General and Comparative Endocrinology 221 (2015) 31–41

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Gonadotropins in European sea bass: Endocrine roles and biotechnological applications

María José Mazón [1], Gregorio Molés [1], Ana Rocha [1], Berta Crespo, Olivier Lan-Chow-Wing, Felipe Espigares, Iciar Muñoz, Alicia Felip, Manuel Carrillo, Silvia Zanuy, Ana Gómez *

Instituto de Acuicultura de Torre la Sal (CSIC), Ribera de Cabanes s/n, 12595 Torre la Sal, Castellón, Spain

ARTICLE INFO

Article history:
Available online 19 May 2015

Keywords:
Gonadotropins
Fsh
Lh
Fish
Reproduction
Recombinant hormones

ABSTRACT

Follicle stimulating hormone (Fsh) and luteinizing hormone (Lh) are central endocrine regulators of the gonadal function in vertebrates. They act through specific receptors located in certain cell types found in the gonads. In fish, the differential roles of these hormones are being progressively elucidated due to the development of suitable tools for their study. In European sea bass (*Dicentrarchus labrax*), isolation of the genes coding for the gonadotropin subunits and receptors allowed in first instance to conduct expression studies. Later, to overcome the limitation of using native hormones, recombinant dimeric gonadotropins, which show different functional characteristics depending on the cell system and DNA construct, were generated. In addition, single gonadotropin beta-subunits have been produced and used as antigens for antibody production. This approach has allowed the development of detection methods for native gonadotropins, with European sea bass being one of the few species where both gonadotropins can be detected in their native form.

By administering recombinant gonadotropins to gonad tissues *in vitro*, we were able to study their effects on steroidogenesis and intracellular pathways. Their administration *in vivo* has also been tested for use in basic studies and as a biotechnological approach for hormone therapy and assisted reproduction strategies. In addition to the production of recombinant hormones, gene-based therapies using somatic gene transfer have been offered as an alternative. This approach has been tested in sea bass for gonadotropin delivery *in vivo*. The hormones produced by the genes injected were functional and have allowed studies on the action of gonadotropins in spermatogenesis.

1. Reproductive cycle of European sea bass

European sea bass (*Dicentrarchus labrax)* belongs to the teleost order Perciformes, family Moronidae. It is essentially a Mediterranean species, and, like other fish species living at moderate latitudes, puberty and adult reproduction are seasonal events that are highly dependent on environmental cues (e.g. photoperiod, temperature). Gonadal growth begins in September–October, with the mitotic proliferation of spermatogonia (Carrillo et al., 2009) in the testis and the beginning of vitellogenesis in the ovaries, which have already undergone primary growth during the summer. Spawning typically takes place in winter (January–March). Males have a long spermiating period overlapping the winter reproductive cycle of females, followed by a sexual resting period (Carrillo et al., 1995; Asturiano et al., 2000). Ovarian development in this species is defined as group-synchronous, i.e. clutches of follicles at different stages of development are simultaneously present in the ovary and are spawned successively in up to four batches (Mayer et al., 1990; Asturiano et al., 2000, 2002). First sexual maturity generally occurs during the second year of life in males and a year later in females, but males can mature as early as at one year of age. Early sexual maturation is strongly influenced by the individual's metabolic/growth status in this species.

In sea bass, as in all vertebrates, gametogenesis is completely dependent on the pituitary hormone follicle-stimulating hormone (Fsh) and steroids locally produced in response to both Fsh and luteinizing hormone (Lh). This dual control has been known for long time, but it is unclear which parts of the gametogenic pathway are regulated by each hormone. Both gonadotropins Fsh and Lh are glycosylated heterodimers formed by the non-covalent association of a common α-subunit (Cga) with a distinct β-subunit (Fshβ or Lhβ), which is what confers hormone specificity (Levavi-Sivan et al., 2010; Pierce and Parsons, 1981).

* Corresponding author. Fax: +34 964 319509.
E-mail address: a.gomez@csic.es (A. Gómez).
[1] These authors contributed equally to this work and are listed in alphabetical order.

http://dx.doi.org/10.1016/j.ygcen.2015.05.002
0016-6480/

2. Tools to measure gonadotropin subunits

In recent decades, different types of assays have been developed to measure gonadotropins in several species. These methods can be grouped into assays based on antigen–antibody recognition, which measure the number of molecules or their mass (e.g. immunoassays), and assays that determine a response of a biological system to stimulation with Fsh or Lh (e.g. bioassays, both *in vivo* and *in vitro*). Traditionally, the assays used to determine gonadotropin levels in fish have been radioimmunoassays (RIA) or enzyme-linked immune sorbent assays (ELISA) based mostly on native gonadotropin subunits purified from fish pituitaries and their specific antibodies (Table 1), and more recently, also on recombinant gonadotropin subunits (Aizen et al., 2007; Molés et al., 2012). The immunological determinations of gonadotropins do not necessarily reflect the biological signal perceived by their cognate receptors (Christin-Maitre and Bouchard, 1996). Pituitary glycoproteins, including gonadotropins, are secreted as highly heterogeneous forms that differ in carbohydrate composition (glycosylation), which affects many of the functional characteristics of these hormones, including their stability and metabolic fate, and the interaction with their cognate receptors, i.e., their bioactivity (Ulloa-Aguirre et al., 2003). Furthermore, differential terminal glycosylation allows for the fine-tuning of the protein properties at the target cell, without having to change the primary sequence (Olivares et al., 2009). More specific studies in mammals have demonstrated that changes in the content of sialic acid affect the bioactivity of Fsh isoforms. In humans and rats, Fsh variants with more acidic/sialylated glycosylation exhibit a longer plasma half-life but lower receptor binding activity and *in vitro* biological potency than their less acidic counterparts (Ambao et al., 2009; Zambrano et al., 1996). In addition, in mammals, it has been observed that the molecular microheterogeneity of intrapituitary FSH may change depending on the age, sexual development, and/or the steroidogenic milieu, provoking a range of biological responses (Ambao et al., 2009; Rulli et al., 1999). Consequently, *in vitro* bioassays constitute an ideal approach to determine some functional aspects of gonadotropins.

Currently, several methods are available for measuring plasma and pituitary gonadotropin levels in European sea bass: one ELISA for Lh derived from the purified native Lhβ subunit (Mateos et al., 2006); two immunoassays for Fsh, a dot-blot (Molés et al., 2011b) and an ELISA (Molés et al., 2012), both based on recombinant Fshβ subunits generated in insect cells and *Pichia pastoris*, respectively; and one *in vitro* bioassay for Fsh based on a HEK-293 cell clone containing the sea bass *fshr* cDNA and a luciferase reporter gene (Molés et al., 2011b). This bioassay, which was the first to be developed and validated in fish to measure Fsh bioactivity in pituitary and plasma samples (Table 2), has provided a more complete vision of sea bass Fsh activity. The combination of both immuno- and bioassay methods makes it possible to evaluate the relative bioactivity of defined amounts of Fsh according to the biological stage of the animal.

3. Gonadotropins in the first year of life of sea bass: gonad differentiation period and first tentative maturation of precocious males

The timing of the appearance of Fsh and Lh-expressing cells in the pituitary during ontogeny appears to be species-specific (Chen and Ge, 2012). The expression profiles of *gonadotropin subunit genes* in the pituitary gland of sea bass during early development, including the period of gonad differentiation (from 50 to 300 days post hatching (dph)), have been studied using female- and male-dominant populations produced by size-grading (Molés et al., 2007). In addition, isolated pituitaries and plasma from those populations – available from 150 dph onwards – were used to analyze gonadotropin content by means of a combination of bio- and immunoassays (Molés et al., 2011b). The expression profile of *cga* was equivalent for both populations. At 150 dph, *fshb* expression was similar in males and females and corresponded to a low content of Fsh in the pituitary, but high plasma levels. At 200 dph *fshb* was up-regulated in males, while it remained constant in females, and in both cases expression stayed high until the final sampling point at 300 dph. Throughout this period, the Fsh content in the pituitary increased without being secreted, whereas Fsh bioactivity in plasma remained high until 200 dph and then gradually decreased in males and females. Additionally, plasma Fsh bioactivity levels were higher in females than in males, and the Fsh biopotency in pituitary (measured as Fsh bioactivity (B):Fsh quantity (I) ratio, B:I) was also higher in females than in males. All these data suggest an important role for Fsh at the time of gonad differentiation in this species, and a possible sexual dimorphism in the synthesis and potency of Fsh at this stage (Molés et al., 2011b). On the other hand, the gonadal expression of *fshr* peaked at 250 days in both males and females (Felip, Zanuy, Gómez, unpublished).

During this period of gonad differentiation *lhb* expression and pituitary and plasma Lh levels were low until 150 dph in both males and females, after which expression levels increased and remained high from 200 dph onwards. From that moment a continuous increase in Lh content in the pituitary was also observed, which reached the highest levels at 300 dph (the end of this experiment). Lh plasma levels in males followed the same trend while in females Lh in plasma dropped at 300 dph (Molés et al., 2007). The expression of *lhcgr* increased at 250 days in male gonads, while no significant differences were found during the period analyzed in females. The increase in Lh content and *lhb*/*lhcgr* expression could be related to the significant number of precocious males that appeared in this population (Papadaki et al., 2005; Felip, Zanuy, Gómez, unpublished).

The expression of the *gonadotropin subunit* genes and levels of gonadotropins in pituitary and plasma of juvenile male sea bass during their first year of life have also been reported in other studies related with research on precocious puberty. It was found that all three subunits have similar expression profiles, peaking during the tentative gonadal maturation period (December–February) (Rodríguez et al., 2005), but *lhb* and *cga* levels were much higher than those of *fshb*, a peculiarity that was not seen in adult males. The pituitary content of Fsh and Lh was higher in juveniles that entered precocious puberty than in those that remained immature at the same age. And in plasma, precocious animals showed higher Fsh levels than non-precocious specimens during testicular growth, including spermiogenesis. This was also observed for Lh, which reached the highest values in precocious males in the spermiation phase (Espigares, Carrillo, Rocha, Gómez, Ameur, Zanuy, unpublished results). On the other hand, the transcription of these genes was completely blocked when fish were exposed to a long (12-month) continuous light treatment, which is known to inhibit the onset of precocious puberty in juvenile sea bass (Felip et al., 2008; Rodríguez et al., 2005). Other light regimes consisting of shorter (4-month) continuous light periods administered before (June–September) or during (October–March) spermatogenesis induced alternative expression profiles, but only for *fshb* (Felip et al., 2008).

4. Gonadotropins in the first maturation of male and female sea bass and in adults

In adult male sea bass, all three pituitary gonadotropin subunits are transcribed throughout the entire reproductive cycle, including