EFFECT OF AMILORIDE ON POTENTIAL DIFFERENCE ACROSS RECTAL MUCOSA IN CYSTIC FIBROSIS PATIENTS

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SUMMARY

This study investigated whether the altered epithelial cell function in cystic fibrosis (CF) concerns channels for passive diffusion of sodium across cell membranes. For this purpose, the potential difference (PD), which arises across the colonic mucosa from electrogenic sodium transport, was compared in CF and non-CF subjects, together with the effect on this PD from bathing the mucosa with the specific Na^+ channel blocker, amiloride. The resting rectal mucosal PD did not differ significantly in the two groups. Amiloride caused a dose-dependent decrease in the PD (lumen negative to skin) in all subjects. The mean amiloride effect was less in the CF population at 10^{-8} , 10^{-7} , 10^{-6} , 10^{-5} , and 10^{-4} M amiloride. The decrease of transmucosal PD in CF patients was significantly less when compared with controls at 10^{-6} M and 10^{-5} M and suggestive of a similar trend at 10^{-4} M.

SPECULATION

Altered sensitivity of rectal PD to amiloride in CF possibly reflects an underlying ion transport abnormality, which could account for decreased Na^+ absorption by the sweat duct in CF, and underlie altered epithelial function in other affected sites.

INTRODUCTION

Salient features of the disease cystic fibrosis (CF) include pathologic change of certain secretory and absorptive epithelia (1) and altered sweat gland function (4, 5). A unified explanation of the protean manifestations of the disease should account for both the epithelial pathology and the elevated sweat electrolytes characteristic of the sweat gland dysfunction (4, 5). Impaired transport of Na^+ and Cl^- from the lumen to the interstitial fluid by sweat duct cells apparently underlies the increased sweat salt, as the ion content of the precursor fluid in the secretory coil is not elevated in CF (14). Net Na^+ transport in the sweat gland duct is thought to transpire by means of passive diffusion of the ion through apical Na^+ channels in response to a concentration gradient established by a basolateral pump (11). A similar mechanism involving a basolateral pump and apical Na^+ channels underlies net Na^+ transport in other epithelia including the colon (7). Because the active pump represented by Na^+/K^+ ATPase of the basolateral plasmalemma appears unaltered in CF (11), attention centers on passive diffusion through Na^+ channels of the luminal plasmalemma as the site of dysfunction resulting in decreased net transcellular transport of ions and elevated sweat electrolytes. A defect in such a fundamental ion transport mechanism could explain both sweat gland dysfunction and pathologic change of secretory and absorptive epithelia. Altered content of fluid and ions consequent to the transport defect, according to this view, would influence unfavorably the solubility and viscoelastic properties of secretory macromolecules in affected sites leading to luminal obstruction and parenchymal injury.

The current view that passive diffusion of sodium ions takes place through specific membrane channels has evolved partially from observations of the action of the diuretic drug, amiloride, which specifically blocks the diffusion of Na^+ through these channels in human kidney, colon, and rectum (9, 13, 15). Current studies aim at assessing Na^+ channel function in CF as measured by amiloride inhibition of Na^+ transport, utilizing a site known to be affected by the disease and to carry out amiloride-sensitive Na^+ transport. The rectal epithelium, as part of the colon, manifests clinical involvement in CF (10). The normal human rectum has been shown to generate a transmucosal electric potential difference (PD) as a consequence of Na^+ resorption from the lumen (8). Amiloride, moreover, has been found to inhibit this electrogenic Na^+ transport as evidenced by a prompt, reversible and dose-related decrease in PD after topical application (13). Increased rectal potential in human subjects (6), as well as rabbits (7), after aldosterone treatment further attests to rectal sodium channels. In the investigation reported here, the effect of increasing concentrations of amiloride on the PD generated across the rectal epithelium by Na^+ transport is compared between CF patients and non-CF controls.

MATERIALS AND METHODS

Subjects: Twenty ambulatory CF patients, whose diagnosis was established by clinical evidence and elevated sweat chloride in standard sweat testing, were examined during a routine follow-up visit at the Cystic Fibrosis Clinic of the Medical University of South Carolina. The patients' disease was well controlled and they were free of acute illness. Ages ranged from 11 months to 23 years. All CF patients were taking enzyme supplements and no other treatment except one patient who was under treatment with INH, Rifampin, and Ethambutol for pulmonary tuberculosis. None of the patients had been treated with steroids within the past two months.

Controls were two volunteers from the Medical University of South Carolina General Pediatric Clinic, children of faculty members and housestaff members. Age range was over 1 to 29 years and, in general, corresponded in individual members with the patient population. None were suffering bowel disease or on antibiotic or steroid medication. Patients and controls were chosen without regard to race or sex. Approval for this study was obtained from the Institutional Review Board of the Human Research Committee at the Medical University of South Carolina. Written consent was obtained from all subjects or from parents in the case of minors. Amiloride usage was governed by IND #18,625.

PD measurement and drug administration: Rectal PD was measured by the method previously described in detail (9). The exploring bridge consisted of a 0.2 cm diameter, 37 cm long polyethylene catheter which was inserted into the rectum and was perfused with normal saline or amiloride HCl in normal saline at one ml/min. Saline in the proximal end of the catheter made contact with a silver chloride coated silver wire of 0.1 cm in diameter and 8 cm in length. The reference electrode consisted of a 2.25 cm² silver chloride coated silver plate, which was wrapped in tissue wetted with normal saline and taped in place on the left buttock over the site of a 0.3-ml intradermal injection of sterile isotonic saline. Previous studies suggest that intradermal saline injection at the reference electrode site suffices to render the skin electrically equivalent to extracellular fluid (9). Other reports demonstrate that the ionic content of the rectal perfusate may vary considerably without altering the measured PD (8); therefore, it was not considered necessary to match exactly the ionic composition of the perfusate and that of the rectal contents.

Electrode symmetry was measured before and after each day's experiments, consisting of examination of one to four patients, and was never more than 3.0 mV. This small fluctuation could add to the variability of results but should not otherwise influence the findings. The Simpson model #461 voltmeter with an input impedance of one $\times 10^{-7}$ ohms and a sensitivity of ± 0.1 mV from one to 200 mV was selected for reasons of convenience.

Variation of perfusion rate was tested for its effect on measured PD and found to have no noticeable influence over the range of 0.5 - 30.0 ml/min. A relatively slow infusion rate was selected to minimize luminal dilution of perfusate. Difficulty in controlling dilution, which depends on peristaltic and absorptive activity as well as the nature of the stool content, was recognized as a potential source of variability; however, this was not expected to obscure the difference between the two groups. Subjects were placed in the prone position. No attempt was made to empty the rectum before the procedure because this has not proven necessary in previous studies (9). After insertion of the rectal catheter to a depth of 10 cm and placement of the reference electrode, infusion of normal saline was begun. The catheter tip was thus inserted routinely beyond the squamo-columnar junction. No attempt was made to correct for age-related differences in bowel anatomy because both groups contained comparable numbers of individuals of various ages. Furthermore, although the colon does show gradually increasing polarization from cecum to rectum, this difference is minimal. A stable potential was usually reached within 3 min. Moving the catheter tip over a distance of several centimeters in a few tests had little influence on the measured potential.

After recording the potential for 5 min during infusion of normal saline, all of the apparatus except for the 37-cm rectal catheter was flushed with the next solution to be infused. After this flushing, infusion of the next solution was begun with an initial rapid flow rate of 30 cc/min for 10 sec to flush the catheter, after which the flow was readjusted to 1-2 ml/min; thus, infusion of amiloride, HCl 10^{-8} M in normal saline, was begun and continued for 3 min, while recording the potential at 60-sec intervals. This step was repeated using amiloride concentrations of one $\times 10^{-7}$ M, one $\times 10^{-6}$ M, one $\times 10^{-5}$ M and one $\times 10^{-4}$ M. Perfusion at one $\times 10^{-8}$ M and one $\times 10^{-7}$ M was omitted in twelve patients and eleven controls. Perfusion at one $\times 10^{-4}$ M was omitted in three patients and five controls. All patients and controls were tested with amiloride concentrations of one $\times 10^{-6}$ M and one $\times 10^{-5}$ M.

RESULTS

The mean resting transmucosal PD was 14.5 ± 5.0 mV (mean $\pm$ SE) for CF patients and 26.5 ± 6.0 mV for controls (lumen negative to skin) (Fig. 1). The mean change in transmucosal PD upon perfusion at each concentration of amiloride is compared between the two groups in Figure 2. Amiloride at 10^{-8} M had no significant effect on the PD measured in either group; and after nine normal and eight CF patients had been studied the use of this concentration of drug was discontinued. At 10^{-7} amiloride, PD was increased in the CF group and decreased in the control group, but the population studied was limited as for the 10^{-8} M concentration. At higher concentrations PD was decreased in both groups. The effect of amiloride on rectal PD was smaller in patients than in controls at each concentration. The difference between the two groups in the magnitude of the amiloride effect was significant at 10^{-6} M ($P < 0.05$) and 10^{-5} M ($P < 0.01$). Differences at 10^{-7} and 10^{-4} M amiloride were suggestive ($P < 0.1$).

DISCUSSION

The dose-related response to amiloride recorded consistently in all subjects examined here confirms previous reports on normal human subjects (9, 13). In addition, the present findings point to the possibility of a lesser inhibition of the potential difference in the rectum of CF compared with non-CF populations. Although the lesser amiloride effect in CF is only suggestive at drug concentration of 10^{-7} M and 10^{-4} M ($P < 0.1$), the difference at 10^{-6} M and 10^{-5} M is statistically significant ($P < 0.05$ and < 0.01 , respectively) despite a wide range of variability among individuals in both diseased and control groups.

Different interpretations of these results can be entertained depending largely on whether the PD across the rectal mucosa is abnormally low in CF. Previous studies of the transmucosal PD in normal human rectum report values consistently ranging from about 20 - 50 mV, lumen negative (6, 9). An early investigation comparing PD between perianal skin and rectal lumen showed a decreased or even reversed resting rectal PD in CF. Although the difference in mean resting potential between the two groups in the present investigation was not statistically significant, it was rather large (14.5 versus 26.5 mV). If indeed a difference in the resting transmucosal PD of normal compared with CF subjects does exist, and is accurately reflected by the present data, then the response of the two groups to amiloride can be considered identical, within experimental error. In both groups, 10^{-6} M amiloride produced 10 - 20%, 10^{-5} M caused 50%, and 10^{-4} M induced 100% inhibition of PD. This would seem to suggest that amiloride-sensitive Na^+ channels are normal in CF and that the difference in resting PD has an alternative explanation. Notably, amiloride produced a considerable effect on the potential difference across the rectal mucosa of the CF subjects examined. Amiloride-sensitive sodium channels are clearly not absent in the disease.

More recent work has shown, however, that the rectal transmucosal PD is probably not different in CF (2, 3, 12). When the reference electrode was placed intravenously rather than on the perianal skin, the previously reported difference was not seen, suggesting that the skin is more negatively polarized in CF instead of the rectal mucosa being less negative. If, then, no real difference in mean transmucosal resting PD exists between the control and CF populations as suggested by these studies, the apparent discrepancy observed here could arise at the reference electrode. In the present study, the reference electrode contacted the skin at the site of intradermal saline injection. If the skin potential were incompletely abolished by intradermal saline injection it would remain negative in CF as compared to normal controls, and account for the apparent difference in resting PD. In the calculation of amiloride effect, however, the reference potential term drops out, so that the observed lesser inhibition of PD by amiloride in the CF group would seem to reflect a true abnormality in amiloride-sensitive sodium transport. A diminished amiloride response in CF appears consistent with the view that channels, which both bind amiloride and mediate net Na^+ transport, are diminished in number or activity in epithelial cells of patients. Such a change could cause altered absorptive activity and, hence, account for clinical manifestations in the colon and, if generally present, would explain elevated sweat electrolytes and, presumably, also the exocrinopathy.

Alternatively, the apparent discrepancy in the resting PD between controls and CF patients could reflect a technical problem with the rectal elec-

trode, attributable to different stool content or motility in the CF patients; however, the CF volunteers were all taking enzyme supplements and none were suffering from steatorrhea or diarrhea at the time of the study. Furthermore, several previous studies using the present technique failed to document an influence of the rectal content on transmucosal PD measurement (2, 3, 12). Additional study of electrolyte transport in the CF colon would be helpful, to identify and perhaps control sources of individual variation and to assess the possible role of other specific transport defects in CF because transport of Na^+ by colon and rectum is known to be aldosterone sensitive. Future studies will be conducted in the General Clinical Research Center where fluid and electrolyte balance can be monitored.

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FIG. 1: Resting potential difference between rectal lumen and cutaneous reference electrode (see Materials and Methods) is compared in 22 cystic fibrosis patients and 20 controls. Although the average potential difference appears less in the cystic fibrosis group, this difference was not statistically significant. Values expressed as mean $\pm$ SE.

FIG. 2: Effect of rectal infusion of increasing amiloride concentrations on rectal transmucosal potential difference in cystic fibrosis patients compared with controls. Values expressed as mean $\pm$ SE.