SIZE HETEROGENEITY OF IMMUNOREACTIVE HUMAN ACTH IN PLASMA AND IN EXTRACTS OF PITUITARY GLANDS AND ACTH-PRODUCING THYMOMA

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Received May 27, 1971

SUMMARY: Immunoreactive ACTH in plasma and in extracts of pituitary glands and ACTH-producing thymoma was demonstrated using Sephadex gel filtration and separatory ultracentrifugation to be composed of at least two major components, one ACTH-like and the other of considerably greater molecular size. The relative distribution between the two major components varied greatly among plasmas. It is concluded that the larger molecular weight fraction originates in the pituitary and represents ACTH bound in covalent linkage to a larger peptide.

Heterogeneity of immunochemical reactivity has been reported for plasma and glandular parathyroid hormone (1). Size heterogeneity has been reported for pancreatic insulin (2), plasma insulin (3), pancreatic glucagon (4) and plasma growth hormone (5), and size and charge heterogeneity has been reported for plasma and tissue gastrins (6,7,8). In the present study, we present evidence for the existence, in plasma and in extracts of pituitary glands and an ACTH-producing thymoma, of immunoreactive ACTH components of larger molecular size than ACTH 1-39, as well as of components resembling ACTH.

METHODS

Plasma samples from patients with Nelson's syndrome, Addison's disease, and Cushing's syndrome due to ectopic ACTH production and from a normal subject stimulated with metyrapone, purified human ACTH and extracts of human pituitary glands and an ACTH-producing thymoma were subjected to gel filtration on Sephadex G50, fine, columns, 1 cm x 50 cm. Columns were equilibrated with hormone-free plasma (outdated blood bank plasma without detectable immunoreactive ACTH) diluted 1:10 in veronal buffer 0.02 M, pH 8.4, containing 0.5% mercapto-

ethanol. One half or one ml of plasma or of hormone-free plasma to which had been added a few microliters of purified hormone or extract was added to the column and eluted with the same plasma-buffer mixture used to equilibrate the column. All samples contained also ^{131}Iodide, ^{125}I-human ACTH and bromphenol blue marker for albumin or ^{131}I-human serum albumin. One ml eluates were collected and counted for radioactivity and their ACTH contents determined by radioimmunoassay (9) using ^{125}I labeled Upton-Lerner human ACTH 8B which was also employed as standard. All standards, plasmas, extracts and eluates were assayed in hormone-free plasma diluted 1:10 in the veronal-mercaptoethanol buffer.

Some plasma samples (with or without additions) were diluted with equal parts of normal saline solution containing 0.5% mercaptoethanol and subjected to ultracentrifugation for 17 hours at 4°C and 37000 rpm in a Spinco Model L fitted with a bucket rotor (SW50.1) containing 6 tubes. After the centrifuge came to rest without braking, portions were removed from different levels of each tube for counting and radioimmunoassay of ACTH. The principles and methods are those described previously for insulin (10) except for the substitution of tubes of 0.5 ml capacity and the removal of four successive 100 microliter portions from the top down.

RESULTS

On gel filtration recoveries ranged from 70 to 100% of immunoreactive ACTH placed on column. Upton-Lerner ACTH emerged as a single component in an elution volume consistent with its molecular weight of 4500 but a standard acetone powder of pooled human pituitary glands and acetone-acetic acid extracts of an ACTH-producing thymoma and of a single human pituitary obtained post-mortem contained larger molecular weight components emerging principally near serum albumin (Fig. 1). On refractionation, the ACTH-like peak ran true (although there was some zone spreading) and the big molecular weight component showed only slight conversion (Fig. 2).

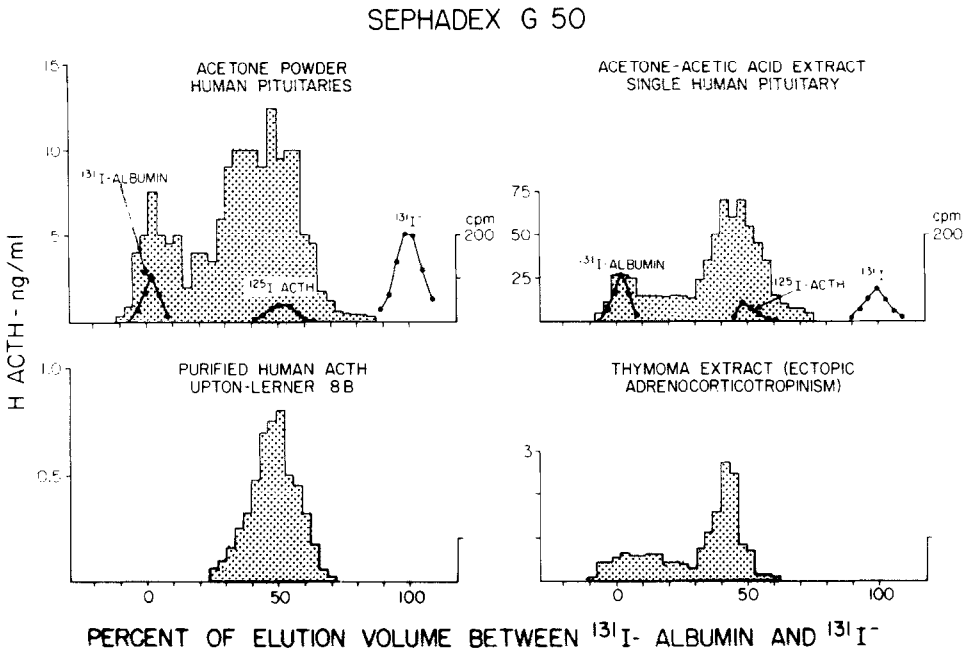

Fig. 1. Sephadex G50 gel fractionation of immunoreactive ACTH in tissue extracts.

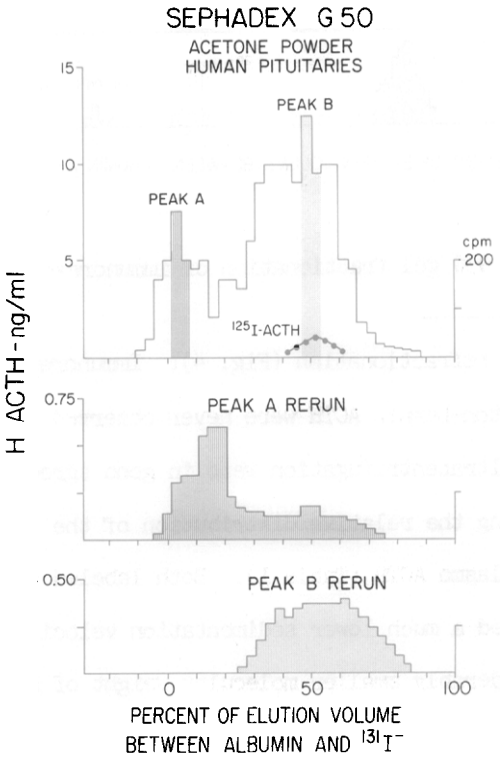

Fig. 2. Refractionation on Sephadex G50 of both immunoreactive components in acetone powder extracts of human pituitaries.

Similar components of endogenous plasma hormone were also observed but there was great variation in the relative distribution between the two components, apparently unrelated to the ACTH concentration (Fig. 3). Each of the plasma

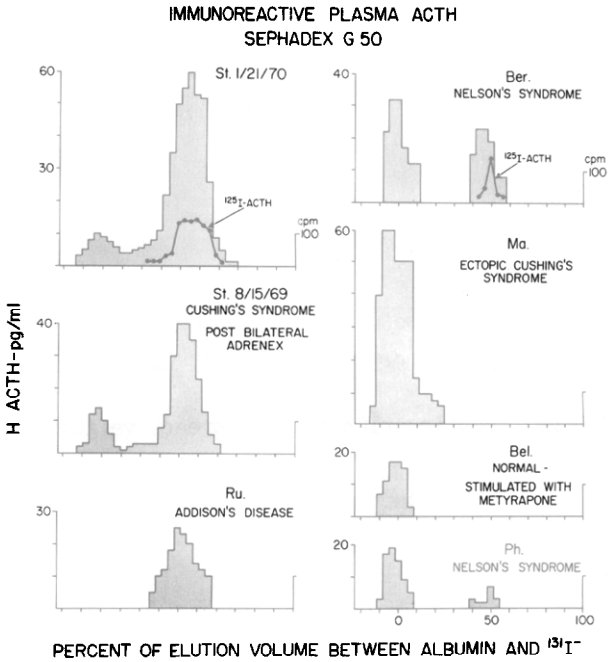

Fig. 3. Sephadex G50 gel fractionation of immunoreactive plasma ACTH.

components ran true on refractionation (Fig. 4). Immunoreactive components emerging later than Upton-Lerner ACTH were never observed.

Results of ultracentrifugation were in good agreement with those of gel filtration regarding the relative distribution of the two principal molecular weight components of plasma ACTH (Table I). Both labeled and unlabeled purified Upton-Lerner ACTH showed a much lower sedimentation velocity than albumin consistent with the considerably smaller molecular weight of ACTH. The sedimentation velocities of immunoreactive ACTH in the plasmas of patients St and Ru, whose gel filtration patterns showed the ACTH-like component to predominate, were similar to that of Upton-Lerner ACTH but much greater sedimentation velocities were shown

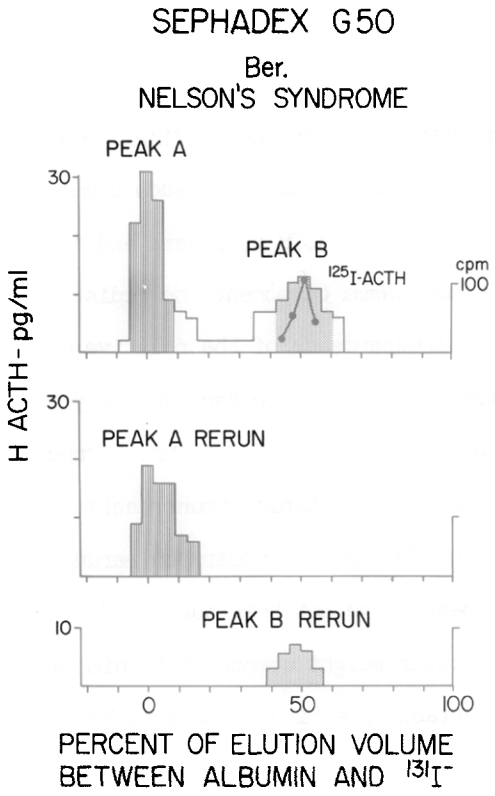

Fig. 4. New fractionation on Sephadex G50 of same sample of plasma from patient Ber shown in Fig. 3 and refractionation of both immunoreactive components.

TABLE 1

CONCENTRATION OF RADIOACTIVITY OR IMMUNOREACTIVE ACTH AT VARIOUS LEVELS IN TUBE AFTER ULTRACENTRIFUGATION EXPRESSED AS PERCENT OF CONCENTRATION IN UNCENTRIFUGED MIXTURE

	^{131}I	^{125}I	Unlabeled	PLASMA ACTH			
	Alb.	ACTH	ACTH	Ru	St 8/15	Ma	Ph
Top 0.1 ml	3	51	70	70	75	17	30
2nd 0.1 ml	25	77	70	80	95	58	60
3rd 0.1 ml	86	92	85	80	95	58	60
4th 0.1 ml	92	99	85	90	95	83	80

by the immunoreactive ACTH in the plasma of patient Ma, in which only the big molecular component was evident on gel filtration, and in the plasma of patient Ph, in which both components were represented.

DISCUSSION

All results indicate that the larger molecular weight component of immunoreactive ACTH is not formed by the binding, in vitro or in vivo, of the ACTH-like component to a plasma protein; no such binding is observed after the addition of unlabeled or labeled ACTH to plasma and there is no evident re-equilibration of the endogenous plasma components on redistribution. For these reasons and also because of the independence of the relative distribution of the two components of concentration, it is also very unlikely that the big molecular weight component is an artifact of the procedures especially since plasma samples from normal subjects show no detectable immunoreactivity in any of the eluates and, as already noted, no "big ACTH" appears on rerun of the ACTH-like peaks. Although the individual peaks have not been subjected to bioassay there is little doubt that the big molecular weight component is biologically active since it comprised the only detectable fraction in patient Ma who was suffering from a well established severe ectopic Cushing's Syndrome (bronchogenic carcinoma) and in normal subject Bel after metyrapone, who showed a normal increase in urinary excretion of 17 ketosteroids. The presence of similar components in plasma and in pituitary and tumor extracts suggests that the hormone is produced at and secreted from its site of origin in at least two forms, the larger probably representing ACTH covalently linked to another peptide originating at or near the site of ACTH synthesis.

ACKNOWLEDGEMENTS

We are indebted to Drs. A. Lerner, V. Upton, T. Lee, B. Pimstone, D. Krieger, L. Gabrilove, J. F. Garcia and J. M. Earll for providing hormone, tissue extracts or plasma samples used in these studies.

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