LIPOTROPIC HORMONE OBTAINED FROM HUMAN PITUITARY GLAND

G.CSEH, L.GRÁF and E.GÓTH

Research Institute for Pharmaceutical Chemistry, Budapest and Jånos Hospital, Budapest

Received 21 October 1968

Specific lipid-mobilizing peptide fractions had been earlier prepared from the pituitaries of various animal species [1–3]. In homogeneous form, however, only β-lipotropin (β-LPH) (sheep [4], bovine [5], pig [6]) and γ-lipotropin (sheep [7]) were isolated. Human pituitaries were also shown to contain lipolytic agents of biological specificity, but isolation of such substances in high purity [8–11] and chemical characterization, respectively [12], have not yet been carried out.

Here we want to report on the isolation and properties of a human pituitary peptide which resembles the β-LPH mentioned in both chemical and biological respect.

Pituitaries removed from cadavers were collected in cold acetone. As the first step, 600 whole glands were extracted at 0°C, after homogenization, with 3.0 l acid acetone including the amounts of acetone used for immersion after the autopsy. (The acetone concentration was made up to 65 per cent containing 0.35 N hydrochloric acid.) The preparative steps following next were identical with those in the procedure of Li et al. [4] for the isolation of fraction D′. Chromatography of the fraction D′ on carboxymethyl-cellulose (CMC) is shown in fig. 1. Of the two fractions found to be the most active on rabbit adipose tissue, fraction 4, lacking of adrenocorticotropic activity, was rechromatographed on carboxymethyl-(CM) Sephadex (C-25) column (fig. 2). It can be seen that the lipolytic activity is concentrated in a small peak. Our observations have shown the quantity of the active component to decrease progressively during the preparative steps and rechromatography (the column chromatographic separations were conducted at room temperature). The first peak obtained from the CM-Sephadex column is also assumed to contain peptides undergone alteration during two consecutive separation steps (e.g. oxidized methionine residues). The polyacrylamide-gel electropherogram of the retarded active component is shown in fig. 3, compared with the starting fraction D′. This active peptide proved to be homogeneous also according to electrophoresis in acid medium. Its yield from 600 pituitaries in lyophilized state amounted to 3.5 mg. When run on a G-50 or G-75 Sephadex Superfine layer in 0.1 N acetic acid and 0.05 M ammonium carbonate, respectively,

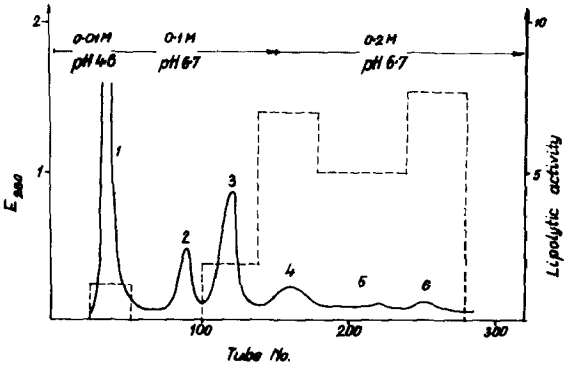

Fig. 1. Chromatography of 950 mg human pituitary concentrate, fraction D′ on CM-cellulose column (60 cm × 2.2 cm); 5 ml per tube; flow rate 60 ml/hour. The column was initially equilibrated with 0.01 M ammonium acetate, pH 4.6. After placing the material on the column a gradient elution was performed by introducing 0.1 M ammonium acetate, pH 6.7 through a 500 ml mixing flask containing the starting buffer. At tube 150 0.2 M ammonium acetate, pH 6.7 was substituted as the buffer flowed through the mixing flask. Tubes belonging to particular fractions were pooled and lyophilized. The solid line (left ordinate) shows the absorbance of the effluent at 280 nm, and the dashed line the lipolytic activities of each fraction as measured *in vitro* on rabbit adipose tissue at a peptide concentration 1 μg/ml and expressed as μeq FFA/g.h.

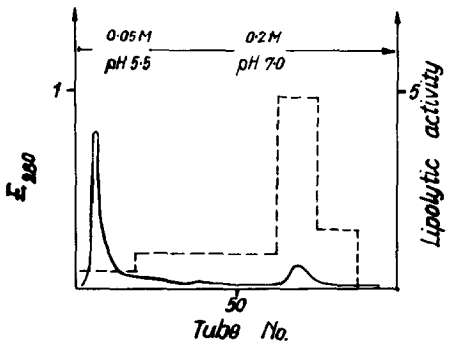

Fig. 2. Chromatography of 20 mg of peak 4 obtained from CM-cellulose column on CM-Sephadex column (50 cm × 1 cm), initially equilibrated with 0.05 M ammonium acetate, pH 5.5. Elution was performed by introducing 0.2 M ammonium acetate buffer, pH 7.0 through 500 ml mixing flask, containing starting buffer. The dashed line (right ordinate) shows the amounts of FFA liberated during 1 hour from 1 g rabbit adipose tissue by 0.1 μg of each lyophilized fraction.

it was subject to a retention equal to that of pig β-LPH (for the procedure see Morris [13]). The molecular weight of the pig β-LPH was shown to be about 11,000 in the sedimentation analysis [6]. Taken into consideration this value and the Try/Tyr ratio 1:3 its amino acid composition is assumed to be as follows: Lys_9 His_2 Arg_5 Asp_6 Thr_4 Ser_5 Glu_{13} Pro_6 Gly_{11} Ala_8 Val_3 Met_2 $Ileu_1$ Leu_8 Tyr_3 Phe_3 Try_1. (The amino acid analysis was performed by a Unichrom Autoanalyzer in Dr. Dévényi's Laboratory, Biochemical Institute, Hungarian Academy of Sciences, Budapest.)

Its lipolytic activity on rabbit retroperitoneal adipose tissue in Krebs-Ringer medium (phosphate) was shown to be 5×10^{-3} μg as given by the minimal effective dose, while that of pig ACTA (Ferring, 84s.c. Sayers U/mg) was 4×10^{-3} μg. Under similar circumstances it did not display any activity at levels less than 10 μg on rat epididymal adipose tissue. Its minimal effective dose on human subcutaneous adipose tissue (surgical specimens) when measured in Krebs-Ringer medium (bicarbonate) containing 5% human albumin proved to be 1×10^{-1} μg. When administered intravenously to rabbits at a dose of 10 μg/kg, it increased the plasma free fatty acid level within 30 min from 400 to 1150 μeq/l. (Tested by Dr. G.Tamási, Research Institute for Pharmaceutical Chemistry, Budapest.) Its *in vivo* steroidogenic activity [14] was 0.02

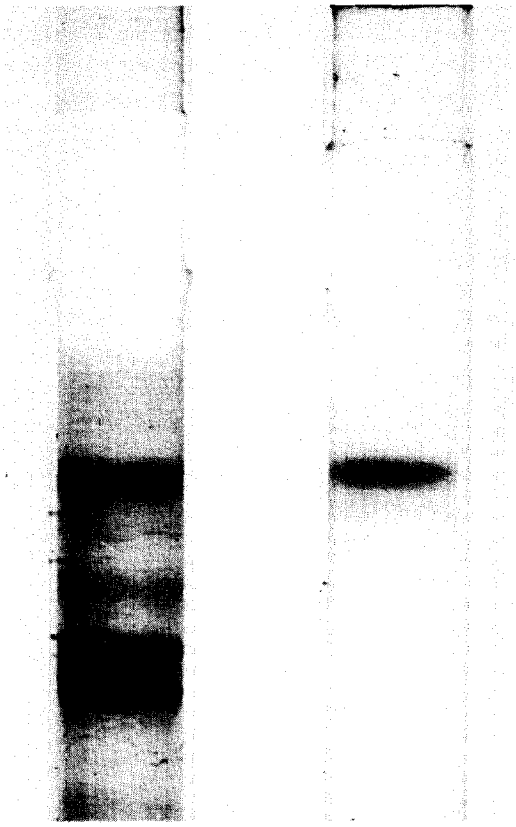

Fig. 3. Disc electrophoresis in 10 per cent gel of human D′ fraction (left) and the purified lipotropin (right) obtained from the CM-Sephadex column. 150 μg and 50 μg, respectively, amounts of substances were polymerized in the sample gel. The electrophoresis was performed at pH 8.5 using a potential gradient 50 V/cm for 25 min. Migration toward anode.

IU/mg. (Measured by Dr. G.Hajós, G.Richter Ltd., Budapest.) The prolactin activity amounted no more than 0.05 IU/mg. (Data from Dr. M.Kurcz, National Institute of Health, Budapest.)

This newly isolated peptide is apparently different from the DLMF-1 peptide of Trygstad recently described [12], both in its molecular weight and lipolytic activity on rabbit and human adipose tissue, respectively, *in vitro*. On the other hand, it may be considered as a human analogue of β-LPH on the basis of its isolation behaviour, molecular size, electrophoretic mobility and effects on adipose tissue of

various species. The lipolytic activity of this peptide on human adipose tissue exceeds that of pig β-LPH [15].

Further investigations are in progress to decide to which sites in the polypeptide chain can be assigned the differences found in amino acid composition as opposed to animal LPH's, furthermore, which role can be attributed to these differences in the species specificity of the biological action.

References

[1] K.J.Anselmino and F.Hoffmann, Klin. Wschr. 10 (1931) 2380.
[2] D.Rudman, F.Seidman and M.B.Reid, Proc. Soc. Exp. Biol. (N.Y.) 103 (1960) 315.
[3] E.B.Astwood, R.J.Barrett and H.Friesen, Proc. Natl. Acad. Sci. U.S. 47 (1961) 1525.
[4] C.H.Li, L.Barnafi, M.Chrétien and D.Chung, Nature 208 (1965) 1093.
[5] P.Lohmar and C.H.Li, Biochim. Biophys. Acta 147 (1967) 383.
[6] L.Gráf and G.Cseh, Acta Biochim. Biophys. (Hung.) 3 (1968) 175.
[7] M.Chrétien and C.H.Li, Can. J. Biochem. 45 (1967) 1163.
[8] H.Friesen, Metabolism 13 (1964) 1214.
[9] L.Kádas and D.Nagy, Endokrinologia 48 (1965) 19.
[10] T.W.Burns, C.N.Hales and A.Stockell Hartree, J. Endocr. 39 (1967) 213.
[11] O.Trygstad, Acta endocr. (Kbh.) 57 (1968) 81.
[12] O.Trygstad, Acta endocr. (Kbh.) 58 (1968) 1.
[13] C.J.R.R.J.Morris, J. Chrom. 16 (1964) 167.
[14] H.D.Purves and N.E.Sirott, Endocrinology 77 (1965) 366.
[15] G.Cseh and L.Gráf, in: Protein and Polypeptide Hormones, Vol. 2, ed. M.Margoulies (Excerpta Medica, 1968) p. 498.