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ALDOSTERONE INDUCED CHANGES IN RNA SYNTHESIS IN RAT INTESTINE

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SUMMARY

After 30 min ^{3}H-aldosterone localized extensively along the intestinal tract of adrenalectomised rats. The localization varied from 0.39 pmol/mg protein in jejunal mucosa to 0.13 pmol/mg protein in rectal mucosa. These values were all higher than those of kidney from the same animals (0.11 pmol/mg protein). Determination of aldosterone binding to cytosol extracts *in vitro* showed a dissociation constant of $6.6 \pm 3.8 \times 10^{-8}$ M and a maximum binding of 2.7×10^{-12} mol/mg protein. Comparable kidney cytosol figures were $1.7 \pm 0.2 \times 10^{-8}$ M and 6.7×10^{-13} mol/mg protein. ^{14}C-Formate was used as a measure of RNA synthesis. Adrenalectomy decreased formate incorporation into the whole homogenate, acid soluble and RNA fractions of mucosa from large and small intestine. Aldosterone (1 μg and 10 μg/100 g body weight) injected 75 min prior to precursor, restored incorporation values to normal in the large intestine and significantly stimulated values in the small intestine. In time course studies maximal stimulation was observed within 3 h of hormone administration. 24 h after administration, incorporation values were not significantly different from adrenalectomy values.

INTRODUCTION

Aldosterone is known to act on a number of target organs in animals, including kidney, sweat glands, intestinal mucosa, salivary glands and bladder [1–5]. In the case of mammalian kidneys and amphibian urinary bladder it has been shown that aldosterone enters the cell and binds to protein receptors. It then caused a stimulation of DNA-dependent RNA synthesis presumably followed by the synthesis of aldosterone induced proteins [6,7]. Although there are several reports concerning the physiological effect of aldosterone on intestine [1, 8–10] little is known about changes in RNA and protein synthesis induced by this hormone. In general it has been found that ion transport rates are increased following aldosterone injection although the details are somewhat confused, with reports of effects on the colon after 90 min [1], the small intestine after 3 h [8, 9] and after a delay of 24 h [10]. We have investigated the effects of aldosterone on the incorporation of precursors into RNA *in vivo* and find an apparent stimulation of RNA synthesis in all sections of the intestine within 3 h.

MATERIALS AND METHODS

Male Hooded Wistar rats (150–200 g) were maintained on drinking water containing 0·5 mg/ml neomycin sulphate. Adrenalectomies were performed by means of bilateral dorsal incisions and animals were subsequently maintained on 1% sodium chloride/0·5 mg/ml neomycin sulphate. Animals were used 5 days after adrenalectomy. 12 h before hormone or isotope administration all rats were given 200,000 units penicillin, 50 mg neomycin sulphate and 24,000 units mycostatin *via* gastric intubation.

Hormones (purchased from Sigma Chemical Co., St.Louis) were injected in 0·9% (w/v) NaCl containing 4% ethanol 75 min before radioisotope injection unless otherwise indicated and were administered intraperitoneally (i.p.) or *via* the tail vein (i.v.). Radioactive precursors were obtained from Radiochemical Centre, Amersham. These were dissolved in 0·9% (w/v) NaCl and injected intraperitoneally 1 h or 2 h before sacrifice. Homogenization and determination of the acid soluble pool and RNA fraction were as described previously [11] except that perchloric acid rather than trichloracetic acid was used throughout.

Characterisation of the radioactive nucleotides was performed by low voltage electrophoresis of material binding to charcoal as described by Markham and Smith[12].

Protein determination was by the biuret method [13], DNA by Burton's method [14] and radioactivity as described by Anderson and McClure[15].

Aldosterone binding to cytosol fractions was measured by a modification of the charcoal assay technique [16].

Animals were sacrificed by spinal dislocation and immediately perfused with 25 ml of buffer A (0·1 M Tris–HCl, 3 mM $CaCl_2$, 0·25 M sucrose, pH 7·4) *via* the abdominal aorta. Tissues were blotted dry, weighed, finely minced and homogenized in 4 vol. (w/v) of ice-cold buffer A using a Teflon glass homogenizer. Cytosol extracts were prepared according to the methods of Herman *et al.* [17].

After the 90 min incubation period with [^{3}H]-aldosterone, a 50 μl suspension of activated charcoal

Table 1. The effect of antibiotic treatment on bacterial content of mucosal homogenate

Treatment	Source	Colonies/ml homogenate
Untreated	Total mucosal scrapings	1.8×10^5
Antibiotic treated	Small intestinal scrapings	1.2×10^3
Antibiotic treated	Large intestinal scrapings	0.8×10^3

The bacterial counts were obtained by plating samples of homogenate on to nutrient agar and incubating at 37° overnight. Values are the means of 5 determinations.

(100 mg/ml, Norit A, Fisher) was added to the 0·5 ml reaction mixture. A new method was devised for quick and simple removal of the charcoal. Glass fibre filters (2·5 cm.) were placed over the mouth of the incubation tube, which was then inverted into a 10 ml plastic centrifuge tube and centrifuged for 8 min at 2500 rev/min at 4° in a bench centrifuge. The cytosol fraction containing only bound [^{3}H]-aldosterone passed rapidly through the filter which retained the charcoal bound (non-protein associated) [^{3}H]-aldosterone of the reaction mix. After removal of the assay tube and filter, a 250 μl sample of the cytosol filtrate was assayed for radioactivity. Blank values obtained in the absence of cytosol were subtracted for each determination.

RESULTS

Rat intestine normally contains large numbers of bacteria (Table 1) and it is essential that these be much reduced if incorporation experiments are to be meaningful. It can be seen that this was achieved in this series of experiments (Table 1) and thus the incorporation is unlikely to result from bacterial contamination.

[^{3}H]-aldosterone (3×10^{-9} mol) was injected into rats which were sacrificed 30 min later. The amount of radioactivity localized along the gastro-intestinal tract is shown in Fig. 1. The extent of localization varied from 0·39 pmol/mg protein in the small intestine to 0·13 pmol/mg protein in the large intestine. These values are all higher than kidney (0·11 pmol/mg protein). Determination of aldosterone binding to cytosol extracts *in vitro* showed a dissociation constant of $6{\cdot}6 \pm 3{\cdot}8 \times 10^{-8}$ M and a maximum binding of $2{\cdot}7 \times 10^{-12}$ mol/mg protein (Fig. 2). This compares with values of $1{\cdot}7 \pm 0{\cdot}2 \times 10^{-8}$M and $6{\cdot}7 \times 10^{-13}$ mol/mg protein for rat kidney in the same series of experiments.

The incorporation of exogenous RNA precursors into rat intestine was generally inefficient. Table 2 shows that [^{3}H]-orotic acid was very poorly incorporated although there was good incorporation into kidney and liver. [^{3}H]-uridine was also poorly incorporated although in this case the incorporation into liver and kidney was also very limited, in keeping

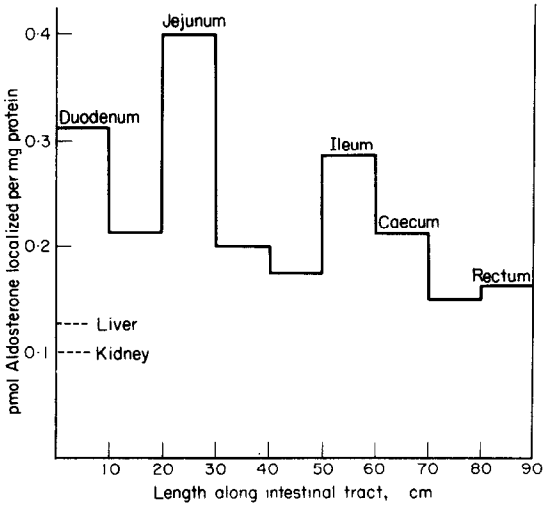

Fig. 1. Localisation of aldosterone along rat gastro-intestinal tract. [^{3}H]-aldosterone (10 μCi, 2 nmol/100 g body weight) was injected and rats were sacrificed 30 min later. The amount localised in successive 10 cm. lengths of intestine and in kidney and liver was then determined.

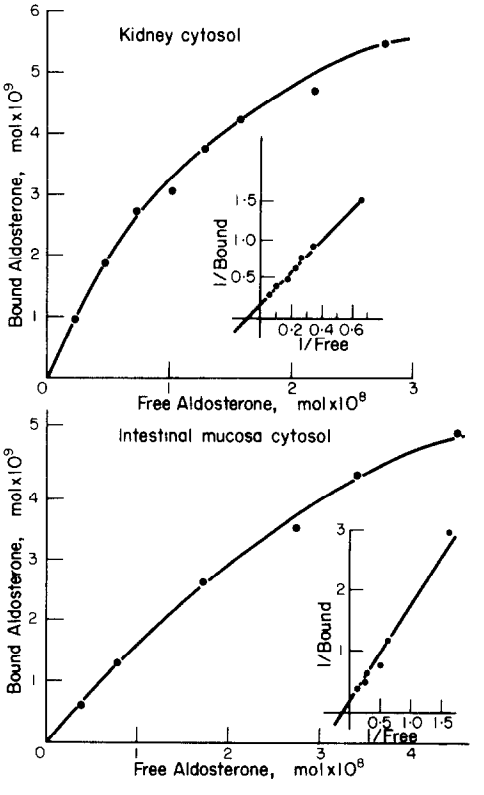

Fig. 2. Determination of the binding constants for [^{3}H]-aldosterone binding to kidney and intestinal mucosal cytosols. Cytosols were incubated with [^{3}H]-aldosterone at the concentration shown for 90 min at 0°. The extent of binding was then determined by the charcoal procedure.

Table 2. Comparison of the incorporation of RNA precursors into various tissues.

Tissue	Uridine 75 μCi/100 g 1 h	Orotic Acid 75 μCi/100 g 1 h	Formate 25 μCi/100 g 2 h
Kidney	0.8 ± 0.2	1401 ± 172	260 ± 40
Liver	0.6 ± 0.2	3282 ± 33	190 ± 25
Small Intestine	0.5 ± 0.2	9 ± 2	1165 ± 75
Large Intestine	0.5 ± 0.2	3 ± 1	613 ± 98

Rats were injected with the RNA precursor shown and killed at the times indicated. The extent of incorporation into RNA was then determined. Specific activities were [5-^{3}H]-uridine, 5 Ci/mmol; [^{3}H]-orotic acid, 1·0 Ci/mmol; and [^{14}C]-formate, 60 mCi/mmol. All values are expressed as pmol/mg DNA.

with earlier results of Kalra and Wheldrake (unpublished observations). The incorporation of [^{3}H]-orotic acid was not improved by starving the rats for 24 h before its injection. On the other hand there was good incorporation of [^{14}C]-formate into the RNA fraction of intestine (Table 2). Although previous workers had used it for the same tissue [18–20] we confirmed that the radioactivity in the RNA fraction was all associated with material which ran as mononucleotides on low voltage electrophoresis (Fig. 3).

Table 3 shows the effect of adrenalectomy and aldosterone on [^{14}C]-formate incorporation into various tissues. It can be seen that on adrenalectomy incorporation into whole homogenate, and soluble and acid insoluble material was reduced. Aldosterone injection 75 min prior to precursor injection stimulated incorporation into all fractions of both large and small intestine. Intravenous injection seemed to have a larger effect than intraperitoneal injection of hormone but the results were more variable and intraperitoneal injection was used for subsequent work.

Incorporation into kidney fractions was also decreased by adrenalectomy and enhanced by aldosterone but no significance tests were done on this data since it was from duplicate experiments only. The effects on liver were small but in the same direction.

In the light of the varying reports concerning the time of action of aldosterone on the intestine [1, 8–10], it was of interest to investigate the time course of aldosterone stimulation of RNA synthesis. It can be seen from Table 4 that the stimulation at 3 h was greater than that at 12 h and by 24 h incorporation had returned to adrenalectomised levels. Table 4 also shows the effect of a lower dose of aldosterone (1 μg/100 g body weight) and this was not distinguishable from our standard dose (10 μg/100 g body weight). Corticosterone also stimulated the incorporation of [^{14}C]-formate into RNA. Its effect in small intestine was similar to aldosterone but in large intestine may have been smaller although the differences are not statistically significant.

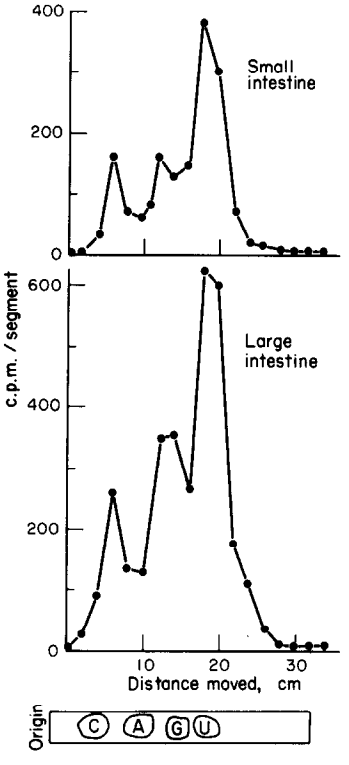

Fig. 3. Characterisation of products of [^{14}C]-formate incorporation into intestinal RNA. The alkaline hydrolysate fraction of intestinal RNA was subject to electrophoresis for 24 h at 4 V/cm. at pH 3·45. The positions of nucleotide markers were as shown.

DISCUSSION

Studies on the effect of hormones on intestinal RNA metabolism are complicated by the possibility of bac-

Table 3. The effect of adrenalectomy and aldosterone administration on [^{14}C]-formate incorporation into rat tissues

Tissue	Fraction	Normal	ADX	ADX + Aldosterone i.p.	ADX + Aldosterone i.v.
Small Intestine	Homogenate	100 (6.45)	51.2[a]	73.6	78.7
	Acid soluble	100 (1.66)	59.2[a]	110.2[c]	92.1
	RNA	100 (1.28)	57.6[a]	72.5[c]	96.2[d]
Large Intestine	Homogenate	100 (2.49)	67.3[a]	82.7[c]	100.4[c]
	Acid soluble	100 (0.69)	90.7	88.8	149.2[c]
	RNA	100 (0.67)	52.2[b]	58.8[d]	96.3[c]
Kidney	Homogenate	100 (2.67)	55.8	70.9	N.D.
	Acid soluble	100 (1.04)	53.8	74.0	N.D.
	RNA	100 (0.26)	50.0	65.4	N.D.
Liver	Homogenate	100 (2.41)	85.9	93.4	N.D.
	Acid soluble	100 (0.55)	101.8	108.9	N.D.
	RNA	100 (0.19)	89.5	94.7	N.D.

[^{14}C]-formate (60 mCi/mmol) was injected intraperitoneally (25 μCi/100 g body wt) and the animals killed 2 h later. Values are expressed as a percentage of normal and the values in parentheses after the normal values are nmoles incorporated/mg DNA.

a = significantly different from normal $P < 0.05$.

b is $0.1 > P > 0.05$ when compared with normal.

c = significantly different from adrenalectomised $P < 0.05$.

d is $0.1 > P > 0.05$ when compared with adrenalectomised.

ADX = adrenalectomised. i.p. = intraperitioneal injection. i.v. = intravenous injection of aldosterone at 10 μg/100 g body weight. N.D. = not determined.

Table 4. The effect of adrenalectomy, aldosterone and corticosterone administration on [^{14}C]-formate incorporation into RNA fraction of rat intestinal mucosa

		Small Intestine	Large Intestine
	Normal	825 ± 104[a]	593 ± 86[a]
	Adrenalectomised (ADX)	273 ± 21	253 ± 22
3 h	ADX + Aldosterone (1 μg/100 g)	442 ± 60[c]	468 ± 72[c]
3 h	ADX + Aldosterone (10 μg/100 g)	404 ± 30[b]	535 ± 81[b]
3 h	ADX + Corticosterone (2 mg/100 mg)	449 ± 63[c]	414 ± 25[c]
12 h	Aldosterone (10 μg/100 g)	340 ± 46	403 ± 42[c]
24 h	Aldosterone (10 μg/100 g)	295 ± 24	287 ± 20

All values compared with adrenalectomised by t-test.
Legend:
a = significantly different for $P < 0.001$.
b = $0.001 < P < 0.01$.
c = $0.01 < P < 0.05$.

terial contamination and by the failure of intestinal mucosal cells to take up certain precursors. The administration of suitable antibiotics lowered bacterial contamination to acceptable levels (Table 1) but the choice of precursor was less simple. Orotic acid was very poorly incorporated (Table 2) although it was a good label for other tissues and uridine was generally unsatisfactory (Table 2). Formate seemed to be the precursor of choice and was readily incorporated into RNA [18–20, Table 3, Fig. 3].

Adrenalectomy caused a decrease in the incorporation of formate into both the acid soluble and RNA fractions of small intestine (Table 3) and the incorporation into large intestine was also decreased Aldosterone restored the incorporation to near normal levels. This is in line with previous studies on kidney [11] and with the results obtained for kidney in this series of experiments. These results make it likely that aldosterone caused a stimulation of RNA synthesis in intestine, which is in agreement with the finding that actinomycin D blocks the aldosterone response [10]. The stimulation of RNA synthesis was seen at both 10 μg/100 g body weight and 1 μg/100 g body weight which suggested that it was a mineralocorticoid and not a glucocorticoid effect. However, corticosterone (2 mg/100 g body weight) also stimulated incorporation suggesting that the effect on RNA synthesis is not highly specific. Table 4 also shows that aldosterone had a greater effect on the large intestine, where it almost abolished the effect of adrenalectomy on RNA synthesis, than on small intestine, where the reversal was less dramatic. (The aldosterone treated adrenalectomised values were not significantly different from normal for large intestine but were for small intestine $P < 0.05$).

The fact that the effect of aldosterone on RNA synthesis was greater at 3 h than at 12 or 24 h accords with the lag period observed by Edmonds and Marriot in large intestine [1] but was much less than the 24 h found by Crocker and Munday for small intestine [10]. As suggested by these workers it is possible that aldosterone was having its early effect in the crypts of Lieberkühn and that the effect on Na^+ transport is only observed after migration of these 'aldosterone primed' cells to a position higher on the villus.

The high level of aldosterone localisation on the gastro-intestinal tract is in keeping with the idea that this tissue is a site of aldosterone action. Previous workers [21, 22] have made similar observations using whole animals and isolated mucosal cells. We have also shown extensive binding of aldosterone to isolated cytosol but the low affinity raises doubts about the physiological importance of the binding described here. Nevertheless the evidence presented tends to support the view that aldosterone acts on intestine in a manner similar to its action on kidney.

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