

Validation and characterization of specific kiss2 antibody: Neuroanatomic study and interactions with kiss-r1 and kiss-r2 in the brain of the European sea bass.

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In vertebrates the kisspeptin system plays a key role in the neuroendocrine control of puberty and reproduction by stimulating the GnRH neurons and the subsequent synthesis and release of gonadotropins. In the European sea bass a marine fish, two ligands and two receptors encoded by four different genes have been identified and molecularly characterized. This study was conducted in the brain and it aimed to map the kiss systems and identify their correlation with kisspeptin receptors (kiss-r1 and kiss-r2). The use of kiss2 specific antibodies, confirms that kiss2 neurons are mainly located in the hypothalamus and widely projected to the subpallium and pallium, the preoptic region, the thalamus, the pretectal area, the optic tectum, the torus semicircularis, the mediobasal medial and caudal hypothalamus and the neurohypophysis. These results were compared to the expression of *kiss-r1* and *kiss-r2* mRNAs by *in situ* hybridization showing a tight correlation between the wide distribution of kiss2-positive fibers and that of *kiss-r2* expressing cells. The *kiss-r2* messengers were highly expressed in ventral telencephalon and the preoptic area where kiss2 projections were also observed. Additionally, in the mediobasal hypothalamus, *kiss-r2* expressing cells were detected in the ventral part of the nucleus of the lateral recess (NRLv), in line with the localization of kiss2 projections and the immunodetection of *kiss2* cells. Instead, *kiss-r1* was more limited to the habenula, the ventral telencephalon and the proximal pars distalis of the pituitary. Finally, as showed by our double staining experiments, GnRH1 neurons in the preoptic area did not appear to express *kiss-r1* or *kiss-r2* messengers. Our findings present detailed information regarding kiss2 fibers localization which suggest that kisspeptins are likely involved in neuronal systems related to several functions in the brain of sea bass. Funded by EU projects LIFECYCLE (FP7-222719-1) and INTEREG TC2N and Generalitat Valenciana (REPROBASS; PROMETEO/2010/003).