CHARACTERIZATION OF THE KISSPEPTIN SYSTEMS IN THE BRAIN OF THE EUROPEAN SEA BASS (*Dicentrarchus labrax*): RELATIONSHIPS WITH OESTROGEN RECEPTORS

Escobar S.[1], Servili A.[2], Felip A.[1], S Zanuy S.[1], Carrillo M.[1] and Kah O[2].

[1]Department of Fish Physiology and Biotechnology, Institute of Aquaculture of Torre de la Sal (IATS), Spanish National Research Council (CSIC), Ribera de Cabanes s/n 12595, Castellón, Spain. E-mail: carrillo@iats.csic.es
[2]Neurogenesis and Œstrogens, UMR CNRS 6026, IFR 140, University of Rennes 1, Campus de Beaulieu, 35042 Rennes cedex, France.

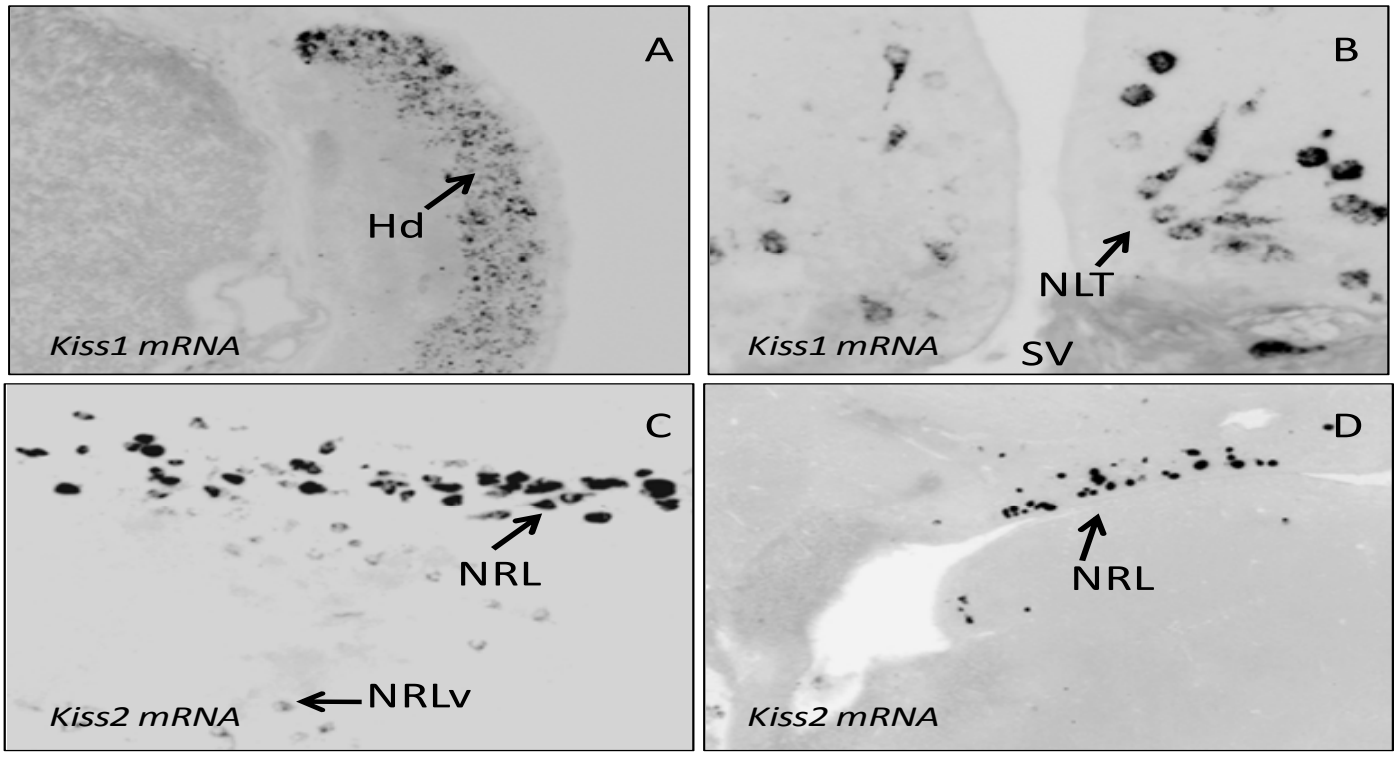
Introduction:

Due to the increasing importance of sea bass (*Dicentrarchus labrax*) in the European aquaculture, puberty control is the focus of intense research. While the GnRH systems have been well described in this species, little information regarding the kisspeptin systems exists. Kisspeptins belong to a family of peptides first identified for their capacity to inhibit tumor metastasis through the receptor GPR54 in mammals. It has been largely demonstrated that the kisspeptin system plays an essential role in the neuroendocrine control of puberty and reproduction by stimulating the GnRH neurons and subsequently releasing gonadotropin hormones. Therefore, the kisspeptin system could be the gatekeeper in the onset of reproduction on fish. In sea bass two ligands and two receptors coded by different genes have been identified and molecularly characterized [1]. Additionally, several sex steroids including œstradiol-17β have been well studied in sea bass and clear evidence exists regarding these hormones play during the period of reproduction. In sea bass, three oestrogen receptors (ERs) have been characterized, named as esr1 (ERα), esr2a (ERβ2) and esr2b (ERβ1) [2, 3]. Here, we report the neuroanatomical distribution of the cells expressing *kiss1* and *kiss2* and their cognate receptors in the brain of adult sea bass and their relationship with the three oestrogens receptors by *in situ* hybridization.

Methods:

Four brains of adult sea bass maintained under a natural of photoperiod were perfused, embedded in paraffin and transversally cut. Specific probes were synthesized using Dig-RNA labeling Mix according to [4]. The relationships between kisspeptin receptors and

Fig 1. Photomicrograph of *kiss1* and *kiss2* expressing cells in the brain of sea bass by *in situ* hybridization. (A) High number of *kiss1* mRNA expressing cells in the habenular nucleus (Hd). (B) Cells located at the caudal lateral tuberal nucleus (NLT) above the saccus vasculosus (VS). In (C-D) a high number of *kiss2* mRNA expressing cells located above the nucleus of the lateral recess (NRL) and a lower expression in cells of the ventral part of nucleus of the lateral recess (NRLv) in the hypothalamus.



the GnRH systems were studied using specific antibodies raised against the GAP portion of the preproGnRH1 [5].

Results and Discussion:

Our findings indicate that *kiss1*-expressing cells are located at the level of the habenular nucleus (Hd) (Fig. 1A) and the mediobasal hypothalamus where a strong expression of *kiss1* messengers is observed just above the pituitary stalk (Fig.1B), which is in line with the *kiss1* messenger distribution in the medaka fish [6]. Additionally, *kiss1* messengers were also detected in the proximal pars distalis of the pituitary gland. In contrast, *kiss2* expressing cells were observed around the nucleus of the lateral recess (NRL) (Fig.1 C-D) and in the anterior preoptic area. Interestingly, similar to what shown in zebrafish [7] the *kiss1r* was also expressed in the habenular nucleus and in the anterior ventral preoptic region. On the other hand, the *kiss2r*-mRNA had a much wider expression in the ventral, lateral and dorsal parts of the telencephalon, the entopeduncular nucleus, the preoptic region and the hypothalamus. Furthermore, a double staining showed that GnRH1 neurons express the *kiss2r*- mRNA in the anterior ventral preoptic area.

Conclusion:

In summary, this work represents the first neuroanatomical analysis of the kisspeptin systems and their relationships with the three oestrogens receptors in the sea bass brain and suggests a critical role of this system in the control of reproduction.

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