NEUROANATOMICAL CHARACTERIZATION OF TWO INDEPENDENT KISSPEPTIN SYSTEMS DERIVED FROM EVOLUTIONARY-ANCIENT KISS GENES IN THE BRAIN OF ZEBRAFISH

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Introduction:

Before kisspeptins became new players in the field of reproductive biology, GnRH was acknowledged in all vertebrates as the major initiator of the hormonal cascade modulating the reproductive axis. Originally identified as a metastasis suppressor in mammals [1], the KISS1 gene produces several peptides named kisspeptins (kisspeptin -54, 14, 13, 10), which activate the KISS1 receptor (GPR54 or KISSR) previously known as an orphan receptor [2]. Recent phylogenetical analyses provided evidence that the number of kiss genes and kiss receptors varies from one class of vertebrate to the other. According to these studies [3] modern mammals have only one KISS gene, monotrems have two, birds would have none, reptiles have one, amphibians have three and fishes have two KISS genes. Similarly, the number of genes encoding GPR54 receptors (or Kissr) also varies from one class to the other. With the aim to enlarge our knowledge on organization and potential functions of Kiss systems in relation to GPR54 receptors in non-mammalian species, so far very poorly investigated, we have focused our interest on the elucidation of these systems in zebrafish, taken as model of study. Zebrafish have two kiss genes, kiss1 and kiss2 and two kiss receptors (GPR54), kiss1r and kiss2r.

Methods:

To be able to provide detailed information regarding the organization of the Kiss1 and Kiss2 systems in zebrafish brain we produced specific antibodies (raised against the C-terminus of zebrafish preproKiss1 and preproKiss2) that unambiguously distinguish zebrafish preproKiss1 from preproKiss2. Immunohistochemical analysis was fully confirmed by *in situ* hybridization and transgenic approaches.

Results and Discussion:

Our findings show that kiss1-expressing neurons are only located in the habenular nucleus, while kiss2-expressing neurons are found in the dorsal and ventral hypothalamus. Kiss1-expressing cells project only to the interpeduncular and raphe nuclei, and strongly expressed the kiss1r receptor. In contrast, kiss2-expressing cells are mostly present in the dorsal and ventral hypothalamus and project widely into the subpallium, the preoptic area, the thalamus, the ventral and caudal hypothalamus and the mesencephalon. All these regions strongly expressed the kiss2r messengers. In the pituitary gland, no proKiss2- positive fibers were detected, while positive cells were observed in the pars intermedia.

Furthermore, the potential relationships between Kiss neurons and GnRH neurons have been investigated. Briefly, Kiss2 fibers profusely innervate the ventral forebrain and notably made close apposition with GnRH3 neurons. Moreover, estrogen treatment of juvenile fish with estradiol causes increase in kiss2 and kiss2r expression.

Conclusion:

In addition of providing a successful strategy to develop antibodies to kisspeptins, this study provides for the first time detailed information on the organization of two separate kisspeptin systems derived from evolutionary-ancient kiss genes in the brain of a vertebrate (see Figure 1 for a schematic representation). These data will be discussed in relation with steroids, melatonin and leptin signaling.

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Fig 1. Schematic representation of the organization of the Kiss1 and Kiss 2 systems in the brain of zebrafish. (Adapted from [4]). Kiss 1 neurons are restricted to the habenula (Hb). They express also kiss1r and project only into the interpeduncular nucleus (IPN) and the raphe (SR). Kiss 2 neurons are located in the dorsal (Hd), lateral and ventral hypothalamus (Hv). These neurons send extensive projections towards the subpallium, the entopeduncular nucleus (EN) the preoptic region (POA), the thalamus, the ventral and caudal hypothalamus and the torus semicircularis (TS). In all these regions, kiss2r are widely expressed. Kiss2 fibers make direct contacts with GnRH3 neurons that project to the pituitary. In contrast, no Kiss fibers were seen in the pituitary (P), whereas Kiss2 positive cells are present in the pars intermedia. CC: Crista cerebellui; LX: vagal lobe; MO; medulla oblongata; OB: Olfactory bulb; OC: Optic chiasma; Pal: Palium; TeO: optic tectum