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EFFECT OF ALDOSTERONE ON RIBONUCLEIC ACID POLYMERASE ACTIVITY IN RAT KIDNEY CORTICAL AND MEDULLARY MITOCHONDRIA

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Abstract—The involvement of mitochondria in the mechanism of action of aldosterone is suggested by the ability of this hormone to alter the activity of certain mitochondrial enzymes. Since the mitochondrion is able to synthesize some of its own macromolecules, we investigated whether the mineralocorticoid effects mitochondrial RNA synthesis in isolated kidney mitochondria. Rat kidney mitochondria were purified and then separated into two populations by sucrose density gradient centrifugation; mitochondria sedimenting at 1.2 M sucrose were predominantly of medullary origin, while mitochondria sedimenting at 1.4 M sucrose were mainly from the cortex. RNA polymerase activity in medullary mitochondria was almost twice that of cortical mitochondria. The activity was characterized as α-amanitin resistant and rifampicin sensitive DNA-dependent RNA polymerase. Adrenalectomy resulted in a 40 per cent decrease in the RNA polymerase activity of cortical mitochondria 5 days later ($P < 0.001$), but no decrease occurred in medullary mitochondrial activity, compared to sham-operated controls. Three hr after aldosterone administration (2 μg/100 g body wt, i.p.) the RNA polymerase activities of medullary and cortical mitochondria were increased 20 and 50 per cent, respectively, above the adrenalectomized values ($P < 0.05$, $P < 0.001$). Spironolactone, an antagonist of aldosterone (7 mg/100 g body wt, i.p., 30 min prior to aldosterone administration), blocked the stimulation of RNA polymerase by aldosterone ($P < 0.05$). Steroid hormones other than aldosterone did not produce significant enhancement of mitochondrial RNA polymerase activity. No change in RNA polymerase activity occurred after direct addition of aldosterone or spironolactone to the assay medium. The results suggest that the mechanism of mineralocorticoid action is at least in part mediated through its effects on mitochondrially synthesized proteins.

The mechanism of aldosterone action on target tissues has been postulated to involve the synthesis of specific proteins [1–3]. Several lines of experimental evidence support this concept. Inhibitors of RNA and protein synthesis block the stimulation of sodium transport by aldosterone [4–6]. Aldosterone has been shown to stimulate nuclear transcriptional activity [7–9] and to increase the incorporation of nucleotides into cellular RNA [6, 10, 11] and of amino acids into tissue protein [6, 12]. However, more detailed information regarding the localization and the function of the proteins is needed to elucidate the connection between the biochemical effects and the physiological response induced by the hormone.

One of the hypotheses regarding the role of aldosterone-induced proteins is that these proteins stimulate oxidative metabolism, yielding an increased supply of energy-rich intermediates for sodium transport [2, 3]. Aldosterone has been shown to alter the redox state of pyridine nucleotides in kidney [13, 14] and to increase the activities of several citric acid cycle enzymes and the level of mitochondrial cytochromes [15–17]. It is not completely clear as yet whether the action of aldosterone at the mitochondrial level is the result of modulation of enzyme activity or of enhanced synthesis of particular mitochondrial enzymes, as in the case of citrate synthase [18]. Although the majority of mitochondrial protein is synthesized extramitochondrially, mitochondria synthesize specific subunits which are essential for the integration of cytoplasmically synthesized subunits into the mitochondrial inner membrane enzymes [19, 20]. In view of the rather short half-life of mRNAs in mitochondria [21] and of aldosterone-induced changes in mitochondrial enzymes, including those of the inner membrane [15–17], it is reasonable to inquire whether aldosterone may act through mitochondrial transcriptional and translational processes. In this report, we present evidence that aldosterone stimulates activity of renal mitochondrial RNA synthesis as indicated by the activity of mitochondrial RNA polymerase.

MATERIALS AND METHODS

Animals and treatment. Male Sprague–Dawley rats (150–200 g) were purchased from Charles River Breeding Laboratories Inc. (Wilmington, MA). Animals were maintained at 22 ± 1° with 12 hr of artificial light from 7:00 a.m. to 7:00 p.m. Purina Laboratory Chow (Ralston Purina Co., St. Louis, MO) was provided *ad lib.* Adrenalectomy and sham-operation, subsequent maintenance of adrenalectomized animals, and the saline preference test were

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the same as described elsewhere [17]. Animals were used 6 days after the operation. Adrenalectomized animals received an intraperitoneal injection of hormone solution (0.2 ml/100 g in saline–ethanol, 95:5, v/v) 3 hr before being killed, unless noted otherwise. Doses of the various hormones administered per 100 g body wt were: aldosterone, 2 μg; corticosterone, 2 mg; 17β-estradiol, 10 μg; hydrocortisone, 2 mg; progesterone, 10 μg; and testosterone, 2 mg. Spironolactone, 7 mg/100 g body wt in 0.2 ml of same vehicle, was given 30 min prior to aldosterone treatment. Sham-operated and adrenalectomized groups received the same volume of vehicle.

Materials. Non-radioactive ribonucleotide triphosphates were purchased from P.L. Biochemicals (Milwaukee, WI) and the ^{3}H-labelled ribonucleotide triphosphates were purchased from New England Nuclear (Boston, MA). Aldosterone was purchased from the Ciba Pharmaceutical Co. (Summit, NJ). Spironolactone, 17β-estradiol, corticosterone, progesterone, testosterone, hydrocortisone and pancreatic DNase and RNase were purchased from the Sigma Chemical Co. (St. Louis, MO). α-Amanitin and actinomycin D were purchased from Cal-Biochem (La Jolla, CA). Rifampicin, provided by Dr. S. T. Jacob, was from Gruppo Lepetit (Milan, Italy). RNase-free sucrose was obtained from Schwarz/Mann (Orangeburg, NY). To minimize possible RNase contamination, all glassware used in tissue preparation and assays was soaked overnight in 0.3 N NaOH, and 1 hr in 0.1 N HCl. Glassware was then rinsed in sequence with 0.1 N HCl, warm tap water, and finally with glass distilled water. It was then dried for 30 min at 210°.

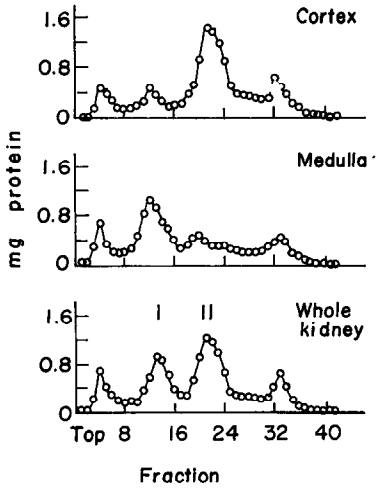

Fig. 1. Sucrose density gradient centrifugation of mitochondria from cortex, medulla and whole kidney. Separation of kidney cortex and medulla, preparation of mitochondria, and conditions of sucrose density gradient centrifugation are described in Materials and Methods. The amounts of mitochondrial protein layered onto each gradient were: cortex, 10.1 mg; medulla, 7.9 mg; and whole kidney, 10.7 mg. The amounts of mitochondria in each fraction are expressed as the amount of protein. The middle two peaks I and II are mitochondrial bands. The sucrose concentrations at the peak of I (medullary mitochondria) and II (cortical mitochondria) were 1.2 M and 1.4 M, respectively.

Preparation of cortical and medullary mitochondria by sucrose density gradient centrifugation. Animals were decapitated between 6:00 and 7:00 a.m. Kidneys were removed immediately and placed in ice-cold buffer A (0.25 M mannitol, 0.07 M sucrose, 0.001 M EDTA and 0.05 M Tris/HCl, pH 7.4). All subsequent steps were performed at 4°. The kidneys were decapsulated and total tissue mitochondria were isolated by the procedure described previously [22], except that tissue was homogenized in buffer A and mitochondria were washed in buffer B (0.25 M mannitol, 0.07 M sucrose and 0.05 M Tris/HCl, pH 7.4). The washed mitochondrial pellet was resuspended in buffer B to a final volume of 0.4 ml/g of initial wet tissue.

One milliliter volumes of mitochondrial suspension were layered over 12-ml 1.0–2.0 M linear sucrose density gradients prepared in buffer B and were centrifuged in a Beckman L5-65 ultracentrifuge at 39,000 r.p.m. for 35 min in an SW40 rotor. The mitochondria from whole kidney were distributed in two bands, located at the 1.2 M (Band I) and 1.4 M (Band II) sucrose regions of the gradients (Fig. 1). Bands I and II mitochondria were recovered separately either by fractionation of the entire gradient utilizing a Beckman fraction recovery system with an attached Gilson Minipuls 2 peristaltic pump, allowing 0.5 ml/fraction, or by puncturing the tube and withdrawing the individual bands with a syringe.

To determine how the mitochondria from cortex and medulla are distributed between Band I and Band II, we carried out experiments using mitochondria prepared simultaneously from the separated cortex and medulla [22]. Like whole kidney, both cortex and medulla produced two bands of mitochondria, but their relative distributions in Band I and Band II differed greatly (Fig. 1). In medulla, Band I mitochondria predominated (80 per cent of total mitochondria on a protein basis), while in cortex, Band II mitochondria comprised the major population (90 per cent). In subsequent experiments, the Band I mitochondria from whole kidney have been designated as medullary mitochondria and Band II mitochondria as cortical mitochondria.

Assays for mitochondrial enzymes. For the characterization of cortical and medullary mitochondria, the purified mitochondria were sonicated and the sonicates were assayed for various enzymes. The sonication was carried out using a Branson Sonifier (W140) operating at medium power for three 15-sec bursts, allowing 15 sec between bursts to dissipate heat. NADP-isocitrate dehydrogenase and malate dehydrogenase were assayed by the method of Cleland *et al.* [23] and Kitto [24] respectively. Alkaline ribonuclease was assayed according to the procedure described by Liu *et al.* [25]. Acid ribonuclease was assayed by a similar procedure in 0.05 M sodium acetate, pH 5.0.

RNA polymerase assay. The mitochondria that were recovered from sucrose gradient centrifugation were diluted with 3 vol. of 0.05 M Tris/HCl, pH 7.4, and centrifuged for 10 min at 9000 g. Mitochondria were swollen [26] and assayed in triplicate by a modification of procedures described by Barsano *et al.* [26] and Jacob *et al.* [27]. The incubation medium in a final volume of 100 μl contained: 50 mM

Tris/HCl buffer, pH 7.9; 10 mM $MgCl_2$; 3 mM $MnCl_2$; 140 mM KCl; 18 mM $(NH_4)_2SO_4$; 10 mM phosphoenolpyruvate; 4 mg/ml pyruvate kinase; 1 mM each of ATP, CTP and GTP; and 50 μM [^{3}H]UTP (0.5 Ci/mmole). The reaction was initiated by adding 30 μl of post-swelling mitochondrial suspension, continued for 30 min at 30°, and terminated with the addition of a 40-fold molar excess of unlabeled UTP in 10 μl. Assessment of [^{3}H]UMP incorporation was measured using the DE81 cellulose filter–disc method [28]. Enzyme activity was expressed as pmoles [^{3}H]UMP incorporated into RNA in 30 min per mg of mitochondrial protein. Protein was determined in triplicate for each sample by the method of Lowry *et al.* [29].

RESULTS

Difference in RNA polymerase activity in the cortical and medullary mitochondria from intact rats. The cortical and medullary mitochondria, as well as the total kidney mitochondria, were assayed for RNA polymerase activity. Panel a of Fig. 2 shows that both the cortical (Band II) and medullary mitochondria (Band I) demonstrated linear incorporation with increasing protein concentration, while the mitochondria in the 9000 *g* pellet which had been washed and had not been subjected to sucrose density gradient centrifugation demonstrated lower activity. The specific activity of RNA polymerase in medullary mitochondria was consistently twice that of cortical mitochondria. The rates of [^{3}H]UMP incorporation in cortical and medullary mitochondria, but not those in the thoroughly washed 9000 *g* pellet, also were linear up to 30 min (Fig. 2, panel b). The consistently lower activity and non-linearity of [^{3}H]UMP incorporation in the whole kidney mitochondria were apparently due to the presence of high RNase activity. We have demonstrated previously that this mitochondrial fraction contains the highest RNase activity (pH 7.8) of any subcellular fraction from rat kidney [22]. In the sucrose gradient fractions, we found that most of the RNase active at pH 7.8 was distributed at the top of the gradient and the RNase active at pH 5.0 was distributed both at the top and at the bottom. Thus, the sucrose density gradient centrifugation step not only separated the cortical and medullary mitochondria but also yielded kidney mitochondria more suitable for the study of *in vitro* RNA synthesis. In addition to RNA polymerase, two other enzymes also demonstrated higher activities in medullary mitochondria when compared to cortical mitochondria. The specific activities of isocitrate dehydrogenase and malate dehydrogenase in medullary mitochondria were, respectively, 2- and 1.5-fold that of the cortical mitochondria.

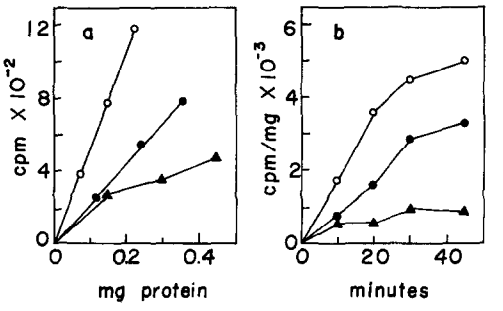

Fig. 2. Comparison of mitochondrial RNA polymerase in different mitochondrial samples. Mitochondria were isolated from whole kidney as described in Materials and Methods and shown in Fig. 1. Assay conditions were as described in Materials and Methods, varying either the amount of mitochondrial protein (panel a) or the incubation time (panel b) using, respectively, 0.29, 0.32 and 0.46 mg protein for medullary mitochondria (○——○), cortical mitochondria (●——●) and whole kidney mitochondria not separated by gradient centrifugation (▲——▲).

Table 1. Characterization of incorporation of radiolabeled ribonucleoside monophosphates into RNA by cortical and medullary mitochondria*

Assay conditions	RNA polymerase activity (pmoles [^{3}H]UMP incorporated/mg protein)	
	Medulla	Cortex
[^{3}H]UTP, complete	14.78 (100)†	8.21 (100)
-CTP	1.02 (6.9)	0.71 (8.7)
-CTP, -GTP	0.29 (2.0)	0.11 (1.3)
Complete + 10 μg RNase‡	0.25 (1.7)	0.02 (0.2)
Complete + 5 μg DNase‡	2.07 (14)	1.80 (22)
[^{3}H]GTP, complete	15.07 (102)	8.17 (100)
[^{3}H]CTP, complete	23.82 (161)	11.53 (140)

* Mitochondrial isolation and RNA polymerase assay are described in the text. The nucleoside triphosphates were all at the same concentration and specific activity (0.05 mM containing 2.5 μCi).

† The numbers in the parentheses are values representing per cent activity remaining.

‡ Additions were made prior to initiation of the reaction by addition of mitochondria.

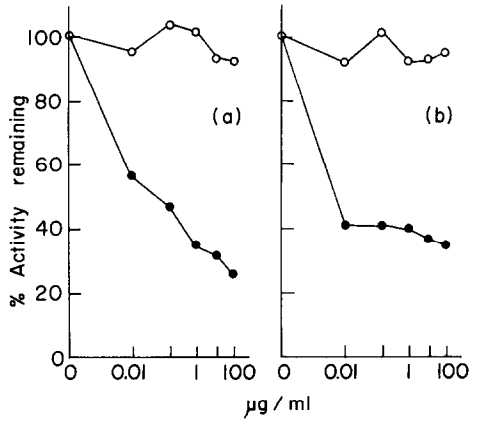

Fig. 3. Effects of α-amanitin and rifampicin on kidney mitochondrial RNA polymerase activity. The enzyme assay was the same as that described in Materials and Methods except that varying amounts of α-amanitin (○——○) or of rifampicin (●——●) were added in the reaction medium. Addition of *N,N*-dimethylformamide (0.65 mM), the solvent used for rifampicin in the reaction mixture, was found to have no effect on RNA polymerase activity. Without the inhibitors, the activities (100 per cent) of medullary and cortical mitochondria were 13.14 and 7.61 pmoles [^{3}H]UMP incorporated/mg protein, respectively. Key: (a) medullary mitochondria; and (b) cortical mitochondria.

Characterization of mitochondrial RNA polymerase assay. The *in vitro* incorporation of [^{3}H]UMP was dependent upon UTP concentration. The amount of [^{3}H]UTP in the assay (0.05 mM) was found to almost saturate the amount of mitochondrial protein (0.1–0.2 mg) normally used in the assay. When CTP was removed from the assay medium, less than 9 per cent of the activity of the complete system was observed in either of the mitochondrial populations (Table 1). There was an even greater loss of activity (less than 2 per cent remaining) upon removal of both CTP and GTP. Thus, the enzymic reaction required the presence of all four ribonucleotide triphosphates. Approximately equal amounts of mononucleotides from [^{3}H]GTP and greater amounts from [^{3}H]CTP were incorporated, compared to the assay using [^{3}H]UTP (Table 1). Addition of pancreatic ribonuclease (100 μg/ml) inhibited [^{3}H]UMP incorporation almost completely in both mitochondrial preparations. Addition of deoxyribonuclease (50 μg/ml) resulted in 78 and 86 per cent reduction in incorporation in cortical and medullary mitochondria, respectively (Table 1).

The addition of α-amanitin, 100 μg/ml (which inhibits nuclear DNA-dependent RNA polymerases I and III), was shown to have no significant effect on the mitochondrial RNA polymerase of either cortex or medulla. However, rifampicin, known to be specifically inhibitory for the bacterial and mitochondrial RNA polymerases [21, 30], was shown to inhibit significantly the reaction in both mitochondrial populations over the range of concentrations used (0.01–100 μg/ml) (Fig. 3, panels a and b). The 70–80 per cent inhibition at this range of rifampicin was comparable to that observed in liver mitochondria [30]. Incomplete inhibition is thought to be at least in part a consequence of limited permeability of the mitochondrial membrane to the inhibitor [30, 31]. Thus, all the results of *in vitro* incorporation of radiolabeled ribonucleotide, using both the cortical and medullary mitochondria, indicated that the activity was characteristic of DNA–dependent RNA polymerase activity.

Effects of adrenalectomy, aldosterone and spironolactone. Adrenalectomy significantly decreased (40 per cent) the [^{3}H]UTP incorporation by cortical mitochondria but had no significant effect on RNA polymerase activity of medullary mitochondria (Table 2). Treatment of adrenalectomized animals with aldosterone 3 hr before they were killed sig-

Table 2. Effects of adrenalectomy and aldosterone and spironolactone treatments on RNA polymerase activity in rat kidney mitochondria*

	RNA polymerase activity (pmoles [^{3}H]UMP incorporated/mg protein)	
Animals	Medulla	Cortex
Sham-operated controls	12.35 ± 0.86 (5)	7.93 ± 0.21 (5)
Adrenalectomized	11.62 ± 0.53 (6)	4.97 ± 0.12† (6)
Adrenalectomized + aldosterone‡	14.03 ± 0.48§ (6)	7.53 ± 0.25‖ (6)
Adrenalectomized + aldosterone + spironolactone‡	11.65 ± 0.33¶ (3)	6.31 ± 0.51¶ (3)

* For the details of animal treatments, mitochondrial isolation, and assays, see the text. Numbers in parentheses indicate number of experiments, each using kidney tissue pooled from four animals. Results are expressed as means ± S.E.M.

† Significantly different from corresponding sham controls ($P < 0.001$) by Student's *t*-test.

‡ Aldosterone, 2 μg/100 g body wt, i.p., was administered 3 hr before the animals were killed. Spironolactone, 7 mg/100 g body wt, i.p. was administered 30 min before aldosterone treatment.

§ Significantly different from correspondong adrenalectomized ($P < 0.05$).

‖ Significantly different from correspondong adrenalectomized ($P < 0.001$).

¶ Significantly different from corresponding adrenalectomized + aldosterone ($P < 0.05$).

nificantly increased (50 per cent) the activity of cortical mitochondria ($P < 0.001$) compared to adrenalectomized animals. Aldosterone treatment of adrenalectomized rats also stimulated the RNA polymerase activity of medullary mitochondria by 20 per cent ($P < 0.05$). Spironolactone, injected 30 min prior to aldosterone administration, blocked the stimulation of RNA polymerase by aldosterone ($P < 0.05$) in both cortical and medullary mitochondria, but did not reduce activity below the basal levels seen in mitochondria from untreated adrenalectomized rats.

The enhancement by aldosterone and the inhibition by spironolactone of both cortical and medullary mitochondrial RNA polymerase activity occurred only when the hormone and the antagonist were administered *in vivo*. Addition of 5 μg aldosterone to the assay mixture produced no increase in [^{3}H]UMP incorporation into mitochondrial RNA of both cortical and medullary mitochondria in the sham control or in the adrenalectomized animals, compared to the assay mixture to which no aldosterone was added. Similarly, 10 μg spironolactone added directly to the assay did not block the effects of aldosterone in the hormone-treated animals.

Effects of other steroid hormones on cortical mitochondrial RNA polymerase. Various other steroid hormones were examined for their *in vivo* effects on cortical mitochondrial RNA polymerase activity (Fig. 4). Only aldosterone had a significant stimulatory effect ($P < 0.05$) on [^{3}H]UMP incorporation into cortical mitochondria. Treatment of adrenalectomized animals with testosterone, 17β-estradiol or progesterone for 3 hr had no effect. A very slight, though not significant, increase was seen with corticosterone, but this was to be expected due to the weak mineralocorticoid effect of this hormone. These results, plus the *in vivo* effect of spironolactone, indicate the specificity of the stimulatory effect of aldosterone on kidney mitochondrial RNA polymerase activity. This specific effect of aldosterone was greater in the mitochondria of cortex than of medulla.

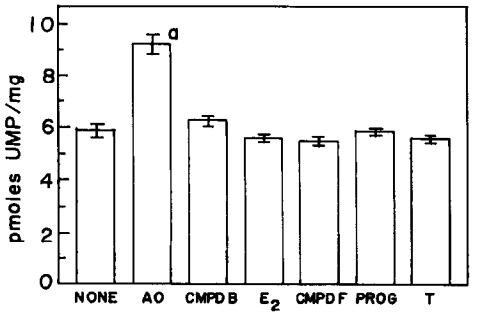

Fig. 4. Effects of various hormones on RNA polymerase activity in cortical mitochondria from kidney of adrenalectomized rats. Animals were treated with various hormones as described in Materials and Methods and, 3 hr later, were killed and cortical mitochondria were isolated. The standard RNA polymerase assay was carried out on the animals treated with the various hormones: NONE (adrenalectomized, no hormone), AO (aldosterone), CMPD B (corticosterone), E_2 (17β-estradiol), CMPD F (hydrocortisone), PROG (progesterone), and T (testosterone). Results are expressed as the means ± S.E.M. for three experiments, using a minimum of three animals per experimental group. The letter "a" denotes significantly different from NONE.

DISCUSSION

A clean separation by dissection of the cortex and medulla of the kidney is not only time consuming but also results in discarding a significant amount of kidney tissue [22]. We have described here a simple procedure to separate mitochondria from kidney cortex and medulla without first having to physically separate the cortex and medulla. Furthermore, the resulting mitochondria are suitable for the study of RNA synthesis *in vitro*. The two populations of mitochondria resemble those separated from whole kidney mitochondria by Ch'ih and Devlin [32] in their sedimentation characteristics; however, these authors did not identify the cortical vs the medullary origin of the two bands. The relative amounts of cortical and medullary mitochondria (Fig. 1), as well as the cytochrome contents of these mitochondria (data not shown), agree with those reported by Kirsten *et al.* [33] who dissected the cortex from the medulla before homogenization. Although a small number of mitochondria from cortex were found at the position where the major portion of medullary mitochondria sedimented and vice versa, this is probably attributable to the fact that a given kidney zone contains predominantly one mitochondrial population, but there are also small numbers of another mitochondrial population present [34].

Without the density gradient centrifugation step we consistently obtained nonlinear kinetics of RNA polymerase activity in whole kidney mitochondria, though not in similarly prepared liver mitochondria. Assays of RNA polymerase activity using increasing amounts of kidney mitochondrial protein or increasing time of incubation not only failed to produce increased incorporation but actually resulted in a decrease, owing to the degradation of labeled products. After the sucrose density gradient centrifugation which separated most of the RNase activity at the top of the gradient tube, both cortical and medullary mitochondria manifested a linear rate of incorporation of [^{3}H]UMP, comparable to or higher than the value reported for liver mitochondria [26].

Mitochondrial RNA polymerase activity, as well as isocitrate dehydrogenase and malate dehydrogenase activity of kidney medulla, was almost twice the activity in the cortex. These results are consistent with the observation that medullary mitochondria were more active than cortical mitochondria in several metabolic parameters [33, 35] and that medullar tissue was more active than cortex in protein synthesis [36].

Our present observations on mitochondrial RNA polymerase cannot be attributed to contamination by nuclear enzymes. The fact that the RNA polymerase activity was completey insensitive to α-amanitin yet sensitive to rifampicin indicates that there was no RNA polymerase of nuclear origin in our purified mitochondria. The effective dose of rifampicin and the kinetics of inhibition of cortical mitochondrial RNA polymerase were very similar

to those observed for Nagarse-treated liver mitochondria [30] (the proteolytic enzyme Nagarse was used to alter outer mitochondrial membranes to facilitate rifampicin entry). Our method of mitochondrial swelling achieved a similar effect.

Our demonstration that aldosterone has a greater effect on cortical than on medullary mitochondrial RNA polymerase activity is consistent with what is known about the site of the physiological action of aldosterone in kidney. The principal site of aldosterone action is believed to be in the distal convoluted tubules [37, 38] and a secondary site is perhaps in the proximal tubules [38]; the majority of both kinds of tubules is located in the cortex. The 40–50 per cent reduction in mitochondrial RNA polymerase activity attributable to adrenalectomy and its complete reversal by aldosterone seem modest, but the comparison is based upon data derived from mitochondria of the whole cortex. The magnitude of the change in the mitochondria of the target cells may be greater. On the other hand, considering that aldosterone exerts its action on the fine adjustment of salt and volume homeostasis (reabsorbing 2 per cent of total sodium being filtered off) [37], one would not expect a drastic change in cellular mechanism on a whole tissue basis.

The stimulatory effect of aldosterone on mitochondrial RNA polymerase in target tissue occurred only when the hormone was administered *in vivo*. The failure of other steroid hormones to stimulate the enzyme activity indicates the specificity of aldosterone action on the target tissue. This and the inhibitory action of spironolactone *in vivo* on the aldosterone-induced RNA polymerase activity are consistent with the currently accepted model of steroid hormone action, e.g. mediated through a hormone–receptor complex which stimulates nuclear gene transcription. We found earlier that aldosterone administration for 3 hr produced maximal nuclear RNA polymerase activity in rat kidney [7]. Whether the increased activity of renal cortical mitochondrial RNA polymerase following aldosterone administration is due to increased synthesis of this enzyme (believed to be synthesized in the cytoplasm [39]) or due to its activation, e.g. resulting from the changing level of regulatory proteins, remains to be investigated.

Regardless of whether aldosterone induced the synthesis of mitochondrial RNA polymerase or caused activation of the enzyme, the fact that aldosterone increased mitochondrial RNA synthesis may explain an important aspect of the mechanism of aldosterone action. Mitochondria synthesize a limited number of protein species [19, 20], and availability of some of them, known to be subunits for the inner-membrane enzymes, is an important factor in the integration of cytoplasmically synthesized subunits. Thus, the intramitochondrial synthesis of these proteins plays an important role in regulating mitochondrial biogenesis. If aldosterone somehow influences the rate of mitochondrial mRNA synthesis, which is known to be very short-lived [21], the hormone then will secondarily affect overall mitochondrial function. This possibility may explain the previous observations that aldosterone affects many aspects of mitochondrial activity. The effect of increased mitochondrial transcription on the synthesis of mitochondrial proteins, and the linkage of these events to sodium retention await further investigation.

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