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FACTORS AFFECTING MUCOSAL WATER AND SODIUM TRANSFER IN EVERTED SACS OF RAT JEJUNUM

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SUMMARY

1. Mucosal water and sodium transfer were measured in everted sacs of rat jejunum.

2. There was no effect on mucosal water and sodium transfer when aldosterone was added to the incubation medium.

3. An 87 % increase in mucosal water and sodium transfer was obtained in sacs prepared from animals maintained on a low sodium diet compared to sacs from animals on a normal diet.

4. Injection of 5 μg aldosterone intraperitoneally to animals maintained on a high sodium diet produced a significant increase in mucosal water and sodium transfer in sacs prepared 27 hr later and this was still present 36 hr after injection.

5. The aldosterone-induced rise in mucosal water transfer was blocked if actinomycin D was administered one hour before the aldosterone injection.

6. It is suggested that aldosterone action on sodium and water transport in rat jejunum is mediated through a primary action on protein synthesis and that the long delay can be explained in terms of gut morphology.

INTRODUCTION

Aldosterone is known to have an important role in sodium homeostasis and has been shown to affect sodium transport in many tissues. There is evidence to suggest that aldosterone increases sodium absorption from the gastrointestinal tract. Adrenalectomy results in impaired sodium absorption from the intestine of rats (Clark, 1939; Stein & Wertheimer, 1940) and dogs (Dennis & Wood, 1940) which can be restored by administration of deoxycorticosterone (Stein & Wertheimer, 1940).

Berger, Kanzaki & Steele (1960) showed that the amount of sodium removed from the intestine when cation exchange resins were administered

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orally was less in patients with conditions in which aldosterone secretion was increased. Laragh (1960) reported that aldosterone decreased the faecal sodium/potassium ratio and this was also found by Wong, Morrison & Hurst (1961) using a 9-α-fluorocortisol. Poutsaika, Thomas & Linegar (1957) showed that in dogs the administration of 9-α-fluorocortisol reduced sodium and increased potassium excretion in the faeces.

More recently Levitan & Ingelfinger (1965) have reported that aldosterone increased sodium absorption from the human colon but no effect on potassium excretion was observed. Edmunds & Marriott (1967) have also shown that aldosterone increased sodium absorption from the rat colon.

No effect of aldosterone on sodium absorption from the small intestine has been reported although Shields, Mulholland & Elmslie (1966) found increased potassium secretion into the lumen of dog small intestine after aldosterone treatment.

The purpose of this study was to investigate whether sodium transport in the rat small intestine was affected by aldosterone.

METHODS

Male Wistar rats (250 g body wt.) that had been starved overnight were anaesthetized with Nembutal (60 mg/kg) and the intestine rinsed with sodium chloride (0·9 g/100 ml.). The intestine was everted and 15 cm sacs from the jejunum were prepared according to the method of Wilson & Wiseman (1954). Each sac was weighed, then filled with Krebs bicarbonate Ringer containing glucose 500 mg/100 ml., tied off, reweighed and incubated in an Erlenmeyer flask. The flask contained 10 ml. of the same Ringer which had been gassed with 5 % carbon dioxide in oxygen. The sacs were incubated for 1 hr at 37° C in a shaking bath at 80 oscillations per minute. At the end of the incubation the sacs were blotted, weighed, then emptied, blotted and reweighed. Samples of the mucosal and serosal solutions were estimated for sodium and potassium in an EEL flame photometer.

Results were expressed as mucosal transfer, that is, the amount of water or sodium leaving the mucosal solution during the hour-long incubation.

Water transfer is described as ml./g initial wet wt. of a 15 cm sac of jejunum/hr and sodium transfer as μ-equiv/g initial wet wt. of a 15 cm sac of jejunum/hr.

Procedures which could affect the water content of the gut and, therefore, its initial wet weight were used in some of the experiments reported in this paper. In such experiments the initial wet weights of the sacs were statistically analysed to determine the effect of the experimental treatments. The results of this analysis are given in Table 1, which shows that there is no significant difference in wet weights of the sacs between groups in each experiment. It is important to establish that there are no such differences because large variations in the water content of the gut could affect the values for mucosal water and sodium transfer calculated on the basis of initial wet weight of the sacs.

Diets

1. *Normal diet.* Animals were fed on diet 41B rat cake and tap water.

2. *High sodium diet.* Rats were maintained on diet 41B but their drinking water was replaced by 2 % saline for a period of 2 weeks before using in an experiment (Hartcroft & Hartcroft, 1953).

3. *Low sodium diet.* Rats were maintained on a special low sodium diet supplied by Rank, Hovis, McDougall Ltd. which had the usual diet 41 B composition without the added salt supplement. They were given tap water for drinking.

Injections

Aldosterone (Aldocorten–Ciba) was diluted with isotonic saline and injected intraperitoneally.

Actinomycin D (a gift from Merck, Sharp & Dohme) was injected intraperitonally dissolved in isotonic saline.

TABLE 1. The effect of various experimental treatments on the initial wet weights of 15 cm sacs of rat jejunum

Results in table	Treatment	Initial wet wt.	S.E.	*P*
4	Normal diet	1·062	0·04	< 0·80
	Low sodium diet	1·032	0·07	
5	High sodium diet	0·897	0·04	< 0·40
6	High sodium diet + aldosterone injection	0·826	0·05	
8	High sodium diet	0·877	0·03	< 0·70
	High sodium diet + aldosterone injection	0·913	0·06	< 0·40
	High sodium diet + aldosterone injection + actinomycin *D* injection	0·828	0·06	
	High sodium diet + actinomycin D injection	0·808	0·03	—

RESULTS

1. *The effect of aldosterone in the incubation medium.* Sacs were prepared from the jejunum of rats maintained on a normal laboratory diet. Control values of mucosal water transfer were obtained by incubating sacs in Krebs bicarbonate Ringer. The effect of aldosterone on mucosal water transfer was investigated after aldosterone at concentrations of 0·1, and 1·0 μg/ml. was added to the mucosal solution bathing the sacs. Table 2 shows that no effect of aldosterone on mucosal water transfer was obtained.

Experiments were also carried out using 1·0 and 10·0 μg/ml. aldosterone in both mucosal and serosal solutions but again no effect on mucosal water transfer was observed (Table 2).

Rats were maintained on the high sodium diet to depress endogenous aldosterone production and jejunal sacs from these animals were incubated in the presence and absence of aldosterone at a final concentration of 1·0 μg/ml. in mucosal and serosal solution. There was no difference in the mucosal water transfer of these two groups of rats (Table 3). It appeared that reducing the levels of endogenous aldosterone did not increase the sensitivity of the jejunum to an *in vitro* action of aldosterone.

2. *The effect of a low sodium diet.* Sacs were prepared from the jejunum of rats that had been fed a low sodium diet for 2 weeks to produce high

levels of circulating aldosterone. The mucosal water transfer of these sacs was compared with values obtained from rats that had been maintained on a normal laboratory diet. Table 4 shows that there was a significant 87 % increase in mucosal water transfer in the sacs from the animals on the low sodium diet compared with animals fed a normal laboratory diet.

This was an indication that increased aldosterone levels could cause a rise in mucosal water transfer and suggested that there might be a delayed action of aldosterone in this system.

TABLE 2. The effect of aldosterone *in vitro* on mucosal water and sodium transfer from rat jejunum

Group	Mucosal water transfer (ml./g wet wt. jejunum/hr)	Mucosal sodium transfer (μ-equiv/g wet wt. jejunum/hr)
Control	0·87 ± 0·08 (5)	89·46 ± 16·00 (4)
With 0·1 μg/ml. aldosterone in mucosal solution	1·05 ± 0·06 (4)	112·40 ± 9·34 (4)
P	< 0·30	< 0·30
Control	0·94 ± 0·12 (11)	103·61 ± 13·48 (11)
With 1 μg/ml. aldosterone in mucosal solution	1·18 ± 0·16 (11)	127·08 ± 15·96 (11)
P	< 0·30	< 0·30
Control	0·74 ± 0·16 (6)	57·39 ± 21·60 (6)
With 1 μg/ml. aldosterone in mucosal and serosal solution	0·67 ± 0·07 (5)	61·12 ± 29·66 (5)
P	< 0·70	< 0·70
Control	1·33 ± 0·28 (3)	192·36 ± 40·83 (3)
With 10 μg/ml. aldosterone in mucosal and serosal solution	1·10 ± 0·11 (3)	165·87 ± 21·60 (3)
P	< 0·50	< 0·50

Results expressed as means ± S.E. of mean; number of animals in parentheses.

TABLE 3. The effect of aldosterone *in vitro* on mucosal water and sodium transfer from jejunum of rats maintained on a high sodium diet

Group	Mucosal water transfer (ml./g wt wt. jejunum/hr)	Mucosal sodium transfer (μ-equiv/g wet wt. jejunum/hr)
Control	0·69 ± 0·09 (6)	100·53 ± 13·75 (6)
With 1 μg/ml. aldosterone in mucosal and serosal solution	0·82 ± 0·05 (6)	122·28 ± 21·60 (6)
P	< 0·30	< 0·50

Results expressed as means ± S.E. of mean; number of animals in parentheses.

3. *The effect of an intraperitoneal injection of aldosterone at varying times before experiment.* Animals were maintained on the high sodium diet and injected with 5 μg aldosterone at 24, 27, 30 and 36 hr before removal of the gut and measurement of mucosal water transfer. There was no difference between the aldosterone-injected group and control group 24 hr after

aldosterone injection. However, there was a significant increase ($P < 0{\cdot}001$) in the mucosal water and sodium transfer of the injected group compared with the control group when aldosterone was administered 27 hr before experiment (Table 5). This increased mucosal water transfer was also observed at 30 and 36 hr after aldosterone injection (Table 6).

It was found necessary to maintain rats on a high sodium diet to observe increased mucosal water and sodium transfer after aldosterone injection since the increase was not obtained with rats fed on a normal laboratory diet (Table 7).

TABLE 4. A comparison of the mucosal water and sodium transfer of jejunal sacs prepared from rats maintained on a normal diet with rats on a low sodium diet

Group	Mucosal water transfer (ml./g wet wt. jejunum/hr)	Mucosal sodium transfer (μ-equiv/g wet wt./hr)
Normal diet	$0{\cdot}73 \pm 0{\cdot}08$ (9)	$81{\cdot}96 \pm 11{\cdot}05$ (9)
Low sodium	$1{\cdot}37 \pm 0{\cdot}21$ (9)	$185{\cdot}86 \pm 34{\cdot}32$ (9)
P	$< 0{\cdot}02$	$< 0{\cdot}02$

Results expressed as means $\pm$ S.E. of mean; number of animals in parentheses.

TABLE 5. The effect of an intraperitoneal injection of 5 μg aldosterone 27 hr before experiment on mucosal water and sodium transfer of rats maintained on a high sodium diet

Group	Mucosal water transfer (ml./g wet wt./hr)	Mucosal sodium transfer (μ-equiv/g wet wt./hr)
Uninjected, high-sodium rats	$0{\cdot}69 \pm 0{\cdot}05$ (12)	$85{\cdot}58 \pm 16{\cdot}40$ (12)
5 μg aldosterone, I.P. to high-sodium rats	$1{\cdot}34 \pm 0{\cdot}11$ (13)	$184{\cdot}07 \pm 22{\cdot}18$ (13)
P	$< 0{\cdot}001$	$\sim 0{\cdot}001$

Results expressed as means $\pm$ S.E. of mean; number of animals in parentheses.

TABLE 6. The effect of 5 μg aldosterone I.P. at varying times prior to experiment on mucosal water transfer of rats maintained on a high sodium diet

Time after injection (hr)	Mucosal water transfer (ml./g wet wt./hr): High sodium	Mucosal water transfer (ml./g wet wt./hr): High sodium + 5 μg aldosterone I.P.	*P*
24	$0{\cdot}73 \pm 0{\cdot}07$ (16)	$0{\cdot}85 \pm 0{\cdot}12$ (13)	$< 0{\cdot}40$
27	$0{\cdot}69 \pm 0{\cdot}06$ (16)	$1{\cdot}28 \pm 0{\cdot}11$ (17)	$< 0{\cdot}001$
30	$0{\cdot}53 \pm 0{\cdot}02$ (6)	$1{\cdot}02 \pm 0{\cdot}11$ (4)	$< 0{\cdot}01$
36	$0{\cdot}46 \pm 0{\cdot}08$ (3)	$1{\cdot}15 \pm 0{\cdot}22$ (3)	$< 0{\cdot}05$

Results expressed as means $\pm$ S.E. of mean; number of animals in parentheses.

4. *The effect of actinomycin D.* It has been observed in all systems where aldosterone increases sodium transport that there is a delay between administration of the hormone and onset of increased sodium transport.

It has been suggested that the delay was due to the time taken for the synthesis of a new protein concerned with sodium transport (Edelman, Bogoroch & Porter, 1963). Williamson (1963) and Edelman *et al.* (1963) have reported that the aldosterone-increased rise in sodium transport can be blocked by the administration of actinomycin D which prevents DNA-directed synthesis of RNA.

The effect of actinomycin D on the aldosterone-increased rise in mucosal water and sodium transfer from sacs of rat jejunum was investigated. Initially it was found difficult to select a suitable dose of actinomycin D since the dose used by Zull, Misztal & DeLua (1965) of 0·66 μg/g body wt. caused many deaths in the high-sodium rats. Small doses of 0·2 μg/g body wt. did not affect the system and eventually a dose of 0·4 μg/g body wt. was selected as satisfactory.

TABLE 7. The effect of 5 μg aldosterone I.P. on the mucosal water and sodium transfer of rats maintained on a normal diet

Group	Mucosal water transfer (ml./g wet wt./hr)	Mucosal sodium transfer (μ-equiv/g. wet wt./hr)
Control	1·09 ± 0·34 (4)	161·00 ± 47·16 (4)
5 μg aldosterone, I.P. at −27 hr	1·12 ± 0·25 (4)	149·42 ± 40·50 (4)
P	< 0·90	< 0·90

TABLE 8. The effect of actinomycin D and aldosterone on the mucosal water transfer of rats maintained on a high sodium diet

Group	Mucosal water transfer (ml./g wet wt./hr)	*P*
High sodium, uninjected	0·86 ± 0·08 (11)	< 0·05
5 μg aldosterone, I.P. at −27 hr	1·18 ± 0·11 (10)	0·05
5 μg aldosterone, I.P. at −27 hr, and actinomycin D 0·4 μg/g body wt., at −28 hr	0·88 ± 0·09 (8)	
Actinomycin D 0·4 μg/g body wt., at −28 hr	0·97 ± 0·12 (8)	—

Results expressed as means ± S.E. of mean; number of animals in parentheses.

Actinomycin D was injected 1 hr before the aldosterone injection and 28 hr before the experiment. Animals that had been maintained on a high sodium diet were divided into four groups:

(i) Control: untreated.

(ii) Aldosterone: 5 μg aldosterone 27 hr before experiment.

(iii) Aldosterone and actinomycin D: 0·4 μg/g body wt. actinomycin D at −28 hr and 5 μg aldosterone at −27 hr.

(iv) Actinomycin D: 4 μg/g body wt. 28 hr before the experiment.

The results of this experiment are given in Table 8, which shows that there was a significant increase in mucosal water transfer after aldosterone injection, but that in the group injected with aldosterone and actinomycin

D there was no increase in mucosal water transfer. It was also shown that in the group treated with actinomycin D alone there was no reduction in the levels of mucosal transfer. These results indicate that the action of aldosterone on rat jejunum causing increased mucosal water transfer is mediated through protein synthesis since the effect is blocked by actinomycin D.

DISCUSSION

It is clear from this study that aldosterone increases sodium and water uptake from the mucosal solution bathing everted sacs of rat jejunum. This effect of aldosterone on small intestine has not been previously reported. The experiments in which aldosterone was shown to produce an approximately 100% increase in water and sodium transfer involved maintaining rats on a high sodium diet. It is possible that this treatment could affect the water content of the gut and so lead to an inaccurate assessment of the results expressed in terms of initial wet weight of sacs. If the increase in water transfer after aldosterone injection resulted solely from a difference in the water content of the gut, then it would be necessary to have a 50% reduction in the water content of the tissue subjected to aldosterone treatment. In fact, a statistical analysis of the initial wet weight of sacs from this experiment showed that there was no significant difference between the two groups (Table 1).

Similarly, the experiments reported in Tables 4 and 8 involved changes in dietary sodium intake which could produce variations in the water content of the gut, but again a statistical analysis has shown that there are no significant differences in sac wet weight between the experimental groups. Therefore, the results for mucosal transfer in this paper do represent actual changes in transfer caused by the experimental treatments.

The work of Shields *et al.* (1966) on dog small intestine showed that aldosterone did not affect sodium transport but that there was an increased secretion of potassium. The latency of aldosterone action on potassium was much shorter (4 hr) in their study than that found in the present study for the effect on sodium. No measurements were made here within the 24 hr after aldosterone injection and although no effect of aldosterone on potassium movement was observed, the possibility of an early action of aldosterone on potassium is not ruled out.

The differences in the findings of this study and that of Shields *et al.* (1966) might be related to the acute conditions of their experiments, since Berger, Berlin & Tulenko (1958) have also reported different actions of aldosterone on sodium and potassium excretion in acute experiments. These workers showed that aldosterone only affected potassium excretion in intact dogs, but that if the dogs were adrenalectomized and the same

dose of aldosterone injected, then sodium retention was observed. There is also evidence that the effect of aldosterone on potassium is independent of its action on sodium from the work of Sonnenblick, Cannon & Laragh (1961), Williamson (1963), and Fimognari, Fanestil & Edelman (1967).

Clark, Miller & Shields (1967) have shown that there is increased absorption of sodium and water from the small intestine of sodium-depleted dogs and these findings are confirmed in the present study since rats maintained on a low sodium diet have higher values of mucosal water transfer than rats maintained on a normal diet.

The latency of aldosterone action on mucosal water and sodium transfer from everted sacs of rat jejunum was found to be 24–27 hr in this study. This latent period is much longer than that found in other biological systems but it is possible that the delay can be explained in terms of gut morphology. The epithelial cells of the small intestine are formed in the crypts of Lieberkühn and migrate up the villus to the tip where they are extruded into the lumen of the intestine. The time taken for this process in rat jejunum is 1·4 days (Widner, Storer & Lushbaugh, 1951).

In addition to this replacement and escalation of the epithelial cells on the villus it has also been shown that there is a zonation of enzymic activity of the epithelial cells on the villus (see Wilson, 1962). The majority of protein synthetic ability occurs in the crypt region but this property disappears as the cells migrate up the villus. Alternatively, many hydrolytic enzymes are present in cells high on the villus but are absent from the crypts. In hamsters, it has been shown that the transport capacity for sugars and amino acids was greatest at the tips of the villi (Kinter, 1961, cited by Wilson, 1962).

There is evidence that aldosterone acts through protein synthesis which would suggest that its primary site of action in the intestine is the crypts of Lieberkühn with their high rate of protein synthesis. Cells modified by aldosterone in the crypts would migrate up the villus and become functionally absorbing cells when positioned near the top. The cell turnover process in rat jejunum takes approximately 34 hr, so that a delay in aldosterone action here would be due both to the initial action on protein synthesis and also to the time taken for the cell to be positioned on the villus.

It is interesting in this context that there is a long delay after vitamin D administration before intestinal calcium transport is increased. Norman (1966) has shown that the length of this delay is dependent to some extent on the dose of vitamin D and was 15–25 hr after 10 i.u. vitamin D was injected intraperitoneally in rachitic chicks. This delay was not due to the time taken for the vitamin to reach its site of action since, after injection of tritium-labelled vitamin D, high levels of radioactivity were found in the

intestine within 1 hr. This vitamin-D-stimulated rise of intestinal transport was blocked by treatment with actinomycin D and Norman (1966) has suggested that the turnover time for the biochemical reactions associated with vitamin-D-mediated calcium absorption is 24–36 hr.

It has been shown in this study that there is a delay of at least 24 hr before aldosterone produced a rise in mucosal water and sodium transfer and that this could be prevented by prior administration of actinomycin D. This suggests that aldosterone is acting through protein synthesis to bring about increased activity of the transepithelial sodium-transporting system of the small intestine.

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