Title of Symposium Glycoprotein Hormones

SERONO SYMPOSIA, USA

POSTER ABSTRACT FORM

Title of abstract (all capitals) ➤ **DIFFERENTIAL ROLES OF THE TERMINAL MONOSACCHARIDES OF RECOMBINANT TSH IN ITS IN VITRO AND IN VIVO BIOACTIVITY.**

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Begin Text ➤

We have previously shown that recombinant human thyrotropin (rhTSH), expressed in Chinese hamster ovary cells is biologically active and due to its highly sialylated oligosaccharide chains, has lower metabolic clearance and higher in vivo bioactivity compared to pituitary hTSH. The specific role of terminal sulfate groups and the subsequent sugar residues in pituitary-derived hTSH could not be assessed individually due to the unavailability of a suitable sulphohydrolase, since the oligosaccharides of pituitary hTSH terminate in NeuAcα2,3(or6)Galβ and SO_4–4GalNAcβ, whereas those in rhTSH terminate only in NeuAcα2,3(or6)Galβ. Due to this type of oligosacharide structure in rhTSH, it is now possible to deglycosylate and assess the role of individual sugar residues in the hormonal activity. The monosaccharides were sequentially removed from rhTSH by exoglycosidase digestions. The removal of sialic acid, galactose or N-acetylglucosamine in that sequence resulted in more than a 10-fold increase in the in vitro bioactivity of rhTSH. As expected, all the derivatives showed higher metabolic clearance rates compared to the intact hormone, but the Gal-removed and GlcNAc-removed derivatives were cleared slower than NeuAc-removed rhTSH. In contrast, the in vivo bioactivity decreased progressively with each monosaccharide removal and the GalNAc-removed derivative showed no activity. Resialylation of incompletely sialylated or desialylated rhTSH using sialyl transferase attenuated the in vitro activity of the hormone and increased the in vivo bioactivity to the level comparable to the untreated TSH. These data demonstrate that sialic acid residues affect both the in vitro and the in vivo bioactivities of rhTSH. In contrast to previous studies on human chorionic gonadotropin, sequential removal of sugar residues does not result in a progressive decrease in the in vitro activity of rhTSH. Thus, glycosidases and glycosyltransferases can be used as powerful tools to study the role of carbohydrate and to alter the in vivo potency of glycoprotein hormones.

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