

Mapping of Glycoprotein hormone-receptor contacts using antibodies and single chain hormones

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The Glycoprotein hormones are interesting models for understanding the structure-function relationship of complex multimeric glycoproteins. The principal objective of the present study is to identify the domains of both hormones and receptors involved in binding and signal transduction using antibodies with different specificities and recombinant expression of hormones and their analogs. Employing hCG polyclonal and monoclonal antibodies it was demonstrated that the antibodies specific for β subunit inhibited binding of the hormone to the receptor and hence the response. Intriguingly, the α subunit specific antibodies did not inhibit binding but inhibited response. It was hypothesized that since both antibodies and receptors bind the hormones specifically and with high affinity, the antigen binding sites of antibodies may share homology with distinct regions of the receptor. Identification of the epitopes in hCG recognized by the antibodies would then allow us to map the contact points between the hormone and the receptor. This hypothesis was tested by generating single chain fragment variables (ScFvs) from antibodies with different epitope specificities and identifying their antigen binding sequences. The precise location of the epitopes present in hCG recognized by these antibodies was carried out using different methods. Comparisons of antigen binding sequences with those of the receptor confirmed that ScFv sequences show homology to the distinct parts of the receptor. The hCG β subunit specific antibody that recognizes the determinant loop in hCG showed homology to the extracellular domain of the receptor, mostly to the leucine rich repeats. The α subunit specific antibody, in contrast, showed homology to the transmembrane domain while a unique heterodimer specific antibody that fails to bind hLH showed homology to the hinge region of the receptor. Single chain hCG, in which the C terminus of the α subunit was fused to the N terminus of the β subunit (hCG $\alpha\beta$) and expressed using Pichia expression system was shown to be immunologically similar to hCG, could bind to the receptor with comparable affinity and elicit response. Replacement of the α subunit in hCG $\alpha\beta$ with another β subunit (hCG $\beta\beta$) resulted in a molecule that could bind to the receptor without eliciting any response, but inhibited hormone action both *in vivo* and *in vitro* suggesting that hCG $\beta\beta$ is a potent inhibitor of the hormone. All these data suggest that in the hormone-receptor complex the β subunit is in contact with the extracellular domain while the α subunit is in close proximity of the transmembrane domain. The initial interaction between the hormone and the receptor probably take place through the β subunit and the extracellular domain, subsequently bringing the α subunit in contact with the transmembrane domain leading to signal transduction. hCG-antibody complexes are interesting mimics of hormone-receptor interactions and can be used to map the contact points in the complex (supported by grants from DBT, ICMR, CSIR and UGC, Government of India, New Delhi, India).