RES N°46

SMALL-SCALE PURIFICATION OF HUMAN PITUITARY THYROTROPIN (hTSH) FOR USE IN RADIOLIGAND ASSAYS.

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Considering the limited disposibility of human pituitaries and the minimal amounts of hormone necessary for radioligand assays, we proposed to employ the large-scale TSH extraction and purification method described by McLean and cols. (in: Beardwell, C. & Robertson, G.L. eds., Clinical Endocrinology, 1981) in a small-scale procedure.
Frozen pituitaries are extracted twice in pH 4.0 buffer and the glycoproteins are precipitated by a two-stage ethanol addition, in the presence of ammonium sulphate. The precipitate is purified in 4 subsequent steps: one molecular sieve, one anion exchange and two cation exchage chromatographies. Immunoreactive hTSH in the eluted fractions was measured by a radioimmunoassay set up with NIADDK reagents.
Starting from 38 glands, the yield in purified hTSH was of 21ug/g of hypophisis, comparable to the 20 to 30ug/g reported for the large-scale method. The immunopotency of this extract was of 580mIU/mg (NIADDK-RP-1/WHO 68/38). The hTSH obtained was used in the preparation of a secondary standard for use in radioassays.
We conclude that the adaptation of McLean's method for hTSH purification in a small scale yields an adequate product for use in radioligand assays.
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