

Elucidating Gonadotropins-receptors interactions using hormone analogues and specific antibodies

Rajan R. Dighe, Gaurav Agrawal, Satarupa Roy, Reema Railkar and Ritankar Majumdar
Molecular Reproduction, Development and Genetics, Indian Institute of Science
Bangalore 560012, India.

Various strategies such as specific antibodies, hormone analogues and mutations in the receptors have been used to determine the roles of different domains of Gonadotropin receptors in hormone binding and signal transduction. Specific antibodies, polyclonal, monoclonal and recombinant, have proved to be excellent tools to map the contact points between Gonadotropins and their receptors. Recently, we purified antagonists of hCG and FSH produced by translationally fusing two β subunits of the hormones (hCG $\beta\beta$ /hFSH $\beta\beta$). These antagonists bind specifically to the receptors with relatively high affinity, inhibit hormone binding and response. Orientation of hCG $\beta\beta$ when it is complexed with the receptor appears to be different from that of the hormone with hormone interacting more with LRR while hCG $\beta\beta$ interacting more with the hinge region of the receptor. Experiments with antibodies against LRR1-6 and the hinge region essentially confirmed these observations. Further, using antibodies specific for different regions of LH receptor, we confirmed that the LRRs4-6 are in contact with hCG through its seat belt. Interestingly, hCG $\beta\beta$ could attenuate the constitutively activated LH receptor found in precocious puberty mutants suggesting inverse agonistic nature of the analogue. Antibodies against different portions of FSH receptors have also provided interesting insights into the roles of different domains of receptors in hormone action. An antibody raised against the hinge region of FSH receptor, while not having any effect on either hormone binding or response, could stimulate the receptor in the absence of the hormone. The stimulatory effect of antibodies was specific since the same antibody failed to stimulate LH and TSH receptors. Through a series of peptide inhibition experiments, in which ability of overlapping fragments of human FSHR to block the stimulatory activity of the antibody was tested, it was found that this antibody recognizes aa296-331 in the hinge region of FSH receptor suggesting importance of this region in signal transduction. This was confirmed by investigating effects of deletions in FSHR on the basal as well as hormone/antibody stimulated response. Deletion of entire ECD resulted in increase in the basal activity, but no further stimulation with the antibody or the hormone. In contrast, deletion of the LRRs led to abolition of response to the hormone, but retention of antibody stimulation. Further deletion of aa296-331 resulted in complete loss of antibody or hormone stimulation confirming that this region is most critical for activation of the receptor by the hormone. These data suggest that the hinge region of the glycoprotein hormone receptors play important role in signal transduction. (Supported by Grants from DBT, CSIR and UGC, Government of India).