PROTEIN AND POLYPEPTIDE HORMONES

PROCEEDINGS OF THE INTERNATIONAL SYMPOSIUM
LIÈGE, MAY 19-25, 1968

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Liège

1969

EXCERPTA MEDICA FOUNDATION
Amsterdam

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Addendum

ON THE ADRENAL SUBCELLULAR DISTRIBUTION OF ^{3}H-ACTH*

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Introduction

Studies performed on the biochemical mechanism of stimulation of adrenal protein synthesis (Scriba and Reddy, 1965) revealed that after ACTH treatment of rats a protein factor of the soluble cell fraction from the adrenals (105,000 × g supernatant) was doubled in activity. This factor was shown to be non-dialysable, heat labile (55°C), inactivated by trypsin and rate limiting for ^{14}C-glycine incorporation by the 15,000 × g soluble cell fraction of adrenal homogenates (Farese and Reddy, 1963; Scriba and Reddy, 1965) and by adrenal polysomes (Scriba and Fries, 1967). This protein factor is probably aminoacyltransferase (Scriba and Reddy, 1965). The stimulation by ACTH *in vivo* of the adrenal protein factor, rate limiting adrenal protein synthesis *in vitro* and the stimulation of corticosterone synthesis *in vivo* appeared to be dissociable 30 hours and 8-14 days after hypophysectomy (Scriba, Fries and Kluge, 1967). Such dissociation after hypophysectomy has been reported for other adrenal effects of ACTH (Harding and Nelson, 1964; Staehelin, Barthe and Desaulles, 1965). With regard to mechanisms of actions of ACTH, it therefore appeared attractive to leave the investigations of events in the adrenal following ACTH and to turn to the problem of the subcellular distribution of ^{3}H-ACTH in the adrenal. Information about adrenal receptor(s) for ACTH might possibly be obtained from such studies.

Methods

Synthetic β^{1-23}corticotropin-23-amide-acetate catalytically labelled with tritium was generously supplied by Farbwerke Hoechst AG. The material was purified using dextran gel filtration (v. Werder, Schwarz and Scriba, 1968) resulting in parallel elution of radioactivity and biological activity upon rechromatography. Purified ^{3}H-ACTH, 300-450 mU ≡ 3-4.5 µg ≡ 1.1-1.65 µCi, in 1.0 ml saline (0.025 N HCl) was infused for 95 sec. into the thoracic aorta of rats 90 min. after hypophysectomy. Corticosterone increment in adrenal vein plasma was assayed fluorimetrically (Scriba *et al.*, 1966), and found to be maximal from 4 to 11 min. after the start of the ^{3}H-ACTH infusion. At times indicated (Table I) both adrenals were removed, carefully cleaned and homogenized for 3 × 5 sec. (Potter-Elvehjem) at 26 mg/ml 0.25 M sucrose-electrolyte solution (Scriba and Reddy, 1965) within 5 min. Preparation of subcellular fractions was achieved by differential centrifugation (Schneider and Hogeboom, 1951) of homogenates: supernatants: nuclear fraction (15 min. × 1,000 × g sed.), mitochondrial fraction (10 min. × 15,000 × g sed.), microsomal fraction (90 min. × 105,000 × g sed.) and soluble cell fraction (SN). Nuclear fractions, averaging 54% of the total adrenal weight, contained 76% 'intact' nuclei, 7% ruptured nuclei, 14% nuclei with adherent cellular material, 3% erythrocytes and practically no unbroken cells.

* Supported by Deutsche Forschungsgemeinschaft and Studienstiftung des Deutschen Volkes.

TABLE I

Adrenal subcellular distribution of ^{3}H-ACTH
a. Activity (CPM) of subcellular fractions given as
b. % of total adrenal activity. Results given as mean ±S.E.

Minutes after start of ^{3}H-ACTH infusion			3	12	40	120
Number of experiments (N)			19	17	17	17
Total activity (CPM) per pair of adrenals			407.8±54.0	337.6±78.0	215.7±31.8	220.0±18.4
Nuclear fraction	*a.*	CPM	105.3±13.6	84.1±12.9	85.4±16.8	78.5± 5.5
	b.	%	27.37±2.38	30.86±2.42	37.95±2.05	37.41±2.41
Mitochondrial fraction	*a.*	CPM	48.2± 9.3	52 1±10.8	35.0± 5.6	17.7± 2.5
	b.	%	13.69±3.04	17.01±1.89	16.54±1.63	8.49±1.16
Microsomal fraction	*a.*	CPM	77.2±18.5	38.7± 7.1	16.9± 2.1	6.9± 1.1
	b.	%	17.93±3.13	12.97±0.84	8.85±1.14	3.39±0.54
Soluble cell fraction	*a.*	CPM	177.2±39.5	162.7±58.2	78.4±14.2	116.8±16.3
	b.	%	41.02±4.21	39.15±3.32	36.67±2.68	50.71±3.03

Colorimetric determination (Scriba and Reddy, 1965) showed an RNA/DNA ratio (Siebert, 1966) of 0.52, and no detectable DNA in the 1,000×g SN. Subcellular fractions were solubilized with NCS and analyzed by liquid scintillation counting at 24% efficiency using the I.S. method for quench correction.

Results

Distribution of adrenal radioactivity was first studied 3 min. after the beginning of ^{3}H-ACTH infusion, corticosterone release into adrenal vein being already stimulated at this time. Subsequently, 12, 40 and 120 min. intervals were chosen, corticosterone release being maximally stimulated at 12 and still elevated at 120 min. The yield of radioactivity in both adrenals, calculated as % of infused radioactive ACTH, was remarkably low and showed a continuous decline (0.134-0.034%) with time. Surprisingly, the actual activities (CPM) per pair of adrenals remained constant from 40-120 min., indicating prolonged retention of the label from ^{3}H-ACTH.

In *nuclear* fractions a remarkably large amount of radioactivity was found. The possibility that the radioactivity in the nuclear fraction (54% of adrenal weight) was due to contamination with soluble cell fraction can be excluded, since the latter was diluted approx. 1:50 during the homogenization-centrifugation procedure. Initial (3 min.) radioactivity in the nuclear fraction may be falsely high due to microsomal contaminations, the radioactivity of the latter however decreased significantly with time. Nuclear radioactivity (CPM) was constant from 12-120 min., but showed a relative (%) increase with time. The % values at 40 and 120 min. were significantly higher than at 3 min. ($p<0.0025$ and $p<0.05$, respectively).

Mitochondrial radioactivity decreased continuously from 12-120 min. ($p<0.0025$) both absolutely (CPM) and relatively (%). The same decrease was found in the *microsomal* fraction (3 vs. 12 min.: $p<0.05$; 12 vs. 40 min.: $p<0.005$; 40 vs. 120 min.: $p<0.0005$). Microsomal radioactivity was surprisingly high at 3 min. Since microsomal material may well be lost to nuclear and mitochondrial fractions, microsomal radioactivity appears to be at this time in excess of microsomal contribution to cell volume. The decline of radioactivity (CPM) in the *soluble cell fraction* parallels the decrease of total adrenal radioactivity and probably represents removal of label from the adrenals. The relative radioactivity (%) remained there-

fore approximately constant from 3-40 min. Slight increases ($p<0.05$) of radioactivity (CPM) in soluble cell fractions at 120 min. may be due to decreases in microsomal and mitochondrial counts.

Discussion

The results present, among others, the following unanswered questions:

1. Is the radioactivity found in adrenal cell fractions (e.g. nuclei) still part of ^{3}H-ACTH or of fragment(s) of ACTH?
2. What is the zonal distribution of ^{3}H-ACTH throughout the adrenal cortex?
3. What is the relation of binding sites and of site(s) of action of ^{3}H-ACTH?
4. Are there more than one adrenal cellular receptor for ACTH and what is their nature?

Summary

Subcellular distribution of ^{3}H-ACTH in the adrenals after infusion for 95 sec. in hypophysectomized rats, was studied 3, 12, 40 and 120 min. after the start of the infusion, using homogenization and differential centrifugation. Considerable amounts of radioactivity (CPM) were still found 40 and 120 min. after ^{3}H-ACTH infusion in nuclear fractions. Nuclear radioactivity (%) increased relatively from 3-40 min. A comparably large amount of radioactivity was detected 3 min. after the start of the ^{3}H-ACTH infusion in the microsomal fraction. The results are to be discussed in relation to mechanisms and sites of actions of ACTH.

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