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ALDOSTERONE STIMULATED AGGREGATE RNA POLYMERASE IN RAT KIDNEY*

R.K. MISHRA, J.F. WHELDRAKE† and L.A.W. FELTHAM

Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, Canada

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1. Introduction

Aldosterone is thought to regulate sodium transport by a process which involves changes in RNA and protein synthesis. At the transcriptional level Fimognari et al. [1], Forte and Landon [2], and Kalra and Wheldrake [3] have observed an increased rate of incorporation of radioactive precursors into RNA in kidneys of aldosterone treated rats. At the translational level Sharp and Leaf [4] and Edelman [5] have shown that puromycin and cycloheximide inhibit the aldosterone-mediated stimulation of sodium transport. It is not known whether the site of aldosterone activation in these systems involves DNA-dependent RNA polymerase (nucleoside triphosphate-RNA nucleotidyl transferase, EC 2.7.7.6). The present studies were conducted to examine the possibility that aldosterone may regulate RNA polymerase activity in kidney. The data reveal that the administration of aldosterone *in vivo* elevated the activities of both Mg^{2+} and Mn^{2+} dependent i.e. forms I and II [6], RNA polymerases. It is concluded, that RNA polymerase is one of the sites involved in the mechanism of action of aldosterone.

2. Materials and methods

2.1. *Chemicals and animals*

Nucleotides, Cleland's reagent and spermine were purchased from Sigma Chemical Company, ^{3}H-UTP (specific activity 22.2 Ci/mmole) was from New England Nuclear Corporation, and d(+)-aldosterone from Calbiochem.

Normal and adrenalectomized female Wistar rats (100–120 g) fed ad libitum on Purina Chow, were used for the experiments. Adrenalectomy was performed 3 days prior to each experiment.

The rats were divided into three groups (6–8 each): normal, adrenalectomized (control), and adrenalectomized + hormone treated. The hormone-treated group received a single injection of aldosterone, 5 μg/100 g, 4 hr before the rats were killed. Normal and control groups received equivalent volumes of 0.9% saline.

2.2. *Assay for RNA-polymerase activity*

Kidney nuclei were isolated essentially by the method of Muramatsu et al. [7].

The incubation mixture for the Mg^{2+} activated RNA-polymerase reaction contained (in a final volume of 0.5 ml): 50 μmoles of Tris-HCl pH 8.2 at 25°, 4 μmoles of Cleland's reagent, 0.05 μmoles of each of GTP, CTP, and UTP, 1.0 μmole of ATP, 2 μCi of ^{3}H-UTP and nuclear suspension representing 200–300 μg of DNA. The $Mn^{2+}/(NH_4)_2SO_4$ dependent reaction differed from the above by the addition of 2 μmoles of $MnCl_2$ and 250 μmoles of $(NH_4)_2SO_4$ adjusted to pH 8.1. In both cases the reaction was initiated by the addition of nuclei. After incubation for 15 min at 37°, the reaction was terminated by transfer to chipped ice, followed by immediate addition of 5 ml of 10% (w/v) trichloroacetic acid. The acid-insoluble material was collected on Whatman GF/C filters, which were then washed three times with 10 ml of 5% trichloroacetic acid containing 0.1 M $Na_4P_2O_7$ (both w/v), and once with ethanol–ether 3:1. The filters were dried under an infra-red lamp and radioactivity was counted in a liquid scintillation counter.

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† Present address: School of Biological Sciences, Flinders University of South Australia, Bedford Park, South Australia.

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3. Results and discussion

Fig. 1 shows the rate of precursor incorporation by the Mg^{2+} and $Mn^{2+}/(NH_4)_2SO_4$ activated RNA polymerases in nuclear fractions from normal, adrenalectomized, and adrenalectomized plus hormone-treated rats. In all cases incorporation is linear for 10 to 15 min. Aldosterone administration *in vivo* resulted in a 2-fold or greater stimulation of both the RNA polymerase activities in the hormone-treated group (table 1). The activities of both RNA polymerases were abolished by inclusion of actinomycin D in the assay systems. The differences in activities of form I and II (table 1) suggest that regulation of nucleolar enzyme may be independent of nucleoplasmic enzyme and the regulatory system for RNA polymerase I may be more sensitive to the hormone than the system for polymerase II.

The changes in the kidney RNA of the rat after adrenalectomy and aldosterone administration [8–11] can be related to the RNA polymerase activity of the kidney nuclei. This is true for both Mg^{2+} and

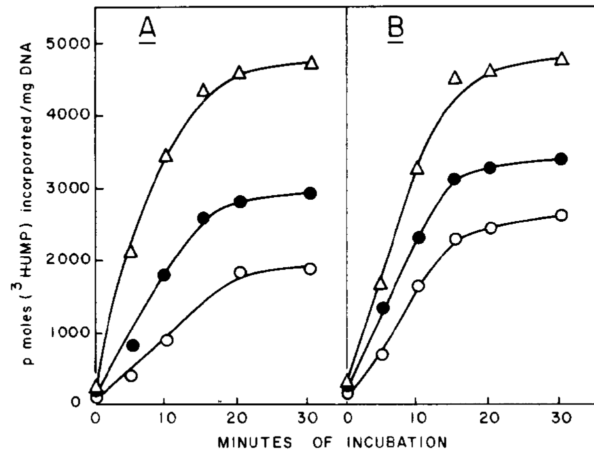

Fig. 1. Kinetics of the RNA polymerase reaction in isolated nuclei of rat kidney. The activities of RNA polymerase I(A) and II(B) were determined as described in Methods and materials. (●—●—●) normal; (○—○—○) adrenalectomized; (△—△—△) adrenalectomized plus aldosterone treated. Each point represents the mean of 12 assays (triplicate determinations of each of 4 animals).

Table 1
The effect of aldosterone administration *in vivo* on the activities of RNA polymerases I and II in isolated kidney nuclear fraction.

Animals	RNA polymerase activity (pmoles of ^{3}H-UMP incorporated/15 min/mg DNA)	
	Polymerase I Mg^{2+} dependent	Polymerase II $Mn^{2+}/(NH_4)_2SO_4$ dependent
Normal	2557 ± 97	3121 ± 194
Adrenalectomized	1563 ± 30	2293 ± 183
% Difference (normal–adrenalectomized)	−63	−36
Adrenalectomized + aldosterone treated	4380 ± 365	4583 ± 170
% Difference (adrenalectomized–adrenalectomized and hormone treated)	+180%	+99%

Values are given as a mean ± SE of 12 assays (triplicate determinations in each of 4 replicate experiments).

increased by d(+)-aldosterone administration. These results also support Edelman's view [12] that the mechanism of action of aldosterone on sodium transport, involves increases in the rate of mRNA and rRNA synthesis. However, it seems unlikely that aldosterone acts solely by modifying the activity of RNA polymerase since there are also reports of changes in the activity of ribosomes [13] and the template capacity of chromatin [14].

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