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Short Communication:

Decreased Cl Permeability as the Basis for Increased Bioelectrical Potentials in Cystic Fibrosis

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INTRODUCTION

We recently suggested that the defect in the sweat gland in Cystic Fibrosis might involve a disturbance in a Cl/HCO_3 exchange (2). More recently we have secured evidence that indicates that the lumen of CF sweat ducts is markedly hyperpolarized (Table 1) even though Na reabsorption in CF sweat glands is greatly decreased (2,3). This additional data strongly indicates that the decreased NaCl reabsorption from CF sweat is due to an inability to reabsorb Cl simultaneously with Na. Thus, these findings add support to the conclusion that the defect in CF glands is due to a problem with anion movements, but they reduce the probability that an electrically neutral anion exchange plays a major role in the defect. Rather, the data are more compatible with a defect which renders the permeability of the CF sweat duct to Cl abnormally low.

METHODS

Five patients with an established diagnosis of Cystic Fibrosis and five control subjects volunteered with informed consent to participate in the study. The age of the patient population ranged from 18 to 36 years and that of the control population ranged from 18 to 34 years. Older subjects participated due to the high level of subject cooperation required to carry out the investigation. All patients were receiving antibiotics and supplemental vitamins. Four of the five patients were receiving bronchodialators with inhalation therapy. No two patients were on the same medical regime. Control subjects had no known health problems and none were receiving any medications.

Four to seven single glands were studied individually in each subject. The volar surface of the forearm was first washed thoroughly with water, rinsed with propanol, and dried. The epidermis was then vigorously rubbed with Sylgard 184 (Dow Corning) in order to render the epidermal surface around the sweat duct pore electrically inert and hydrophobic. A small spot was stimulated iontophoretically with a solution containing 2% acetylcholine plus 0.1% mecholyl. (We note that most of the conductivity through the skin is through the pores of the sweat duct (5) which are therefore probably the major routes of drug movement through the skin during iontophoresis.) Immediately after stimulation, the area was blotted and a plastic well filled with mineral oil was fitted around the stimulated area. Sweat from single glands could be seen to emerge under the oil as discrete droplets on the skin. The electrical PD between the sweat droplets and the interstitial fluid was measured by inserting one of a pair of electrodes in a sweat droplet with the other electrode contacting the subdermal interstitial space through a small abrasion in the skin. The electrode in the sweat droplet was a glass microelectrode filled with saturated KCl connected to a high impedence electrometer via a Ag/AgCl electrode. The reference electrode was a saline wetted wick applied over the skin abrasion and connected to the electrometer ground via a saturated KCl-Ag/AgCl junction. Electrode assymetries were determined regularly by placing the glass electrode in a small droplet of saline applied over a second small abrasion in the skin made in the vicinity of the stimulated sweat droplets. Asymmetries were usually less than ± 2 mV and were subtracted from the measured potential.

Immediately after measuring the electrical potential, the sweat droplet from the single gland was collected and the rate of sweat secretion, the concentration of Na in the sweat, and the rate of Na reabsorption were determined as described previously (6,7). Since the rate of Na reabsorption is highly dependent on sweat rate glands secreting at similar rates in both populations were used to compare electrical potentials and Na reabsorption.

The methods used in this study may appear similar to those used several years ago to study transcutaneous skin potentials in CF and control subjects (8). In those studies, a wet "wick" electrode was placed on the skin surface with a metal reference electrode inserted subcutaneously. The measured potential was assumed to be generated by sweat glands. The approach differs considerably from the present methods in that 1.) the measuring electrode was not restricted to single sweat droplets, but rather it was placed directly on the skin surface with no attempt to electrically isolate the epidermis from the sweat pore, 2.) rather large potentials were associated with the reference electrode, and 3.) since the sweat was not collected simultaneously with the electrical measurements, the potentials were not correlated with Na reabsorption. Even so those studies were the basis of the first suggestion that Cl may be impermeable in the CF sweat duct. Unfortunately, the suggestion seems not to have been followed by additional work until now.

It should also be pointed out that in an earlier study (9), methods almost identical to those employed here were used to record potentials in a population of normal subjects. The PD values reported were very similar to those reported here for control subjects.

RESULTS AND DISCUSSION

Perhaps the most recognized and well established abnormality in Cystic Fibrosis is the abnormally high concentrations of NaCl in the sweat of affected individuals. However, the basis of this abnormality has not been previously determined. The present data not only confirm the abnormality, but also show that the rate of Na reabsorption in the CF duct is only about 30% of that observed in controls when the simultaneously measured potential associated with the CF duct is 200% more negative than in controls. That is, in the normal glands when the average electrical potential was only -33 mV, the average Na concentration measured simultaneously in the sweat droplets was 26 mM indicating a rate of net NaCl reabsorption of 228 pM/min/gland at an average sweat rate of 1.95 nl/min/gland. In contrast, in the CF glands the average electrical potential was -66 mV when the average Na concentration measured simultaneously was 99 mM indicating a rate of net NaCl reabsorption of only 89 pM/min/gland at an average sweat rate of 1.82 nl/min/gland (Table I). Since the secretory rates are almost identical, the differences in reabsorptive rates cannot be due to differences in secretory Na loads on the duct. It is clear from these data that the more negative bioelectric potentials cannot be due to increased rates of Na transport (reabsorption) in the CF duct.

On the other hand, the data can be explained by assuming that in the sweat duct, as in certain other Na absorptive epithelia (frog skin, distal tubule, colon, urinary bladder), NaCl transport occurs by active transport of a Na cation which is passively followed by a Cl anion. This "pulling" of the anion by the cation results in a separation of charge which creates an electrical PD across the epithelium. If Cl cannot follow Na readily, the separation of charge and the transepithelial PD increases, but the overall process of net NaCl reabsorption decreases, since Na must be reabsorbed with Cl due to the requirement for electroneutrality. Hence, a relative impermeability to Cl explains both the decrease in NaCl reabsorption and the increase in transductal potential in the CF sweat duct.

Recently, Knowles et al. (1) reported hyperpolarized respiratory epithelia in Cystic Fibrosis. These workers felt that the more negative potentials in CF were due principally to an acceleration of Na reabsorption. Even though this view is attractive because it provides a logical basis in terms of increased fluid absorption for what seems to be an increase in viscosity and solid content in sputum collected from CF patients (10,11), it may neglect important characteristics of other affected organs. Since the disease is genetic, exocrine defects should involve a common derangement, which make it difficult to reconcile the postulated increased Na transport in the lung with known defects in the sweat gland and pancreas. In CF sweat, Na (and Cl) concentrations are 3-5 times higher than in normal sweat which is due to decreased Na transport in the duct of the gland (ref. 4, Table 1). In the CF pancreas, secretions are low in fluid and HCO_3 content. Since pancreatic fluid secretion depends upon the transport of electrolytes, the observed abnormality again is apparently due to a decrease in ion transport in the duct (12). Thus, it is difficult to understand how a decrease in electrolyte transport can be the basis of the defect in the sweat gland and pancreas in this disease while an increase in transport is the basis of the defect in the respiratory tract.

In view of these considerations, it seems to us more likely that the more negative potentials observed in the respiratory tract may be similar in nature to those in the sweat duct and that net NaCl absorption in the respiratory tract is also reduced. It may help to keep in mind that both tissues are sensitive to amiloride (1,6) and that in general amiloride affects aldosterone sensitive, Na savengering epithelia which do not move large volumes of fluid (distal nephron, colon, urinary bladder, sweat duct), while it has little effect on those epithelia which transport relatively large volumes of fluid (small intestine, gall bladder, proximal tubule). Thus, the suggestion that respiratory problems in CF are due to enhanced fluid absorption in the lung are not entirely consistent with the nature of the observed abnormality and the physiological characteristics of the epithelium.

Furthermore, if the defect in the lung is also due to Cl impermeability with an associated decrease in net NaCl absorption, as in the sweat duct, the application of amiloride as a therapeutic agent (1) would decrease Na reabsorption further and may, thereby, exacerbate rather than ameliorate the condition.

In conclusion, we feel that present evidence argues that Cl permeability in the CF sweat duct is abnormally low and that the defect explains the high concentrations of NaCl in CF sweat. The present observations of electrical potentials associated with the sweat duct and the explanation of its basis are consistent with observations in CF respiratory tissue, which may indicate that Cl impermeability may be characteristic of tissues affected in Cystic Fibrosis.

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Table 1

	Potential Difference (mV)	Sweat Na Concentration (mM/L)	Single Gland Sweat Rate (nl/min/gland)	Single Gland Na Reab Rate (pM/min/gland)
Normal Subjects	-33 ± 4	26 ± 10	1.95 ± 0.55	228 ± 43
CF Patients	-66 ± 6*	99 ± 8*	1.82 ± 0.19	89 ± 20*

Table 1. Parameters of Single Sweat Glands in CF and Normal Sweat Glands. The electrical potential difference between a microelectrode inserted into sweat droplets formed under oil on the skin and a reference electrode contacting the interstitial fluid are given in the first column. The sweat Na concentration, sweat rate, and rate of Na reabsorption determined simultaneously with the PD for each sample are given the subsequent columns. Results are expressed as the average of the mean of measurements from 4-7 glands each for 5 control subjects and 5 CF patients. Errors are standard errors of the mean.

* $p < .01$

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