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PURIFICATION OF GLYCOSYLATED AND NONGLYCOSYLATED RECOMBINANT HUMAN PROLACTIN PRODUCED BY CHINESE HAMSTER OVARY CELLS IN A HOLLOW FIBER BIOREACTOR

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Recombinant human Prolactin (rec-PRL) was produced in CHO cell using an expression system based on the selectable gene marker (dhfr). This vector after three steps of methotrexate (MTX) amplification (20, 50 and 100 nM MTX) provided some clones which were able to secrete up to 30 µg hPRL/10^6 cells/day. The obtained secretion levels are higher then those reported for this hormone in other eukaryotic systems. CHO-derived rec-hPRL contained approximately 10-15% of the glycosylated form (G-PRL), a value that, up to a point, agrees with those reported for hPRL purified from the pituitary or from transformed murine C-127 cell.

We have utilized a new method for growing transformed CHO cells in useful quantities, which significantly reduces cost, time and space requirements. This involves cell culture in hollow fibers bioreactors, wherein cells grow on tube-shaped, semi-permeable membrane, producing about 1.2 mg of rec-PRL/day (200µg/mL), determined by radioimmunoassay.

The glycosylated (G-PRL) and nonglycosylated (NG-PRL) rec-PRL were purified by a two-step purification process modifying the methodology described by Price et al. (Endocrinology 1995;136:4827). Rec-hPRL was recovered in a single peak by size exclusion chromatography on Sephacryl S-100 (90% yield) with a purity of approximately 70%. After the second chromatography on an SP-Sepharose high performance column (cation exchange) the NG-PRL (23 kDa) and G-PRL (25 kDa) were separated with the use of a buffer containing ethylene glycol and n-butanol.

After SDS-PAGE and Western Blot analysis purified G-PRL was estimated to contain less than 5% NG-PRL, whereas purified NG-PRL contained less than 2% G-PRL.

An isoform of prolactin (22 kDa PRL_{11-199}) was identified and characterized for the first time. Studies are in progress in order to understand if the bioreaction conditions are responsible for its formation.

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