiod manipulation was used to generate ovarian tissue samples with largely differing stages of maturation. Specific real-time, quantitative (rtq) PCR assays revealed up to 40-fold (*fshr*) and up to 350-fold (*lhr*) changes in ovarian expression levels, which correlated well with the differences in ovarian weight, histology, and circulating oestrogen levels recorded in January and June, respectively. Vitellogenic ovaries were used to localise receptor-expressing cells by in situ hybridization. Granulosa cells of small and large vitellogenic follicles were positive for both receptors. Also theca cells of small and large vitellogenic follicles expressed *fshr* mRNA, while only in large vitellogenic follicles theca cells were (weakly) positive for *lhr* mRNA. While only ovulatory Lh levels seem high enough to cross-activate the Fshr, expression by both receptors by granulosa and theca cells suggests that homologous ligand receptor interaction will prevail.

© 2009 Elsevier Inc. All rights reserved.

1. Introduction

Two gonadotropins are present in tetrapod vertebrates and the duality of gonadotropins has become an accepted principle also for fishes, the largest group of vertebrates. Heterodimeric glycoproteins with a common α-subunit and Fsh- and Lh-specific β-subunits (or the respective mRNAs) have been found in all major fish groups including, representative species of phylogenetically ancient groups such as shark or sturgeon (Quérat et al., 2000, 2001), primitive (e.g. salmoniform and anguilliform fish; Itoh et al., 1988; Quérat et al., 1990; Yoshiura et al., 1999) and evolved (e.g. perciform fish; Tanaka et al., 1993; Mateos et al., 2003) teleost fish, and lungfish (Quérat et al., 2004) that together with the tetrapods constitute the vertebrate group of sarcopterygians. It appears that the regulation of reproductive processes by two distinct pituitary gonadotropins is a phylogenetically ancient principle among vertebrates. Accordingly, two gonadotropin receptors, belonging to either the Fsh receptor (Fshr) or Lh receptor (Lhr) type, are found from mammals to teleost fish (Bogerd et al., 2005).

Information on specific gonadotropin functions in mammalian models has become available from studies using recombinant gonadotropins, from the molecular characterization of pathological conditions associated with mutations in gonadotropin or gonadotropin receptor genes (Huhtaniemi and Themmen, 2005), or from murine phenotypes following targeted mutagenesis of these ligand and receptor genes (Layman and McDonough, 2000). This information, together with results from specific quantification systems for circulating gonadotropins for a number of mammalian species, has led to the following general model: Fshr and Lhr bind their respective ligands specifically and show little cross-activation (0.01–0.1%), resulting in functional specificity in the presence of

* Corresponding author. Address: Utrecht University, Science Faculty, Department Biology, Division Endocrinology & Metabolism, Kruyt Building, Padualaan 8, NL-3584 CH Utrecht, The Netherlands. Fax: +31 30 2532837.
E-mail address: r.w.schulz@uu.nl (R.W. Schulz).

0016-6480/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygcen.2009.05.001

physiological hormone concentrations. The Fshr is expressed by granulosa (ovary) and Sertoli (testis) cells, the somatic cell types that are in direct contact with germ cells and facilitate their survival and development. In the ovary, special theca cells, preovulatory granulosa and postovulatory corpus luteum cells express Lhr. In males, Lhr is expressed by the interstitial Leydig cells in the testis. In both genders, these steroidogenic cells provide the sex steroids required for the development and functioning of the reproductive organs and for gametogenesis. However, a certain functional interplay appears to exist between gonadotropins since, for example, Lh-stimulated steroids are required to regulate functions of the germ cell-supporting Sertoli (de Gendt et al., 2004) and granulosa cells (Hu et al., 2004). Fsh, on the other hand, can directly stimulate bioconversion of steroids (increase aromatase activity) in granulosa and Sertoli cells, and indirectly stimulate steroid production by paracrine stimulation of expression of Leydig cell proteins involved in steroidogenesis (Lejeune et al., 1996; Sriraman et al., 2004). The Fsh-mediated increase in *lhr* gene expression also contributes to the apparent functional interaction of Fsh and Lh (Nakamura et al., 1991).

Comparatively little information is available regarding specific gonadotropin functions in nonmammalian species, such as fish, which is regrettable for two reasons. First, the control of reproduction by the brain–pituitary system is a general characteristic for vertebrates, so that it is interesting to ask from an evolutionary point of view if a functional (accompanying the structural) separation of the gonadotropin ligand–receptor pairs known from mammals can also be observed in species at the basis of the vertebrates. Second, studies on the regulation of fish reproduction are relevant for aquaculture biotechnology. An important aspect in this context is that puberty in some species occurs at an earlier age under aquaculture rearing conditions than is observed naturally and can reduce growth performance and product quality, while in other species delayed age of puberty occurs.

For studies on gonadotropin functions in the context of pubertal maturation, Atlantic salmon (*Salmo salar*) are a suitable model. Salmonids are the only order of teleost fish where substantial information on circulating gonadotropin levels has been published at different stages of the reproductive cycle (Oppen-Berntsen et al., 1994; Borg et al., 1998; Gomez et al., 1999; Campbell et al., 2003).

Previous studies on gonadotropin receptors from representatives of three orders, siluriform (Kumar et al., 2001a,b; Bogerd et al., 2001; Vischer and Bogerd, 2003; Vischer et al., 2003), cypriniform (Kwok et al., 2005; So et al., 2005), and anguilliform fish (Kazeto et al., 2008), have indicated that Fshr's show a preference for Fsh but also respond to Lh, while Lhr's specifically respond to Lh. However, studies on receptors from salmonid fish, the amago salmon (Oba et al., 1999a,b) and the rainbow trout (Sambroni et al., 2007), came to the conclusion that the Lhr, but not the Fshr, responded to both gonadotropins. Intriguingly, ligand binding data from coho salmon gonad tissue showed that, similar to catfish, eel, and zebrafish, coho salmon Fshr bound both Fsh and Lh, while the Lhr bound only Lh (Yan et al., 1992; Miwa et al., 1994). Maugars and Schmitz (2006) have reported the cDNA cloning of Atlantic salmon gonadotropin receptors, including a construction of a phylogenetic tree and in silico analysis of some of the functional domains of the receptors. However, gonadotropin binding properties or the identity of receptor-expressing cells were not studied. In order to contribute to the understanding of the functional specificity of fish gonadotropins, we report here the pharmacological characterization of the Atlantic salmon gonadotropin receptors, provide for the first time information on the cellular localization of the mRNAs of both receptors in the ovary, and validate receptor mRNA quantification systems.

2. Materials and methods

2.1. Maintenance, photoperiod treatment, and sampling of fish

Prepubertal salmon were maintained in sea cages at the Institute of Marine Research (Matre, Norway; 61°N) under ambient, normal light (NL) conditions for 19 months until the start of the trial. All fish were treated and killed according to Norwegian National Legislation for laboratory animals.

In the course of the molecular studies, we have developed PCR-based quantification systems for the gonadotropin receptor transcripts and tested them methodologically (see Section 2.3). In addition, we carried out a physiological evaluation by analysing gonadotropin receptor expression in ovarian tissue at largely different stages of oocyte maturation. To this end, we collected ovarian tissue from 3-year-old female Atlantic salmon. The first (initial control) sample was collected on January 8. On February 1, half of the animals were exposed to additional constant light (LL), while the other half remained under NL conditions, as described previously (Schulz et al., 2006). The second sampling took place on June 11, i.e. 18 weeks after commencing LL exposure.

The fish were netted from the cages, immediately anaesthetized with 6ppt metomidate (Syndel, Victoria, B.C.) and weighed (total body weight). Blood (5 ml) was collected in heparinized syringes from the caudal veins, and gonads were excised and weighed for gonado-somatic index (GSI) determination (GSI = gonad weight (g) $\times$ 100/total body weight (g)). A few ovarian lamellae were isolated by transversal cuts with a scalpel blade from each female. One piece of tissue was fixed in phosphate buffered 4% paraformaldehyde for histological analysis or in situ hybridisation, and another piece was immediately frozen in liquid nitrogen and stored in −80 °C until analyzed for gonadotropins receptor expression.

2.2. Cloning and molecular characterization of salmon fshr and lhr

Based on partial cDNA sequences we deposited at NCBI in 2001 (AY049953 and AY049956), we have cloned the respective full length Atlantic salmon (*Salmo salar*) gonadotropin receptor cDNAs (*fshr*, DQ837298; *lhr* DQ837299). Others have reported the full-length cDNA cloning of these receptors previously (Maugars and Schmitz, 2006). Our experimental procedures used for the cloning differ from those used by Maugars and Schmitz (2006) and are described briefly in the Supplementary data section, where also our approach to the modelling of the extracellular domain of the Fshr is described, and where predictions resulting from the modelling are discussed that differ from those made by Maugars and Schmitz (2006).

2.3. Localization and quantification of receptor expression

Localization of receptor expression by in situ hybridization was done as described previously (Weltzien et al., 2003), using 12 μm cryo-sections prepared from paraformaldehyde-fixed ovarian tissue from fish sampled during vitellogenesis in June. Sense and antisense cRNA probes were used for detection of *fshr* or *lhr* mRNA, covering nucleotides 374–743 of the *fshr* sequence DQ837298, and nucleotides 1872–2397 of the *lhr* sequence DQ837299, respectively.

Primers and TaqMan fluorogenic probes (see Table 1), specific for Atlantic salmon *fshr*, *lhr*, and for the endogenous control (Atlantic salmon elongation factor *ef1α*; AF321836) were designed with Primer express software (Applied Biosystems), according to the manufacturer's guidelines, for real-time, quantitative (rtq) PCR analysis of receptor expression. Primers were purchased from

Download English Version:

https://daneshyari.com/en/article/2801334

Download Persian Version:

https://daneshyari.com/article/2801334

Daneshyari.com