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ALTERATION IN THE LABELLING OF RENAL RIBOSOMAL PROTEIN BY ALDOSTERONE

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SUMMARY

The present study is an investigation of the effect of aldosterone on the relative extents of labelling of the renal cortical ribosomal proteins from adrenalectomized rats. It was noted that between 30 and 90 min of incubation of the renal cortical slices at 37°C with radioactive leucine (between 2·5 and 3·5 h following aldosterone administration to the adrenalectomized animals) the hormone did not alter the extent of labelling of the total ribosomal protein population. On the basis of double-isotope labelling it was found that 3·5 h following aldosterone administration there was a relative decrease in the extent of labelling of a renal cortical ribosomal protein(s) whose molecular weight was between 57,000 and 62,000. This decrease in labelling could be due to either a decreased synthesis or a decreased exchange of the ribosomal protein(s). When the rats were first pretreated with actinomycin D the effect of aldosterone on the ribosomal protein(s) was lost. Also, the administration of dexamethasone alone to the adrenalectomized animals did not alter the [^{3}H/^{14}C] ratios of the ribosomal protein(s). This molecular alteration may be physiologically significant since we had found earlier that the functional capacities of these same ribosomes were increased 3·5 h after aldosterone treatment, and since pretreatment of adrenalectomized animals with actinomycin D is known to abolish the antinatriuretic effect of aldosterone while dexamethasone has no sodium retention activity at all on adrenalectomized animals.

INTRODUCTION

Very little had been known until recently about the effect of aldosterone on protein biosynthesis. Edelman and coworkers [1] reported from their experiments *in vivo* that a subcutaneous injection of aldosterone significantly increased the incorporation of [^{3}H]-leucine into the proteins of the microsomal fraction of the rat kidney. Following this, others could show no effect on protein biosynthesis *in vivo* after aldosterone administration [2, 3]. Very recently Lahav and Dietz[4] using the toad bladder system with labelled leucine, have presented results which are consistent with an aldosterone effect on protein synthesis but do not exclude changes in the leucine pool. The results of Porter and Kimsey[5] are also consistent with a mineralocorticoid effect on protein synthesis. Our recent results [6, 7] substantiate the idea that aldosterone and other mineralocorticoids influence protein synthesis in the rat kidney at the translational level.

Further evidence consistent with aldosterone stimulation of ribosome capacity has been provided by Chu and Edelman[8]. Recent experiments indicate that RNA polymerase I functions in the synthesis of nucleolar RNA [primarily ribosomal RNA (rRNA)]; RNA polymerase II is more widely involved in the transcription of nucleoplasmic genes to synthesize many classes of messenger RNA (mRNA) and heterogeneous nuclear RNA [9]. Chu and Edelman[8] showed that aldosterone increased nucleolar polymerase activity (rRNA synthesis) relative to nucleoplasmic polymerase activity (mRNA synthesis) in the adrenalectomized rat kidney.

In view of recent findings concerning the discrete roles of ribosomal proteins in protein biosynthesis [10], the present study was undertaken to investigate the effect of aldosterone in the adrenalectomized rat on the relative extents of labelling of the renal cortical ribosomal proteins. These experiments were designed to explore the possibility that aldosterone specifically altered either the relative extent of synthesis or exchange [11] of a ribosomal protein(s) which might then perhaps be the molecular basis for the increased functional capacities of the ribosomes already reported [6, 7], since we had demonstrated that aldosterone administration to adrenalectomized rats altered the phosphorylation [12] and acetylation [13] of specific renal cortical ribosomal proteins. Covalent modification of ribosomal protein(s) could be one way to produce alterations in the structure and function of the ribosomal apparatus. If such an effect on a ribosomal protein(s) were to be found we would then also have to determine whether or not it had any relevance to the antinatriuretic action of aldosterone by seeing if actinomycin D, which is known to abolish the antinatriuretic effect of aldosterone [14], would also abolish the influence of aldosterone on the relative extent of labelling of this particular ribosomal protein(s). As well, dexamethasone, which is without sodium retention activity [15] should also not affect the ribosomal protein(s) in question if this protein(s) is somehow related to the antinatriuretic effect of aldosterone.

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EXPERIMENTAL

Treatment of rats

The rats were treated as already described [12]. Usually the experimental and control groups each contained 6 rats. When the effect of actinomycin D was studied, 100 μg of the antibiotic was administered subcutaneously per 100 g of weight 20 min. before the aldosterone or the diluent for aldosterone. The influence of the glucocorticoid, dexamethasone, was also investigated after a single subcutaneous injection of 75 μg of this hormone; the control rats received 10% ethanol in saline. Two hours after the hormone or control injections the rats were decapitated, the kidneys were excised and decapsulated, and cortical slices (0·5 mm) were rapidly prepared with a Stadie–Riggs tissue slicer. The tissues were kept on ice for several minutes and moistened with Krebs–Ringer bicarbonate buffer until used for incubations.

Incubation of kidney cortex slices

The slices from each of the two groups were incubated separately with constant shaking at 37°C in Krebs–Ringer bicarbonate buffer, pH 7·4 containing 200 mg glucose and 500 mg albumin per 100 ml (KRBGA buffer) and labelled leucine, as indicated, in stoppered flasks with an atmosphere of 95% O_2–5% CO_2. The weight to volume ratio used in the incubations was 1 g of slices to 3 ml of buffer. Each flask contained either 50 μCi of L-[4,5-^{3}H]-leucine (31 Ci/mmol) or 15 μCi of L-[U-^{14}C]-leucine (250 mCi/mmol) per ml of buffer. Generally there were approximately 2·5 g of slices for each group of six rats. After the 90 min incubation the slices were chilled on ice and rinsed several times with large volumes of cold KRBGA buffer. Finally the rinsed slices were pooled, frozen in an acetone–dry ice bath and stored overnight at −80°C.

Preparation of ribosomes

Ribosomes were prepared and assayed for their purity as described [7, 12].

Removal of nascent peptides

(i) Portions of several of the ribosome preparations were incubated as already described [6] to remove the nascent peptides and endogenous mRNA. The ribosomes were then reisolated [6] and dissolved in 1 ml of 0·01 M sodium phosphate buffer, pH 7·2, containing 0·5 M urea and 1·0% sodium dodecyl sulfate (SDS). (ii) As well, portions of other ribosome preparations were dissolved in medium containing 0·033 M EDTA, 0·05 M Tris–HCl, pH 7·4, 0·01 M KCl and 0·002 M Mg acetate in order to prepare ribosomal subunits and thus release the nascent peptides [3, 16]. The ribosome suspensions were homogenized and incubated at 37°C for 10 min. Following this the suspensions were centrifuged at 400,000 *g* for 5 h in the SB-405 rotor of the International B-60 ultracentrifuge and the pellets obtained, containing both the large and small subunits, were dissolved in 1 ml of 0·01 M sodium phosphate buffer, pH 7·2, containing 0·5 M urea and 1·0% SDS.

Estimation of the incorporation of [^{3}H]- *and* [^{14}C]-*leucine into ribosomal proteins*

The purified ribosomes which were dissolved in 0·01 M sodium phosphate buffer, pH 7·2, containing 0·5 M urea and 1·0% SDS were heated at 70°C for 3 min. Protein quantities were determined by the method of Lowry *et al.*[17] using BSA as a standard. Aliquots of ribosome suspensions containing 5 μg of ribosomal protein were each placed into separate scintillation vials containing 1 ml of 10% hydrogen peroxide and 1 drop of 1·0 N NaOH. Following incubation at 55°C overnight, 10 ml of Instagel (Packard) was added to each vial. The vials were stored in the dark at 4°C for at least 2 days to allow the low levels of background chemiluminescence to subside. Hydrogen peroxide and NaOH were added to all counting vials since these same agents had to be added to the polyacrylamide gel slices in order to dissolve them so that the [^{3}H]- and [^{14}C]-labelled ribosomal proteins in the slices could be effectively counted (see section below on "polyacrylamide gel electrophoresis of double-labelled ribosomal proteins"); this was done in order to maintain the same counting conditions throughout the entire investigation. Radioactivity was measured in an automatic refrigerated liquid scintillation spectrometer. Under our experimental conditions [^{3}H] was measured at approximately 10% efficiency and [^{14}C] at 45% efficiency. Spillover of [^{14}C] counts into the [^{3}H] channel was 12% and was corrected in each case. There was no spillover of [^{3}H] counts into the [^{14}C] channel.

In all our experiments the range of incorporation of [^{3}H]-leucine and [^{14}C]-leucine into the ribosomal proteins was 8 to 30 c.p.m./μg of ribosomal protein for both [^{3}H] and [^{14}C]. Usually the specific radioactivity of the ribosomal proteins was 15 c.p.m./μg ribosomal protein for both [^{3}H] and [^{14}C]. In other words we usually secured about 75 c.p.m./sample for [^{3}H] or [^{14}C].

Time course of incorporation of [^{3}H]*-leucine into ribosomal proteins: effect of aldosterone*

In these experiments incubations of kidney cortex slices from hormone-treated and control adrenalectomized rats were carried out separately for 30, 60 and 90 min using [^{3}H]-leucine. In each group, tissues from each of six rats contributed equally to each time period of incubation. At the end of the incubations the slices were chilled on ice and rinsed several times with large volumes of cold KRBGA buffer containing 0·01 M unlabelled leucine. The rinsed slices from each of the six incubation flasks were then processed separately. The ribosomes were isolated and purified, and the specific radioactivity of the ribosomes was determined as described above.

The cytosol was represented by the supernatant obtained by centrifugation at 100,000 *g* for 2 h. The quantity of protein in this fraction was determined by the method of Lowry *et al.*[17]. To determine the

amount of radioactivity associated with [^{3}H]-labelled protein precursors (e.g. [^{3}H]-amino acids), the protein in this cytosol fraction was precipitated in the cold with a final concentration of 15% trichloroacetic acid (TCA) and the radioactivity of an aliquot of this deproteinized fraction was counted using Instagel. The acid-soluble radioactivity was then calculated with reference to the amount of protein in this cytosol fraction.

Polyacrylamide gel electrophoresis of double-labelled ribosomal proteins

Disc gel electrophoresis was performed with stacking gels on 0·5 × 8 cm separating gels according to a slight modification of the method of Warner[12, 18]. 0·3% and 1·5% ethylene glycol diacrylate was used as the cross-linker in the separating and stacking gels, respectively. About 300 μg of ribosomal protein was heated at 70°C for 3 min before it was added to the stacking gel.

Electrophoresis was carried out at room temperature for about 5·5 h at a constant current of 7 mA/gel. During this time the tracking dye (bromphenol blue) had migrated 6·5 cm along the separation gel.

As shown by Warner[18] and others [19, 20] the ribosomal constituents disaggregate fully in these conditions and they electrophorese independently in the gels. The rRNA present does not interfere with the electrophoresis and was not detected on the stained gels. To confirm that under our experimental conditions rRNA was indeed excluded from entering the gels we have in another investigation [12] extracted rRNA from [^{32}P]-labelled renal cortical ribosomes and then tried to separate the [^{32}P]-labelled RNA by SDS-urea polyacrylamide gel electrophoresis. The extracted rRNA gave no appreciable radioactivity in the gel. The protein–dodecyl sulfate complexes are polyanionic and they migrate toward the positive electrode. In SDS it has been shown previously that all ribosomal proteins migrate toward the anode [18, 19]. Various workers have shown that the electrophoretic mobilities of protein–dodecyl sulfate complexes are inversely proportional to the logarithms of the polypeptide chain molecular weights [19–21] and this has been confirmed in the conditions of these experiments (see Fig. 3).

Following electrophoresis the gels were removed from the tubes and soaked for 3 h at room temperature in a solution containing 5% TCA and 5% sulphosalicylic acid to fix the protein and remove the SDS. It was found to be important to remove the SDS from the gel before staining [20]. The gels were stained as described by Weber and Osborn[21]. The gels were destained according to the method of Vesterberg[22]. These procedures gave gels free from artifacts due either to loss of stain bound to protein or to background coloration. The gels were stored in 7·5% acetic acid. Densities of the stained protein bands were recorded by a Vitatron UR linear recorder.

For radioactivity measurements the gels were cut into 2 mm slices with a device (developed from a Hamilton syringe) which gave 30 equal slices from the origin of the separation gel to the dye front (distance of 6·5 cm). The slices were placed into separate scintillation vials containing 1 ml of 10% hydrogen peroxide and 1 drop of 1·0 N NaOH. The vials were then processed as described above and the counting efficiencies were found to be the same as before. [^{3}H/^{14}C] ratios were determined only on gels in which the lowest net c.p.m. (after correction for background and spillover) for either isotope exceeded 40.

Relation between relative electrophoretic mobility and molecular weight of protein

Twenty micrograms of each of these five proteins of known molecular weights were treated with SDS and urea at 70°C for 3 min. The electrophoresis was carried out exactly as mentioned above for the ribosomal proteins. The relative mobilities of the stained protein bands were calculated as described by Weber and Osborn[21]. Figure 3 is the average of six standard curves.

RESULTS

Time course of incorporation of [^{3}H]*-leucine into ribosomal proteins*

Table 1 shows the time course of [^{3}H]-leucine incorporation into the total isolated and purified renal cortical ribosomes. The kinetics of [^{3}H]-leucine incorporation were similar in tissues from aldosterone-treated and control adrenalectomized animals.

Since the amount of radioactivity associated with the acid-soluble [^{3}H]-labelled protein precursors in the cytosol fraction can influence the labelling of the ribosomes, the extents of incorporation of [^{3}H]-leucine into the ribosomes were determined not only in terms of their specific activities (c.p.m. of [^{3}H] incorporated per mg of ribosomal protein), but also in terms of these specific activities relative to the amount of radioactivity of the acid-soluble protein precursors in the cytosol fraction (c.p.m. acid-soluble [^{3}H]/mg of cytosol protein). With this measure there were no significant differences in the corrected specific activities of the ribosomes between the control and aldosterone-treated rats as calculated by the unpaired *t*-test. Each time point is the mean of five separate incubations for each group of animals.

Comparison of the labelling of ribosomal proteins from aldosterone-treated and control adrenalectomized animals by SDS-polyacrylamide electrophoresis

Total ribosomes were resolved into at least 14 Coomassie blue-stained protein bands. The protein banding patterns of ribosomes from control and aldosterone-treated animals were the same. Each of these protein bands probably represents several protein species, since eukaryotic ribosomes are known to contain 70 or more distinct proteins [20, 23].

The labelling of individual structural proteins of ribosomes was compared in aldosterone-treated and control adrenalectomized rats by a double-labelling technique. In some experiments kidney cortex slices

Table 1. Time course of incorporation of [^{3}H]-leucine into ribosomal proteins of kidney cortex slices of aldosterone-treated and control adrenalectomized rats

Duration of incubation (min)	Experimental group	Specific activity of ribosomes (c.p.m./mg ribosomal protein × 10^{-3})	S.A.* (c.p.m. acid soluble cytosol/mg cytosol protein × 10^{-4})
30	Aldosterone-treated	7·60 ± 0·45†	0·180 ± 0·028‡
	Control	8·50 ± 0·55	0·189 ± 0·033
60	Aldosterone-treated	8·95 ± 0·30	0·225 ± 0·039‡
	Control	9·55 ± 0·35	0·222 ± 0·042
90	Aldosterone-treated	10·45 ± 0·75	0·235 ± 0·051‡
	Control	11·80 ± 0·90	0·225 ± 0·047

* Specific activity of ribosomes (S.A.) corrected for by the amount of radioactivity associated with the acid-soluble [^{3}H]-labelled protein precursors in the cytosol (100,000 *g*) fraction.

† Each time point is the mean value ±1 S.D. of five separate incubations.

‡ Differences between aldosterone-treated and controls are not significant as calculated by the unpaired *t* test ($P < 0·40$).

from aldosterone-treated animals were labelled with [^{14}C]-leucine, those from the controls with [^{3}H]-leucine. In other experiments the radioactive labels were reversed. The tissues were pooled and the ribosomes were isolated, purified and electrophoresed together. The effect of the hormone treatment on ribosomal protein labelling could then be assessed by the [^{3}H/^{14}C] ratios in different slices of the gels. This ratio per gel slice is an indication of the relative extents of labelling of the proteins in that fraction. If all ribosomal proteins from the aldosterone-treated rats were labelled to the same extent as the corresponding proteins from the controls, all the [^{3}H/^{14}C] ratios would be the same.

The variability in [^{3}H/^{14}C] ratios inherent in the experimental design was determined by labelling the ribosomes in kidney cortex from control adrenalectomized animals with [^{3}H]-leucine and [^{14}C]-leucine separately, pooling the tissues, and isolating and electrophoresing the ribosomes as for the experimental group. In order to present succinctly the consistent changes in these ratios due to aldosterone treatment we have computed the averages of the standardized ratios determined on many control and experimental gels, by taking the [^{3}H/^{14}C] ratio in each gel slice and dividing by the ratio of total [^{3}H] radioactivity to total [^{14}C] radioactivity recovered from the gel. In this way [^{3}H/^{14}C] ratios from different gels could be compared.

Figure 1a shows the radioactivity profile of the first series of double isotope experiments. This study represents six separate experiments in which the renal cortical slices from the aldosterone-treated adrenalectomized rats were incubated continuously for 90 min with [^{14}C]-leucine while the slices from the non-treated adrenalectomized (control) rats were incubated with [^{3}H]-leucine. Fourteen electrophoretic gels were run. There were at least two gels for each separate experiment. This profile is the average of all these gels and is quite representative of each individual profile. In all of these studies the levels of radioactivity per slice was calculated after the background radioactivity had been subtracted and corrections made for spillover. Quenching was the same in all gel slices. Figure 1b depicts a densitometric trace of a representative gel. Since the curves in (a) are superimposable over those in (b), it is seen in Fig. 1b that slices 8, 9 and 10 fall on the descending limb of the first large protein peak in the gel.

The [^{3}H/^{14}C] ratios were calculated for all the slices of the 14 gels. In all 14 gels it was found that slices 8, 9 and 10 had [^{3}H/^{14}C] ratios that were significantly and constantly greater than those of the other gel slices (Fig. 2, ●). Even when the nascent peptides were removed from the ribosomes as indicated in the Experimental, the [^{3}H/^{14}C] ratios for slices 8, 9 and 10 were always significantly and constantly greater than those of the other slices. The mean value ±1 S.D. of the standardized [^{3}H/^{14}C] ratios for slices 8, 9 and 10

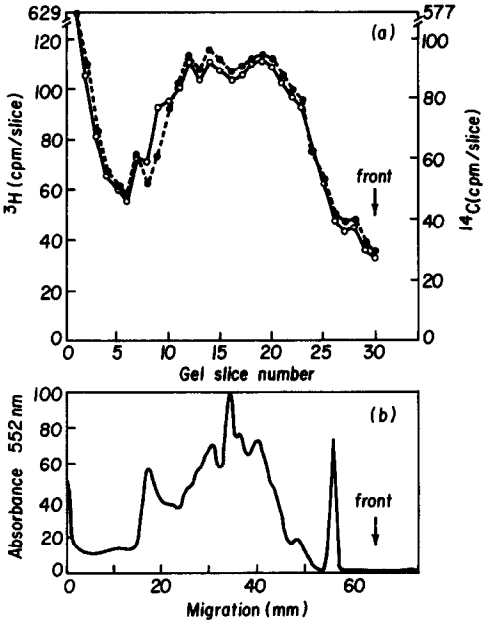

Fig. 1. Mean radioactivity profile (a) and densitometer tracing (b) of stained ribosomal proteins separated on polyacrylamide–urea-SDS gels. Renal cortical slices from adrenalectomized rats which had been treated with aldosterone (●, broken line) or only the diluent for aldosterone (○, solid line) for a period of 2 h were incubated continuously at 37°C for 90 min. with [^{14}C]-leucine (●, broken line) or [^{3}H]-leucine (○, solid line), respectively. The proteins of the isolated and purified ribosomes from the pooled slices were then separated on the gels. Migration was from left to right.

were 1·18 ± 0·12, 1·30 ± 0·28 and 1·12 ± 0·10, respectively. These ratios were significantly different from their respective control ratios (see Fig. 2, ▲) at $P < 0.005$, $P < 0.005$ and $P < 0.01$ as calculated by the unpaired t-test. Removal of the nascent peptides indicated that the protein(s) whose extent of labelling, because of aldosterone treatment, was relatively less from those in the other gel slices was most probably a structural protein component of the ribosome. This suggests, that after aldosterone administration, there is relatively less biosynthesis or exchange [11] of the ribosomal protein(s) in slices 8, 9 and 10 compared to the ribosomal proteins in all the other slices.

In the second series of investigations four separate experiments were performed again; only this time the radioactive labels were reversed. This was done as a further check to obviate any isotope effect. The nascent peptides were removed again too. If aldosterone administration to the adrenalectomized rats differentially altered the extent of labelling of the ribosomal proteins in slices 8, 9 and 10, then in this series of experiments the aldosterone treatment should produce a mirror profile of the curve depicting the [^{3}H/^{14}C] ratio values for these slices in the first series of experiments. Slices 8 and 9 fall on the descending limb of the first large protein peak in the gel—as they did in Fig. 1.

In Fig. 2 we see the curve which is the average of the standardized [^{3}H/^{14}C] ratios for all the slices of the 9 gels (see, ○). There were again at least 2 gels for each separate experiment. Each individual ratio was calculated as described above. The mean value ±1 S.D. of the standardized [^{3}H/^{14}C] ratios for slices 8 and 9 were 0·90 ± 0·08 and 0·88 ± 0·09, respectively. These ratios were significantly lower from their respective control ratios (see Fig. 2, ▲) at $P < 0.005$ as calculated by the unpaired t-test. This may indicate that after aldosterone administration, there is relatively less biosynthesis or exchange [11] of the ribosomal protein(s) in slices 8 and 9 compared to the structural proteins in all other slices.

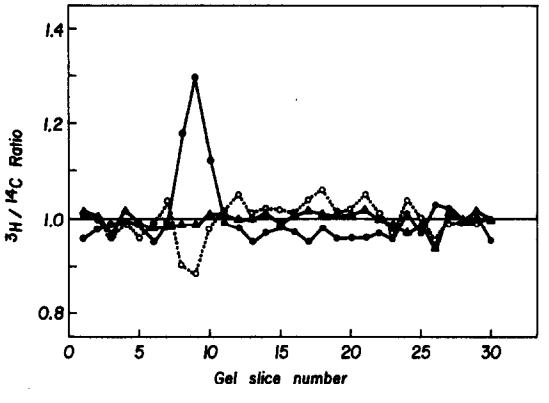

Fig. 2. Influence of aldosterone on renal cortical ribosomal proteins analyzed by the double-labelling technique. This study examines the mean standardized [^{3}H/^{14}C] ratios of all the gel slices in the first (●), second (○) and third (▲) series of experiments. In order to present succinctly the consistent changes in these ratios due to aldosterone treatment we have computed the averages of the standardized ratios determined on many control and experimental gels, by taking the [^{3}H/^{14}C] ratio in each gel slice and dividing by the ratio of total [^{3}H] radioactivity to total [^{14}C] radioactivity recovered from the gel. In this way [^{3}H/^{14}C] ratios from different gels could be compared. The coefficients of variation of the mean gel ratio of 1·00 (computed from the mean standardized [^{3}H/^{14}C] ratios) on the gels from the first (●), second (○), and third (▲) series of experiments are ±2·0%, ±2·8% and ±1·7%, respectively.

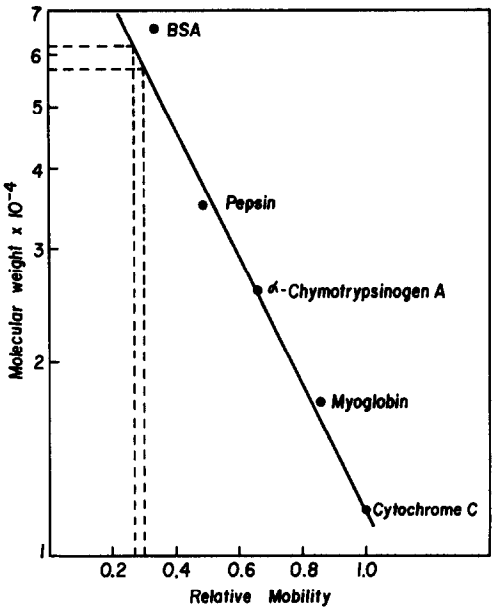

Fig. 3. Calibration curve of the polyacrylamide–urea-SDS gel. A semi-log plot of the known molecular weights of standard proteins against their mobility relative to the tracking dye, bromphenol blue. The dashed lines indicate the range of the mobility of the altered ribosomal protein(s) and the range of its calibrated molecular weight(s). BSA—Bovine serum albumin.

In order to establish the variability in [^{3}H/^{14}C] ratios inherent in the experimental design, tissue slices from control adrenalectomized rats were incubated separately in two groups with either [^{3}H]-leucine or [^{14}C]-leucine. Three separate experiments were carried out in this third series of incubations. Nascent peptides were again removed.

Again the mean standardized [^{3}H/^{14}C] ratios were computed for all the slices of the 7 gels (see Fig. 2, ▲). There were at least two gels for each separate experiment. For each individual gel there were no systematic variations in the standardized [^{3}H/^{14}C] ratios that were evident. There were no significant ratio changes in slices 8, 9 and 10. This curve indicates the error of ratios (deviation from the standardized 1·00) obtained in such a control experiment. The coefficient of variation of the averaged standardized [^{3}H/^{14}C] ratios from the mean gel ratio (i.e. 1·0) on control gels is ±1·7%. It is obvious that there are no significant ratio changes in gel slices 8, 9 or 10 unless the adrenalectomized animals are treated with aldosterone.

Calculation of molecular weight of altered ribosomal protein(s)

The molecular weight of the altered ribosomal protein(s) in gel slices 8 and 9 was determined according to the method of Weber and Osborn[21]. Calculating the relative mobility of the altered ribosomal protein(s) in gel slices 8 and 9 it is seen that its molecular weight, according to Fig. 3, is between 57,000 and 62,000.

Effect of actinomycin D *on the aldosterone-induced alteration of ribosomal protein(s) labelling: effect of dexamethasone on ribosomal protein labelling*

When the effect of actinomycin D was investigated, 100 μg of the antibiotic was administered subcutaneously per 100 g weight 20 min before the aldosterone or the diluent for aldosterone. In the first series of investigations six separate experiments were carried out in which the renal cortical slices from the aldosterone-treated adrenalectomized rats were incubated continuously for 90 min with [^{14}C]-leucine while the slices from the non-treated adrenalectomized (control) rats were incubated with [^{3}H]-leucine. Eight electrophoretic gels were run. There was a minimum of 1 gel for each separate experiment. Figure 4a shows the mean standardized [^{3}H/^{14}C] ratios profile calculated from all of the slices of the 8 gels (see, ●). In the second series of studies four separate experiments were performed again; only this time the radioactive labels were reversed. Figure 4a depicts the [^{3}H/^{14}C] standardized ratios of these 4 experiments and are the average values for 7 separate gels (see, ○). There were 2 gels for each of 3 separate experiments. Included in Fig. 4a is the mean standardized [^{3}H/^{14}C] ratios profile from the control (no aldosterone, no actinomycin D, see ▲) experiments carried out in the previous set of investigations with aldosterone (same as ▲, Fig. 2). We found that there were no significant ratio changes in gel slices 8, 9 and 10 when the animals were first pretreated with actinomycin D. As a matter of fact there were no significant alterations in the ratios of all gels slices when one takes into account the control measure of reversing the labels (to obviate any isotope effect).

The influence of the glucocorticoid, dexamethasone (in 10% ethanol in saline), was also investigated after a single subcutaneous injection of 75 μg of this hormone. The control adrenalectomized rats received 10% ethanol in saline. The procedure for this set of experiments was identical to that for aldosterone. In the first series of investigations five separate experiments were performed in which the renal cortical slices from the dexamethasone-treated adrenalectomized rats were incubated continuously for 90 min with [^{14}C]-leucine while the slices from the non-treated adrenalectomized (control) rats were incubated with [^{3}H]-leucine. Two gels were run for each of the five separate experiments. Figure 4b shows the mean standardized [^{3}H/^{14}C] ratios profile computed from all of the slices of the 10 gels (see, ●). In the second series of studies six separate experiments were carried out again; only this time the radioactive labels were reversed. In Fig. 4b we see the standardized [^{3}H/^{14}C] ratios of these six experiments which are the average values from 12 gels (see, ○). There were two gels for each of the six separate experiments. Included in Fig. 4b is the mean standardized [^{3}H/^{14}C] ratios profile from the control (no aldosterone, no dexamethasone, see ▲) experiments performed in the previous set of investigations with aldosterone (same as ▲, Fig. 2). No significant ratios changes were found in gel slices 8, 9 and 10 when the animals were treated with dexamethasone. As well there were no significant alterations in the ratios of all gel slices when we consider the control measure of reversing the labels.

DISCUSSION

Since it was evident from our previous investigations [6, 7] that aldosterone had in some way altered the

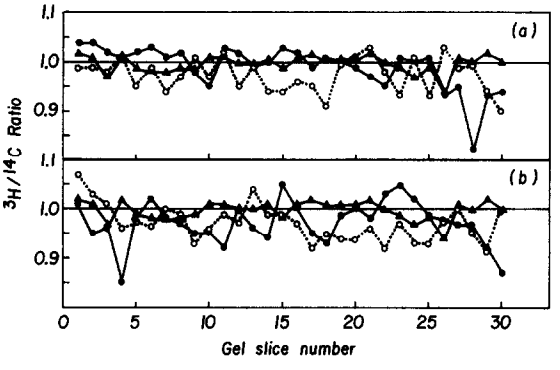

Fig. 4. Effect of aldosterone and actinomycin D (a) and dexamethasone (b) on renal cortical ribosomal proteins analyzed by the double-labelling technique. This first investigation (a) examines the mean standardized [^{3}H/^{14}C] ratios of all the gel slices from adrenalectomized rats which had been pretreated with actinomycin D. In the first series of studies (●) renal cortical slices from adrenalectomized rats which had been treated with aldosterone or only the diluent for aldosterone for a period of 2 h were incubated continuously at 37°C for 90 min with [^{14}C]-leucine or [^{3}H]-leucine, respectively. In the second series of studies (○) the radioactive labels were reversed. Included in Fig. 4a is the mean standardized [^{3}H/^{14}C] ratios profile for the control (no aldosterone, no actinomycin D, see ▲) experiments carried out in the previous set of investigations (see ▲ Fig. 2). The coefficients of variation of the mean gel ratio of 1:00 (computed from the mean standardized [^{3}H/^{14}C] ratios) on the gels of the first series (●), second series (○) and controls (▲) are ±4·5%, ±3·6% and ±1·7%, respectively. The second investigation (b) examines the influence of dexamethasone on the mean standardized [^{3}H/^{14}C] ratios of all the gel slices from adrenalectomized rats. In the first series of studies (●) renal cortical slices from adrenalectomized rats which had been treated with dexamethasone or only the diluent for dexamethasone for a period of 2 h were incubated at 37°C for 90 min. with [^{14}C]-leucine or [^{3}H]-leucine, respectively. In the second series of studies (○) the radioactive labels were reversed. Included in Fig. 4b is the mean standardized [^{3}H/^{14}C] ratios profile for the control (no aldosterone, no dexamethasone, see ▲) experiments carried out in the previous set of investigations (see ▲, Fig. 2). The coefficients of variation of the mean gel ratio of 1·00 (computed from the mean standardized [^{3}H/^{14}C] ratios) on the gels of the first series (●), second series (○), and controls (▲), are ±4·6%, ±3·7% and ±1·7%, respectively. The standardized ratio value represents the [^{3}H/^{14}C] ratio in a given gel slice divided by the ratio of the total [^{3}H] radioactivity to total [^{14}C] radioactivity in the gel.

functional capacities of the renal cortical ribosomes from adrenalectomized rats, and since we had also demonstrated [12] that the hormone altered the phosphorylation of specific renal cortical ribosomal proteins which might in turn alter ribosome function [24], the present experiments were designed to explore the possibility that aldosterone produced a specific effect on the relative extent of incorporation of labelled leucine into a ribosomal protein(s) which might then perhaps be another possible molecular basis for the increased functional capacitites of the ribosomes. Since we had investigated the functional capacities of the renal cortical ribosomes 3·5 h after hormone administration [6, 7], the same time period was employed in these present studies as well. If such an effect on a ribosomal protein(s) were to be found we would also have to determine whether or not it had any relevance to the antinatriuretic action of aldosterone in adrenalectomized rats.

Hirsch and Hiatt[25] have already shown that the average half-life of ribosomal proteins is 5 days while Dice and Schimke[11] have demonstrated that the incorporation of [^{3}H]-leucine into ribosomal proteins reaches a maximum specific activity at 12 h. There were no significant differences in the labelling of the total ribosomal proteins of the control and aldosterone-treated rats when the incorporation of [^{3}H]-leucine into the total ribosomal proteins was corrected for by the acid-soluble [^{3}H]-labelled protein precursors in the cytosol fraction.

Our present investigations show quite conclusively that aldosterone administration to adrenalectomized rats effected a relative decrease in the extent of incorporation of labelled leucine into a particular renal cortical ribosomal protein(s) whose molecular weight is between 57,000 and 62,000. This conclusion is based on the fact that the [^{3}H/^{14}C] ratio was altered only for this protein(s) after aldosterone treatment. Even after correction for quenching and the [^{3}H/^{14}C] ratios were calculated on the basis of d.p.m. we obtained essentially the same results.

These investigations also revealed that when the rats were first pretreated with actinomycin D the effect of aldosterone on the ribosomal protein(s) was lost. It has also been shown by several investigators [14] that pretreatment of adrenalectomized animals with actinomycin D will also abolish the action of aldosterone on the Na^+/K^+ ratios in the urine. As well, we found that administration of dexamethasone to the adrenalectomized rats did not alter the [^{3}H/^{14}C] ratios of the ribosomal proteins. Dexamethasone is a glucocorticoid and is known to be without sodium retention activity [15].

Dice and Schimke[11] have recently reported that the different protein constituents of rat liver ribosomes are synthesized and degraded at different rates and that most of the ribosomal proteins are exchangeable *in vitro* with similar proteins in the cytoplasm. They suggest that alterations in the ability of ribosomes to carry out protein synthesis need not require synthesis of entire ribosomes, but only changes in the types or concentrations of specific ribosomal proteins which may then be incorporated preferentially into ribosomes through exchange. The rapidity with which ribosomal proteins exchange leaves little doubt that rapid changes in ribosome function may occur.

In this report the altered [^{3}H/^{14}C] ratio after aldosterone administration for the protein(s) whose molecular weight is between 57,000 and 62,000 could be due to either a decreased synthesis or a decreased exchange of this protein(s). If the altered [^{3}H/^{14}C] ratio after aldosterone administration is due to exchange of a protein(s), then the results and Chu and Edelman[8] concerning rRNA synthesis may be pertinent in that perhaps a new species of rRNA is synthesized after the mineralocorticoid treatment, and that it is this new species of rRNA which affects how the ribosomal proteins exchange. We know, too, that actinomycin D preferentially inhibits rRNA synthesis [26], and that actinomycin D will abolish the antinatriuretic effect of aldosterone and also the effect of aldosterone on the altered [^{3}H/^{14}C] ratio of this particular ribosomal protein(s).

The possibilities raised by these results are very exciting. Could the decreased biosynthesis or exchange of this protein(s) be the molecular basis for the augmentation in the functional capacities of the ribosomes after aldosterone administration [6, 7] and subsequently for the antinatriuretic effect of the mineralocorticoid?

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