KISSPEPTIN SIGNALING AND REPRODUCTION IN FLATFISH

Mechaly, A.S.*, Viñas, J. , Piferrer, F.***

* Institut de Ciències del Mar, Consejo Superior de Investigaciones Científicas (CSIC), Passeig Marítim, 37-49, 08003 Barcelona, Spain. piferrer@icm.csic.es

** Laboratori d'Ictiologia Genètica, Departament of Biology, University of Girona, Spain.

Introduction:

The recently discovered decapeptide kisspeptin and its G-protein coupled receptor form a signaling system expressed ubiquitously and are implicated in a variety of still poorly characterized functions [1]. In the brain, kisspeptin is secreted by specific neurons and its receptor is localized in GnRH neurons [2]. Kisspeptin signaling has been fully established in the control of the onset of puberty in vertebrates, from fish to mammals [3-6]. To obtain a better understanding of the mechanisms associated with kisspeptin signaling within the reproductive axis we used Senegalese sole (*Solea senegalensis*), an important valuable flatfish species for aquaculture, as a model. We aimed at the study of the expression of kisspeptin and its receptor in the different components of the reproductive axis in relation to other important genes, according to sex and during a full reproductive cycle.

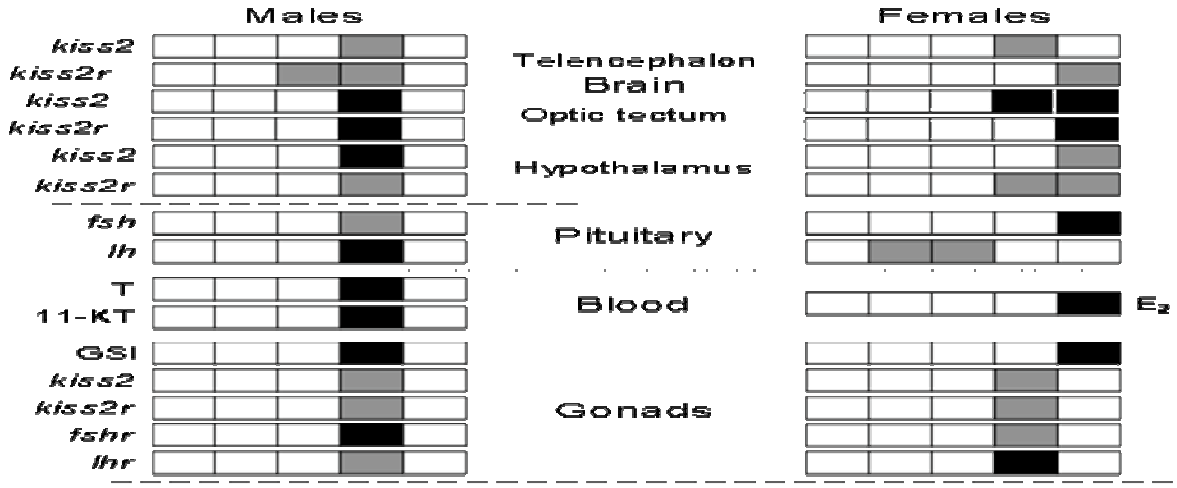
Methods:

Senegalese sole (F1 generation) were reared from eggs spawned by different stocks of wild fish (F0) and acclimated to captivity. Seasonal changes in plasma levels of testosterone (T) and 11-ketotestosterone (11-KT) were determined in males, whereas estradiol-17ß (E2) plasma levels were determined in females, using commercially available enzyme immunoassay (EIA) kits. The expression patterns of *kiss2* and *kiss2r* in different brain areas (including hypothalamus, telencephalon and optic tectum) and gonads, mRNA levels of *lh* and *fsh* in pituitary, and *lhr* and *fshr* in gonads were analyzed by quantitative real-time PCR (qRT-PCR) in males and females at the five different samplings periods (spring, summer, fall, winter and a second spring) comprising a full reproductive cycle. To calculate relative changes in gene expression, we analyzed the data using the comparative Ct method (also known as the DDCt method). Fold change (the relative quantification, RQ) was calculated from the DDCt and normalized by the endogenous reference gene *ßactin*.

Results:

In males, plasma levels of T and 11-KT followed the same pattern as the GSI, with a clear and significant peak in winter (Fig. 1). In females, E2 plasma levels remained low until the fall and then increased through winter and the following spring. Regarding the gene expression analysis, in the telencephalon, *kiss2* increased after summer and peaked in winter in both males and females. In contrast, *kiss2r* mRNA levels were highest during the fall and winter in males but not until the following spring in females. In the optic tectum of males, significant changes in both *kiss2* and *kiss2r* mRNA levels were observed, with a clear peak of expression in winter. In females, in contrast, *kiss2* mRNA levels started to increase in the fall and reached

Fig. 1. Summary of the changes observed in the variables measured in this study. The five boxes correspond, from left to right, to: spring, summer, fall, winter and the following spring. The level of expression is related to the intensity of shadowing in each box: white, low expression; grey, high expression (but not significantly different); black, highest expression with significant differences.



maximum levels in winter, and then started to slightly decrease, whereas *kiss2r* mRNA levels kept increasing until they reached maximum values in the following spring. In the hypothalamus, a similar pattern was observed, i.e., low levels of both *kiss2* and *kiss2r* in males throughout except in winter, when a clear peak was observed in males but only a progressive increase in females. In the gonads, both *kiss2* and *kiss2r* shared a similar expression pattern, with maximum mRNA levels in the winter, although no significant differences were observed. Regarding the expression levels of gonadotropin genes in the pituitary, *lh* in males peaked in winter and *fsh* of females in the second spring. Non-statistically significant changes were observed in *fsh* mRNA levels in males and *lh* mRNA levels in females. In the gonads, mRNA levels of *fshr* and *lhr* remained low during most part of the study, but were consistently higher in winter. However, the inverse situation was found with respect to the levels of mRNA for *fsh* and *lh* observed in the pituitary, i.e., significant differences were observed for *fshr* in the testis and *lhr* in the ovaries (Fig. 1).

Conclusion:

Analysis of the temporal and spatial changes in expression of kisspeptin, gonadotropins and their respective receptors in the Senegalese sole during a full reproductive cycle shows that, in males, *kiss2* agrees with what one would expect according to its proposed role as a major regulator of the onset of reproduction. However, in females the situation is not so clear, since *kiss2* and *kiss2r* expression was highest either before or during the reproductive season. The origin and physiological significance of these differences, which could also apply to other fish, deserve further investigation.

References:

[1]TENA-SEMPERE, M. 2006. GPR54 and kisspeptin in reproduction. Hum. Reprod. Update, 12: 631–639.

[2]OAKLEY, A.E., CLIFTON, D.K., STEINER, R.A. 2009. Kisspeptin signaling in the brain. Endocr. Rev., 30: 713–743.

[3]TARANGER, G.L., CARRILLO, M., SCHULZ, R.W., FONTAINE, P., ZANUY, S., FELIP, A., WELTZIEN, F.A., DUFOUR, S., KARLSEN, O., NORBERG, B., ANDERSSON, E., HANSEN, T. 2009. Control of puberty in farmed fish. Gen. Comp. Endocrinol., 165: 483–515.

[4]MECHALY, A.S., VIÑAS, J., PIFERRER, F. 2009. Identification of two isoforms of the kisspeptin-1 receptor (kiss1r) generated by alternative splicing in a modern teleost, the Senegalese Sole (*Solea senegalensis*). Biol. Reprod., 80: 60–69.

[5]MECHALY, A.S., VIÑAS, J., MURPHY, C., REITH, M., PIFERRER, F. 2010. Gene structure and regulation of the Kiss-1 receptor-2 (Kiss1r-2) in the Atlantic halibut: Insights into the evolution and regulation of Kiss1r genes. Mol. Cell. Endocrinol., 317: 78–89.

[6]MECHALY,A.S.,VIÑAS,J.,PIFERRER,F. 2011. Gene structure analysis of kisspeptin-2 (Kiss2) in the Senegalese sole (*Solea senegalensis*): Characterization of two splice variants of Kiss2, and novel evidence for metabolic regulation of kisspeptin signaling in non-mammalian species. Mol. Cell. Endocrinol., 339: 14-24.