Abstracts

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1 BINDING STUDIES OF GROWTH HORMONE TO RAT LIVER CELLS

M.C. Postel-Vinay.
Unité de Recherche sur les Maladies du Métabolisme chez l'Enfant, Hôpital des Enfants Malades, Paris, FRANCE.

The interaction of human growth hormone (HGH) with isolated liver cells from normal rats has been studied. Cells prepared with the method described by Berry and Friend were incubated at room temperature with ^{125}I-labeled HGH. Free and cell-bound hormone were separated by filtration or centrifugation. The binding of ^{125}I-HGH to liver cells is a specific, time dependent, saturable and reversible process. At a hormone concentration of 5×10^{-11}M, equilibrium of binding is achieved in 120 minutes. The bound labeled HGH is rapidly dissociated by addition of an excess of unlabeled hormone. The specific binding of ^{125}I-HGH is saturable at a hormone concentration of 6×10^{-10}M. 1500 molecules of HGH are specifically bound per cell. Native HGH inhibits binding of ^{125}I-HGH to cells ; half maximal inhibition occurs at 4×10^{-9}M. No significant inactivation or degradation of ^{125}I-HGH or of the specific receptors occurs. Preliminary data indicate that those receptor sites are biologically meaningful.

2 BINDING OF HUMAN GROWTH HORMONE TO RAT LIVER CELL MEMBRANES : PROPERTIES AND EFFECT OF AGE

M.V.L. Du Caju and B. Desbuquois.
Unité de Recherche sur les Maladies du Métabolisme chez l'Enfant Hôpital des Enfants Malades, Paris, FRANCE.

A method has been devised for measuring the specific interaction of ^{125}I-labeled human growth hormone (HGH) with microsomal membranes of rat liver. The HGH-membrane complex is isolated by centrifugation. Binding is time and temperature dependent, reversible, and saturable. In adult female rats, the maximal binding capacity of the membranes is about 0.4 pmoles HGH per mg of protein, and the dissociation constant (Kd.) of the interaction (as calculated from equilibrium data) is about 5.0×10^{-10}M. No significant inactivation of HGH occurs during the binding process. The pH optimum for binding is about 6.5.

Membranes of young (3 days-old) rats bind 5 to 6 times less HGH than do membranes of adult (105 days-old) rats. Early data suggest that this difference with age results from a change in the number of binding sites rather than from an alteration in the affinity of the interaction.

3 STRUCTURE-FUNCTION RELATIONSHIPS IN THE HUMAN GROWTH HORMONE MOLECULE: IMPORTANCE OF MOLECULAR CONFORMATION IN RADIORECEPTOR ASSAY AND RADIOIMMUNOASSAY ACTIVITY.

M.L. Aubert, T.A. Bewley, M.M. Grumbach, and S.L. Kaplan, Dept. Pediatrics and Hormone Res. Lab., Univ. Calif. San Francisco, Ca.

The conformational requirements for activity in hGH have been evaluated by comparing the activity of different derivatives and fragments of this hormone in two radioreceptor assays (RRA) and in several types of radioimmunoassays (RIAs). With derivatives of hGH which contained almost the entire primary structure, but which had undergone certain chemical modifications, good parallelism was found between the retention or loss of conformation of these derivatives (CD spectrum, rate of tryptic digestion), their biological potenties and their activities in the RRA or the RIAs. hGH derivatives with reduced and tetra-S-carbamidomethylated cysteine residues (RCAM-hGH) or with the single tryptophan in position 86 blocked with an o-nitrophenylsulfenyl group (NPS-hGH) or a hGH molecule lacking the hexapeptide 135-146 (Lewis α_3-hGH), all retained a native conformation and substantial biological potency and exhibited good retention of activity in the RRA or in the RIAs. Contrariwise, performic acid oxidized hGH, which has a very disorganized tertiary structure, had no activity in any of the systems tested. Cyanogen bromide cleaved or tryptic digest fragments of hGH did not exhibit substantial activity. Similar results were obtained with derivatives and fragments of hCS. We conclude that there is good correlation between molecular conformation and these functions of hGH; the lack of activity of the different hGH fragments may be attributed to their inability to fold into the required conformation or to stabilize such a conformation.

4 HETEROGENEITY OF PITUITARY AND ENDOGENOUS PLASMA HGH FROM FETUSES, PREMATURES AND FULL-TERM NEW BORNS

R. Eshet, S. Assa and Z. Laron
Institute of Pediatric and Adolescent Endocrinology, Beilinson Hospital, Petach Tikva, and Dept. of Physiology Sackler School of Medicine, Tel Aviv University, Israel.

Fetal and adult HGH extract from human pituitaries as well as plasma from premature and fullterm newborns and children were submitted to chromatography on Sephadex G-75 and the IR-HGH in the elution fractions was determined quantitatively by radioimmunoassay.

Three fractions of IR-HGH could be differentiated according to the elution pattern: "big-big", "big" and "little" IR-HGH.

In fetal pituitary extract only "big-big"and "little" IR-HGH were found whereas in adult pituitary extract all three forms of IR-HGH were present. In the plasma samples studied no "big-big" form was found; only "big" and "little" IR-HGH appeared. In addition, plasma samples of premature newborns had a smaller proportion of the "big" IR-HGH than did those of the fullterm newborns and older children. It is suggested that the "big" forms of the IR-HGH are pro-hormones.