RES N°45

PURIFICATION AND CHARACTERIZATION OF HUMAN PROLACTIN EXTRACTED FROM FROZEN PITUITARIES AND ITS APLLICATION TO THE PREPARATION OF RIA REAGENTS.

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Purification of pituitary extracts was carried out in our laboratory to obtain pure hPrl for use in radioligand assays. The extraction and purification procedure was adapted from the method of McLean & cols (St Bartholomew's Centre for Clinical Research, London), wich involves the following steps: I. Extraction of frozen pituitaries in buffers of pH 4.0, 7.0 and 10.0. II. Purification by Hidrophobic Interaction chromatography on Phenyl-Sepharose CL-4B in the presence of Acetonitrile. III.Purification by Anion Exchange chromatography on DEAE-Sepharose CL-6B. Prolactin was recovered from 58 pituitaries with an overall yield of 286,5ug. Purify of the hormone was analyzed on 7% polyacrylamide gel electrophoresis. hPrl-IPEN presented a single band with RM=0.505, very close to the RM=0.503 of NIADDK-hPrl-I-7. In the hPrl RIA, the standard curves obtained with hPrl-IPEN and NIADDK hPrl RP-1 showed a significant parallelism (P-0.05). By specific RIA, this hPrl-IPEN presented only 2.3% of immunoreactive hGH. The purification method is considered effective to obtain a hPrl of the purity needed for radioassay purposes knowing the additional advantage of rapidity and simplicity.

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