Abstract

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Site-directed mutagenesis of the human thyrotropin receptor: role of asparagine-linked oligosaccharides in the expression of a functional receptor.

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OBJECTIVE: We studied the role of glycosylation in the expression of a functional human TSH receptor.

METHODS: Oligonucleotide-directed mutagenesis was used to replace, separately or together, the Asn codons with Gln in each of the six potential glycosylation sites in the receptor.

RESULTS: Recombinant wild-type and mutated TSH receptors were stably expressed in Chinese hamster ovary cells. High affinity TSH binding and the cAMP response to TSH stimulation were abolished in the receptor mutated at Asn77 as well as in the receptor mutated at all six potential glycosylation sites. In the receptor mutated at Asn113, the affinity of TSH binding was markedly decreased (Kd, 2.6 x 10(-8) 3.3 x 10(-10) M in the wild-type receptor). This affinity was too low to permit the transduction of a signal, as measured by an increase in intracellular cAMP generation. Substitution of Asn at positions 99, 177, 198, and 302 did not appreciably affect the affinity of the TSH receptor for TSH binding or its ability to mediate an increase in intracellular cAMP levels.

CONCLUSION: Therefore, either these four potential glycosylation sites are not glycolysated, or alternatively, oligosaccharide chains at these positions do not play a major role in the folding, intracellular trafficking, stability, or expression of a functional receptor on the cell surface. Conversely, our data suggest that N-linked glycosylation of Asn77 and Asn113 does play a role in the expression of a biologically active TSH receptor on the cell surface.

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