Actions of Aldosterone on rRNA and Na^+ Transport in the Toad Bladder†

P. A. Wilce,‡ B. C. Rossier,§ and I. S. Edelman*

ABSTRACT: Aldosterone increased methylation by [*methyl*-^{3}H]methionine of nuclear ribosomal ribonucleic acid (rRNA) sedimenting at 18S, 28S, and 40S within 90 min and of 28S cytoplasmic RNA within 240 min of continuous exposure to the precursor and the hormone, in the toad bladder. In addition, incorporation of [^{14}C]uridine into cytoplasmic 4S transfer RNA, and 18S and 28S rRNA was enhanced after 240 min of continuous exposure to the precursor and the hormone. Aldosterone had minimal effects on the ^{3}H or ^{14}C-labeled, acid-soluble pools. These results suggest that aldosterone augments the synthesis of rRNA at the transcriptional level. Ribosomes isolated 240 min after treatment of the toad bladder with aldosterone showed increased incorporation of [^{3}H]-phenylalanine into peptides with both endogenous messenger RNA (mRNA) and exogenous mRNA (i.e., poly(uridylic acid)) in an in vitro assay. Inhibition of reinitiation with NaF or poly(AUG) reduced the rate of amino acid polymerization by 45% but the aldosterone to control ratio remained significantly high. These results imply an increase in active ribosomes and perhaps in endogenous mRNA content. Our findings, however, do not distinguish between a steroid-dependent increase in the total number of active ribosomes and an increase in translational activity per ribosome.

The role of accumulation of specific mRNAs[1] in the actions of steroid hormones has received considerable attention in recent years (King and Mainwaring, 1974). Evidence that the primary effects of steroids on protein synthesis are mediated by regulation of the synthesis of mRNA continues to be adduced (Rhoads et al., 1971, Chan et al., 1973, Schutz et al., 1973; Palmiter and Carey, 1974). Recently Rossier et al. (1974) reported that aldosterone increased the incorporation of [^{3}H]uridine into 9 to 18S RNA (with little or no methyl content) of toad bladder. Wilce et al. (submitted for publication) found that aldosterone augmented labeling of 7, 12, and 18S poly(A)(+)-RNA during the latent period with respect to the action on Na^+ transport across the toad bladder. These findings are in accord with the inference that aldosterone enhanced the synthesis of mRNA in this system.

There is, in addition, abundant evidence that steroids also promote the synthesis of rRNA or the precursors of rRNA relatively early in the course of hormone action (King and Mainwaring, 1974). Indirect evidence that aldosterone also promotes the synthesis of rRNA has also appeared. Aldosterone increased endogenous RNA polymerase I activity (Liew et al., 1972) and the RNA polymerase I/II activity ratio in rat kidney nuclei (Chu and Edelman, 1972). Accordingly, we elected to assess the effects of aldosterone on incorporation of labeled uridine and methyl groups into cytoplasmic rRNA, and on translational activity of isolated ribosomes.

Experimental Section

Materials

The incubation medium (frog Ringer's solution) for the hemibladders contained (all in mM), 90 NaCl, 3 KCl, 25 $NaHCO_3$, 0.5 $MgSO_4$, 0.5 KH_2PO_4, 1 $CaCl_2$, and 6 glucose, pH 7.6 (gassed with 3% CO_2); osmolality was 230 mOsm. Gentamycin was added to the medium (final concentration was 10 μg/ml) and then filtered through 0.45 μm Millipore filters. All glassware and other solutions were heat sterilized. Aldosterone-*d* was obtained from Calbiochem Corp., oligo(dT)-cellulose (T_2) from Collaborative Research, Inc., bovine pancreatic DNase, type I (RNase-free), was from Worthington, and poly(U) and poly(AUG) were from Miles Laboratory. All of the conventional reagents were either reagent grade or spectroquality. Aquasol, [U-^{14}C]uridine (420 mCi/mmol), and *l*-[*methyl*-^{3}H]methionine (12 Ci/mmol) were purchased from New England Nuclear Corp., and *l*-[^{3}H]-phenylalanine (1.2 Ci/mmol) was from Amersham (Bucks, U.K.).

Methods

A. Physiological and Labeling Procedures. Colombian, female toads (*Bufo marinus*) obtained from Tarpon Zoo, Fla., were stored partially immersed in 75 mM NaCl for 48 h at room temperature before use. The details of the mounting, incubation, and monitoring of the hemibladders have been described previously (Walser 1969; Rossier et al., 1974). In brief, the bladders were removed and mounted as sacs on plastic cannulas, mucosal side outside, and filled with 5 ml and immersed in 90 ml of frog Ringer's solution. The assembly was

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[1] Abbreviations used: mRNA, tRNA, and rRNA, messenger, transfer, and ribosomal ribonucleic acid, respectively; oligo(dT), oligo(deoxythymidylate); poly(U), poly(uridylic acid); poly(A)(+)-RNA, polyadenylated ribonucleic acid isolated by oligo(dT)-cellulose chromatography; pd, spontaneous electrical potential difference across the wall of the toad bladder; scc, the current required to clamp the pd to zero (i.e., the short-circuit current) measured in μA/hemibladder; SEM, standard error of the mean; Tris, tris(hydroxymethyl)aminomethane; C-T medium, medium consisting of 50 mM Tris-HCl, 60 mM KCl, 8 mM magnesium acetate, and 6 mM mercaptoethanol (pH 7.8) (Clemens and Tata, 1972).

maintained at a constant temperature of 25 °C, both sides of the sac were oxygenated continuously with 97% O_2–3% CO_2, and the transepithelial potential differences (pd) and short-circuit currents (scc) were measured at 30-min intervals as described by Walser (1969). The scc is a convenient and accurate measure of the rate of transepithelial active Na^+ transport both in the presence and absence of aldosterone (Porter and Edelman, 1964). Four hours after mounting, aldosterone (final concentration 7×10^{-8} M) or the diluent was added to both the mucosal and serosal solutions. The following protocols were used to assess incorporation of labeled compounds into epithelial RNA: (1) Paired hemibladders (aldosterone and control) were exposed continuously to *l*-[*methyl*-^{3}H]-methionine (15 μCi/ml) added to the serosal media for 30 or 90 or 240 min. (2) Paired hemibladders (aldosterone and control) were exposed continuously to both *l*-[*methyl*-^{3}H]-methionine (6 μCi/ml) and [U-^{14}C]uridine (0.5 μCi/ml) added to the serosal media for 240 min. (3) To assess translational activity of the ribosomes, paired hemibladders (aldosterone (7×10^{-8} M) and control) were incubated for 240 min, without any labeled compounds. The epithelial cells were then harvested for preparation of the ribosomal fractions.

B. Sucrose Gradient Analysis of Labeled Nuclear and Cytoplasmic RNA. The labeling periods were terminated by washing the hemibladders with 2 volumes of ice-cold frog Ringer's solution. All subsequent steps were carried out at 0–4°C. The collection of the epithelial cells, preparation of the nuclear and cytoplasmic fractions, and sucrose density gradient analyses were performed as described previously (Rossier et al., 1974). The epithelial nuclei were isolated and RNA was extracted by the method of Penman (1966). The cytoplasmic RNA was extracted as described by Rossier et al. (1974). The nuclear and cytoplasmic precipitates were dissolved in 0.1 M NaCl–0.05 M sodium acetate (pH 5.2)(sodium acetate buffer) and stored at −60 °C. The acid-soluble pools of *l*-[*methyl*-^{3}H]-methionine and [^{14}C]uridine were measured as described previously (Rossier et al., 1974). The DNA contents of the homogenates were estimated by the method of Burton (1956).

C. Oligo(dT)-Cellulose Chromatography. Cytoplasmic RNA was fractionated on the basis of poly(A) content by the procedure of Aviv and Leder (1972). In brief, the RNA extracts resuspended in 0.5 M KCl–0.01 M Tris HCl (pH 7.6) (i.e., the high salt buffer) were applied to the oligo(dT) columns and the bound RNA was then eluted with 0.01 M Tris-HCl (pH 7.6) (i.e., the low salt buffer). The procedure was carried out at room temperature with sterile glassware and buffers. Within the limits of detectability, these columns completely separated methylated RNA from nonmethylated poly(A)(+)-RNA (Wilce et al., submitted for publication).

D. Preparation of the Ribosomal Fractions and Translational Assay. 1. Ribosomes. Epithelial scrapings were suspended in ice-cold frog Ringer's solution and washed twice by sedimenting at 600*g*. All subsequent steps in the preparation of the ribosomes were also at 0–4 °C. The washed cells were resuspended in 2 ml of C-T medium consisting of (all in mM): 50 Tris-HCl, 60 KCl, 8 magnesium acetate, and 6 mercaptoethanol (pH 7.8) (Clemens and Tata, 1972). The cells were homogenized with 15 strokes of a motor-driven, Teflon–glass Potter-Elvejhem homogenizer. The homogenates were centrifuged at 10 000*g* for 10 min in a Sorvall RC-2B refrigerated centrifuge. The supernatants were treated with 1/9th volumes of Triton X-100, stirred for 5 min, layered on 2 ml of 1 M sucrose–C-T medium, and centrifuged at 150 000*g* for 2.5 h in a 4.4-ml swinging bucket rotor (SB 405) in a B-60 International Ultracentrifuge. The pellet of ribosomes was resuspended in 1.0 ml of C-T medium.

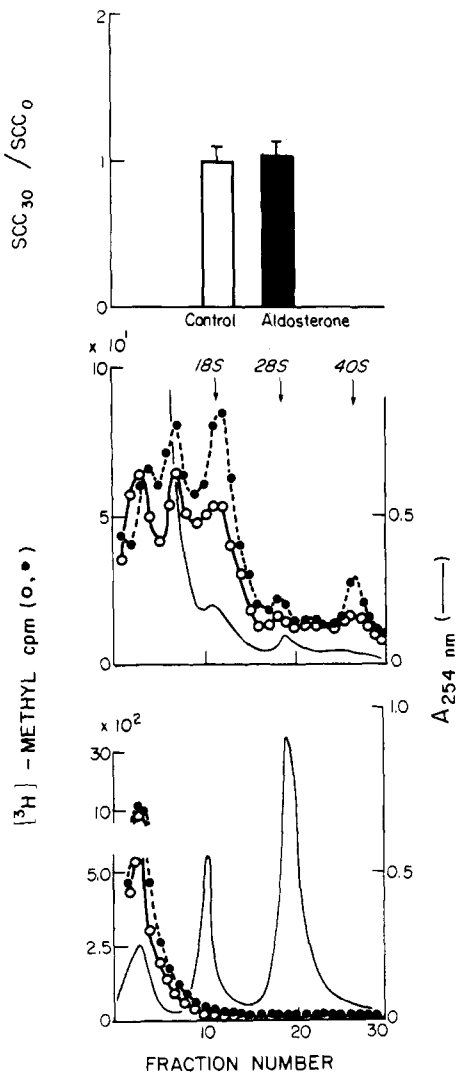

FIGURE 1: Effects of aldosterone on Na^+ transport and on the incorporation of [*methyl*-^{3}H] into nuclear and cytoplasmic RNA labeled for 30 min. (Upper Panel) Na^+ transport measured by the ratio of scc at time t = 30 min (scc_{30}) divided by the scc at time zero (scc_0) is indicated by the ordinate on the left. The bar indicates the mean and the vertical + 1 SEM. (Middle and Lower Panels) Nuclear RNA (middle panel) and cytoplasmic RNA (lower panel) analyzed by linear 5–20% sucrose gradient centrifugation. ^{3}H activity is indicated by the ordinate on the left. (●) Aldosterone treated; (○) control. The counting rates are normalized to the area under the A_{254nm} absorbance profile that is indicated by the ordinate on the right.

2. Charged tRNA–Crude Protein Factors. Transfer RNA charged with [^{3}H]phenylalanine and the crude protein factors were prepared from toad liver cell sap by the method of Mainwaring and Wilce (1973), except for the use of the C-T medium as suggested by Clemens and Tata (1972).

3. Translational Assay. The assay medium consisted of 0.5 ml of (all in mM) 50 Tris-HCl, 60 NH_4Cl, 10 mercaptoethanol, 5 $MgCl_2$, and 0.1 GTP, (pH 7.8), 75 μg of [^{3}H]phenylalanine-tRNA, 375 μg of liver cell sap protein, and ribosomes equivalent to 100 μg of ribosomal RNA. Inhibitors and polynucleotides were added at zero time, as given in Table II. The system was incubated at 25 °C for 40 min and terminated by the addition of 2 ml of 5% Cl_3CCOOH containing 1 mg/ml of unlabeled phenylalanine. The samples were cooled in an ice bath, then heated to 90 °C for 10 min, and filtered through

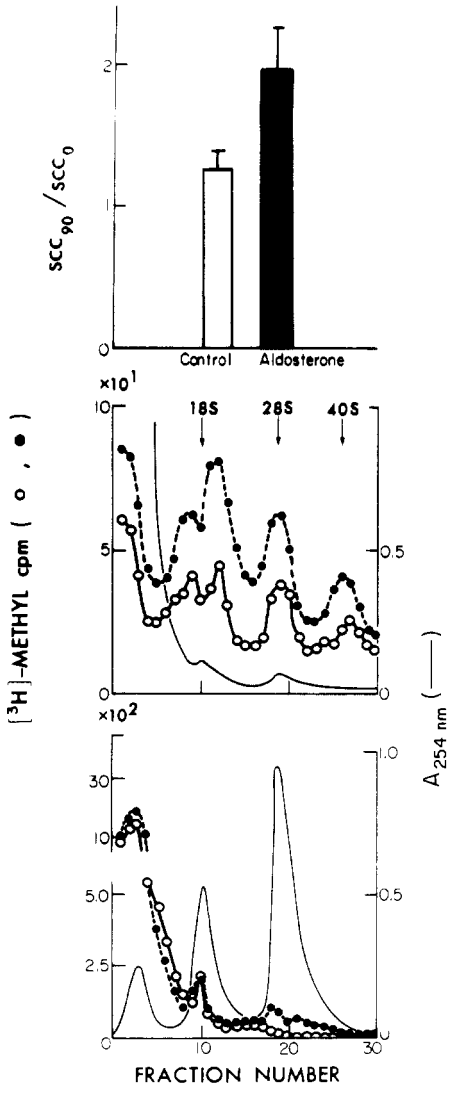

FIGURE 2: Effects of aldosterone on Na^+ transport and on the incorporation of [*methyl*-^{3}H] into nuclear and cytoplasmic RNA labeled for 90 min. The conventions used in this figure are described in the legend of Figure 1. (Upper Panel) Na^+ transport measured by the scc method. (Middle and Lower Panels) Nuclear RNA (middle panel) and cytoplasmic RNA (lower panel) analyzed by linear 5–20% sucrose gradient centrifugation.

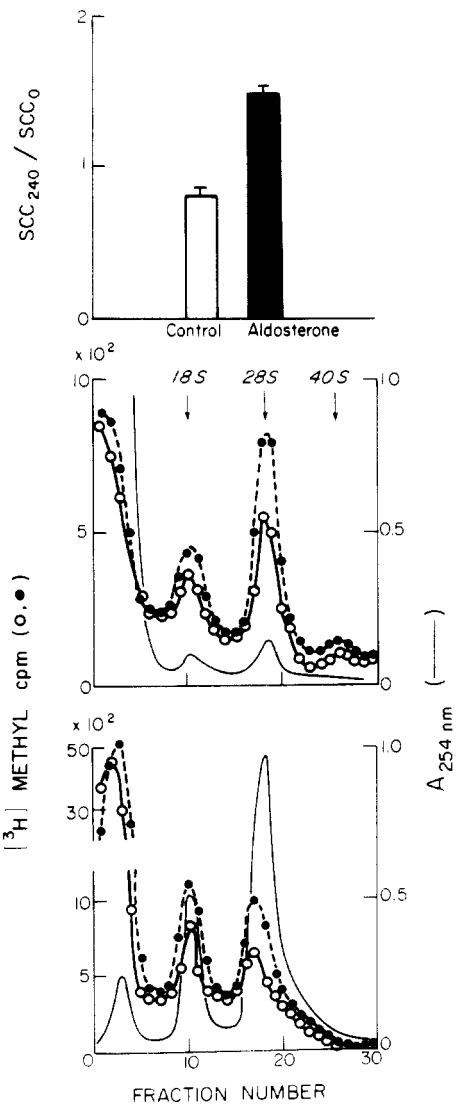

FIGURE 3: Effects of aldosterone on Na^+ transport and on the incorporation of [*methyl*-^{3}H] into nuclear and cytoplasmic RNA labeled for 240 min. The conventions used in this figure are described in the legend of Figure 1. (Upper Panel) Na^+ transport measured by the scc method. (Middle and Lower Panels) Nuclear RNA (middle panel) and cytoplasmic RNA (lower panel) analyzed by linear 5–20% sucrose gradient centrifugation.

glass-fiber filters (Whatman GFA). The filters were washed twice with 10 ml of 5% Cl_3CCOOH followed by 10 ml of 100% ethanol. Each filter was dropped into 10 ml of Aquasol and assayed for ^{3}H content in a liquid scintillation spectrometer.

Results

A. Time-Course of Incorporation of [^{3}H]Methyl into Nuclear and Cytoplasmic RNA. Nuclear and cytoplasmic extracts were prepared from toad bladder epithelium that had been incubated in *l*-[*methyl*-^{3}H]-methionine plus aldosterone or the diluent for 30, 90, or 240 min. These extracts were analyzed by sucrose density gradient centrifugation; the results are shown in Figures 1–3. The absorbance patterns of the nuclear fraction (middle panels of Figures 1–3) contain two small but distinct A_{254} peaks at 18 S and 28 S, and a very large diffuse peak at the top of the gradient, indicated by the incomplete vertical line. The large, diffuse peak was due to contaminating DNA that had not been removed from the extracts. No attempt was made to extract the contaminating DNA from the preparations for two reasons. (1) In preliminary experiments in which the DNA was removed by the method of Joel and Hagerman (1969), the large diffuse peak was deleted with no change in the relative RNA and radioactivity profiles. In the process, however, significant quantities of nuclear RNA were lost, thereby limiting the utility of removing the DNA. (2) It is unlikely that the epithelial cells (which are nondividing cells) would incorporate significant quantities of the [^{3}H]methyl group into DNA (Kappler, 1970).

Although cytoplasmic rRNA may be present, the radioactivity profile associated with the A_{254} peaks in the middle panels of Figures 1–3 probably represents primarily nuclear rRNA since the specific activity of the nuclear RNA (i.e., cpm/A_{254}) is tenfold greater than that of the cytoplasmic rRNA (cf. the middle and lower panels of Figures 2 and 3).

Figure 1, middle panel, shows the pattern of methylation of nuclear RNA after 30 min of incubation with *l*-[*methyl*-^{3}H]-methionine. At this time, aldosterone had no effect on scc. Several species of nuclear RNA were methylated during the interval, including two unidentified peaks that sedimented more slowly than the 18S rRNA species. The 18S rRNA was

TABLE I: The Effect of Aldosterone on the Acid-Soluble Pool of [*methyl*-^{3}H]Methionine and [^{14}C]Uridine.[a]

Incubation Conditions	Acid-Soluble Fraction (cpm/μg of DNA)					
	Aldosterone		Control		*A/C*	
	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
90 min incubation, [*methyl*-^{3}H]methionine (15 μCi/ml), n = 10	1650		1436		1.15	
240 min incubation, [*methyl*-^{3}H]methionine (6 μCi/ml), [^{14}C]uridine (0.5 μCi/ml), n = 16	754	688	769	807	0.98	0.85

[a] Aliquots of homogenates from the experiments summarized in Figure 2 and Figures 4 and 5 were precipitated and washed twice with 2 ml of 5% Cl_3CCOOH. The combined washings were analyzed for ^{3}H and ^{14}C content and the precipitates were analyzed for DNA content. n = 2 for each pool.

heavily labeled: the 28S rRNA contained minimal ^{3}H activity as did a small peak at 40 S, which is probably the precursor of 18S and 28S rRNA (Perry et al., 1970). Even in this brief period, aldosterone augmented the labeling of the nuclear 18S and 40S species. Incubation with the precursor for 90 min increased the specific activity of the 28S peak and to a lesser extent of the 40S peak, relative to that of the 18S peak. After 90 min, the aldosterone-treated hemibladders showed higher rates of Na^+ transport (not quite statistically significant, however) and augmented labeling of all of the peaks in the gradient. After 240 min of incubation, Na^+ transport was significantly increased by the hormone. After 240 min of continuous labeling, the radioactivity and A_{254} patterns are proportionate across the gradient, probably because methylation is at equilibrium by this time. Stimulation of labeling by aldosterone is particularly evident in the 28S peak.

The effects of aldosterone on cytoplasmic rRNA cannot be evaluated during the first 90 min of exposure, owing to the minimal amounts of labeling achieved by this time (lower panels of Figures 1 and 2). The cytoplasmic 4 S (tRNA) was heavily labeled in the 30- and 90-min incubations, but was not affected by aldosterone. After 240 min of incubation, aldosterone stimulated incorporation of [^{3}H]methyl groups in the 18 and 28S cytoplasmic peak with no change in the 4S peak (Figure 3, lower panel).

The early enhancement of the labeling of nuclear rRNA, especially evident in 90 min, and the later appearance of this effect in cytoplasmic rRNA imply hormonal regulation of either the synthesis or the processing of rRNA, if the specific activity of the precursor methionine pool did not increase significantly. To obtain information on this issue, the effect of aldosterone on the acid-soluble *l*-[*methyl*-^{3}H]methionine pool was measured relative to the DNA content of the epithelial homogenates (Table I). After 90 min of incubation, the ^{3}H-labeled, acid-soluble content was 15% higher in the aldosterone-treated tissues. After 240 min of incubation, the ^{3}H-labeled, acid-soluble content was 2% less in the hormone-treated hemibladders. A precise evaluation of effects mediated by changes in the precursor pool requires definition of the specific activity of the methyl donor. Nevertheless, the lack of any change in the acid-soluble ^{3}H content suggests that the aldosterone-stimulated changes in nuclear or cytoplasmic RNA are probably not a consequence of effects on the specific activity of the precursor.

B. Comparison of Incorporation of [*methyl*-^{3}*H*]- *and* [^{14}*C*]*Uridine.* The findings summarized above are compatible with hormonal stimulation either of transcription of the rRNA precursors or of methylation of the post transcriptional products. To obtain information on this question, double-label experiments with *l*-[*methyl*-^{3}H]methionine and [^{14}C]uridine were completed. The uridine-labeled mRNA (i.e., poly(A)(+)-RNA) fraction was separated from the remainder of the newly synthesized RNA by oligo(dT)-cellulose chromatography. Hemibladders were incubated, with and without aldosterone, for 240 min in the presence of both labeled compounds. Cytoplasmic RNA extracts were analyzed by sucrose density gradient centrifugation both before and after fractionations on oligo(dT)-cellulose. The RNA in the high salt eluates of the oligo(dT) columns (containing tRNA and rRNA) were precipitated with 2 volumes of 100% ethanol overnight at −20 °C. The precipitates were washed twice with ethanol–sodium acetate buffer (2:1) and then dissolved in the sodium acetate buffer (~20 A_{254} units/ml). The results are shown in Figures 4 and 5. Aldosterone increased the scc significantly (Figure 4, upper panel) and enhanced [*methyl*-^{3}H] labeling of the 28S rRNA peak but had a marginal if any effect on the 4 and 18S peaks. It should be noted, however, that Figure 4 presents one of three experiments, all of which gave similar results. The integral 18S peak (fractions 9–11) contained 21 and 12% more ^{3}H in the middle and lower panels, respectively, after aldosterone. The increase in the labeling of the 28S peak was particularly marked after oligo(dT)-cellulose chromatography (cf. Figure 4, middle and lower panels). As shown in Figure 5, upper panel, aldosterone augmented incorporation of [^{14}C]uridine into unfractionated cytoplasmic RNA at 4 S, in the 4–18 S region and at 28S. These results are in accord with our earlier findings (Rossier et al., 1974). On fractionation by oligo(dT)-cellulose chromatography, the broad component in the 4–18S region was deleted and stimulation of incorporation of [^{14}C]uridine into 4 (tRNA), and 18 and 28 S (rRNA) was preserved (Figure 4, middle panel). The 4–18S region also contains many species of poly(A)(+)-RNA which were recovered in the low salt eluates. The poly(A)(+)-RNA fraction was also analyzed by sucrose density gradient centrifugation. As shown in Figure 5, lower panel, aldosterone increased uridine labeling of the poly(A)(+)-RNA over a broad region (12–20 S). These results imply that integrated over a 240-min period, aldosterone augments the syn-

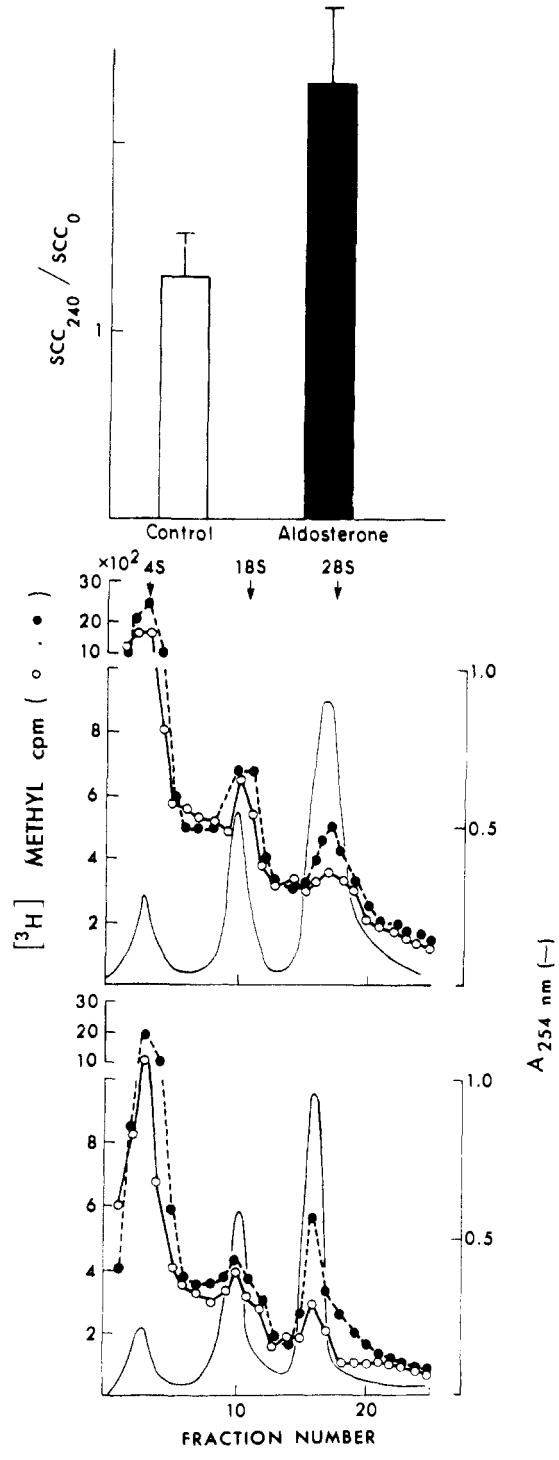

FIGURE 4: Effects of aldosterone on Na^+ transport and on the incorporation of [*methyl*-^{3}H] into cytoplasmic RNA labeled for 240 min. (Upper Panel) Na^+ transport was measured by the scc method. See legend of Figure 1 for definitions. (Middle and Lower Panels) Extracts of total cytoplasmic RNA (middle panel) and of the high salt (0.5 M KCl–0.01 M Tris-HCl) eluates of cytoplasmic RNA fractionated by oligo(dT)-cellulose chromatography (lower panel) analyzed by linear 5–20% sucrose gradient centrifugation. See legend of Figure 1 for definitions.

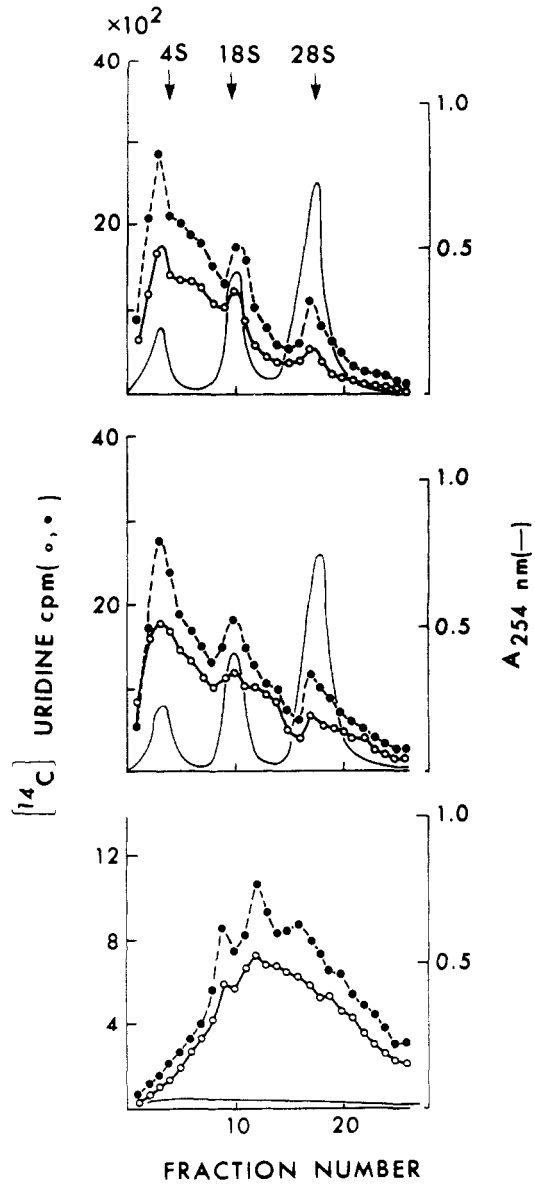

FIGURE 5: The effect of aldosterone on incorporation of [^{14}C]uridine into cytoplasmic RNA labeled for 240 min. See legend of Figure 1 for definitions. (Upper and Middle Panels) Extracts of total cytoplasmic RNA (upper panel) and of the high salt (0.5 M KCl–0.01 M Tris-HCl) eluates of cytoplasmic RNA fractionated by oligo(dT)-cellulose chromatography (middle panel) analyzed by linear 5–20% sucrose gradient centrifugation. (Lower Panel) Low salt (0.01 M Tris-HCl) eluates of cytoplasmic RNA fractionated by oligo(dT)-cellulose chromatography analyzed by linear 5–20% sucrose gradient centrifugation.

thesis of both poly(A)(+)-RNA and of rRNA and that the increased methylation may be a consequence of the latter effect, although other factors may also play a role in this process. No evidence was adduced for the participation of increased cellular uptake of the labeled compound in the effect of aldosterone on the incorporation of [^{14}C]uridine into 18 and 28S RNA, as the hormone had no effect on the acid-soluble ^{14}C content of the homogenates (A/C = 0.85, Table I).

C. Translational Activity of the Ribosomes. To assess whether aldosterone modified ribosomal activity, as a possible index of the physiological consequences of modulation of rRNA synthesis, a translational assay was used in which the variables of formation of the aminoacyl-tRNA precursors and the activity of cell sap factors were minimized (Table II). Without added polynucleotides, the aldosterone-treated epithelia yielded ribosomal fractions of higher translational activity (A/C = 1.39). The difference was less marked with an excess of the exogenous homopolymer poly(U) as the messenger, but was still present when reinitiation was inhibitied with 10 mM NaF or with an excess of a polymer of one of the initiation codons, poly(AUG).

Discussion

Earlier studies implicated RNA and protein synthesis in the action of aldosterone on active Na^+ transport in the toad bladder (Fanestil and Edelman, 1966). Recently, Rossier et al. (1974) provided evidence for enhanced synthesis of 9–12S RNA (with little or no methyl content) by aldosterone. Wilce et al. (submitted for publication) extended these observations and found significant increases in incorporation of uridine and adenosine into cytoplasmic poly(A)(+)-RNA in the first 30 min of exposure to the hormone. These results are in accord with the inference that accumulation of specific mRNAs is involved in the response to mineralocorticoids.

Many, if not all, of the steroid hormones apparently promote rRNA synthesis, in addition to the well-documented effects on accumulation of specific mRNAs (King and Mainwaring, 1974). Our results indicate that aldosterone promotes the methylation of 18, 28, and 40S nuclear RNA; this effect was clearly evident after 90 min of continuous exposure to the hormone and the methyl donor, [^{3}H]methionine (Figure 2). These results imply modulation of the formation of the precursor of rRNA (40 S) and of the processed products (18 and 28 S). After a more extended period of labeling (240 min), cytoplasmic rRNA, particularly the 28S species, was more intensely labeled with the methyl donor and showed increased incorporation of [^{14}C]uridine. Aldosterone, therefore, apparently stimulates rRNA synthesis. This inference is supported by the minimal changes in acid-extractable, ^{3}H or ^{14}C content of the epithelium on administration of aldosterone (Table I). Alternative explanations for the enhanced labeling of nuclear and cytoplasmic rRNA under the influence of aldosterone include primary effects on processing of the precursors of rRNA, e.g., methylation or reduced losses in conversion of precursors to mature rRNA, or on the rates of degradation of the rRNA after formation. The latter explanation is unlikely owing to the relatively long half-life of eucaryotic rRNA (half-life of 2–5 days in rat liver (Hadjiolov, 1966)).

If, as seems probable, aldosterone stimulates de novo synthesis of rRNA, the magnitude of the effect is small compared with that of the morphogenetic steroids, e.g., estrogen or testosterone. The latter produce measurable increases in bulk tissue rRNA (Billings et al., 1969; Mainwaring and Wilce, 1973) and aldosterone does not do so (Porter and Edelman, 1964). In the case of aldosterone, the proposed link between the accumulation of rRNA and the hyperplastic or hypertrophic response induced by growth promoting steroids can be discounted (Tata, 1970). In the toad bladder or in the mammalian kidney epithelium, neither hypertrophy nor hyperplasia has been noted in response to aldosterone (Edelman, 1975). In these systems, aldosterone selectively increases Na^+ transport with a rapid onset and release from the effect, implying fairly rapid turnover of a small population of the mediators. Moreover, the surface cells of the epithelium of the toad bladder are virtually devoid of mitotic activity (Danon et al., 1974). Nevertheless, changes in rRNA metabolism may play a role in the physiological action of aldosterone. To inquire into this possibility, we assessed the effects of aldosterone on the performance of ribosomes in protein synthesis in a reconstituted system. The increased rate of protein synthesis with ribosomes isolated from aldosterone-treated epithelia (Table II) may be attributed to: (1) increased initiation of peptide synthesis; (2) increased rate of translocation of the ribosomes along the messenger; or (3) augmentation of the pool of active ribosomes. When initiation is blocked by specific inhibitors, e.g., NaF or poly(AUG), the number of active ribosomes engaged in completion of the polypeptide chains can be estimated. As shown in Table II, ribosomal preparations from aldosterone-treated epithelia yielded higher endogenous translational activity that was preserved in the presence of inhibitors of initiation. These effects are in accord with the inference that aldosterone increased the pool of active ribosomes. The following lines of evidence support this inference. (1) The rate of translocation depends on the activity of the protein factors from the cell sap and these were provided in excess from toad liver. (2) The downward shift in the *A/C* ratio on saturation of the ribosomes with poly(U) may indicate that more ribosomes were available for initiation in the control group, in that more endogenous messenger was bound to the ribosomes of the aldosterone-treated group. (3) The evidence cited above of a probable increase in mRNA synthesis evoked by aldosterone, i.e., enhanced labeling of poly(A)(+)-RNA, could signify steroid-induced accumulation of mRNA and engagement of more ribosomes in protein synthesis.

TABLE II: Effects of Initiation Inhibitors on Incorporation of [^{3}H]Phenylalanine by Ribosomes Isolated from Control and Aldosterone-Treated Tissue.[a]

Additions	Aldosterone	Control	*A/C*
None	916	656	1.39
+ 100 μg of poly(U)	1356	1139	1.19
+ 10 mM NaF	475	344	1.38
+ 100 μg of poly(AUG)	505	377	1.33

[a] Paired hemibladders were incubated in aldosterone (7×10^{-8} M) or the diluent for 240 min and ribosomal fractions were prepared as described in the text. The results are in units of cpm of [^{3}H]/100 μg of ribosomal RNA. n = 10 pairs of hemibladders.

The higher yield of translational activity in ribosomal fractions from aldosterone-treated toad bladder agrees closely with similar observations in the rat kidney reported by Nandi-Majumdar and Trachewsky (1971). Hill and Trachewsky (1974) also noted changes in phosphorylation of ribosomal proteins after administration of aldosterone and suggested that modification of the ribosomal protein assembly may play a role in the augmented functional capacity of the ribosomes.

The role of the induced rRNA and ribosomes in the response to aldosterone remains obscure in the toad bladder system at this stage. The response of some eucaryotic cells to regulatory factors, however, has, under some circumstances, been attributed to changes in the activity of ribosomes. In a recent review, Shields (1975) outlined the evidence that the first effects of serum factors on fibroblasts is the binding of free mRNA to ribosomes and the formation of polysomes, an effect ascribed to changes in characteristics of the ribosomes. It is possible that the rRNA induced by aldosterone in the later stages of the response act in a similar way, controlling the translation of the induced mRNA. Other steroids have been shown to regulate not only the synthesis but also the rate of degradation of induced mRNAs. Withdrawal of estrogen, for example, results in a rapid loss of hormone-induced translatable mRNA from the chick oviduct (Palmiter and Carey, 1974), implying that the hormone provided an environment that stabilized the induced ovalbumin-mRNA. In the response of the toad bladder to aldosterone, the incorporation of [^{3}H]uridine into poly(A)(+)-RNA was greater during the first 30 min of exposure to the hormone than after 2.5–3 h (Wilce

et al., submitted for publication). Thus, the induced ribosomes may stabilize the poly(A)(+)-RNA synthesized immediately after hormone administration and thereby maintain translation of the induced mRNA and the physiological response.

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Nuclear Estradiol Receptor in the Adult Rat Uterus: a New Exchange Assay†

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ABSTRACT: A protamine exchange assay has been developed to measure uterine nuclear estrogen receptor in mature rats exposed to estradiol (E). After ovariectomized-adrenalectomized mature rats are injected with E, estrogen receptor (RnE) is extracted from uterine nuclei with 0.6 M potassium chloride, diluted, and quantitatively precipitated with protamine sulfate. The precipitate is subjected to a ligand exchange with radiolabeled estradiol (E*), with or without unlabeled diethylstilbesterol, to determine nonspecific binding. At 37 °C complete exchange of E* for E in RnE is observed at 2.5 h; virtually no receptor degradation occurs up to at least 5 h. Exchange does not occur at 4 °C. Using the protamine assay, the depletion of cytoplasmic estrogen receptor (Rc) and the accumulation of RnE were studied at various doses of E at specific time points. Increasing doses of E result in a decrease of Rc with an equal increase of RnE. At the highest dose of E (10 μg) Rc is completely depleted within 10 min, by 6 h it is 25% replenished, and by 24 h returns to slightly above control levels. Within 10 min after the injection, RnE increases to 80-90% of the original cytoplasmic level of receptor (~2-3 pmol/mg of DNA or ~1.5 pmol/100 mg of uterus). At 6 h RnE is 75% depleted and it is completely absent at 24 h. The protamine assay permits precise quantitative studies of nuclear estrogen receptor and avoids the problems of receptor degradation and excessive nonspecific binding often found in exchange reactions at elevated temperatures.

Estrogen administration to mature ovariectomized-adrenalectomized rats results in rapid binding of hormone to specific receptor molecules in the cytoplasm of target organs. This is followed by rapid nuclear accumulation of the receptor-hormone complex (Gorski et al., 1968; Anderson et al., 1973; Giannopoulous and Gorski, 1971; Tchernitchin and Chandross, 1973). Well defined uterotrophic responses such as RNA and protein synthesis (Hamilton, 1971), increased uterine weight, and increased cell division (Anderson et al., 1974) are all associated with nuclear entry of the receptor-hormone complex.

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