PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, **147** 396-398 (1974)

Effect of Aldosterone on Acetylation of Ribosomal Proteins in Outer and Inner Zones of Kidney (38349)

DANIEL TRACHEWSKY

Laboratory of Molecular Biology, Montreal Clinical Research Institute, 110 Pine Avenue West, Montreal H2W 1R7, Quebec, Canada

Acetylation of histones is one of the earliest chemical events in the process of gene activation for RNA synthesis in various systems (1). In liver responding to stimulation by cortisol, histone acetylation is clearly correlated with patterns of RNA synthesis (1). Ribosomal proteins can be rapidly acetylated not only in rat liver but in heart, kidney, and thymus as well (2). In addition we (3) and others (4, 5) have found that aldosterone treatment of adrenalectomized rats produces a very early increase of renal histone acetylation.

In this paper we show a differential effect of aldosterone administration on the acetylation of ribosomal proteins in the outer and inner zones of the kidney of the adrenalectomized rat. This is of interest because Edelman and coworkers (6) have recently shown that the Type I mineralocorticoid receptors are probably equally distributed in the outer cortex and medullary papilla. These receptors are believed to initiate the intracellular sequence of reactions ultimately resulting in the physiologic effect of the hormone (7).

Materials and Methods. Male bilaterally adrenalectomized hooded rats weighing approximately 150 g (six per Expt) each received an intravenous injection of 4 μg of *d*-aldosterone in saline. 3.25 hr later each rat received 1.5 mCi of sodium [^{3}H]acetate (sp act 2.2 Ci/mmole) iv and 15 min later was decapitated. Liew and Gornall (2) have already shown that the incorporation of [^{3}H]acetate into the ribosomal proteins increased between 5 and 15 min of labeling; the amount of radioactive acetate in the ribosomal proteins declined after 15 min of labeling but remained relatively constant from 30 to 60 min. The control adrenalectomized rats (six per Expt) received injections of saline and of 1.5 mCi of sodium [^{3}H]acetate. Seven separate experiments were performed.

The kidneys were divided immediately after death into three gross anatomical regions: cortex, outer medulla, and inner medulla. Ribosomes from these three layers were isolated and purified in medium of high ionic strength (8). The ribosomal proteins were then extracted with urea and LiCl (9). After the mixture had stood overnight at 4°, the RNA precipitate was removed by centrifugation at 1,000*g* for 20 min. Urea–LiCl extraction is known to precipitate all the ribosomal RNA from the supernatant (10). The ribosomal proteins extracted by urea–LiCl were precipitated by 7.5% trichloroacetic acid and then washed with 7.5% trichloroacetic acid, ether, and ethanol. These washes were to eliminate the possibility of incomplete dissociation of ribosomes from the membrane by the detergents used (Lubrol WX and deoxycholate), and also to exclude the possibility that the radioactivity found in the ribosomal proteins was due to the incorporation of [^{3}H] acetate into nucleotides or the lipids of the endoplasmic reticulum. After these treatments the radioactivity remained in the ribosomal proteins.

The presence of radioactive labile acetyl groups in the ribosomal proteins was verified by acid hydrolysis and steam distillation of volatile [^{3}H] acetate as described by Allfrey (1). The quantity of ribosomal protein was determined by the method of Lowry *et al.* (11). Under our experimental conditions [^{3}H] was counted at approximately 25% efficiency.

Results and Discussion. Table I shows that 3.5 hr after aldosterone administration there was a significantly (60%) lower acetylation of the ribosomal proteins in the renal cortex and a significantly (77–94%) higher acetylation of the ribosomal proteins in the renal medulla. The

Printed in U.S.A.

TABLE I. Effect of Aldosterone on the *in Vivo* Acetylation of Renal Ribosomal Proteins 3.5 hr after the Hormone Administration.

Kidney portion	Rats	Sodium [^{3}H] acetate incorporated into volatile acetyl groups of ribosomal proteins[a] (dpm/mg-ribosomal protein)	P[c]	Ratio[d] Aldosterone-treated control
Cortex	Aldosterone-treated	3,860 ± 905[b]	<0.0025	0.40
	Control	9,620 ± 3,589		
Outer medulla	Aldosterone-treated	2,210 ± 281	<0.005	1.77
	Control	1,250 ± 194		
Inner medulla	Aldosterone-treated	1,870 ± 378	<0.005	1.94
	Control	964 ± 196		

[a] Pulse-labeling for 15 min.

[b] Specific activity of volatile acetyl groups of ribosomal proteins. Mean value ± 1 standard deviation (seven separate experiments).

[c] Calculated by unpaired *t* test.

[d] Specific activity of the volatile [^{3}H] acetyl groups of the ribosomal proteins from the aldosterone-treated animals divided by the specific activity of the same groups from the control rats.

specific activity of the labile [^{3}H] acetyl groups of the ribosomal proteins was two to nine times as great in the cortex as in the medulla. We found that 75%, 30%, and 37% of the incorporated [^{3}H] acetate was in the form of labile acetyl groups in the cortex, outer medulla, and inner medulla, respectively.

Just as the phosphorylation of ribosomal proteins may play a part in altered ribosome function (12–16), the acetylation of ribosomal proteins may likewise have a role in the regulation of protein synthesis. Both positive (12–14) and negative correlations (15) between ribosomal protein phosphorylation and protein synthesis have been documented. We have just recently reported (17) that 3 hr after aldosterone administration to adrenalectomized rats the phosphorylation of at least two renal cortical ribosomal proteins of high molecular weights increased, while that of at least two others of lower molecular weights decreased. We have shown (8,18) that 3.5 hr after aldosterone administration to adrenalectomized rats the functional capacity of ribosomes from the renal cortex is increased, so that in this tissue we have a negative correlation between ribosomal protein acetylation and protein synthesis. However, we should also bear in mind that Eil and Wool (19) recently found no appreciable and consistent difference in the *in vitro* functional capacity of phosphorylated and nonphosphorylated rat liver ribosomes. They may not, however, have tested the proper function, or it may be possible that liver ribosomes are already maximally phosphorylated before they are isolated, so that *in vitro* phosphorylation is only a small fraction of what can occur.

Gornall and coworkers (20) report a 31% lower acetylation of whole kidney ribosomal protein from adrenalectomized rats 2.5 hr after administration of aldosterone. In agreement with this, appropriate calculation from our figures gives a 38% decrease for whole kidney ribosomal proteins 3.5 hr after aldosterone administration, and this yields a negative correlation with protein synthesis when we remember the earlier demonstration (21) of higher incorporation of [^{14}C] leucine into whole kidney protein after aldosterone administration. It will now be interesting to determine whether this negative correlation holds for medulla as well as cortex by determining whether the functional capacity of medullary ribosomes is lower 3.5 hr after aldosterone administration.

Summary. The role of acetylation of ribosomal proteins in the regulation of protein synthesis was investigated by examination of the effect of aldosterone injection into adrenalectomized rats on the *in vivo* incorporation of [^{3}H] acetate into the ribosomal proteins of kidney cortex and medulla (outer and inner zones) separately. Incorporation of acetate into acetyl groups of the ribosomal proteins of adrenalectomized rats was highest in the cortex. Three and

a half hours after aldosterone administration, acetate incorporation in the cortex was 60% lower and in the medullary zones 77–94% higher than in the saline injected controls. This decrease for the cortex, and a calculated 38% decrease for whole kidney, are negatively correlated with increased protein synthesis after hormone administration.

We gratefully acknowledge the support of this investigation made possible by grant MT-3294 to D.T. by the Canadian Medical Research Council.

The excellent technical assistance of Sandra Lawrence and Janie Prosmanne is appreciated.

1. Allfrey, V. G., in "Regulatory Mechanisms for Protein Synthesis in Mammalian Cells" (A. San Pietro, M. R. Lamborg and F. T. Kenney, eds.), p. 65. Academic Press, New York (1968).

2. Liew, C. C., and Gornall, A. G., J. Biol. Chem. **248,** 977 (1973).

3. Trachewsky, D., and Lawrence, S., Proc. Soc. Exp. Biol. Med. **141,** 14 (1972).

4. Libby, P. R., Biochem. J. **134,** 907 (1973).

5. Liew, C. C., Suria, D., and Gornall, A. G., Endocrinology **93,** 1025 (1973).

6. Funder, J. W., Feldman, D., and Edelman, I. S., Endrocrinology **92,** 994 (1973).

7. Feldman, D., Funder J. W., and Edelman, I. S., Amer. J. Med. **53,** 545 (1972).

8. Trachewsky, D., Nandi Majumdar, A. P., and Congote, L. F., Eur. J. Biochem. **26,** 543 (1972).

9. Gestland, R. F., and Staehelin, T., J. Mol. Biol. **24,** 149 (1967).

10. Leboy, P. S. , Cox, E. C., and Flaks, J. G., Proc. Nat. Acad. Sci. U.S.A. **52,** 1367 (1964).

11. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193,** 265 (1951).

12. Blat, C., and Loeb, J. E., F.E.B.S. Letters **18,** 124 (1971).

13. Walton, G. M., Gill, G. N., Abrass, I. B., and Garren, L. D., Proc. Nat. Acad. Sci. U.S.A. **68,** 880 (1971).

14. Correze, C., Pinell, P., and Nunez, J., F.E.B.S. Letters **23,** 87 (1972).

15. Monier, D., Santhanam, K., and Wagle, S. R., Biochem. Biophys. Res. Commun. **46,** 1881 (1972).

16. Kabat, D., Biochemistry **9,** 4160 (1970).

17. Hill, A. M., and Trachewsky, D., J. Steroid Biochem. (1974) in press **5**.

18. Nandi Majumdar, A. P., and Trachewsky, D., Can. J. Biochem. **49,** 501 (1971).

19. Eil, C., and Wool, I. G., J. Biol. Chem. **248,** 5130 (1973).

20. Liew, C. C., Suria, D., and Gornall, A. G., Abstract 370, IV International Congress of Endocrinology, Washington, D. C. Excerpta Medica Foundation, Amsterdam (1972).

21. Edelman, I. S., and Fanestil, D. D., *in* "Biochemical Actions of Hormones" (G. Litwack, ed.), Vol. 1, p. 321. Academic Press, New York (1970).

Received April 4, 1974. P.S.E.B.M., 1974, Vol. 147.