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Permalink

<https://escholarship.org/uc/item/7802f5tb>

Journal

MOLECULAR BIOLOGY OF THE CELL, 3

ISSN

1059-1524

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Publication Date

1992-09-01

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Peer reviewed

Glucocorticoids Accelerate proTRH Processing in Cultured Anterior Pituitary Cells. E.A. Nillni, T.O. Bruhn, S. S. Huang and I.M.D. Jackson. Division of Endocrinology, Brown University, RI Hospital, Providence, RI 02903.

We have reported that anterior pituitary (AP) cells in long-term culture synthesize proTRH-derived peptides and that dexamethasone (DEX) markedly increases proTRH gene expression. Since glucocorticoids affect the levels of peptide processing enzymes, we evaluated the effect of DEX on proTRH processing in the AP as well as in the AtT₂₀ pituitary tumor cell line transfected with a cDNA encoding preproTRH. These cells lack the TRH promoter and, therefore, provide a model system to study posttranslational processing of proTRH without interference from changes in biosynthesis.

AP cells derived from 15 days old rats were cultured for up to 18 days in a L-15/DMEM medium containing 10% fetal calf serum (FCS). AtT₂₀ cells were grown for up to seven days in DMEM containing 10% FCS. AP or AtT₂₀ cells pretreated with 10 nM DEX were radiolabelled with ³H-leucine for 18 hours. Peptides were extracted, immunoprecipitated with an antibody that recognizes the 26kd MW TRH prohormone and its intermediate product of processing, a 15kd peptide, and analyzed by SDS-PAGE followed by counting or RIA's for the detection of proTRH-derived peptides.

Exposure of AP cells to 10 and 100 nM DEX increased the cellular content of preproTRH₈₃₋₁₀₆, a final processing product of proTRH, 2.8 and 6-fold, respectively, while AtT₂₀ cells showed no change. Since peptide content does not necessarily reflect processing, this was monitored by radiolabelling. DEX pretreatment caused a pronounced shift of radiolabel in both AP and AtT₂₀ cells from the 26 kd intact TRH precursor (AP: 93%, AtT₂₀: 23% decrease) to an intermediate product, a 15kd peptide (AP: 246%, AtT₂₀: 138% increase).

Conclusion: glucocorticoids not only increase transcription but also profoundly affect posttranslational processing of the TRH prohormone in cultured AP cells.

Supported in part by DK 34540.