Primate FSH Glycoforms

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Human pituitary FSH is comprised of numerous isoforms because its four N-glycosylation sites are decorated with oligosaccharides possessing 2 to 4 branches. Each branch is potentially capped with a negatively charged sialic acid residue, providing the basis for most differential charge separation methods. Sialic acid contents should range from 8-16, yet the actual content ranges from 1.5-13.5, in part because not all branches are terminated with sialic acid. Follicle-stimulating hormone was the first gonadotropin demonstrated to exhibit glycosylation changes associated with changing endocrine physiology. Because carbohydrate is necessary for full expression of gonadotropin activity, a hypothesis developed that changes in gonadotropin glycosylation modulate biological activity. We demonstrated the existence of a novel hFSH glycoform, di-glycosylated hFSH, which was glycosylated only on the common alpha subunit and which expressed greater biological activity than receptor-binding activity. Mixtures of di-glycosylated hFSH and tetra-glycosylated hFSH exhibited biological activities comparable to their receptor-binding activities. Our studies compared the relative abundance of these two glycoforms to determine if this was altered by gonadal feedback.

Pooled pituitary extracts, postmenopausal urinary gonadotropin preparations, and immunopurified FSH preparations from individual pituitaries were characterized by Western blotting, protein sequencing, mass spectrometry, gel filtration, and receptor binding.

Tetra-glycosylated FSH was the more adundant glycoform present in pooled pituitary extracts (>95%) and postmenopausal urine gonadotropin preparations (75-95%). However, in individual pituitaries its abundance was only 35-40%, with di-glycosylated FSH the more abundant glycoform. This suggested that gonadal feedback selectively inhibited FSH beta glycosylation. We compared hFSH from pituitaries obtained from women in their 20s with hFSH from women in their 70s. FSH obtained from younger women was 35% tetra-glycosylated hFSH, while in the older women its abundance ranged from 35-50%. Thus, the high abundance of tetra-glycosylated hFSH in traditional sources of the gonadotropin did not appear to be related to physiological processes, but may represent a bias in FSH isolation. A better understanding of effects of glycosylation on FSH action requires characterization of the oligosaccharide structures at each glycosylation site. In order to obtain detailed characterization of FSH glycosylation changes we obtained glycopeptides from hFSH representing all 4 N-glycosylation sites: NIT, KNVT, INTT, and NHT. These were analyzed by mass spectrometry.

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