

Ligand-selective determinants in gonadotropin receptors

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Glycoprotein hormones (GpHs) and glycoprotein hormone receptors (GpHRs) constitute an interesting example of co-evolution. Glycoprotein hormones are heterodimers consisting of a common α -subunit and a receptor-specific β -subunit. GpHRs are G protein-coupled receptors characterized by a large N-terminal, extracellular domain (ECD) responsible for the specific recognition and binding of the hormones. The ECD of a particular GpHR contains the molecular determinants to bind and select the 'correct' GpH from the 'bouquet' of potential GpHs present in the circulation in order to elicit the correct physiological response. In this way, LH and hCG bind to the same LH/hCG receptor (LHR), whereas TSH and FSH bind to the TSH receptor (TSHR) and the FSH receptor (FSHR), respectively.

Functional characterization of fish gonadotropin receptors revealed that their FSHRs displayed promiscuous gonadotropin-signaling behaviour (*i.e.* binding both FSH and LH), whereas mammalian GpHRs bind GpHs with high selectivity (*i.e.* virtually no cross-reactivity between GpHs and the heterologous GpHRs).

The observed difference in gonadotropin selectivity allowed us to design a gain-of-function strategy in order to identify the LH/hCG-selective determinants for the human gonadotropin receptors. Domain-exchange experiments, followed by single amino acid substitutions, for various LHR-ECD domains using the FSHR as 'host' receptor revealed that only two amino acids function as LH/hCG-selective determinants (*i.e.* Asn¹⁰⁴, a positive, LHR-derived determinant, and Lys¹⁷⁹, a negative, FSHR-derived determinant), apart from numerous low-impact, common GpH contact sites.

Recent data on the FSH-selective determinants, in relation to the three-dimensional structure of FSH bound to the ECD of its receptor, will also be discussed.

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