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CONTROL OF ADRENAL MEDULLARY PROTEIN SYNTHESIS BY CORTICOSTEROIDS

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SUMMARY

It has previously been shown that hypophysectomy decreases the activity of the adrenal medullary enzyme, phenylethanolamine *N*-methyltransferase, which catalyzes the conversion of norepinephrine to epinephrine. This effect and the consequential decrease in adrenal epinephrine content and secretion can be reversed by treatment with adrenocorticotrophic hormone (ACTH) or glucocorticoids. This report examines the effect of glucocorticoids on adrenomedullary protein synthesis in general. Hypophysectomy causes a profound disaggregation of the polysomes in the rat adrenal and decreases ability of adrenal ribosomes to incorporate amino acids into peptide chains *in vitro*. Both effects are restored by *in vivo* treatment with dexamethasone, a potent synthetic glucocorticoid. The reaggregation of adrenal polysomes precedes the phenylethanolamine *N*-methyltransferase activity.

INTRODUCTION

The conversion of norepinephrine to epinephrine in the mammalian adrenal medulla is catalyzed by the enzyme phenylethanolamine *N*-methyltransferase[1]. This enzyme is controlled by glucocorticoid hormones delivered to the medulla *via* the adrenocortical venous effluent[2]: when the rat[3,4] or dog[5] is hypophysectomized, adrenal phenylethanolamine *N*-methyltransferase activity declines, as do the levels of epinephrine within the medulla[5,6] and the amounts of catecholamine secreted basally or in response to insulin hypoglycemia[5]. These parameters of medullary function can be restored to normal by the administration of "replacement" doses of adrenocorticotrophic hormone (ACTH), or of very high doses of natural or synthetic glucocorticoids[4–6].

The present study is concerned with the effects of pituitary and adrenocortical hormones on adrenomedullary protein synthesis in general. It will be shown that

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Abbreviation: ACTH, adrenocorticotrophic hormone.

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hypophysectomy is associated with disaggregation of adrenal polyribosomes and a decrease in *in vitro* protein synthesis. Both reactions are normalized within several days of treatment with dexamethasone, a potent synthetic glucocorticoid.

MATERIALS AND METHODS

Chemicals

A ^{3}H-labeled amino acid mixture (relative proportions as found in typical algae protein hydrolysate) was purchased from the New England Nuclear Corp., Boston; dexamethasone was obtained from Star Pharmacy in Cambridge, Mass. All other chemicals used in our experiments were of reagent grade. Most of the solutions were mixed in Tris–$MgCl_2$–KCl buffer (50 mM Tris–HCl, pH 7.8, 25 mM KCl and 5 mM $MgCl_2$).

Experimental animals

Normal, hypophysectomized, and sham-operated Sprague–Dawley female rats (Charles River Laboratories, Wilmington, Mass.) weighing 150 g were used in all our experiments. The animals were kept in a light environment (Vita-Lite, Duro-Test Mfg., North Bergen, N. J.) for 12 h daily, and had access *ad libitum* to Big Red Lab Chow and water. Dexamethasone treatment was initiated (1.8 mg per rat) 14 days after hypophysectomy; control animals received an equal volume of saline. The animals were decapitated at various intervals after surgery or the start of hormone treatment, as indicated in the text.

Isolation of polysomes and cell sap enzymes

The medullas from seven rats were pooled and homogenized in 2 vol. of 0.37 M sucrose prepared in Tris–$MgCl_2$–KCl buffer, pH 7.8, with a 0.01-inch clearance Teflon homogenizer[7]. After the solution was centrifuged at $20\,000 \times g$ for 20 min, the sediment was discarded and the supernatant was treated with 1/10 vol. of 10 % deoxycholate and layered on a discontinuous sucrose gradient of 0.5 sucrose over 2 M sucrose prepared in Tris–$MgCl_2$–KCl buffer, pH 7.8. The discontinuous gradients were centrifuged at $105\,000 \times g$ for 4 h. The pellet obtained at the bottom of the tube (c-ribosomes) was suspended in 0.2 ml of Tris–$MgCl_2$–KCl buffer and stored at -40 °C.

To obtain the pH 5 enzyme fraction, we centrifuged the postmitochondrial supernatant (which was prepared as described above but not treated with deoxycholate) at $105\,000 \times g$ for 3 h; this filtered out the microsome, leaving a supernatant cell sap. The cell sap was then passed through a column of Sephadex G-25 that had been equilibrated with Tris–$MgCl_2$–KCl buffer at pH 7.6.

Determination of catecholamine content and phenylethanolamine N-methyltransferase activity

Tissues were homogenized in 3 vol. of 1 M KCl containing 10^{-4} M EDTA, using a Teflon homogenizer. An aliquot of the original homogenate was rehomogenized in a glass homogenizer with an equal volume of 0.8 M $HClO_4$. After centrifugation at $15\,000 \times g$ for 30 min, an aliquot of the supernatant fluid was taken to determine radioactive tyrosine[8]; catecholamines in the remaining supernatant fluid were

separated by chromatography on alumina columns[9], and assayed by the spectrophotofluorimetric method of von Euler and Lishajko[10]. Another aliquot of the original homogenate was centrifuged at $105\,000 \times g$, and portions of the resulting supernatant fluid were assayed for phenylethanolamine *N*-methyltransferase activity by the method of Axelrod[1] as modified by Wurtman *et al.*[11].

Amino acid incorporating system

The reaction mixture in total volume of 0.5 ml contained: 50 mM Tris–HCl, pH 7.8 (at 0 °C); 80 mM NH_4Cl, 5 mM $MgCl_2$, 2 mM ATP, 2 mM dithiothreitol, 0.5 mM GTP, 0.5 μCi generally ^{3}H-labeled L-amino acid mixture, 500 μg of enzyme protein (pH 5), and 500 μg of polysome protein. These amounts of enzyme and polysome protein have been shown to be conducive to incorporation of labeled amino acids into protein[7]. Incubation was carried out at 37 °C for 1 h; the residue left after treatment with hot trichloroacetic acid was measured with a Packard liquid scintillation spectrophotometer to determine the radioactivity incorporated into protein[7].

Polysome profiles

For the polysome profiles the same amount of protein either from the postmitochondrial supernatant or from c-ribosomes was layered over a linear gradient of 10–40 % sucrose in Tris–$MgCl_2$–KCl buffer[7]. The gradient was centrifuged at 38 000 rev./min in SW-50 rotor of the Spinco Model L2 Ultracentrifuge for 90 min, and the absorption profile for 260 nm was automatically recorded using a flow cell device in a Gilford Model 2000 spectrophotometer.

Estimation of protein

The protein content in tissue extracts containing enzymes and polysomes was determined by the method of Lowry *et al.*[12], with bovine serum albumin as the standard. Ribosome concentrations were computed from $A_{260\ \mathrm{nm}}$, assuming $E^{1\%}_{260\ \mathrm{nm}}$ to be 145.

RESULTS

Effects of hypophysectomy and dexamethasone on adrenal phenylethanolamine N-methyltransferase activity and epinephrine content

In confirmation of previous findings[2–4], adrenals of hypophysectomized rats contained low phenylethanolamine *N*-methyltransferase activity and 32 % less epinephrine content than adrenals from intact rats (Table I). An elevation of both could be seen 72 h after dexamethasone treatment.

Effect of hypophysectomy and dexamethasone on cell-free medullary protein synthesis

Polysomes isolated from adrenals of control, hypophysectomized or hypophysectomized–dexamethasone-treated rats were incubated with pH 5 enzyme preparation from the medullary cell cap of similar glands in the presence of ^{3}H-labeled amino acid mixture. Table II shows that there was considerable incorporation of ^{3}H into peptides in this system. The ribosomal and pH 5 enzyme activity derived from control rats (Tube 1) was 5-fold greater than when they were prepared from the medullas of hypophysectomized animals of the same age (Tube 2). When the control

TABLE I

EFFECTS OF HYPOPHYSECTOMY AND DEXAMETHASONE ON ADRENAL CATECHOLAMINES

Tissues from groups of 7 rats were taken 14 days after hypophysectomy and 72 h dexamethasone and assayed individually. Results are given as mean ± S.E. in all tables. PNMT, phenylethanolamine *N*-methyltransferase.

Treatment	*PNMT activity (units/gland per h)*	*Total catecholamine (μg/gland)*	*Percent epinephrine*
Control	3.34±0.91	19.03±0.4	91.5±2.1
Hypophysectomy	0.73±0.12	12.23±0.24	78.0±2.0
Hypophysectomy and dexamethasone	2.83±0.81	17.26±1.15	87.0±2.0

ribosomes were incubated with the pH 5 enzyme fraction from hypophysectomized animals (Tube 3), incorporation was less than that obtained with control enzyme fraction. In contrast, the pH 5 enzyme from control animals and the ribosomes of hypophysectomized rats (Tube 4) did not significantly improve incorporation above the levels obtained with enzyme harvested after hypophysectomy. Thus hypophysectomy mainly affects the ribosome, which appears to be the limiting factor for the impaired incorporation.

Ribosomes from hypophysectomized rats treated with dexamethasone showed some recovery of activity when incubated with pH 5 enzyme prepared from the same medullas (Tube 5) or from the medullas of hypophysectomized (Tube 6) or control rats (Tube 7). This implies partial restoration of ribosome activity by the hormone treatment. Dexamethasone also improved the capacity of the pH 5 enzyme fraction, as its addition to the control ribosomes resulted in full incorporation (Tube 8); but it failed to stimulate amino acid incorporation by ribosomes from hypophysectomized animals (Tube 9).

Effect of hypophysectomy on medullary polysome aggregation

In view of the preceding results showing that ribosomes from hypophysectomized rats are much less active in protein synthesis under cell-free conditions than ribosomes from normal rats, we examined the polyribosome distributions in c-ribosomes prepared from medullas of intact, hypophysectomized, and hypophysectomized–dexamethasone-treated rats. The polysomes from intact rats displayed the usual pattern of abundant heavy aggregates with some oligosomes and a peak of monosomes similar to the profiles obtained from other tissues actively synthesizing proteins (Fig. 1). Rats hypophysectomized one week before being killed displayed major changes in polyribosomal profiles: there were almost no large aggregates, but considerable peaks of monosomes and disomes. The loss of heavy aggregates was quite sufficient to account for the reduced capacity of ribosomes to incorporate amino acids in a cell-free system (Table II). The effect of dexamethasone in restoring cell-free amino acid incorporation (Table II) was paralleled by a reaggregation of polysomes in the medulla: 24 h after being hypophysectomized, rats received the synthetic corticosteroid there was little change in the adrenal polysome profiles; but at 36 h there was some reaggregation, and at 72 h considerable polysome aggregation, with a sharp reduction in the monosome population (Fig. 2). This finding was compatible

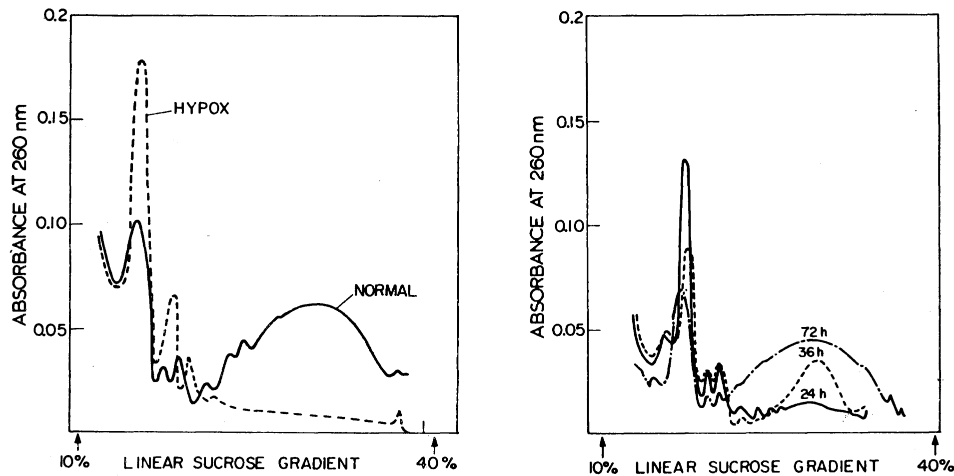

Fig. 1. Comparison of adrenal medullary polysome profiles of normal and hypophysectomized animals. The adrenals from four animals were pooled in each group and homogenized as described in Materials and Methods. The postmitochondrial supernatant was initially treated with deoxycholate and then diluted 1 : 3 with Tris–$MgCl_2$–KCl buffer. An aliquot of 0.1–0.2 ml was layered over 10–40 % linear sucrose gradient and centrifuged. Hypox, hypophysectomized.

Fig. 2. Effect of administration of a single dose of dexamethasone (1.6 mg per rat) on polysome profiles obtained from hypophysectomized animals. The postmitochondrial supernatants were prepared from a group of four hypophysectomized animals, each at 24, 36, and 72 h after dexamethasone treatment. They were treated with deoxycholate and the profiles were prepared as described in Fig. 1.

TABLE II

INCORPORATION OF ^{3}H-LABELED AMINO ACIDS BY ADRENAL POLYSOMES

The ribosomes and pH 5 enzyme in each group were prepared from pooled adrenals of seven rats. Each reaction mixture in 0.5 ml contained 50 mM Tris–HCl, pH 7.8, 80 mM NH_4Cl, 5 mM $MgCl_2$, 2 mM dithiothreitol, 0.5 mM GTP, 0.5 μCi ^{3}H-labeled L-amino acid mixture, 500 μg of pH 5 enzyme protein, and 500 μg of ribosomes. Incubations were carried out at 37 °C for 30 min. Each tube contained the pooled adrenals from 7 rats. Hypox = hyophysectomized; Dexa = dexamethasone.

Tube No.	*Ribosome*	*Enzyme*	*^{3}H-labeled amino acids incorporated (cpm)*
1	Control	Control	2170
2	Hypox	Hypox	420
3	Control	Hypox	1040
4	Hypox	Control	515
5	Dexa	Dexa	1370
6	Dexa	Hypox	1000
7	Dexa	Control	1540
8	Control	Dexa	2800
9	Hypox	Dexa	520

with the improved activity of ribosomes in the cell-free system, which was also tested for amino acid incorporation 72 h after hormone administration.

The time course for the increases in phenylethanolamine *N*-methyltransferase activity and polysome reaggregation after a single dose of dexamethasone are compared in Fig. 3. Polysome reaggregation, as indicated by an increasing polysome–monosome ratio, is evident at 24 h, about 50 % complete at 36 h, and is almost as great as in control animals (ratio = 1.9) by 72 h. In comparison, phenylethanolamine *N*-methyltransferase activity has not risen detectably by 24 h after dexamethasone, and increases slowly, thereafter remaining lower than in the glands of intact animals 72 h after dexamethasone (Fig. 3). This suggests that polysome reaggregation (and protein synthesis) precedes the increase in phenylethanolamine *N*-methyltransferase activity.

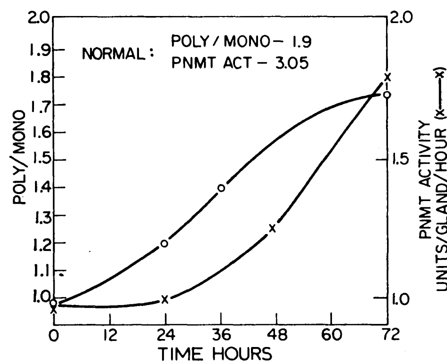

Fig. 3. Comparison of polysome/monosome ratio and phenylethanolamine *N*-methyltransferase (PNMT) activity of hypophysectomized animals 24, 36, 48, and 72 h after dexamethasone (1.6 mg/rat intraperitoneally) treatment. The normal polysome/monosome ratio is 1.9 and phenylethanolamine *N*-methyltransferase activity is 3.05 units per gland per h. Results are the mean average of three experiments.

DISCUSSION

On the basis of studies on actinomycin D or puromycin Wurtman and Axelrod[3] suggested that the stimulation of phenylethanolamine *N*-methyltransferase activity by glucocorticoids results from new enzyme synthesis. The experiments described here show that cell-free systems prepared from adrenals of hypophysectomized rats have a markedly reduced capacity to incorporate amino acids into peptides *in vitro*. This is largely the result of impaired ribosomal activity: the ribosome remains the rate-limiting factor for *in vitro* protein synthesis. Our observations also indicate that hypophysectomy causes an extensive but steroid-reversible disaggregation of adrenal polysomes. The poor *in vitro* performance of ribosomes from the medullas of hypophysectomized rats can thus be attributed to the lack of aggregated polysomes bearing incomplete peptide chains that can still be charged with amino acids. Regeneration of polysomes reverses this defect. The restoration of phenylethanolamine *N*-methyltransferase activity (and of adrenal epinephrine content) follows the course of polysome regeneration.

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