THE EFFECT OF CYCLOHEXIMIDE ON THE GLYCOSYLATION OF RECOMBINANT HUMAN PROLACTIN SYNTHESIZED IN CHO CELLS

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Human prolactin (hPRL) is a 199aa protein hormone (23,000 Da) with a wide spectrum of biological activities being, however, best known for its stimulation of lactation and development of the mammary gland. This protein contains only one potential asparagine-linked glycosylation site (Asp^{31}-Leu-Ser-Ser), which is partially (~10%) occupied when then protein is synthesized in eucaryotic cells. We describe the effect on human prolactin glycosylation of different culture conditions i.e. with or without cycloheximide, an inhibitor of protein synthesis that favours glycosylation. Prolactin is an ideal model for these studies because it exhibits a simple type of macroheterogeneity: one protein population produced with and one without a single N-linked oligosaccharide. We utilized in our studies CHO cells transfected with the hPRL cDNA (Soares CRJ, Biotechnol. Appl. Biochem., 2000:32,127-135). Western blots obtained upon incubation with cycloheximide showed a remarkable increase in glycosylated prolactin (G-hPRL), together with a substantial decrease in non-glycosylated prolactin. The maximum G-hPRL production was obtained with 0.6µg/mL of cycloheximide: ~5µg/mL (>50% of total prolactin), against 0.6µg/mL obtained without cycloheximide (~10% of total prolactin). Our results show that cycloheximide can be an important tool for G-hPRL production, facilitating the purification and characterization of this important isoform of prolactin, whose physiological action has not been defined.

Key words: prolactin, glycosylation, CHO

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