Identification of the key binding residues for insulin-like peptide 3 (INSL3) in its receptor, LGR8

D.S. Scott, T. Wilkinson, G.W. Tregear and R.A.D. Bathgate , Howard Florey Institute, University of Melbourne, Vic 3010, Australia.

Insulin-like peptide 3 (INSL3), a member of the relaxin peptide family, is essential for testicular descent and has important roles in oocyte maturation and male germ cell survival. The relaxin and INSL3 receptors, LGR7 and LGR8 respectively, are GPCRs similar to the glycoprotein hormone receptors and contain large ectodomains with 10 leucine-rich repeats (LRRs). Relaxin is able to bind to LGR8 with high affinity whereas INSL3 exhibits very poor affinity for LGR7. Ligand binding is mediated within the LRRs which typically form arced structures with β-strands lining the concave surface of the arc. It is these β-sheets that likely form the binding interface. To determine the INSL3 binding interface in LGR8 the LRR β-strands of LGR7 and LGR8 were identified first by alignment to their closest homolog with a known structure, the Nogo-66 receptor. Multiple species alignment of these putative β-strands revealed blocks of residues conserved in either LGR7 or LGR8 alone, along with a conserved relaxin binding site. It is probable that some of the conserved residues specific to LGR8 are imperative for high affinity INSL3 binding. A combination of “gain of function” mutations in LGR7 and “loss of function” mutations in LGR8 were used to characterize the relevance of these residues. LGR7 mutants with significantly increased [125I]-INSL3 binding over LGR7, and LGR8 mutants with decreased [125I]-INSL3 binding highlighted residues that are crucial for INSL3 binding to LGR8. This approach has provided us with a basic model of INSL3 interaction with LGR8 which has been combined with peptide mutations to give a clear picture of the peptide and receptor residues responsible for interaction. These studies will allow the design of INSL3 mimetics which may prove to be very useful as fertility regulators.