Journal of Steroid Biochemistry, Vol. 9, pp. 739–742

0022-4731/78/0801-0739$02.00/0

ALDOSTERONE INDUCED CHANGES IN PROTEIN SYNTHESIS IN RAT INTESTINE

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(*Received* 1 *September* 1977)

SUMMARY

Adrenalectomy decreases the incorporation of [^{14}C]-leucine into the acid insoluble fraction of both the small and large intestinal mucosa of adrenalectomised rats. Aldosterone injection (1 μg and 10 μg/100 g body weight) restores incorporation values to normal. Deoxycorticosterone and corticosterone do not show such an effect. In the case of large intestine there is a greater stimulation of incorporation into cytosol than into other sub-cellular fractions. No effect of hormones could be demonstrated on protein synthesis by bladder.

INTRODUCTION

Aldosterone effects on target tissues are thought to be mediated via the synthesis of new species of RNA and protein. A number of studies showing abolition of the aldosterone response on Na^+ transport by inhibitors of RNA synthesis [1–4], stimulation of precursor incorporation into RNA by aldosterone in a variety of tissues [5–13] and stimulation of DNA dependent RNA synthesis [4, 14, 15] provide strong support for the view that changes in RNA synthesis occur as a response to aldosterone treatment in a variety of tissues.

On the other hand the evidence for an effect of aldosterone on amino acid incorporation into protein is unconvincing [16]. Although three reports have appeared suggesting a stimulation of amino acid incorporation into protein in rat kidney or toad bladder [7, 17, 18] these have not been confirmed [16]. In addition Trachewsky and co-workers [19–21] have produced evidence for a specific mineralocorticoid effect on kidney cortical ribosomes.

Although colon and small intestine are known to be target tissues for aldosterone [22–26] little is known about its effect at the biochemical level. We have recently shown that aldosterone 'receptors' exist in intestinal mucosal cytosol and that aldosterone stimulates precursor incorporation into RNA in both small and large intestine [13]. In this paper we have examined the effect of aldosterone on leucine incorporation into protein in large and small intestine and in bladder of rats.

MATERIALS AND METHODS

Animals were treated as described previously [13]. Hormones were administered by i.p.* injection in 0.9% sodium chloride containing 4% ethanol 60 min prior to isotope injection. Radioactive precursors were dissolved in 0.9% sodium chloride and injected i.p. 60 min prior to sacrifice. In double label experiments rats from one group were injected with [^{3}H]-leucine and from another group with [^{14}C]-leucine. The intestinal mucosal scrapings of one rat from the [^{14}C]-group and one rat from the [^{3}H]-group were mixed, homogenized in 4 volumes of 0.25 M sucrose, 3 mM $CaCl_2$, 0.1 M Tris/HCl pH 7.4 and fractionated by the method of Haussler and Norman [27]. Samples were subject to oxidation in a Packard Model 306 Sample Oxidiser and the separated $[^3H]_2O$ and $[^{14}C]O_2$ counted.

In other experiments the radioactivity incorporated into the acid insoluble protein fraction was determined by adding 2 ml 10% TCA containing 0.1% leucine to 0.4 ml of homogenate, collecting the pellet by centrifugation, redissolving it in 2.5 ml 0.3 M KOH and reprecipitating with 2.5 ml 10% TCA/0.1% leucine. This washing procedure was repeated twice and the pellet finally redissolved in 0.3 M KOH. An aliquot (0.4 ml) was taken for radioactivity determination as described by Anderson and McClure [28]. Acid soluble radioactivity was determined by combining the initial supernatant with the first wash.

Protein and DNA determinations were as described previously [13].

RESULTS

The effect of adrenalectomy and subsequent aldosterone treatment on [^{14}C]-leucine incorporation into the TCA soluble and insoluble fractions of small intestine is shown in Fig. 1. Adrenalectomy causes a 59% decrease in incorporation into protein ($P < 0.001$) but has a smaller effect (27% decrease; $P < 0.05$) on incorporation into the soluble fraction. Aldosterone at 10 μg/100 g body weight restores incorporation into protein to 113% of normal and at 1 μg/100 g body weight has a lesser effect, with incor-

* Abbreviations: TCA, trichloracetic acid; i.p., intraperitoneal.

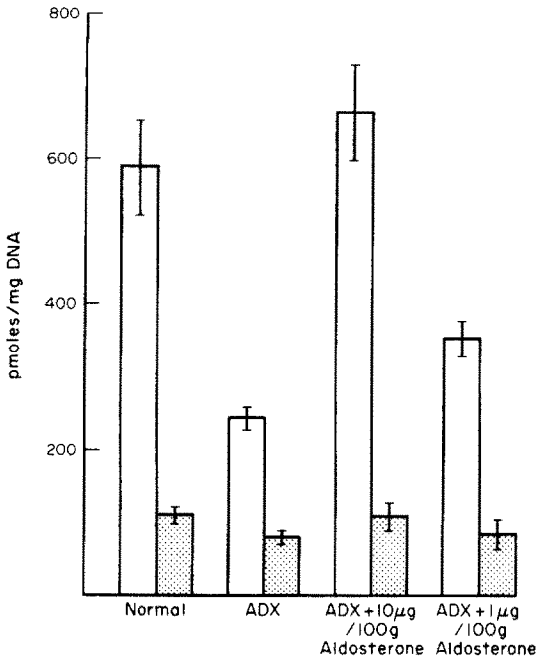

Fig. 1. The effect of adrenalectomy (ADX) and subsequent administration of aldosterone at doses shown on the incorporation of [^{14}C]-leucine (59 mCi/mmol) into the TCA soluble and insoluble fractions of rat small intestinal mucosa. Aldosterone was injected 60 min before isotope administration (10 μCi/100 g). Rats were sacrificed 2 h later. Values shown represent the mean ± S.E.M. for six rats. Open columns: acid insoluble fraction; hatched columns: acid soluble fraction.

poration 60% of normal. In both cases the values are significantly increased when compared to adrenalectomised animals ($P < 0.001$ and $P < 0.002$ respectively). However the values obtained after treatment with 1 μg/100 g body weight are significantly less than the values obtained with normal (not adrenalectomised) animals ($P < 0.05$). The values for the soluble fraction are 105% and 82% of normal respectively ($P < 0.05$ and $P = 0.14$ compared to adrenalectomised animals).

Figure 2 shows the situation in large intestine. Adrenalectomy causes a 52% reduction in incorporation into protein ($P < 0.05$). Treatment with 1 μg/100 g body weight increased incorporation to 76% of normal and with 10 μg to 94% of normal. In both cases the increase was significant when compared with the adrenalectomised values ($P < 0.002$ and

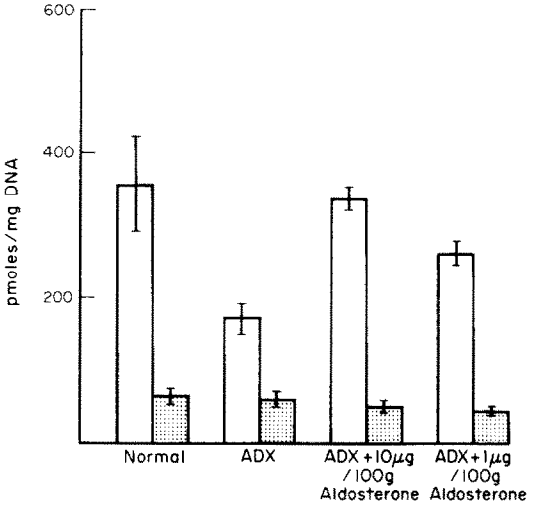

Fig. 2. Similar conditions to Fig. 1. The large intestinal mucosa was isolated from the same groups of animals.

$P < 0.001$ respectively) but neither of the values for aldosterone treated animals was significantly different from normal. No significant changes in uptake into the soluble fraction were detected and, in fact, the hormone treated animals were lower than either adrenalectomised or normal animals.

Deoxycorticosterone and corticosterone have no stimulatory effect in either large or small intestine at 10 μg/100 g body weight (Table 1). At a much larger dose corticosterone apparently has a small stimulatory effect in both tissues but this is much less than the effect of aldosterone (cf. Figures 1, 2), and is not significant ($P = 0.87$ and 0.73 respectively).

We also examined various sub-cellular fractions from both large and small intestine to determine if a specific effect on any particular fraction could be detected (Table 2). In the case of small intestine all fractions seemed to be affected equally. However in large intestine there does seem to be a more marked effect on the cytosol, as demonstrated by an increase in the ratio in Group A and a decrease in Group C. Group C is significantly different from both groups A and B ($P < 0.02$ and $P < 0.005$ respectively).

Attempts to demonstrate increased synthesis of individual protein bands on polyacrylamide gels were unsuccessful because of the relatively low incorporation of radioactivity into intestinal proteins.

Recently aldosterone has been implicated in the

Table 1. The effect of corticosterone and deoxycorticosterone on protein synthesis in small and large intestine. Conditions as for Fig. 1

Hormone	Dose	Incorporation into acid insoluble fraction (pmol/mg DNA) Small intestine	Large intestine
Corticosterone	2 mg/100 g	272 ± 53	201 ± 60
Corticosterone	10 μg/100 g	192 ± 2	145 ± 13
Deoxycorticosterone	10 μg/100 g	165 ± 4	123 ± 5
ADX	—	243 ± 13	169 ± 20

Table 2. Relative incorporation ratios for sub-cellular fractions of large and small intestine

	Small intestine			Large intestine		
	Cytosol	Mitochondria	Microsomes	Cytosol	Mitochondria	Microsomes
Group A	1.10(2)	1.25(2)	1.25(2)	1.67 ± 0.31	0.83 ± 0.03	1.06 ± 0.31
Group B	1.12 ± 0.06	1.32 ± 0.14	1.00 ± 0.11	1.17 ± 0.07	1.20 ± 0.09	1.13 ± 0.13
Group C	1.08 ± 0.32	1.08 ± 0.06	0.90 ± 0.04	0.43 ± 0.11	1.11 ± 0.11	1.01 ± 0.04

In Group A adrenalectomised animals were injected with [^{3}H]-leucine and aldosterone treated with [^{14}C]-leucine. The relative incorporations were determined in each fraction as a $^{14}C/^3H$ ratio for that fraction and then these ratios were related to the ratio found in the nuclear fraction. In this way an aldosterone induced stimulation in a particular fraction shows up as a number greater than 1. Group C was similar, except that the labels were reversed and hence an increased incorporation would be represented by a number less than 1. Group B was a control in which neither group of animals received hormone. $n = 3$ except where shown.

control of active Na^+ transport in the rabbit urinary bladder [29]. We failed to demonstrate any aldosterone effect on protein synthesis in the rat urinary bladder. Whole bladders were homogenized, so that subtle mucosal changes may have been lost in the whole tissue homogenate.

DISCUSSION

The inference that aldosterone action requires the synthesis of new protein was initially based on the observation that interference with ribosomal mechanism by puromycin or cycloheximide inhibited the response of toad bladder epithelia to aldosterone [2]. However since then there has been very little direct evidence to support such a theory. Only preliminary reports [16] and unconfirmed reports [7, 17] have observed enhanced amino acid incorporation after aldosterone treatment.

The difficulty in demonstrating such an effect probably lies in the target tissue used. The rat kidney has been used most extensively, but with little success, perhaps because the percentage of aldosterone responsive cells is low so that changes in protein synthesis within these cells is difficult to detect. We have previously shown that the intestine is an alternative system in which to study aldosterone action [13] and it seemed likely that a mucosal scraping would provide a greater percentage of aldosterone responsive cells.

Figures 1 and 2 show that aldosterone at a high concentration (10 μg/100 g) and a more physiological (1 μg/100 g) significantly stimulates the incorporation of leucine into the protein fraction of large and small intestinal mucosa. Corticosterone even at high doses (2 mg/100 g) and deoxycorticosterone failed to stimulate protein synthesis to the same extent (Table 1). In fact, corticosterone and deoxycorticosterone, at a concentration of 10 μg/100 g depressed leucine incorporation in both large and small intestine, but this effect is only small compared to the stimulation observed in aldosterone treated animals. This decreased [^{14}C]-leucine incorporation could be due to a corticosteroid stimulated uptake of endogenous non-radioactive leucine, resulting in an apparent depression of protein synthesis, or alternatively the effect may be real, in that corticosteroids may inhibit protein synthesis as is the case in activated human lymphocytes [31]. The absence of a corticosterone effect argues against the aldosterone stimulation being attributable to its glucocorticoid effect, as does the fact that significant stimulation is observed at low doses (1 μg/100 g).

These effects cannot be caused solely by a change in the uptake of radioactive precursor since the incorporation into the soluble fraction is increased to a lesser extent than the incorporation into the insoluble fraction in the case of small intestine (Fig. 1) and not increased at all in the case of large intestine (Fig. 2).

The physiological dose of aldosterone restores the rate of protein synthesis in the large intestine of ADX rats. However in the small intestine although this dose significantly stimulates protein synthesis, it does not restore it to normal levels. This is in keeping with previous RNA synthesis studies [13] and also physiological evidence that the large intestine is the main site of aldosterone action in the alimentary tract [27].

Table 2 shows that there is a preferential stimulation of incorporation into the cytosol of large intestine, which is compatible with the finding by Scott and Saperstein[30] that there is a specific effect of aldosterone on the synthesis of soluble proteins in toad urinary bladder. However, no such preferential stimulation was found in small intestine, despite the stimulation of protein synthesis observed in this tissue. However although these results show an aldosterone mediated effect on protein synthesis they do not allow a distinction between a primary effect on the synthesis of a small number of proteins and a more general, possibly indirect, effect.

Acknowledgements—We would like to thank Mr. J. Marshall for valuable technical assistance.

REFERENCES

1. Williamson H. E.: Mechanism of the antinatriuretic action of aldosterone. *Biochem. Pharmac.* **12** (1963) 1449–1450.
2. Edelman I. S., Bogaroch R. and Porter G. A.: On the mechanism of action of aldosterone on sodium transport: the role of protein synthesis. *Proc. natn. Acad. Sci., U.S.A.* **50** (1963) 1169–1177.

3. Fanestil D. D. and Edelman I. S.: On the mechanism of action of aldosterone on sodium transport: effects of inhibitors of RNA and of protein synthesis. *Fedn. Proc.* **25** (1966) 912–916.
4. Chu L. H. and Edelman I. S.: Cordycepin and α-amanitin: inhibitors of transcription as probes of aldosterone action. *J. memb. Biol.* **10** (1972) 291–310.
5. Castles T. R. and Williamson H. E.: Stimulation *in vivo* of renal RNA synthesis by aldosterone. *Proc. Soc. expl. Biol. Med.* **119** (1965) 308–311.
6. Castles T. R. and Williamson H. E.: Mediation of aldosterone induced anti-natriuresis via RNA synthesis *de novo*. *Proc. Soc. expl. Biol. Med.* **124** (1967) 717–719.
7. Fimognari G. M., Fanestil D. D. and Edelman I. S.: Induction of RNA and protein synthesis in the action of aldosterone in the rat. *Am. J. Physiol.* **213** (1967) 954–963.
8. Forte L. and Landon E. J.: Aldosterone-induced RNA synthesis in the adrenalectomised rat kidney. *Biochim. biophys. Acta* **157** (1968) 303–309.
9. Porter G. A., Bogaroch R. and Edelman I. S.: On the mechanism of action of aldosterone on sodium transport. *Proc. natn. Acad. Sci., U.S.A.* **52** (1964) 1326–1333.
10. DeWeer P. and Crabbé J.: The role of nucleic acids in the sodium retaining action of aldosterone. *Biochim. biophys. Acta* **155** (1968) 280–289.
11. Rousseau G. and Crabbé J.: Stimulation by aldosterone of a rapidly labeled RNA fraction in toad bladder tissue. *Biochim. biophys. Acta* **157** (1968) 25–32.
12. Kalra J. and Wheldrake J. F.: Evidence that the stimulation of precursor incorporation into RNA of rat kidney by aldosterone is mainly an effect on uptake. *FEBS lett.* **25** (1972) 298–300.
13. Watts R. W. and Wheldrake J. F.: Aldosterone induced changes in RNA synthesis in rat intestine. *J. steroid biochem.* **7** (1976) 263–266.
14. Liew C. C., Liu D. K. and Gornall A. G.: Effect of aldosterone on RNA polymerase in rat heart and kidney nuclei. *Endocrinology* **90** (1972) 488–495.
15. Mishra R. K., Wheldrake J. F. and Feltham L. A. W.: Aldosterone stimulated aggregate RNA polymerase in rat kidney. *FEBS lett.* **24** (1972) 106–108.
16. Ludens J. H. and Fanestil D. D.: The mechanism of aldosterone function. *Pharmac. Ther.* **2** (1976) 263–266.
17. Simone P. G. and Solomon S.: Aldosterone: effect on incorporation of leucine into the trichloracetic acid precipitable fraction of rabbit renal corticol tissue. *Experientia* **26** (1970) 656–657.
18. Benjamin W. B. and Singer I.: Aldosterone-induced protein in toad urinary bladder. *Science* **186** (1974) 269–271.
19. Majumdar N. A. P. and Trachewsky D.: Protein synthesis by ribosomes from rat kidney cortex: Effect of aldosterone and bilateral adrenalectomy. *Can. J. Biochem.* **49** (1971) 501–509.
20. Trachewsky D., Majumdar N. A. P. and Congote L. F.: Effects of aldosterone and other steroids *in vivo* on rat kidney cortex. *Eur. J. Biochem.* **26** (1972) 543–552.
21. Trachewsky D. and Yang H. Y.: Alteration in the labelling of renal ribosomal protein by aldosterone. *J. steroid Biochem.* **6** (1975) 1157–1164.
22. Edmonds C. J. and Marriott J.: The effect of aldosterone and adrenalectomy on the electrical potential difference of rat colon and on the transport of sodium, potassium, chloride and bicarbonate. *J. Endocr.* **39** (1967) 517–531.
23. Spät A., Suligo M., Stwez J. and Solyom J.: Effect of aldosterone on the intestinal transport of sodium and potassium in rats. *Acta physiol. Hung.* **24** (1964) 465–469.
24. Hill G. L. and Clarke A. M.: The effect of aldosterone on mucosal transfer of sodium, potassium and water in the jejunum and ileum of the rat *in vitro*. *Proc. Univ. Otago Med. Sch.* **47** (1969) 76–77.
25. Crocker A. D. and Mundy A.: Effect of aldosterone on sodium and water absorption from rat jejunum. *J. Endocr.* **38** (1967) 25–26.
26. Edmonds C. J.: Effect of aldosterone on mammalian intestine. *J. steroid Biochem.* **3** (1972) 143–149.
27. Haussler M. and Norman A.: The subcellular distribution of physiological doses of vitamin D_3. *Archs biochem. Biophys.* **118** (1974) 145–153.
28. Anderson L. E. and McClure W. O.: An improved scintillation cocktail of high solubilising power. *Analyt. Biochem.* **51** (1973) 173–179.
29. Lewis S. and Diamond J.: Active sodium transport by mammalian urinary bladder. *Nature, Lond.* **253** (1975) 747–748.
30. Scott W. and Sapirstein V.: Identification of aldosterone induced proteins in the toad's urinary bladder. *Proc. natn. Acad. Sci., U.S.A.* **72** (1975) 4056–4060.
31. Rosenberg J. C., Lysz K., Strefling A. and Rosenberg S. A.: Steroid suppression of protein synthesis of activated human lymphocytes. *J. Surg. Res.* **20** (1976) 255–260.