QUALITATIVE CHANGES IN NUCLEAR RNA FROM RAT KIDNEY CORTEX AFTER ALDOSTERONE TREATMENT

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SUMMARY:

RNA extracted from nuclei, microsomes and the 100,000xg supernatant of rat kidney cortex cell homogenates was methylated "in vitro" with labelled dimethyl sulfate and hybridized with differentially renaturing DNA base sequences isolated by hydroxyapatite chromatography. 40 Minutes after intraperitoneal injection of aldosterone into adrenalectomized rats, a specific increase in the hybridization of nuclear RNA with repetitive DNA was observed.

INTRODUCTION:

The stimulation of RNA synthesis by aldosterone in its target tissues has been considered as an important step in the mechanism of action of this hormone (1). Although the theories proposed on this subject suggest a specific change in the RNA synthesized under the influence of aldosterone, attempts to detect any kind of specific changes were so far unsuccessful (2). This report presents the first evidence for a qualitative change in rat kidney cortex RNA after "in vivo" administration of the hormone.

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ABBREVIATION-

SSC: 0.15 M sodium chloride and 0.015 M sodium citrate, pH 7.0

MATERIALS AND METHODS:

Nuclei were isolated from rat kidney cortex according to the method of Avdalovic and Kochakian (3), with the modification that the main centrifugation was performed in 1.7 M sucrose for one hour at 200,000xg. The nuclei were free of erythrocytes and had a DNA:protein ratio of about 1:4. Microsomes were isolated from the tissue homogenate in the same medium of Avdalovic and Kochakian, with the homogenization being performed by only one stroke of a teflon pestle in a glass homogenizer, in order to prevent nuclear breakage. After centrifugation at 700xg and 15,000xg for 20 minutes, respectively, in order to eliminate nuclei and mitochondria, the supernatant was centrifuged for one hour at 100,000xg. Both the pellet (microsomes) and the 100,000xg supernatant were used for RNA extractions. These were carried out using the extraction medium of Schütz et al. (4), without bentonite, by the phenol-chloroform-sodium dodecyl sulfate method (5) at 65°C and 25°C, successively. The crude RNA fractions were further purified by passage through a column of hydroxyapatite (6) and Sephadex G-50. The nuclear RNA was extracted by the method of Shütz et al. (4) at 25°C and 65°C and purified by DNase treatment (30-40 µg enzyme/ml, 30 min at room temperature) and gel filtration through Sephadex G-50 (7).

RNA methylation was carried out by the method of Singer and Fraenkel-Conrat (8). RNA was dissolved in 3mM diethyloxidiformate and 20 mM EDTA (pH 7.0) at a concentration of 3.3 mg RNA/ml. 5 µl Diemethyl sulfate solution in ethanol (2.5 µC/µl, specific activity 2.3 C/mmole, New England Nuclear) or 10 µl (when the specific activity was 980 mC/mmole)were added to 100 µl RNA solution and the mixture shaken in closed vials for 6 hours at 25°C. After incubation the RNA was extracted 4 times with ether to remove the excess dimethyl sulfate (9,10) and precipitated three times with ethanol. When enough quantities of RNA were available, two of the three ethanol precipitations were replaced by gel filtration through Sephadex G-50.

DNA was extracted from purified rat liver nuclei (11) according to the method of Marmur (12), supplemented with further purification steps,

such as double ribonuclease treatment, incubation with pronase and washings with norite (7) and bentonite. DNA was sonicated for three minutes using the conditions of Melli and Bishop (13). The sedimentation coefficient of the sheared DNA in sucrose gradients was about 3.5-4 s. Rat liver DNA was fractionated by hydroxyapatite chromatography according to the method of Britten and Kohne (14). The fractions were characterized by their Cot values (DNA concentration in nucleotides per liter multiplied by the time in seconds that denatured DNA is exposed to renaturing conditions). Two fractions were used in these experiments, having Cot<0.6 and Cot>150, respectively. These fractions differ not only in their adsorption properties to hydroxyapatite but also in their hyperchromicity and hybridization properties with RNA.

RNA was hybridized with denatured DNA in solution for 48 hours at 37°C in 30% formamide/2xSSC (v/v) (15). A standard hybridization mixture contained 128 µg rat RNA/ml, 16 µg denatured DNA/ml and 400 µg E.coli RNA/ml (13). Incubations with Clostridium perfringens DNA were run simultaneously as controls. After annealing, the samples were filtered through nitrocellulose filters (Schleicher and Schuell, Type B-6). Each filter was exhaustively washed with 200 ml of 4xSSC, dried, and counted in a scintillation spectrometer. The amount of DNA present in the filters was determined by the method of Burton (16) using "in vitro" methylated, radioactively labelled, rat liver DNA as standard. Caesium sulfate density gradient centrifugations were carried out by the method of Marks and Spencer (17).

RESULTS AND DISCUSSION:

Hybridization experiments require highly labelled RNA. While "in vivo" labelling of rat kidney cortex RNA does not give sufficiently high specific activities, "in vitro" labelling with dimethyl sulfate offers the possibility of obtaining a highly labelled RNA with a random distribution of radioactivity in all RNA populations. Smith et al (6) have proven that"in vitro" methylation of RNA does not change the hybridization properties of the molecule, and the method has been successfully applied by several authors in different systems (9,18,19). Since all RNA types are equally labelled, the hybridization

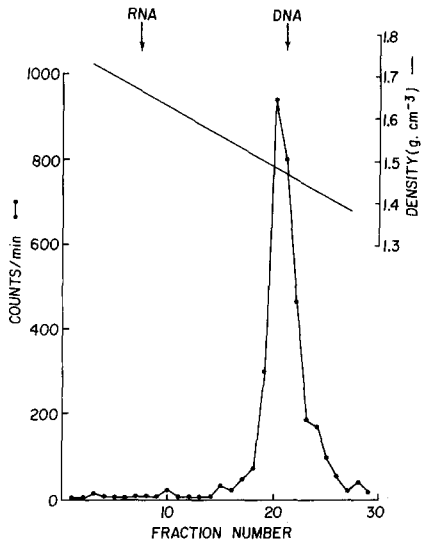

Fig. 1. Cs_2SO_4 density centrifugation of hybrids between kidney cortex nuclear RNA and repetitive DNA (Cot<0.6). After annealing, the hybridization mixture was dialyzed against 2xSSC, treated for one hour at 37°C with pancreatic ribonuclease, passed through Sephadex G-100, mixed with Cs_2SO_4 and centrifuged at 25°C for 72 hours and 35,000 rpm in the SB-405 rotor of a B-60 International centrifuge (17). The fractions were collected on nitrocellulose filters, washed with 2xSSC and counted in a scintillation spectrometer. The densities were calculated from the refraction indices of the fractions. The arrows indicate the densities of kidney cortex nuclear RNA and rat liver nuclear DNA, respectively. The specific activity of the RNA was 8,300 counts.min^{-1}. µg RNA^{-1}. The gradient contained 0.6 optical density units of nucleic acids (A_{260nm}). —— density of Cs_2SO_4 in 2xSSC.

is less specific for rapidly labelled RNA than the "in vivo" labelling. In this report we have improved the specificity and resolution of the reaction using RNA isolated from different subcellular fractions and different kinds of DNA. Since the hybridization was carried out in solution, the reaction products could be analyzed by Cs_2SO_4 density gradient centrifugation. One of these gradients is shown in Fig. 1. Here nuclear RNA was hybridized with rapidly reannealing, repetitive DNA (Cot<0.6). The hybridization product was treated with ribonuclease (5 µg/ml) for one hour at 37°C, separated from nucleotides and oligonucleotides by chromatography in Sephadex G-100 and analyzed in Cs_2SO_4 gradients (17). The sedimentation properties of this ribonuclease-resistant hybridization product corresponds to a hybrid where only a small portion of the DNA has annealed with the RNA. The hybridization levels of this product correspond to those found using the standard procedure described

in Materials and Methods, showing the reliability of the hybridization system used. The complete absence of hybridization with heterologous DNA (from Clostridium perfringens) is further evidence of the specificity of the reaction.

The "in vitro" methylated rat kidney cortex RNA from adrenalectomized

TABLE I
Effect of aldosterone on the hybridization of rat kidney cortex RNA

Groups of 15 to 28 adrenalectomized rats were injected with 1 ml 0.14 M NaCl per animal (controls) or 1 ml 0.14 M NaCl, containing 4 µg aldosterone. The animals were decapitated 40 min after injection; the RNA was prepared from the different cell fractions and hybridized with nuclear DNA as described in Materials and Methods. The final volume of the hybridization samples was usually 250 µl (for nuclear RNA) and 500 µl (for the cytoplasmic fractions). Specific activities of the RNA used: between 3,000-8,000 counts. min^{-1}. µg RNA^{-1}. The radioactivity measurements were (according to the specific activities of the RNA samples) about 500-2000 counts/min (nuclear RNA) and 200-600 counts/min (cytoplasmic fractions) per sample.

RNA source	DNA fraction	Hybridization (10^{-4} x $\frac{\mu g\ RNA}{\mu g\ DNA}$ ±S.E.)	
		controls	aldosterone
Nuclei	Cot<0.6	204 ± 26 (12)*	306 ± 40 (11)*
	Cot>150	358 ± 52 (12)	292 ± 33 (11)
Microsomes	Cot<0.6	40 ± 35 (4)	40 ± 21 (5)
	Cot>150	66 ± 9 (13)	83 ± 18 (12)
100,000xg supernatant	Cot<0.6	70 ± 20 (4)	67 ± 26 (5)
	Cot>150	71 ± 20 (4)	69 ± 20 (5)

* P<0.05 according to the unpaired t-test. The number of experiments is indicated in parentheses.

rats showed some changes in its hybridization properties 40 min after intraperitoneal injection of aldosterone. The experimental data and the results are summarized in Table I. A significant increase in the hybridization between nuclear RNA and rapidly reannealing DNA (Cot<0.6) could be seen. No significant changes were observed in the hybridization properties of all other

fractions after hormone administration. However, it is interesting to see how the hybridization of nuclear RNA with the DNA fraction of Cot>150 seemed to be lower, while that of microsomal RNA with the same DNA fraction was higher than the controls after aldosterone treatment. Although this DNA fraction contains low repetitive and unique sequences, as has been established in reassociation studies of rat liver DNA (20,21) it is evident that the hybridization conditions used do not allow annealing of RNA with unique sequences of DNA. Thus the hybridization is with those moderately repetitive regions present in the DNA fraction of Cot>150. The role of RNA hybridizing with these sequences is not known. It is possible that they represent in part messenger RNAs, since some of these seem to be transcribed from reiterated sequences (22).

The observed increase in the hybridization of nuclear RNA with highly repetitive DNA after aldosterone treatment shows that the hormone qualitatively changes the RNA populations of rat kidney cortex. The role of the RNA transcribed from highly repetitive sequences is still unknown. It could be involved in the regulation of transcription (19,23,24), or like poly A, it may play a role in transport processes from nucleus to cytoplasm or represent some kind of specific signals for translation (25).

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