

Charged amino acids in the peptide 20-30 of FSHR contribute to the FSH antagonistic activity

Madhavi Dupakuntla, Jeevan Ghosalkar and Smita Mahale
Division of Structural Biology, National Institute for Research in Reproductive Health (Indian Council of Medical Research), Mumbai, India

FSH mediates its specific physiological functions through its cognate receptor. Like other glycoprotein hormone receptors (GPHRs), FSH receptor has a characteristically large extracellular domain (ECD) that is responsible for specific high affinity hormone binding. The N terminal region 9-30, the sequence of which is unique to FSHR, has been shown to have a role in hormone binding, signal transduction and receptor trafficking. We had earlier undertaken a detailed study to identify a specific functional domain within the region 9-30 from rat FSHR. Antipeptide antibodies specific to the peptide 9-30 were generated and using overlapping peptides, 20-30 was identified as the immunodominant region. This is also the smallest reported peptide that acts as an effective hormone antagonist. The region 20-30 of FSHR has 3 charged residues, namely, E²², D²⁶ and R²⁹ that are specific to FSHR and are conserved in mammals. We synthesized three peptide analogs, each having an alanine substitution for a corresponding charged residue. These peptides were then tested for their ability to affect hormone binding and signal transduction by means of the radioreceptor assay and the estimation of cAMP. None of the analog peptides were able to inhibit hormone binding or the subsequent signal transduction. However, the antigenicity of the epitope was found to be due to the charged residues. The antipeptide antibodies could recognize 20-30 and to lesser extent 20-30 E²²A. The change in functionality led us to verify whether there was a structural change as well. CD spectra of all the peptides were recorded and the aminoacid substitutions led to a marginal conformational shift. Thus, being the smallest peptide with the ability to act as a hormone antagonist, electrostatic interactions of the charged residues contribute to the activity of 20-30 FSHR. However, the exact molecular mechanisms involved in the interaction are yet to be resolved.

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