Biological Functions of N- and O-linked Oligosaccharides of Equine Chorionic Gonadotropin and Lutropin/Chorionicgonadotropin Receptor

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Members of the glycoprotein family, which includes CG, LH, FSH and TSH, comprise two noncovalently linked α- and β-subunits. Equine chorionic gonadotropin (eCG), known as PMSG, has a number of interesting and unique characteristics since it appears to be a single molecule that possesses both LH- and FSH-like activities in other species than the horse. This dual activity of eCG in heterologous species is of fundamental interest to the study of the structure-function relationships of gonadotropins and their receptors. CG and LH β genes are different in primates. In horse, however, a single gene encodes both eCG and eLH β-subunits. The subunit mRNA levels seem to be independently regulated and their imbalance may account for differences in the quantities of α- and β-subunits in the placenta and pituitary. The dual activities of eCG could be separated by removal of the N-linked oligosaccharide on the α-subunit Asn 56 or CTP-associated O-linked oligosaccharides. The tethered-eCG was efficiently secreted and showed similar LH-like activity to the dimeric eCG. Interestingly, the FSH-like activity of the tethered-eCG was increased markedly in comparison with

the native and wild type eCG. These results also suggest that this molecular can implay particular models of FSH-like activity not LH-like activity in the eCG/indicate that the constructs of tethered molecule will be useful in the study of mutants that affect subunit association and/or secretion. A single-chain analog can also be constructed to include additional hormone-specific bioactive generating potentially efficacious compounds that have only FSH-like activity.

The LH/CG receptor (LH/CGR), a membrane glycoprotein that is present on testicular Leydig cells and ovarian theca, granulosa, luteal, and interstitial cells, plays a pivotal role in the regulation of gonadal development and function in males as well as in nonpregnant and pregnant females. The LH/CGR is a member of the family of G protein-coupled receptors and its structure is predicted to consist of a large extracellular domain connected to a bundle of seven membrane -spanning a-helices.

The LH/CGR phosphorylation can be induced with a phorbol ester, but not with a calcium ionophore. The truncated form of LHR also was down-regulated normally in response to hCG stimulation. In contrast, the cell lines expressing LHR-t631 or LHR-628, the two phosphorylation -negative receptor mutant, showed a delay in the early phase of hCG-induced desensitization, a complete loss of PMA-induced desensitization, and an increase in the rate of hCG-induced receptor down-regulation. These results clearly show that residues 632-653 in the C-terminal tail of the LHR are involved in PMA-induced desensitization, hCG-induced desensitization, and hCG-induced down-regulation. Recently, constitutively activating mutations of the receptor have been identified that are associated with familial male-precocious puberty. Cells

expressing LHR-D556Y bind hCG with normal affinity, exhibit a 25-fold increase in basal cAMP and respond to hCG with a normal increase in cAMP accumulation. This mutation enhances the internalization of the free and agonist-occupied receptors ~2- and ~17-fold, respectively. We conclude that the state of activation of the LHR can modulate its basal and/or agonist-stimulated internalization. Since the internalization of hCG is involved in the termination of hCG actions, we suggest that the lack of responsiveness detected in cells expressing LHR-L435R is due to the fast rate of internalization of the bound hCG. This statement is supported by the finding that hCG responsiveness is restored when the cells are lysed and signal transduction is measured in a subcellular fraction (membranes) that cannot internalize the bound hormone.