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P.6 .A comparison between the oligosaccharide structures of native and recombinant glycosylated human prolactin obtained from CHO cells

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Human prolactin (hPRL) is a pituitary polypeptide hormone mainly involved in mammary gland development and lactation. It has a unique N-glycosylation site, only partly occupied (~10%), being therefore possible to separate non-glycosylated (NG-hPRL) from glycosylated prolactin (G-hPRL). The goal of the present work was, therefore, to study, in comparison, the N-glycan structures present in the native, pituitary form (provided by the National Hormone and Pituitary Program of the US) and in the recombinant, CHO-derived form, synthesized and purified in our laboratory. Recombinant G-hPRL was obtained in spinner flasks from suspension-adapted CHO cell cultures, to which 0.6 µg/ml cycloheximide were added, a medium modification favoring glycosylation and that provided ~50% of G-hPRL on the total amount of the hormone. This artifact greatly facilitated purification, which was carried out via a two-step chromatographic purification process: a cationic exchanger (SP-Sepharose fast flow) and a preparative reversed-phase HPLC (RP-HPLC). The overall yield was approximately 37,5%, the purity of recombinant G-hPRL >95%, as confirmed by Western blotting, SDS-PAGE, RP-HPLC and high-performance size-exclusion chromatography (HPSEC), while its biological activity, determined via the classical Nb2 in vitro bioassay was, as expected, ~17 IU/mg. N-glycosylation profiling, determined via mass spectrometric techniques, identified 28 different N-glycan structures in pituitary and 14 N-glycan structures in recombinant G-hPRL. The most abundant structures were biantennary, in both cases: N2GN2F2SO₄ (33.8%) and N2GN2F1(SO₄)₂ (28.1%) in the former and N2G2S2F1 (56%) and N2G2S1G1 (12%) in the latter. The difference in sialic acid content was even greater than expected: 0.03 moles/mole of G-hPRL in the pituitary and 1.4 moles/mole in the recombinant. This is quite relevant since sialic acid is known to influence in vivo bioactivity. Considering the abundance of each N-glycan and the well-known molecular mass for G-hPRL of 25,000 d, a total carbohydrate content of 7.13% (pituitary G-hPRL) and of 8.5% (recombinant G-hPRL) was obtained by stoichiometric calculations. These data were found in very good agreement with the values of 7.7% and 8.3% obtained, respectively, via MALDI-TOF-MS.