Oral Presentation

Identification of novel hypothalamic G protein-coupled receptors involved in the control of reproduction

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Gonadotropin-releasing hormone (GnRH) secreted from the hypothalamus is the master hormone regulator of the hypothalamus-pituitary-gonadal axis, which controls puberty, fertility and reproduction. Several genes have been shown to impact GnRH activity, defects in these causing reproductive dysfunction including hypogonadotropic hypogonadism (HH) but the full spectrum of inputs into this system is still unknown.

Blood from a cohort of European HH patients was collected and exome sequenced. Mutations in GPCR genes were identified and shortlisted to those expressed in the hypothalamus. Using bioinformatics tools, several GPCRs of interest were identified. Two of these, the oxytocin receptor (OXR) and neuropeptide Y4 receptor (NPY4R) were selected for in vitro analysis. These mutant GPCRs were cloned into a mammalian N-terminal epitope-tagging expression vector and their functionality tested *in vitro*. An ELISA assay was utilized to compare WT and mutant receptor cell surface expression and indicated a significant difference in cell surface expression of both OXR and NPY4R mutants compared to WT. Cell signalling assays were then used to compare WT and mutant receptor cell signalling and highlighted a decrease in signalling potency of an OXR mutant, and little to no signalling of an NPY4R mutant, confirming their disrupted functionality.

Characterization of novel GPCR mutations implicated in HH will provide insight into the physiological and pathophysiological roles of these receptors in the neuroendocrine control of reproduction.

References

1. Caligioni CS, Oliver C, Jamur MC, Franci CR. Presence of oxytocin receptors in the gonadotrophin-releasing hormone (GnRH) neurones in female rats: A possible direct action of oxytocin on GnRH neurones. J Neuroendocrinol. 2007;19(6):439–48.
2. Gimpl G, Fahrenholz F, Gene C. The Oxytocin Receptor System: Structure, Function, and Regulation. 2001;81(2):629–83
3. Lee H, Caldwell H, Macbeth A, Tolu S, Young W. A conditional knockout mouse line of the oxytocin receptor. Endocrinology. 2008;149(7):3256–63.
4. Millar RP. GnRHs and GnRH receptors. Anim Reprod Sci. 2005;88(1–2 SPEC. ISS.):5–28.
5. Pedragosa-badia X, Sliwoski GR, Nguyen ED, Lindner D, Stichel J, Kaufmann KW, et al. Pancreatic Polypeptide Is Recognized by Two Hydrophobic Domains of the Human Y 4 Receptor Binding Pocket. 2014;289(9):5846–59.
6. Sainsbury A, Schwarzer C, Couzens M, Jenkins A, Oakes SR, Ormandy CJ, et al. Y4 receptor knockout rescues fertility in ob/ob mice. Genes Dev. 2002;16(9):1077–88.
7. Silveira LFG, Trarbach EB, Latronico AC. Genetics basis for GnRH-dependent pubertal disorders in humans. Mol Cell Endocrinol