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Aldosterone Metabolism and Transepithelial Potential Difference in Normal and Cystic Fibrosis Subjects

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ABSTRACT. The transepithelial potential difference (PD) is raised across cystic fibrosis (CF) respiratory epithelia. This raised voltage reflects active sodium absorption across a relatively chloride impermeable membrane. Because relatively little is known about the regulation of the rate of sodium absorption across mammalian airways, we assessed the possible contribution of aldosterone to the PD in normal and CF respiratory epithelia. Aldosterone excretion in five CF patients was 12.2 ± 0.9 μg/24 h, a mean value not different from normal control subjects (13.6 ± 1.5 μg/24 h, $n = 5$). Despite similar aldosterone excretion rates, nasal PD was more than 2-fold greater in the CF patients (−53.6 ± 6.4 mV) than normal subjects (−21.3 ± 1.4 mV). The effect of an aldosterone antagonist, spironolactone, on aldosterone excretion and nasal and rectal PD was evaluated in four CF patients and five normal subjects. During spironolactone administration, aldosterone excretion increased (2- to 4-fold) and rectal PD decreased in both groups. However, nasal PD was unchanged in each group (CF = −52.1 ± 4.3 mV pre, −53.6 ± 1.4 mV during; normal = −21.2 ± 3.1 mV pre, −21.6 ± 3.2 mV during). We conclude that neither increased aldosterone secretion rates nor organ sensitivity to aldosterone can account for the abnormally raised PD that characterizes the respira-

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tory epithelium of subjects with CF. (*Pediatr Res* 19: 676–679, 1985)

Abbreviations

CF, cystic fibrosis
PD, potential difference
CRU, Clinical Research Unit
BUN, blood urea nitrogen

CF is characterized by thick and tenacious secretions which tend to obstruct exocrine ducts and epithelial-lined organ passages (1). Because systematic assessment of the mucin component of respiratory "secretions" in CF patients has failed to identify a unique abnormality in the mucus glycoproteins (2), and because the secretions of the gut, reproductive tract, and airways of CF patients appear to have a reduced water content (3), we have recently explored abnormalities in the epithelial ion transport processes that regulates the salt and water content of respiratory secretions of CF patients.

The transepithelial electric PD, a parameter that reflects active ion transport and passive ion permeability, is raised across the nasal and lower airway epithelium of CF patients as compared to healthy and disease control subjects (4). Recent *in vitro* studies have suggested that the rate of active Na^+ absorption across CF respiratory epithelia is raised (5) and that the Cl^- permeability of CF respiratory epithelium is reduced by ~50% compared to tissue from normal subjects (6). Other studies have demonstrated a reduction of PD with amiloride, a selective inhibitor of Na^+ transport, and support the notion that Na^+ is actively absorbed across CF airway surfaces *in vivo* (7). Thus it appears that active Na^+ absorption persists at a significant rate across CF airways *in vivo* despite the relatively dehydrated surface liquid.

Relatively little is known about the *in vivo* regulation of Na^+ absorption across mammalian airways. Aldosterone, a potent mineralocorticoid that plays an important role in regulating the balance of Na^+ and K^+ transport across many epithelia, is one candidate for modulating Na^+ absorption across respiratory epithelia. Aldosterone has been shown to increase the rate of Na^+ absorption in the kidney (8), the gastrointestinal tract (9), and the sweat duct (10). The change in the rate of aldosterone-dependent Na^+ transport in the colon is associated with a parallel change in the transepithelial PD (11). It has been suggested that excessive sensitivity to aldosterone may contribute to the gastrointestinal dysfunction in CF (12).

In the present study, we investigated the contribution of aldosterone to the raised nasal PD in CF. First, the relationship between absolute aldosterone excretion and nasal PD was compared in CF and normal subjects. Second, the sensitivity of CF nasal epithelial PD to the effects of aldosterone was investigated by administering spironolactone, an aldosterone antagonist, to a small group of CF and normal subjects. Because spironolactone has been shown to reduce PD in tissues known to be aldosterone sensitive (13), *e.g.* the colon, the effect of this antagonist on rectal PD in CF and normal subjects was measured for comparison.

METHODS

Basal aldosterone excretion rates and nasal PD. Five normal subjects (three females and two males, 24.4 ± 1.4 yr, weight 56.8 ± 0.6 kg) and five adult CF patients (three females and two males, 27.8 ± 4.7 yr, weight 55.6 ± 0.8 kg) had measurements of aldosterone excretion and nasal PD between June and September in an air-conditioned CRU. The diagnosis of CF was established by clinical criteria and elevated concentrations of chloride in sweat (>80 mEq/liter). None of the CF patients had evidence of cor pulmonale, hepatic insufficiency, or significant oxygen desaturation (O_2 saturation > 88%). Each of the subjects had normal baseline renal function. Two of the CF patients were taking antibiotics and three were taking pancreatic enzyme supplements. Twenty-four-hour urinary aldosterone excretion was measured by radioimunoassay (Bioscience, Van Nuys, CA) while each subject was on a daily Na^+ intake of 150 mEq. Each of the CF patients had by dietary history an *ad libitum* intake of >150 mEq of Na^+ per day during the 2 wk before the study. Nasal PDs were measured at the conclusion of the 24-h urine collection.

Effect of spironolactone on aldosterone excretion and epithelial PDs. Four adult CF patients and five normal subjects were studied (December and September–October, respectively) to assess the effect of spironolactone on aldosterone metabolism and transepithelial PD (CF patients = three females and one male, 22.5 ± 2.0 yr, 54.8 ± 1.2 kg; normal subjects = three females and two males, 26.2 ± 1.5 yr, 55.2 ± 0.7 kg). Two of the CF patients and two of the normal subjects were in the group studied in the summer (above). None of the patients had evidence of cardiac or hepatic dysfunction and each had normal baseline renal function and urinalysis. During the study, three patients were taking antibiotics and three were taking pancreatic enzymes. Each subject was admitted to the CRU for 3 days for a physical examination, baseline studies, and initiation of spironolactone. Baseline measurements included serum electrolytes, BUN, and creatinine; 24-h urinary excretion of Na^+, K^+, creatinine (North Carolina Memorial Hospital laboratory) and aldosterone (Bioscience, radioimmunoassay); and nasal and rectal PDs (see below). Dietary histories for the 2 wk prior to admission to the CRU disclosed that each subject had a routine daily intake of >150 mEq of Na^+. During the study, each subject continued an *ad libitum* daily intake of >150 mEq of Na^+ (210.0 ± 31.9 mEq/day). After a baseline was established, spironolactone (gift of Searle, Chicago, IL) was administered orally at 200 mg/day in divided doses. After 48 h of observation and measurements of serum electrolytes, BUN, and creatinine, the subjects were discharged for 7–10 days of spironolactone therapy. Study subjects were instructed to avoid potassium supplements and excessive intakes of food with high potassium content. Serum electrolytes and BUN were measured every 48–72 h during the period of drug administration. Then subjects were readmitted to the CRU, blood was drawn 3 h after the last dose of spironolactone and assayed by gas chromatography (Searle) for canrenone, the active metabolite of spironolactone, to assess drug absorption, and the remaining spironolactone tablets were counted to assess compliance. Clinical chemistry and PD measurements were then repeated.[1]

All procedures were approved by the Committee on the Rights of Human Subjects at the University of North Carolina and informed consent was obtained.

Measurement of nasal and rectal epithelial PDs. PDs were measured between a Ringer-perfused exploring bridge and a reference bridge in the subcutaneous space of the forearm. Nasal PDs were measured under the inferior turbinate as previously described (4). A Ringer-perfused (0.4 ml/min) polyethylene-50 catheter was used as an exploring bridge to record rectal PD through a sigmoidoscope at sites 5 and 10 cm caudal to the anal sphincter.

Data analysis. Measurements of nasal PD were expressed as the mean and maximal PD, as previously described (4). The rectal PD was calculated by the method of Rask-Madsen *et al.* (14) as the mean of the highest stable (>5 s) value recorded at each site. Changes induced during drug administration were assessed by paired *t* analysis. All values shown represent the mean ± SEM unless otherwise indicated.

RESULTS

Basal aldosterone excretion (summer measurements) of the five CF patients (12.2 ± 0.9 μg/24 h) was similar to that of the

[1] Spironolactone was discontinued after 60 h in one asymptomatic CF subject because the BUN had increased from 9 to 28 mg/dl, despite an *ad libitum* Na^+ intake of 270 mEq/day. The BUN returned to baseline after spironolactone was discontinued and parenteral saline was administered. Measurements of aldosterone excretion and nasal and rectal PDs are included for that subject after 60 h of drug.

five control subjects (13.6 ± 1.5 μg/24 h) and within the normal range. Despite similar aldosterone excretion, the mean nasal PD of the CF patients (−53.6 ± 6.4 mV) was more than 2-fold greater than the PD of the normal subjects (−21.3 ± 1.4 mV). The magnitude of this difference is similar to that described for a larger group of CF and normal subjects (4).

The effects of spironolactone on aldosterone excretion and nasal and rectal PD in CF and normal subjects are summarized in Table 1. During the control period, the urinary aldosterone excretion was normal for each subject and not different from the values measured during the summer. The baseline nasal PD of CF patients was again 2-fold greater than the voltage of normal subjects. Values for nasal PDs in both the CF and control groups were unchanged by spironolactone administration (7.0 ± 2.1 and 6.7 ± 0.3 days, respectively). Although the number of subjects we studied is small, the magnitude and variance of the rectal PD values are similar to those of other CF and normal subjects with normal aldosterone excretion (14). After spironolactone the voltage across the rectal epithelium of CF and normal subjects was reduced. Urinary aldosterone excretion increased 4-fold in CF patients and nearly 3-fold in normal subjects. The mean serum K^+ and BUN tended to increase in CF (4.0 to 4.5 mEq/liter and 10.8 to 18.3 mg/dl) and normal subjects (4.2 to 4.5 mEq/liter and 14.8 to 17.4 mg/dl). Spirometry was unchanged in CF subjects; this parameter was not measured in normal subjects.

Pill counts showed each CF and normal subject removed the appropriate number of tablets from the prescription bottle during the period of self-administration of the drug. Canrenone was detected in the plasma of each study subject. The plasma concentration of canrenone 3 h after spironolactone administration was 363.4 ± 23.5 ng/ml in normal subjects and 254.0 ± 28.5 ng/ml in CF patients.

DISCUSSION

Our data do not support the notion that aldosterone contributes to the raised PD noted across nasal epithelia in CF. We found no relationship between baseline aldosterone excretion and nasal PD when CF patients were compared with normal subjects. Aldosterone excretion rates of the CF patients were within the normal range of our reference laboratory and were not different from values obtained under similar conditions in age-matched healthy control subjects.

Several factors suggest our study accurately assessed aldosterone metabolism. First, 24-h aldosterone excretion rates yield good estimates of average circulating aldosterone concentrations (8). Second, all subjects studied in the summer were on standard diets with fixed Na^+ and K^+ intakes, thus minimizing the effect of differences in salt and volume status in our two subject groups. Third, all urine collections were performed in a CRU and 24-h creatinine measurements suggested complete collections.

The normal values for aldosterone excretion rates are also congruent with previous experience in CF patients who do not have far-advanced disease (14–16). Although some CF patients are presumed to have hyperaldosteronism secondary to cardiopulmonary, renal, or hepatic disease and the loss of salt via the kidney or sweat ducts (17–19), our patients demonstrate several characteristics which are associated with the maintenance of normal aldosterone metabolism: 1) "mild" clinical disease with no evidence of cardiac or hepatic insufficiency, 2) high *ad libitum* Na^+ intake, and 3) normal renal function. Consequently, it appears unlikely that excessive aldosterone secretion has a role in the interplay of processes in proximal airways that leads to "dehydrated" airway surface liquid in CF.

We also found no evidence to support the hypothesis that nasal epithelia in CF are excessively sensitive to normal circulating levels of aldosterone. Specifically, administration of the competitive aldosterone antagonist, spironolactone, induced no change in the magnitude of the nasal PD in CF (or normal) subjects. The strength of this evidence rests on the efficacy of spironolactone to antagonize the action of aldosterone. Several considerations suggest that spironolactone was effective in this regard. First, the nominal daily dose (200 mg) is "maximal" for 55-kg subjects. Second, gastrointestinal absorption, as reflected by the plasma concentration of the spironolactone metabolite canrenone, was normal in the CF subjects. Third, the frequency and duration (~7 days) of drug administration would be expected to induce maximal effects. Finally, two physiologic effects of spironolactone were evident in both CF and normal subjects: a 2- to 4-fold increase in aldosterone excretion and a fall in rectal PD.

In summary, our data suggest that neither increased aldosterone secretion nor organ sensitivity to the hormone is likely to induce the raised PD in CF nasal epithelia that may be linked to increased or inappropriately maintained salt and water absorption. This finding is congruent with other recent observations. Nasal PDs of several patients with raised circulating levels of aldosterone and increased rates of aldosterone excretion (primary and secondary hyperaldosteronism) were not raised (4, 20). In addition, exposure of airway epithelia excised from salt-loaded rabbits to a supramaximal concentration of aldosterone did not affect bioelectric properties or ion transport (21). Whereas the findings of others in the rat may indicate some species variability (22), our results suggest that most airway epithelia, including human, are not a target for aldosterone.

Since aldosterone does not appear to regulate the rate of Na^+ absorption across CF or normal nasal epithelia, the mechanism by which Na^+ flow is regulated remains unresolved. If, indeed, inappropriate salt and water transport across CF airway surfaces is responsible for dehydration of surface liquid, the elucidation of the normal regulation of active ion transport and passive ion permeabilities may hold the key to a therapeutic approach on a manifestation of the disease that favors the development of ultimately fatal respiratory infections.

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Table 1. *The effect of spironolactone on aldosterone excretion and nasal and rectal PD in CF and normal subjects (mean ± SEM)*

	CF			Normal		
	n	Control period	During spironolactone	*n*	Control period	During spironolactone
Aldosterone (μg/24 h)*	4	15.5 ± 1.7	59.5 ± 5.6†‡	5	13.8 ± 1.4	33.7 ± 6.2†
Nasal PD (mV)	4			5		
Mean		−52.1 ± 4.3‡	−53.6 ± 1.4‡		−21.2 ± 3.1	−21.6 ± 3.2
Maximal		−70.1 ± 5.1‡	−73.3 ± 1.7‡		−30.6 ± 3.8	−30.3 ± 3.3
Rectal PD (mV)	3	−51.9 ± 12.3	−26.6 ± 10.9†	3	−46.3 ± 9.5	−31.7 ± 7.4

* Normal = 2–25 μg/24 h.

† Different from control period ($p < 0.05$).

‡ Different from normal ($p < 0.05$).

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Whole Body Protein Synthesis and Energy Expenditure in Very Low Birth Weight Infants

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ABSTRACT. The aim of the present work was to study whole body protein synthesis and breakdown, as well as energy metabolism, in very low birth weight premature infants (<1500 g) during their rapid growth phase. Ten very low birth weight infants were studied during their first and second months of life. They received a mean energy intake of 114 kcal/kg·day and 3 g protein/kg·day as breast milk or milk formula. The average weight gain was 15 g/kg·day. The apparent energy digestibility was 88%, *i.e.* 99 kcal/kg·day. Their resting postprandial energy expenditure was 58 kcal/kg·day, indicating that 41 kcal/kg·day was retained. The apparent protein digestibility was 89%, *i.e.* 2.65 g/kg·day. Their rate of protein oxidation was 0.88 g/kg·day so that protein retention was 1.76 g/kg·day. There was a linear relationship between N retention and N intake ($r = 0.78$, $p < 0.001$). The slope of the regression line indicates a net efficiency of N utilization of 67%. Estimates of body composition from the energy balance, coupled with N balance method, showed that 25% of the gain was fat and 75% was lean tissue. Whole body protein synthesis and breakdown were determined using repeated oral administration of ^{15}N glycine for 60–72 h, and ^{15}N enrichment in urinary urea was measured. Protein synthesis averaged 11.2 g/kg·day and protein breakdown 9.4 g/kg·day. Muscular protein breakdown, as estimated by 3-methylhistidine excretion, contributed to 12% of the total protein breakdown. There was a positive correlation ($r = 0.68$, $p < 0.05$) between protein synthesis and protein gain, as well as between resting energy expenditure and protein gain ($r = 0.58$, $p < 0.01$). The slope of the regression line indicated that 1 g of protein gain required the synthesis of five times more protein (5 g) and resulted in an extra energy expenditure of 10 kcal. Thus, the net cost of protein synthesis in these very low birth weight infants was 2 kcal/g. (*Pediatr Res* 19: 679–687, 1985)

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Abbreviations

3-MHis, 3-methylhistidine