TWO ACTH SPECIES IN RAT PITUITARY GLAND

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1. Introduction

In recent times heterogeneity of nearly all known peptide hormones in endocrine glands, tumors and plasma has been reported [1–10]. An immunoreactive ACTH-component, considerably larger than the well-characterized 1–39 ACTH peptide, has been identified in human plasma and human pituitary gland extracts and because of its conversion to authentic ACTH by tryptic digestion, the substance is supposed to be a corticotrophin precursor [11]. In a cloned murine pituitary tumor cell line and in normal mouse pituitaries, two ACTH moieties have been demonstrated, one of which represented 1–39 ACTH, while the other one had a molecular weight of about 7800 [12].

In preliminary experiments dealing with the biosynthesis of rat ACTH we found in addition to the well-known corticotrophic hormone, which corresponds to the 1–39 amino acid peptide, a second component with similar immunological and biological properties. The molecular weight of this substance differed only slightly from the normal ACTH and was estimated between 5000–6000.

2. Material and methods

Male albino rats 200–300 g body weight (strain FW 49/Kirchberg/Lemgo/Biberach) were sacrificed by decapitation. The anterior pituitary was removed immediately after separation from the posterior lobe and either processed or frozen at –20°C. Usually 10–20 glands were combined for extraction and grounded with an automatically driven Teflon pestle in 0.1 N HCl. After freezing and thawing, the homogenates were centrifuged at 30 000 *g* for 30 min under refrigeration. Aliquots of the supernatant were supplied to a Bio Gel P6 column 90 × 1.5 cm (Bio Rad Laboratories, Richmond, California, U.S.A.) and elution was performed with 0.1 N HCl containing 1% bovine serum albumin by descending flow rate of about 20 ml/hr. Dextran blue 2000 and [^{3}H]-phenylalanine was added simultaneously to mark void volume and salt peak, respectively. Fractions of 1.3 ml were collected and frozen until assays were carried out. In two cases the extracts subjected to gel filtration contained 8 M urea. ACTH was assayed biologically according to the isolated adrenal cell technique of Sayers et al. [13]. Samples were neutralized with 0.1 N NaOH and at least two doses of every sample were compared with 6 doses of standard ACTH (Isactid, Ferring, Malmo, 150 I.U./mg). Each dose was given in duplicate.

Assays of immunoreactivity were performed using methods previously reported from our laboratory [14]. Highly purified porcine ACTH was used for iodination and as reference standard. (Isactid, Ferring, further purified by Dr. Schleyer, Ulm).

3. Results and discussion

In order to determine, whether or not the adrenocorticotrophic activity of the pituitary gland extracts was due to a single molecular species or the results of different ACTH moieties, the extracts were subjected to molecular sieving on Bio Gel P6, a gel material having an operating range from 1000–6000 mol.wt. The column has been calibrated previously

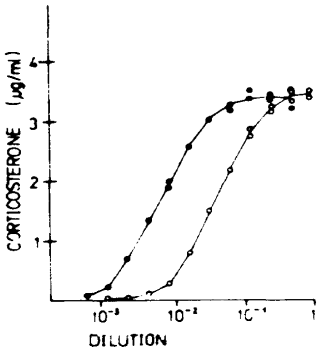

Fig. 3. Corticosterone production of isolated adrenal cells vs log dose of several dilutions of ACTH-bioactivity of peak I (●) and II (○) from original fractionation demonstrated in fig. 1b.

cannot be provided. From gel filtration characteristics a mol.wt. of 5000–6000 is suggested. However, the anomalous behaviour of peptides on gel filtration has to be considered in assigning the molecular weight on this basis.

The nature and function of the peptides are still unclear. We have found that it is not disaggregated on gel filtration in the presence of extreme acid pH or 8 M urea and it therefore seems unlikely to represent an aggregate of ACTH. It might be a true ACTH precursor or the intermediate product of a step in ACTH biosynthesis, released from a 'Big ACTH' by proteolytic enzymes and subsequently converted into 'Small ACTH'. An alternative explanation is that the rat synthesizes two different ACTH's, one of which is larger than the various types of ACTH thus far encountered in other species. Investigations in these directions are in progress.

Acknowledgements

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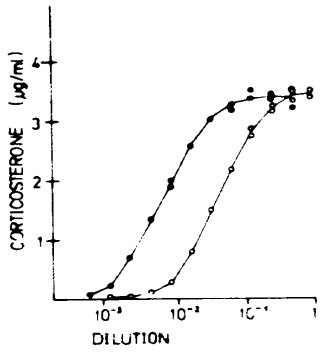
References

[1] Berson, S.A. and Yalow, R.S. (1968) J. Clin. Endocrinol. Metab. 28, 1037.
[2] Steiner, D.F., Cunningham, D., Spiegelman, L. and Aten, B. (1967) Science 157, 697.
[3] Roth, J., Gordon, P. and Pastan, I. (1968) Proc. Natl. Acad. Sci. U.S. 61, 138.
[4] Yalow, R.S. and Berson, S.A. (1970) Gastroenterology 58, 609.
[5] Bala, R.M., Ferguson, K.A. and Beck, J.C. (1970) Endocrinology 87, 506.
[6] Berson, S.A. and Yalow, R.S. (1971) in: Les Adenoms Hypophysaires Secretanes: Endocrinopathies et Immunologie, 239, Masson et Cie, Paris.
[7] Yalow, R.S. and Berson, S.A. (1971) Biochem. Biophys. Res. Commun. 44, 439.
[8] Habener, J.F., Kemper, B., Potts, Jr., J.T. and Rich, A. (1972) Science 178, 630.
[9] Hellerstrom, C., Howell, S.L., Edwards, J.C. and Andersson, A. (1972) FEBS Letters 27, 97.
[10] O'Connor, K.J., Gay, A. and Lazarus, N.R. (1973) Biochem. J. 134, 473.
[11] Yalow, R.S. and Berson, S.A. (1973) J. Clin. Endocrinol. Metab. 36, 415.
[12] Orth, D.N., Nicholson, W.E., Shapiro, M. and Byyny, R. (1970) Program of Endocrine Soc., 52nd Meeting, St. Louis, 140 (Abstract).
[13] Sayers, G., Swallow, R.L. and Giordano, N.D. (1971) Endocrinology 88, 1063.
[14] Voigt, K.H., Fehm, H.L. and Peiffer, E.F. (1971) Horm. Metab. Res. 3, 313–317.
[15] Schwyzer, R., Schiller, P., Seelig, S. and Sayers, G. (1971) FEBS Letters 19, 229.



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[3] Roth, J., Gordon, P. and Pastan, I. (1968) Proc. Natl. Acad. Sci. U.S. 61, 138.
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[5] Bala, R.M., Ferguson, K.A. and Beck, J.C. (1970) Endocrinology 87, 506.
[6] Berson, S.A. and Yalow, R.S. (1971) in: Les Adenoms Hypophysaires Secretanes: Endocrinopathies et Immunologie, 239, Masson et Cie, Paris.
[7] Yalow, R.S. and Berson, S.A. (1971) Biochem. Biophys. Res. Commun. 44, 439.
[8] Habener, J.F., Kemper, B., Potts, Jr., J.T. and Rich, A. (1972) Science 178, 630.
[9] Hellerstrom, C., Howell, S.L., Edwards, J.C. and Andersson, A. (1972) FEBS Letters 27, 97.
[10] O'Connor, K.J., Gay, A. and Lazarus, N.R. (1973) Biochem. J. 134, 473.
[11] Yalow, P.S. and Berson, S.A. (1973) J. Clin. Endocrinol. Metab. 36, 415.
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[13] Sayers, G., Swallow, R.L. and Giorcano, N.D. (1971) Endocrinology 88, 1063.
[14] Voigt, K.H., Fehm, H.L. and Peiffer, E.F. (1971) Horm. Metab. Res. 3, 313–317.
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