Aldosterone and thyroid hormone interaction on the sodium and potassium transport pathways of rat colonic epithelium

C. J. Edmonds and C. L. Willis

Endocrinology Research Group, Division of Clinical Sciences, Clinical Research Centre, Watford Road, Harrow HA1 3UJ

REVISED MANUSCRIPT RECEIVED 20 July 1989

ABSTRACT

The effect of hypothyroidism on potassium adaptation (shown by increased potassium secretion in response to potassium loading) and on the action of aldosterone on potassium secretion and sodium fluxes was examined in the rat distal colon.

Potassium adaptation, particularly the response to an acute potassium load, was impaired by hypothyroidism which also considerably reduced the rise of transepithelial electrical potential difference (p.d.) of total and transcellular (active) lumen-to-plasma sodium fluxes and of potassium secretion normally produced by aldosterone. These changes were, in part, corrected by a short period (3 days) of tri-iodothyronine replacement. Moreover in aldosterone-treated hypothyroid rats, amiloride in the lumen was considerably less effective in reducing the p.d. and sodium fluxes than in aldosterone-treated normal rats.

The intracellular sodium transport pool was greater in the hypothyroid than in the normal rats (5.0 ± 1.1 (S.E.M.) nmol/mg dry weight compared with 2.9 ± 0.2 nmol/mg dry weight; P<0.02). Aldosterone increased the pool in the normal but not in the hypothyroid rats while amiloride had little effect on the pool in the aldosterone-treated hypothyroid rats but almost abolished it in aldosterone-treated normal rats.

Aldosterone plays a major part in the adaptation of colonic sodium and potassium transport to sodium depletion or potassium excess; these adaptations were much impaired in hypothyroid animals. The present results are consistent with a deficiency in aldosterone induction of potassium- and amiloride-sensitive sodium pathways in the apical membrane of colonic epithelial cells in hypothyroid rats, a deficiency which limits the stimulant effect of aldosterone on sodium and potassium transport.

Journal of Endocrinology (1990) 124, 47-52

INTRODUCTION

When animals are depleted of sodium or are fed a potassium-rich diet, adaptive changes take place in the sodium and potassium transport systems of the colon which are analogous to those which occur in the kidney. Thus, in the rat distal colon, sodium depletion leads to increased active sodium absorption (Edmonds, 1967) while potassium excess stimulates potassium secretion (Fisher, Binder & Hayslett, 1976). The importance of aldosterone to these adaptive changes has been demonstrated in a variety of species (Edmonds & Marriott, 1967; Frizzell & Schultz, 1978; Thomas, Skadhauge & Read, 1979; Foster, Jones, Hayslett & Binder, 1985). In the rat distal colon, aldosterone induces amiloride-sensitive sodium channels in the apical membrane of the epithelial cells and a small expansion of the intracellular sodium transport pool as the sodium absorption rate increases (Will, Lebowitz & Hopfer, 1980; Sandle, Hayslett & Binder, 1984; Edmonds & Mackenzie, 1987). The Na,K-ATPase activity of the basolateral membranes also rises in epithelia stimulated by aldosterone (Jorgensen, 1972; Charney, Silva, Besarab & Epstein, 1974; Botella, Paris & Lahlou, 1986; Paccolat, Geering, Gaeggeler & Rossier, 1987). As regards potassium secretion, the principal factors in the action of aldosterone appear to be an increase of potassium-selective channels in the apical membrane and of Na,K-ATPase activity in the basolateral membrane (Kashgarian, Taylor, Binder & Hayslett, 1980; Sandle, Foster, Lewis et al. 1985; Edmonds & Willis, 1988).

Previous work showed that colonic adaptation to a sodium-restricted diet was, like renal adaptation, impaired when the rats were hypothyroid (Edmonds, Thompson & Marriott, 1970). Recent studies have shown, moreover, that tri-iodothyronine (T3) has a significant influence in potentiating the action of
aldosterone on the cells of the renal collecting tubules (Barlet-Bas, Khadouri, Marsy & Doucet, 1988). In the present work, we have further investigated the interrelationship of aldosterone and thyroid hormones in the rat distal colon, particularly to examine the potassium-secreting mechanism and effects on the induction of the amiloride-sensitive sodium channels and on the intracellular sodium transport pool.

**MATERIALS AND METHODS**

Male albino rats (250–350 g) were used and fed a diet based on a standard rat pellet. The standard pellet diet contained 0.2 mmol potassium/g. In the two other diets used, the potassium content was enriched by preliminary soaking of pellets in either 0.1 mol KCl/l or 0.3 mol KCl/l. Subsequently they were dried to their original form. Pellets having a potassium content about 0.35 mmol/g and 0.7 mmol/g were thereby obtained. All these diets were consumed in similar amounts, averaging about 6–7 g/100 g body weight per day.

Hypothyroidism was induced by thyroid ablation by intraperitoneal injection of 9 MBq 131I (Amersham International plc, Amersham, Bucks, U.K.) and subsequently the rats were given propylthiouracil (Sigma Chemical Co. Ltd, Poole, Dorset, U.K.; 100 mg/l) in drinking water for 6 weeks. The hypothyroid animals were maintained in a constantly warmed room for 6 weeks until the colonic perfusion studies. They remained in good general health but their weight gain was markedly less than that of the intact rats.

The methods used for measuring transport function in the distal colon were as previously described in full (Edmonds & Mackenzie, 1987). In brief, anaesthesia was obtained by i.p. injection of sodium pentobarbitone (6 mg/100 g body wt) well away from the distal colon. A segment of distal (descending) colon about 3 cm long was rinsed clean with warm saline and cannulated. The transepithelial electrical potential difference (p.d.) was measured periodically during an experiment using a high input impedance millivoltmeter connected through calomel half cells and saline bridges to the lumen and serosal surface of the colon.

In the first series of experiments, to examine the potassium secretion response to an acute potassium load, the rats had an i.v. polythene cannula implanted into the external jugular vein. The potassium load was given as a solution of KCl (120 mmol/l), KHCO₃ (30 mmol/l) using a 10 ml syringe driven by a Sage constant infusion pump (270 µl/min for the first 10 min, thereafter 190 µl/min). Potassium secretion rate was measured by collecting the effluent during perfusion of the colonic segment with a solution containing NaCl (50 mmol/l) and mannitol (200 mmol/l) at 37°C and at a rate of 1 ml/min using a Watson-Marlow H.R. Flow Inducer. The concentration of sodium was chosen as being similar to that of faecal fluid in this part of the colon; mannitol was added to render the solution isotonic. Perfusion was always commenced 15 min before collections were made; this allowed the potassium secretion rate to become steady. For measurement of the sodium flux rates, 0.5 ml of a solution of a composition similar to that above but containing in addition 22Na (2 Bq/µmol sodium; Amersham International plc) was introduced into the lumen of the cannulated segment and allowed to remain there for 10 min. It was then rapidly rinsed out with a solution of mannitol (300 mmol/l) and collected. The total sodium flux from lumen-to-plasma (Jms) across intestinal epithelium comprises a transcellular component which appears to depend on active sodium transport and a passive diffusive component which is probably through the paracellular pathway. In the present experiments we used the method described previously (Edmonds & Mackenzie, 1987) to determine Jms from the loss of 22Na from the lumen and from the mean specific activity (22Na/23Na ratio); the transcellular (active absorptive) lumen-to-plasma flux was estimated from the observed values of Jms, the net sodium flux and the transepithelial p.d. To determine the epithelial sodium transport pool (Edmonds & Mackenzie, 1987) a similar solution, but containing considerably higher radioactivity (22Na, 200 Bq/µmol sodium), was left in the lumen for 10 min. This was then rapidly washed out, the segment removed, blotted and scrapings taken of the epithelium, the whole procedure being completed within 1 min.

Aldosterone (Sigma Chemical Co. Ltd) was given by osmotic minipumps (Alzet Corporation, Palo Alto, CA, U.S.A.) inserted into the peritoneal cavity through a small lateral abdominal incision with sterile precautions. Antibiotics were not used and no problems of infection were encountered (Edmonds & Willis, 1988). A supramaximal dose of about 50 µg/day per 100 g body wt was given for the 3 days preceding the colonic perfusion. In the experiments in which hypothyroid rats were given T₃, this was added to the solution in the osmotic minipump and 1 µg/day per 100 g body wt was delivered. In some experiments, amiloride (100 µmol/l) was added to the solution in the lumen. All solutions were freshly prepared before an experiment.

Samples of venous blood were taken from the inferior mesenteric vena cava immediately before the end of the experiment.

Sodium and potassium concentrations were measured by flame photometry and 22Na by gamma counting. Flux results are expressed per cm² of mucosal area, obtained by measuring the length of the segment used on a standard glass tube after its removal at the end of an experiment. The results are given as
RESULTS

Potassium adaptation and hypothyroidism

When taking the standard diet, the normal and hypothyroid rats had a similar rate of potassium secretion into the lumen of the colon (Fig. 1). In both groups, potassium secretion rate was about trebled ($P < 0.001$) when the animals were fed the potassium-rich diet (0.7 mmol/g). When the acute i.v. potassium load was given a clear difference between the normal and hypothyroid groups (P<0.05). The transepithelial p.d. was similar in the normal and hypothyroid rats and, as found previously (Edmonds & Willis, 1988), unaffected by the potassium-rich diet or acute potassium infusion. The plasma potassium concentration of rats fed the potassium-rich diet was similar in the normal and hypothyroid groups averaging $4.3 \pm 0.2$ mmol/l ($n = 9$). By the end of the intravenous potassium infusion, the plasma potassium concentration had risen to $9.3 \pm 0.7$ mmol/l ($n = 9$). Again, there was no significant difference between the normal and hypothyroid animals.

Effect of aldosterone on sodium fluxes and potassium secretion

Preliminary studies showed that 3 days of administration of aldosterone to rats taking the standard diet produced a marked fall in plasma potassium to $3.2 \pm 0.2$ mmol/l ($n = 4$) compared with $4.4 \pm 0.3$ mmol/l ($n = 4$) in untreated rats ($P < 0.02$). In all the subsequent experiments, therefore, a diet based on the standard diet but having a higher potassium content (0.35 mmol/g) was used. On this diet, both normal and hypothyroid rats maintained their plasma potassium concentration satisfactorily during aldosterone administration ($3.8 \pm 0.1$ mmol/l ($n = 8$) after 3 days compared with $4.1 \pm 0.1$ mmol/l ($n = 8$) in untreated rats on this diet).

Neither the sodium fluxes nor the potassium secretion rate were significantly different between the untreated normal and hypothyroid rats (Fig. 2). After 3 days of aldosterone administration the sodium lumen-to-plasma fluxes had increased in both the normal and hypothyroid rats ($P < 0.01$ and $P < 0.05$ respectively); however, the rates recorded in the hypothyroid rats were significantly lower than in the normal rats with the total transepithelial flux being 64% and the estimated transeptileal flux being only 50% of that observed in the normal rats. Potassium secretion rate increased in both groups after aldosterone treatment but only significantly in the normal rats ($P < 0.05$). The transepithelial p.d. was nearly doubled in the normal animals ($P < 0.01$) but was unaffected in the hypothyroid group. There was no significant difference between the p.d. of the untreated normal and that of the hypothyroid rats but following treatment with aldosterone the p.d. was considerably greater ($P < 0.001$) in the normal than in the hypothyroid rats.

Effect of amiloride and $T_3$

The segment of colon was perfused initially for 5 min with the standard solution containing amiloride (100 μmol/l). This had been previously established as long enough for amiloride to bind and block the amiloride-sensitive sodium channels in the apical membrane. The flux rates were then measured using solutions which also contained amiloride. In the aldosterone-treated normal rats, amiloride reduced the total transepithelial flux ($J_{se}$) by nearly half while the transegellular sodium flux was almost completely abolished (Fig. 3). The transepithelial p.d. was reduced to nearly zero. The potassium secretion rate was unaffected. In the aldosterone-treated hypothyroid rats, by contrast, amiloride did not significantly influence the total transepithelial sodium

---

**FIGURE 1.** Secretion of potassium and transepithelial electrical potential difference (p.d.) in the distal colon of normal and hypothyroid rats fed (A) the standard diet, (B) a potassium-rich diet (0.7 mmol potassium/g) or (C) the potassium-rich diet and infused with an acute potassium load. Values are means ± s.e.m.; groups of five and four rats. *$P < 0.05$ compared with corresponding column of the normal rats (two-tailed Student's $t$-test).
flux and the reduction in the transcellular flux was markedly less than observed in the aldosterone-treated normal rats. Amiloride also reduced the transepithelial p.d. significantly \((P < 0.05)\) but again less effectively than in the normal rats. Potassium secretion was not significantly affected by amiloride.

Although the administration of \(T_3\) with aldosterone to the hypothyroid rats appeared to increase the sodium fluxes, the change was not significant. The increase of potassium secretion was, however, significant as was the increase of the p.d. \((P < 0.02)\). Moreover, the p.d. became largely amiloride-sensitive as shown by further experiments on three hypothyroid rats treated with \(T_3\) in which the p.d. fell from \(44 \pm 7\) mV to \(5 \pm 1\) mV when amiloride was added to the luminal solution.

**Changes in the sodium transport pool**

The sodium transport pool, determined from the \(^{22}\)Na content of the epithelial scrapings following 10-min luminal exposure to \(^{22}\)Na-containing solution of high specific activity, was recorded in the variously treated groups (Table 1). In the normal rats, aldosterone treatment produced a significant \((P < 0.01)\) increase in the sodium transport pool.

In the untreated hypothyroid rats, the pool was also expanded \((P < 0.02)\) by comparison with the untreated normal rats but was not significantly affected by aldosterone. Furthermore, amiloride did not reduce the sodium transport pool in the aldosterone-treated hypothyroid rats, a finding which contrasted with its
TABLE 1. Effect of various procedures on the epithelial sodium transport pool in normal and hypothyroid rats. Values are means ± S.E.M.; there were four rats in each group

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sodium transport pool (nmol/mg dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Normal: 2.9 ± 0.2, Hypothyroid: 5.0 ± 1.1</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>Normal: 4.2 ± 0.3, Hypothyroid: 3.3 ± 0.3</td>
</tr>
<tr>
<td>Aldosterone + amiloride</td>
<td>Normal: 0.6 ± 0.1, Hypothyroid: 2.3 ± 0.6</td>
</tr>
<tr>
<td>Aldosterone + T₃</td>
<td>Normal: 6.0 ± 0.7</td>
</tr>
</tbody>
</table>

Amiloride was given for 3 days at 50 µg/day per 100 g body wt. Aldosterone was given for 3 days at 1 µg/day per 100 g body wt.

The administration of T₃ over 3 days seemed principally to influence events in the apical membrane which regained the characteristics found in the aldosterone-treated normal rats; namely, considerable elevation of the transepithelial p.d. and sensitivity of the p.d. amiloride. The sodium transport pool remained, however, relatively elevated, suggesting that, despite the recovery of the apical membrane, the basolateral Na,K-ATPase had not fully recovered. In the case of potassium secretion, the deficient response to aldosterone in the hypothyroid rats was corrected by T₃ administration and, as potassium secretion

effect on aldosterone-treated normal rats in which the sodium transport pool was much reduced (P < 0.001). Finally, in the hypothyroid rats treated with T₃ and aldosterone, the sodium transport pool was greater than either that observed in the normal rats (P < 0.05) or in the hypothyroid rats treated with aldosterone alone (P < 0.025).

DISCUSSION

In the rat distal colon, the potassium secretion rate increases when the animal takes a potassium-rich diet (Fisher et al. 1976) and there is an enhanced response to an acute potassium load (Edmonds & Willis, 1988). The present results showed that the colonic adaptation to potassium intake, like that to restricted sodium intake (Edmonds, Thompson & Marriott, 1970) was impaired in hypothyroidism. Both adaptation to sodium restriction and potassium excess depend on aldosterone (Edmonds & Marriott, 1969; Foster et al. 1985) and our experiments in which aldosterone was administered chronically by osmotic minipumps showed that the action of aldosterone on colonic sodium and potassium transport was considerably impaired in hypothyroid animals.

Aldosterone in influencing sodium and potassium movements affects both apical and basolateral membranes of the epithelial cells. The earliest changes, well developed within a few hours of stimulation (Clauss & Skadhauge, 1988) involve alteration in sodium and potassium selective pathways in the apical membrane. Sodium diffusion channels, which can be blocked by amiloride at low concentration (100 µmol/l), appear and the amiloride-insensitive sodium pathway, by which sodium is absorbed in the unstimulated epithelium, is suppressed (Will et al. 1980; Edmonds & Mackenzie, 1987). Associated with this change is a rise of the transepithelial p.d. and of the active transcellular sodium flux together with a small increase in the intracellular sodium transport pool. All these effects were found to be impaired in the hypothyroid rats. The relatively small effect of amiloride in the aldosterone-treated hypothyroid rats showed that the amiloride-sensitive sodium pathway was only partly induced and the amiloride-insensitive sodium pathway was little reduced despite the high rate of aldosterone administration. Transcellular sodium transport was much less increased by aldosterone than in the intact rats, while the transepithelial p.d. was not increased, reflecting the low level of induction of sodium diffusion channels in the apical membrane.

In addition to changes in the apical membrane, changes in the plasma Na,K-ATPase activity of the basolateral membrane will also influence sodium transport. Thus variations in the intracellular sodium transport pool would be expected to depend on the combined effects of changes in the apical and basolateral membrane. Previous work has, however, shown that alterations in the sodium transport pool are relatively small or even undetectable despite considerable changes in transepithelial sodium absorption (Eaton, 1981; Edmonds & Mackenzie, 1987; Krattenmacher & Clauss, 1988) indicating some mechanism of coordinating apical inflow and basolateral outflow of sodium. The present measurements were in accord with these previous observations showing in general only small changes in the sodium transport pool. In the hypothyroid rats, however, there did appear to be a small expansion of the pool possibly reflecting reduction in basolateral membrane Na,K-ATPase previously demonstrated in both renal and colonic epithelium (Thompson & Edmonds, 1974; Ismail-Beigi & Edelman, 1977). The most striking finding, however, was in the effect of amiloride which almost abolished the pool in the aldosterone-treated normal rats but had relatively little effect in the aldosterone-treated hypothyroid rats. This finding was consistent with the results of the sodium flux and p.d. measurements in indicating that, in hypothyroid rats, aldosterone was much less effective both in inducing amiloride-sensitive and in suppressing the amiloride-insensitive pathways of the apical membrane.
probably depends largely on augmentation of the potassium channels in the apical membrane (Wills & Biagi, 1982; Sandle et al. 1985; Binder, McGlone & Sandle, 1989), this recovery presumably also indicated a relatively rapid restoration of the apical membrane response to aldosterone.

Precisely how thyroid hormones influence the action of aldosterone was not defined in the present studies. Several possibilities exist. Aldosterone requires specific cytosol receptors for mediation of its interaction with the nucleus and these may be affected by hypothyroidism. Moreover, aldosterone and thyroid hormones appear to have their primary action on the genome (Rossier, Paccolat, Verrey et al. 1985; Oppenheimer, Schwartz, Mariash et al. 1987) and recent work has shown that the steroid and thyroid hormone receptors which bind to DNA have a common structure (Evans, 1988). These observations suggest that the interaction between thyroid hormones and aldosterone may well be at the genome itself.

REFERENCES


