Histone Acetylation and Hormone Action

EARLY EFFECTS OF ALDOSTERONE ON HISTONE ACETYLATION IN RAT KIDNEY

By PAUL R. LIBBY

Roswell Park Memorial Institute, 666 Elm Street, Buffalo, N.Y. 14203, U.S.A.

(Received 19 January 1973)

Histone acetylation in the kidney of the adrenalectomized rat was increased 5 min after a physiological dose of aldosterone. The increase was exclusively in the histone F2a1. The increase in histone acetylation was given by 9 α -fluorocortisol, deoxycorticosterone and cortisol, but not by progesterone. The increase in histone acetylation was abolished by prior treatment of the animals with the anti-mineralocorticoid agent, SC14266 [potassium 3-(3-oxo-17 β -hydroxy-4,6-androstadien-17-yl) propionate]; the basal degree of histone acetylation was unaffected. Pretreatment of the animals with actinomycin D or cycloheximide did not affect the stimulation, showing the stimulation was not due to increased synthesis of enzyme.

In recent years several instances have been found where an increase in histone acetylation has been found before an increase in RNA synthesis occurs. Several groups have shown that phytohaemagglutinin treatment of human lymphocytes results in an increase in histone acetylation before an increase in RNA synthesis (Pogo *et al.*, 1966; Mukherjee & Cohen, 1969; Ono *et al.*, 1969; Monjardino & MacGillivray, 1970), although Ono *et al.* (1969) demonstrated that in this system cortisol could dissociate the increase in histone acetylation from the increase in RNA synthesis. Monjardino & MacGillivray (1970) found the increase in histone acetylation with one preparation of phytohaemagglutinin, but not with another preparation, and suggested that histone acetylation might be caused by an impurity in the phytohaemagglutinin.

In addition to these results on human lymphocytes Pogo *et al.* (1968) found increased histone acetylation in the liver remaining after partial hepatectomy and this preceded the increase in RNA synthesis also noted. Allfrey *et al.* (1966) reported that cortisol administration to adrenalectomized rats caused increases in the acetylation of liver histones and an early increase followed by a decrease in the acetylation of thymus histones. Takaku *et al.* (1969) reported increased histone acetylation in mouse spleen after erythropoietin administration and Libby (1972*b*) reported an increase in uterus histone acetylation after oestradiol administration.

The kidney of the adrenalectomized rat contains enzymes for the acetylation of exogenous histones, which are stimulated *in vitro* by mineralocorticoids (Libby, 1972*c*). It was decided to determine if the kidney of the adrenalectomized rat responds *in vitro* to mineralocorticoids by a change in histone acetyla-

tion. The present study demonstrated that a sharp increase in the acetylation of one histone fraction is an early response of the kidney to mineralocorticoids.

Materials and Methods

Reagents

Sodium [^3H]acetate was purchased from the International Chemical and Nuclear Corporation, Irvine, Calif., U.S.A. Unlabelled sodium acetate was added to give a final specific radioactivity of 50 mCi/mmol. Cycloheximide was purchased from the Sigma Chemical Co., St. Louis, Mo., U.S.A., actinomycin D from Calbiochem, La Jolla, Calif., U.S.A., ethylene diacrylate from the Borden Chemical Co., Philadelphia, Pa., U.S.A., aldosterone from Schwartz/Mann, Orangeburg, N.Y., U.S.A., and Sepraphore III from the Gelman Co., Ann Arbor, Mich., U.S.A. 9 α -Fluorocortisol was a generous gift from the Upjohn Co., Kalamazoo, Mich., U.S.A., and SC14266 [potassium 3-(3-oxo-17 β -hydroxy-4,6-androstadien-17-yl) propionate] was a generous gift from G. D. Searle Co., Chicago, Ill., U.S.A.

Animals

Adrenalectomized female rats, 75-100g, were purchased from the Charles River Corp., Wilmington, Mass., U.S.A. The animals were shipped 1 day after the operation was performed and were received the next day. The animals were maintained on Purina rat chow and 0.9% NaCl drinking water *ad libitum* and were used for experiments 3 or 4 days after the operation. Animals were injected intraperitoneally with 0.5 ml of 0.9% NaCl containing 1 mCi of labelled

sodium acetate. When animals were given hormones, 0.01 ml of an ethanol solution of the hormone was added to the solution of acetate in saline; 0.01 ml of ethanol was added to the solution injected into control animals.

Preparation of chromatin and proteins

Animals were killed by cervical dislocation at various times after injection. Kidneys were removed, frozen on solid CO₂ and stored at -20°C. Both kidneys from one animal were pooled, thawed and homogenized in a Teflon-glass, Potter-Elvehjem homogenizer with about 10 ml of 0.075 M-NaCl-0.024 M-EDTA, pH 8.0 (Bonner *et al.*, 1968). All procedures were carried out at 0-4°C unless otherwise stated. The homogenate was forced through nylon gauze and centrifuged at 3500 rev./min (Sorvall rotor SM-24) for 10 min. The pellet was washed once with the above buffer followed by two washes with 0.05 M-Tris, pH 8.0. The pellet was then suspended in 1.7 M-sucrose and centrifuged at 55000 rev./min for 30 min in a Spinco Ti 65 rotor. The pellet was washed twice with 0.05 M-Tris, pH 8.0, to remove sucrose. Histones and non-histone chromatin proteins were extracted and analysed as previously described (Libby, 1972b).

Results

Time-course of incorporation of acetate into kidney chromatin proteins

Incorporation of acetate into the histones of the kidney is essentially complete within the first 3-5 min after intraperitoneal injection. These results are given in Table 1. This is similar to previous findings on the incorporation of acetate into uterus histones (Libby, 1972b). In contrast with the incorporation of acetate

into histones, the incorporation into non-histone chromatin proteins continues over the course of the experiment (Table 1).

Table 1 also shows the effect of a physiological dose of aldosterone (0.34 µg/kg) on the acetylation of chromatin proteins. Neither the acetylation of the histone F1 nor of the non-histone chromatin proteins was affected by the aldosterone at any time-period. However, as shown in Table 1, the acetylation of the histone band F2+F3 at 5 min was strongly stimulated by the dose of aldosterone. By 20 min after the injection the experimental values had returned to control values similar to the situation in the uterus (Libby, 1972b).

In both the livers of partially hepatectomized rats (Pogo *et al.*, 1968) and in the uterus of rats treated with oestradiol (Libby, 1972a) the major effect on histone acetylation was on the acetylation of the histone fraction F2a1. Portions of histones from the 5 min time-point of Table 1 were subjected to polyacrylamide-gel electrophoresis and the results are shown in Fig. 1. Obviously aldosterone only affects the acetylation of the histone F2a1 and has no effect on other histones at this time.

Effect of aldosterone dose on histone acetylation

Rats were injected with various doses of aldosterone with the acetate and were killed 5 min after injection. Table 2 shows that a dose of 0.034 µg/kg caused a significant increase in the incorporation of acetate into the F2+F3 histone band. Doses of 0.34 and 3.4 µg/kg caused further increases in the incorporation of acetate, but the dose of 34 µg/kg had no further effect. There was no significant effect of aldosterone on the incorporation of acetate into the histone F1 at any dosage (results not shown).

Table 1. *Incorporation of acetate into rat kidney chromatin proteins at various times after injection of 1 mCi of sodium [³H]acetate*

Four rats in each group were injected with [³H]acetate in 0.5 ml of sterile saline as described in the text and killed at the time-intervals shown. Proteins were analysed as described in the text. For the experiment, each rat received 0.34 µg of aldosterone/kg in the [³H]acetate solution. Results are the mean ± s.d. * *P* < 0.01 from control value.

Time (min)	Incorporation of acetate (pmol/mg of protein)					
	Histone F1		Histone F2+F3		Non-histone chromatin proteins	
	Control	Experiment	Control	Experiment	Control	Experiment
3	43.2 ± 23.1	32.0 ± 15.4	27.6 ± 17.6	33.7 ± 15.3	36.3 ± 2.5	43.3 ± 12.7
5	48.5 ± 16.2	44.7 ± 18.7	29.0 ± 5.1	49.2 ± 4.8*	45.4 ± 7.9	47.4 ± 11.0
10	51.3 ± 5.4	56.6 ± 14.4	40.5 ± 5.5	58.3 ± 5.1*	60.9 ± 5.5	67.5 ± 11.5
20	28.7 ± 13.4	20.3 ± 8.0	31.7 ± 5.9	28.5 ± 6.2	97.8 ± 12.6	94.3 ± 9.2

Fig. 1. [^3H]Acetate incorporation into histone fractions

Portions of histones from the 5min time-point of Table 1 were subjected to electrophoresis on polyacrylamide gels. Conditions of electrophoresis and analysis are as described by Libby (1972b). The bars denote the S.D.

Effect of anti-mineralocorticoid on histone acetylation

The compound SC14266 is a water-soluble spironolactone of high anti-mineralocorticoid activity (Porter, 1968). Animals were injected with this compound 30min before injection of sodium acetate. For comparison a second group of animals was given either acetate alone or acetate plus aldosterone, but not SC14266 (Fig. 2). Neither aldosterone, SC14266, nor the combination of the two, had any effect on the acetylation of the histones F1. Aldosterone caused the anticipated rise in the acetylation of the histone band F2+F3, SC14266 caused a slight decrease in the acetylation of this band and aldosterone did not cause any increase in the acetylation of this histone band in the animals pretreated with the anti-mineralocorticoid.

Effect of antibiotics on histone acetylation

Several steroid hormones induce new protein synthesis in their target tissues (Barker, 1967; Rosen & Nichol, 1963; O'Malley *et al.*, 1969). It is possible

Table 2. Effect of various doses of aldosterone on acetate incorporation into kidney histones 5min after treatment

Animals were injected intraperitoneally with the dose of aldosterone in 0.5ml of 0.9% NaCl containing 1mCi of sodium [^3H]acetate as detailed in the text. The number of animals in the group is given in parentheses. * $P < 0.05$ from control; ** $P < 0.001$ from control.

Dose ($\mu\text{g/kg}$)	Incorporation into histones F2+F3 (pmol/g)
0	32.8 ± 6.2 (8)
0.034	$40.8 \pm 4.9^*$ (4)
0.34	$49.1 \pm 4.1^{**}$ (8)
3.4	$51.4 \pm 4.6^{**}$ (4)
34	$48.4 \pm 3.2^{**}$ (4)

that the increase in acetylation of the histone F2a1 is due to the rapid induction of an enzyme specific for the acetylation of this one histone. (Such specificities of histone acetokinases have been described by Gallwitz, 1972.) To rule out this possibility animals were pretreated with either actinomycin D or cycloheximide. At zero time they were then injected with either [^3H]acetate or [^3H]acetate plus aldosterone and killed 5min later. Table 3 presents the results of this experiment and compares the results with those obtained from animals not so pretreated. Two conclusions may be drawn from these results. First, both cycloheximide and actinomycin D pretreatments caused a marked diminution of acetate incorporation into the various histones, as seen in the uterus (Libby, 1972b). Secondly, pretreatment caused no change in the stimulation caused by aldosterone on the incorporation of acetate into the histone band F2+F3, suggesting that the stimulation of histone acetylation is not due to enzyme induction. In these experiments the extent of inhibition of either protein synthesis or RNA synthesis was assessed by the injection of either L-[^{14}C]leucine or [^{14}C]orotic acid. Cycloheximide inhibited protein synthesis in the kidney by greater than 98% and actinomycin D inhibited RNA synthesis by 84% (results not shown).

Effect of aldosterone on the incorporation of acetate into liver histones

Aldosterone in high concentrations has glucocorticoid activity (Schuler *et al.*, 1954) and the kidney is capable of gluconeogenesis (Cohn *et al.*, 1951). It was therefore decided to examine the effect of aldosterone on the liver, the other organ concerned with gluconeogenesis but not concerned with salt transport. Table 4 presents results on the incorporation of

Fig. 2. Effect of spironolactone on kidney histone acetylation

Animals received 2mg of SC14266 in 1 ml of saline intraperitoneally 30min before injection of [3 H]acetate or [3 H]acetate plus 0.34 μ g of aldosterone/kg. (a) Incorporation into histone F1. The bars show the s.d. (b) Incorporation into histone band F2+F3. The difference between control and aldosterone treated is significant at the 1% level.

Table 3. Effect of antibiotics on histone acetylation

Animals treated with cycloheximide received 5mg/kg intraperitoneally in 1 ml of 0.9% NaCl 3h before injection of sodium [3 H]acetate. Animals treated with actinomycin D received 2mg/kg intraperitoneally 2h before injection of sodium [3 H]acetate. All animals were killed 5min after injection of sodium [3 H]acetate. There were four animals in each group. Aldosterone dose was 0.34 μ g/kg. Values are given as the mean $\pm$ s.d. * $P < 0.02$ from control. † $P < 0.05$ from cycloheximide. ‡ $P < 0.01$ from actinomycin D.

	Incorporation of acetate (pmol/mg)	
	Histone F1	Histone F2+F3
Control	90 $\pm$ 50	201 $\pm$ 76
Aldosterone	60 $\pm$ 6	342 $\pm$ 21*
Cycloheximide	98 $\pm$ 65	107 $\pm$ 49
Cycloheximide and aldosterone	125 $\pm$ 18	176 $\pm$ 24†
Actinomycin D	63 $\pm$ 16	101 $\pm$ 23
Actinomycin D and aldosterone	46 $\pm$ 22	163 $\pm$ 17‡

acetate into the histones of kidney and liver in the presence and absence of aldosterone. Whereas aldosterone markedly increases the acetylation of

Table 4. Effect of aldosterone on histone acetylation in kidney and liver

Animals were given 1 mCi of [3 H]acetate and killed 5min after injection. Experimental rats received 0.34 μ g of aldosterone/kg. There were four animals in each group, and values are means $\pm$ s.d. * $P < 0.01$ from control value.

Tissue	Acetate incorporation into histone (pmol/mg)	
	F1	F2+F3
Kidney Control	48.5 $\pm$ 16.2	29.0 $\pm$ 5.1
Kidney Aldosterone	44.7 $\pm$ 18.7	49.2 $\pm$ 4.8*
Liver Control	23.8 $\pm$ 8.7	15.7 $\pm$ 5.0
Liver Aldosterone	19.4 $\pm$ 5.2	16.4 $\pm$ 6.5

kidney histones F2+F3, it has no effect on the incorporation of acetate into the liver histones.

Effect of other steroids on histone acetylation in kidney

Several other adrenalcortical steroids also possess mineralocorticoid activity, as do some synthetic steroids. Table 5 shows that several steroids also increase the acetylation of the kidney histones to extents comparable with those caused by aldosterone. Progesterone, however, is an hormonal steroid with

Table 5. *Effect of steroids on acetylation of kidney histones*

Animals were killed 5 min after injection. There were four animals in each group and values are means $\pm$ S.D. * $P < 0.05$, ** $P < 0.02$.

Steroid	Incorporation (pmol/mg of histone F2+F3)
Expt. 1	
None	32.4 $\pm$ 6.1
Aldosterone (0.5 μ g)	54.9 $\pm$ 11.8**
9 α -Fluorocortisol (1 μ g)	65.0 $\pm$ 23.3*
Expt. 2	
None	52.8 $\pm$ 13.9
Aldosterone (1.0 μ g)	102.8 $\pm$ 20.6
Cortisol (10 μ g)	77.8 $\pm$ 3.6*
Expt. 3	
None	19.3 $\pm$ 2.6
Progesterone (10 μ g)	15.0 $\pm$ 1.1
Deoxycorticosterone (1 μ g)	26.4 $\pm$ 3.6*

little or no mineralocorticoid activity and this hormone has no effect on histone acetylation in the kidney.

Discussion

The above results show that one of the early actions of aldosterone on the kidney is to increase the acetylation of one histone fraction in the kidney chromatin. Similar results have been reported for two other steroid hormone target tissues, the liver and the uterus. Libby (1972b) reported that oestradiol causes an early and transient increase in uterine histone acetylation and Allfrey *et al.* (1966) reported that cortisol increased the amount of acetate incorporation into liver histones 15 min after injection. In addition, cortisol causes an increase in histone acetylation in the cortisol-sensitive lymphosarcoma P1798, but has no effect on histone acetylation in the resistant strain, P1798R (P. R. Libby, unpublished work). Two recent reports partially confirm our observations on the aldosterone effect on the kidney. Trachewsky & Lawrence (1972) found a rise in histone acetylation 15 min after hormone administration, an effect which was somewhat later than that which we found (Table 1); however, these workers used much higher doses of aldosterone (1 mg/rat). Liew *et al.* (1972) investigated the acetylation of kidney histones at later time-periods after aldosterone treatment. At 45 min after administration (the earliest time examined), aldosterone stimulated the incorporation of acetate into the histone fraction F3. Since this time is approaching the time when aldosterone has gross effects on sodium

transport in the rat kidney (Fimognari *et al.*, 1967), it is likely that the stimulation of acetylation of the histone F3 is a late effect of aldosterone. All of these observations suggest that a general effect of steroid hormones on target tissues may be to increase the extent of acetate incorporation into the histones of the target-tissue chromatin.

In any event, the results above demonstrate that the increase in histone acetylation in kidney chromatin is a true physiological, mineralocorticoid effect of aldosterone. Thus this effect is given by physiological doses of aldosterone and only a slight additional effect is apparent when animals are given much higher doses. It is given by other steroids with potent mineralocorticoid activity, but not by progesterone at a comparable dose. The experiments with the anti-mineralocorticoid agent SC14266 show a specific inhibition of the specific increase in histone acetylation caused by aldosterone, since there was no effect on the incorporation of acetate into the histone F1 under any circumstance and no effect on the incorporation of acetate into the histone band F2+F3 in animals not treated with aldosterone; there was only an inhibition of the stimulation which aldosterone caused in the incorporation of acetate into the histone band F2+F3 (Fig. 2).

The mechanism or mechanisms by which aldosterone causes this change in the histone acetylation are at present unknown. The experiments given in Table 3 probably rule out the possibility that this is a case of rapid enzyme induction. Although both actinomycin D and cycloheximide pretreatment lowers the basal extents of incorporation of acetate into histones (an effect noted also in the uterus by Libby, 1972b), they are unable to suppress the stimulation caused by a 5 min treatment with aldosterone. Since both protein synthesis and RNA synthesis were almost completely inhibited in the kidney we feel that there is no possibility of the synthesis of a specific enzyme.

A second mechanism for such an increase is an aldosterone-mediated increase in the nuclear pool of acetyl-CoA. I am inclined to rule this out on the basis of the results presented in Fig. 1 and Table 1. All histone fractions are acetylated, as well as the non-histone chromatin proteins, and although the ϵ -amino groups of lysyl groups of F1 histones are apparently not acetylated (Candido & Dixon, 1972), several workers have observed the incorporation of acetate into the histones F1 of several tissues (Pogo *et al.*, 1966; Pogo *et al.*, 1968; Shepherd *et al.*, 1972; Libby, 1972b). Since aldosterone affects only the acetylation of the histone F2a1, the effect of aldosterone on pool size does not seem to be important. These results are not conclusive, however, since the possibility remains that the enzyme which acetylates the histone F2a1 is not saturated with acetyl-CoA under normal conditions, but an increase in pool size due to aldosterone

causes an increase in acetylation. The presence of at least two histone acetokinases in rat kidney with different specificities has been reported (Gallwitz, 1971), but Michaelis constants were not measured. In this laboratory, we have studied three histone acetokinases from calf uterus with different specificities for histones, but with approximately the same K_m values for acetyl-CoA (S. Harvey & P. R. Libby, unpublished work), suggesting that similar results are possible in rat kidney.

The third possibility is that aldosterone directly stimulates the histone F2a1 acetokinase in the kidney nucleus. There is some evidence that this possibility might be the correct one. Kidney cytosol contains a histone acetokinase activity, which is stimulated by physiological amounts of aldosterone *in vitro* (Libby, 1972c). This stimulation showed certain characteristics similar to those shown above, in that other mineralocorticoids (deoxycorticosterone, 9 α -fluorocortisol and cortisol) also caused stimulations *in vitro* and incubation in the presence of aldactone, another spironolactone, abolished the stimulation, but had no effect on the basal values. On this basis I suggest that aldosterone directly stimulates a histone acetokinase, which causes the increased acetylation *in vivo* detailed above. Such an increase in histone acetylation may lead to a greater template capacity of the kidney chromatin; Allfrey *et al.* (1966) demonstrated such an effect of histone acetylation. Increased template capability may lead to the synthesis of a new species of RNA and increased RNA synthesis and increased protein synthesis has been implicated in the action of aldosterone on kidney (Congote & Trachewsky, 1972; Castles & Williamson, 1965; Fanestil & Edelman, 1966), as well as in the action of oestrogen on the uterus (Hamilton, 1968).

This work was supported in part by Grant no. P-612 of the American Cancer Society.

References

- Allfrey, V. G., Pogo, B. G. T., Pogo, A. O., Kleinsmith, L. J. & Mirsky, A. E. (1966) in *Histones*; Ciba Foundation Study Group no. 24 (De Reuck, A. V. S. & Knight, J., eds.), p. 42-67, Little, Brown and Co., Boston
- Barker, K. L. (1967) *Endocrinology* **81**, 791-797
- Bonner, J., Chalkley, G. R., Dahmus, M., Famborough, D., Fujimora, F. F., Huang, R. C., Huberman, J., Jensen, R., Marushige, K., Ohlenbusch, H., Olivera, B. & Widholm, J. (1968) *Methods Enzymol.* **12**, 3-65
- Candido, E. P. M. & Dixon, G. H. (1972) *J. Biol. Chem.* **247**, 3868-3873
- Castles, T. R. & Williamson, H. E. (1965) *Proc. Soc. Exp. Biol. Med.* **119**, 308
- Cohn, C., Katz, B. & Kolinsky, M. (1951) *Amer. J. Physiol.* **165**, 423-428
- Congote, L. F. & Trachewsky, D. (1972) *Biochem. Biophys. Res. Commun.* **46**, 957
- Fanestil, D. D. & Edelman, I. S. (1966) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **25**, 912-916
- Fimognari, G. M., Fanestil, D. D. & Edelman, I. S. (1967) *Amer. J. Physiol.* **213**, 954-962
- Gallwitz, D. (1971) *FEBS Lett.* **13**, 306-308
- Gallwitz, D. (1972) *Biochim. Biophys. Acta* **263**, 315-328
- Hamilton, T. H. (1968) *Science* **161**, 649
- Libby, P. R. (1972a) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **31**, abstr. 456
- Libby, P. R. (1972b) *Biochem. J.* **130**, 663-669
- Libby, P. R. (1972c) in *Estrogen Target Tissue and Neoplasia* (Dao, T. L., ed.), p. 85-100, University of Chicago Press, Chicago
- Liew, C. C., Suria, D. & Gornall, A. G. (1972) *Int. Congr. Endocrinol. 4th: Excerpta Med. Found. Int. Congr.* Abstr. no. 370
- Monjardino, J. P. & MacGillivray, A. J. (1970) *Exp. Cell Res.* **60**, 1-15
- Mukherjee, A. B. & Cohen, M. M. (1969) *Exp. Cell Res.* **54**, 257-260
- O'Malley, B. W., McGuire, W. L., Kohler, P. O. & Korenman, S. G. (1969) *Recent Progr. Horm. Res.* **25**, 105-160
- Ono, T., Terayama, H., Takaku, F. & Nakao, K. (1969) *Biochim. Biophys. Acta* **179**, 214-220
- Pogo, B. G. T., Allfrey, V. G. & Mirsky, A. E. (1966) *Proc. Nat. Acad. Sci. U.S.* **55**, 805-812
- Pogo, B. G. T., Pogo, A. O., Allfrey, V. G. & Mirsky, A. E. (1968) *Proc. Nat. Acad. Sci. U.S.* **59**, 1337-1344
- Porter, G. A. (1968) *Mol. Pharmacol.* **4**, 224-287
- Rosen, F. & Nichol, C. A. (1963) *Vitam. Horm. (New York)* **21**, 136-214
- Schuler, W., Desaulle, P. & Meier, R. (1954) *Experientia* **10**, 142
- Shepherd, G. R., Walters, R. A., Hardin, J. M. & Nolan, B. J. (1972) *Arch. Biochem. Biophys.* **149**, 175-182
- Takaku, F., Nakao, K., Ono, T. & Terayama, H. (1969) *Biochim. Biophys. Acta* **195**, 396-400
- Trachewsky, D. & Lawrence, S. (1972) *Proc. Soc. Exp. Biol. Med.* **141**, 14-20