Suggestion of an Abnormal Anion Exchange Mechanism in Sweat Glands of Cystic Fibrosis Patients

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Summary

The physiological basis for the defect in NaCl reabsorption in cystic fibrosis (CF) sweat glands is not known. We have systematically followed the concentrations of the three major inorganic electrolytes in sweat as a function of single gland sweat rate in patients with CF and normal individuals. The result demonstrates that (1) at low sweat rates, sweat glands of CF individuals can reduce NaCl concentrations to normal levels and (2) that the concentration of organic anions and/or HCO_3^- in CF sweat is substantially less than in normal sweat.

Speculation

Present data and other findings suggest that Na/K transport and a postulated Na/H exchange are normal, but that a postulated anion exchange may be defective in cystic fibrosis sweat glands. These observations suggest an underlying transport genetic abnormality, which might explain observed abnormalities in cystic fibrosis exocrine gland secretions in general.

Almost without exception, the fluid secreted by exocrine glands in cystic fibrosis (CF) are either altered in volume or composition. For example, the volume of pancreatic secretions formed under similar conditions of stimulation is only about 10% of normal (11). On the other hand, the volume of submaxillary salivary secretions is normal but the concentrations of Na and K are elevated in these fluids (18).

Of the known abnormalities in exocrine secretions, an elevated concentration of NaCl in sweat from cystic fibrosis individuals is one of the most characteristic expressions of the disease. In children, a sweat Cl concentration greater than 60 meq/liter almost without exception indicates a positive diagnosis while a Cl concentration less than 50 meq/liter almost as certainly rules out the diagnosis (5). Even though this alteration has proven most useful in diagnosis and has been the subject of much investigation, the underlying basis of the physiologic defect remains unknown.

With few exceptions (14, 17, 26), almost all previous investigations of the sweat defect in cystic fibrosis have studied whole sweat collected from the body surface. Most of these studies were limited to the analysis of one of two of the major electrolytes present in sweat. These investigations have the advantage of presenting the average function of numerous glands, but suffer from the disadvantages of being unable to: (1) account for the behavior of individual glands and (2) to follow the presence of the several major components of sweat simultaneously.

With the present study, we have examined secretions of single glands from both cystic fibrosis and normal individuals, using techniques of x-ray microanalysis.

MATERIALS AND METHODS

Subjects. Informed consent forms were obtained from four normal volunteers, ages 22-28 years, and from 5 individuals

diagnosed with cystic fibrosis and followed at the UCLA cystic fibrosis clinic with ages 20-34 years.

Collection. Sweat was collected from the volar surface of the forearm of all subjects. Glands were stimulated to secrete by iontophoresing a solution of 1% bethanecol into an area of approximately 40 cm² with a current of 1.8 mA applied for 5 min. In order to collect sweat secretions from single glands, the stimulated area was then washed thoroughly with distilled water, wiped briefly with ethanol, dried thoroughly, and coated with water-saturated mineral oil. A small well was then strapped to the arm and filled with water-saturated mineral oil. With the aid of a dissecting microscope, individual sweat droplets were visualized and collected in constant bore capillaries (66 μm inside diameter) between oil blocks. Samples were collected continuously from two or three glands separately until sweating became too slow to obtain sufficient volumes for collection. Sample volumes ranged from about 5 nl to more than 50 nl and were collected at 2-5 min intervals, depending on secretory rate. Usually maximal secretory rates occurred within 5 min of stimulation and declined spontaneously thereafter to rates of less than 1 nl/min within 45 min. Secretory rates were determined by dividing the measured volumes of collected secretion by the period of time required for each collection.

Analysis. The content of the collected sweat samples was determined by energy dispersive x-ray spectrometry analysis of liquid microdroplets (20). Samples were analyzed for Na, Mg, P, S, Cl, K and Ca. The analysis does not permit the detection of organic constituents or of elements with atomic weights less than Na.

Standardization. Secretory rates of individual glands vary over a wide range even among glands from the same subject. In order to compare the function of all glands, each measured secretory rate was standardized by dividing the rate of each collected sample by the maximum rate measured for that gland. The maximum rate measured for each gland was therefore always represented as 100%. Curves of concentration *versus* rates were fitted by the method of least squares. Statistical significance was determined using Student *t*-test.

RESULTS

Rates. There was no significant difference between the average maximal secretory rates of glands from the two groups of subjects. The mean maximal rate of glands from normal individuals was 6.7 ± 3.3 nl/min ($n = 10$) and 7.9 ± 7.9 nl/min ($n = 13$) for glands from CF individuals ($P > 0.35$).

Minor components. Of the elements assayed, Mg, P, S, and Ca were generally present at concentrations of less than 1.0 mM. Hence, these elements were neglected in the calculation of organic anion concentration, and the only major electrolytes considered were Na, K, and Cl.

Sodium. Figure 1 shows that Na concentration increases as a function of sweat rate for both CF and control groups. The mean Na concentration of sweat from glands secreting at 100% observed

maximal rate was 97 ± 24 meq/liter ($n = 13$) for CF glands and 44 ± 20 meq/liter ($n = 10$) for normal glands ($P < 0.01$). As secretory rates among CF glands fell to below 35–40% maximal rate, Na concentrations were observed to fall below 60 meq/liter *i.e.*, below the level diagnostic for cystic fibrosis clinically. At secretory rates less than 20% maximal, Na concentrations fell below 20 meq/liter. The range of Na concentrations in sweat from single normal glands was similar to that reported for whole sweat collected from normal individuals (28). Notably, however, there is remarkably little overlap of Na concentrations between the two groups when compared at similar secretory rates.

Chloride. The pattern of Cl concentration as a function of secretory rate largely mimics that of Na for both groups (Fig. 2). The average Cl concentration (95 ± 23 meq/liter) at 100% maximal secretion was about the same as Na in fluid from CF glands. However, in sweat from normal glands, the chloride concentration (24 ± 12 meq/liter) was about 20 meq/liter lower than Na concentration. There is little overlap between Cl concentrations from the two groups when compared at similar secretory rates ($P < 0.01$ at 100% maximal rate). In normal glands, Cl concentrations drop to relatively low levels (less than 20 meq/liter) at relatively high secretory rates (50% maximal) while in CF glands, Cl concentrations were not found to fall below 20 meq/liter at even the lowest observed rates.

Potassium. The concentration of K in both groups is inversely related to secretory rate. At very low rates K may exceed 50 meq/liter, but at higher rates the concentrations stabilize at values that are slightly higher than plasma (Fig. 3). The average concentration of K (10.8 ± 5.8 meq/liter) at 100% maximal secretory rate was significantly higher in CF glands than the K concentration (6.3 ± 1.6 meq/liter) in sweat from control glands ($P < 0.05$). There was considerable overlap of K values at similar rates throughout the range of sweat rates (Fig. 3), but K concentrations were generally higher in CF glands than in normals.

Anion deficit. Because the sum of the Na and K concentrations

Fig. 1. Sweat sodium concentration as a function of % maximal rate. The concentration of Na in sweat secretions from single glands is plotted as a function of the % of maximal secretory rate observed for 11 glands from normal individuals (●) and 13 glands from cystic fibrosis patients (○). The consistently higher Na values in CF sweat shows that ductal reabsorption of Na in CF glands is inhibited. Note that even though CF sweat Na values reach "clinically normal levels" (below 50 meq/liter) at low secretory rates, the Na in CF sweat is higher than normal at comparable rates. Curves were drawn by the method of least squares for the best fit of second degree polynomials.

Fig. 2. Sweat chloride concentration as a function of % maximal rate. Chloride concentration values are plotted against % maximal rate on the same samples recorded in Figure 1. Again CF values (○) are well separated from normal values (●). Curves fitted as in Figure 1.

Fig. 3. Sweat potassium concentration as a function of % maximal rate. K concentration values are plotted against sweat rate on the same samples recorded in Figure 1. Although CF sweat K values (○) are generally higher than normals (●), there is considerable overlap of values between the two populations at all sweat rates. Curves fitted as in Figure 1.

almost always exceeds the concentration of Cl in sweat samples, another anion is required to satisfy electroneutrality. With the present x-ray analytical methodology we cannot detect HCO_3^- or organic components, but HCO_3^- and lactate are the only two anions known to be present in sweat at concentrations greater than a few mM. Lactate comprises about 0–15 meq/liter of the deficit with the remainder due to HCO_3^- (23). Because lactate is produced by the secretory coil, but not by the duct (24), most of the changes in the size of the anion deficit (X^-) are probably due to changes in HCO_3^- concentration in the ducts.

The anion deficit was calculated as $X^- = Na + K - Cl$. The pattern of X^- excretion as reflected by this calculation is shown in Figure 4. Although there is some overlap of values at similar rates among CF and normal secretions, the anion deficit in CF sweat generally is substantially lower than that of normal sweat throughout the range of secretory rates. Furthermore, the X^- concentration in normal secretions increases much more rapidly with increasing sweat rate than in CF secretions. At maximal rates, the mean X^- concentration was 13.2 ± 10.5 meq/liter in CF sections and 30.9 ± 10.0 meq/liter in normal gland secretions ($P < 0.01$).

DISCUSSION

Components of Reabsorption. Additional mechanisms or processes may be involved in ductal reabsorption, but our interpretation of available data suggest that electrolyte reabsorption in the eccrine sweat duct may require at least three integrated processes: (1) active Na/K exchange at the basal membrane of ductal cells, (2) Na absorption and H secretion at the apical membrane, and (3) Cl reabsorption and HCO_3^- or organic anion (X^-) secretion at the apical membrane. The present studies suggest that this latter process, Cl/ X^- exchange, may be defective in CF sweat glands.

Na/K pump. As in other ion transporting epithelia, electrolyte transport in the sweat duct is dependent upon a Na/K exchange pump involving Na/K ATPase in the basal membrane of the ductal cells. The pump in several other systems generally has been shown to extrude three Na ions in exchange for the uptake of 2 K ions and to be inhibited by ouabain, a specific inhibitor of Na/K ATPase (8). This enzyme has been localized in the basal lateral membranes of isolated, perfused human sweat glands (22). Local intradermal injections of ouabain block Na reabsorption *in vivo* (7, 25), and in microperfused segments of sweat ducts, Na reabsorption is blocked when ouabain is applied to the serosal, but not luminal, side (21).

Even though profiles of Na *versus* rate (Fig. 1) showed diminished Na reabsorption in CF glands, it seems unlikely that the Na/K pump is defective for at least three reasons: (1) in the assay of Na/K ATPase activity, Gibbs *et al.* (9) found no significant difference between CF and normal sweat glands; (2) likewise, Quinton and Tormey (22) found no detectable differences in

ouabain binding patterns; and (3) if there were a defect in an ion pump as ubiquitously as Na/K ATPase, it is very difficult to understand why only exocrine glands express transport abnormalities in the disease.

The elevated K concentration in CF sweat (Fig. 3) may, as suggested previously (28), be related to sustained high concentrations of Na. That is, the higher Na loads on the distal segments of the reabsorptive duct may stimulate the Na/K pump and thereby enhance K secretion in CF glands. At the same time, intracellular K may tend to equilibrate with ductal fluid resulting in high K in both CF and normal sweat when sweat rates are very low (Fig. 3).

Na/H exchange. Although there is direct evidence that Na reabsorption across the apical membrane is coupled to H secretion in some systems such as the proximal tubule and intestine (2, 16, 19), direct evidence that Na and H transport are linked in the sweat is not available. However, sweat Na and H are inversely related as a function of secretory rate. At low rates, Na concentration is low while H concentration is high, and the relative concentrations are reversed at high rates (6, 28). In addition, the dependence of Na reabsorption on HCO_3^- (23) suggest a possible role for H in Na transport in the duct.

The present data may also suggest a Na/H exchange because X^- increases as a function of sweat rate in normal glands and X^- remains relatively low in CF sweat (Fig. 4). Because sweat precursor fluid is isotonic with extracellular fluid (26, 27), the concentration of HCO_3^- entering the duct should be about 25 mM. The decrease in final sweat X^- concentration at lower rates in normal glands must be due to either an increase in the fractional reabsorption of X^- or to an increase in the fractional neutralization of X^- by H secreted in the duct, presumably via Na/H exchange. (30). It seems unlikely that X^- is reabsorbed by the duct, because this would simply increased reabsorption of X^- in CF glands, which are characterized by defective Na and Cl reabsorption. Therefore, we conclude that a Na/H exchange may be a functioning part of the reabsorptive mechanism in both normal and CF sweat ducts. If the Na/H exchange were defective in CF, we would anticipate that less of the HCO_3^- entering the duct would be consumed and X^- would remain equal to, or greater, than X^- in normal sweat at comparable rates.

Cl/ X^- Exchange. Recent investigations of a number of absorptive systems have implicated an anion exchange such as Cl/ HCO_3^- as being involved in Cl absorption. Examples of such systems include the gall bladder (13), gill (12), urinary bladder (4), colon (1), and the submaxillary main excretory duct (3). Two aspects of the present data suggest the presence of Cl/ X^- exchange in the sweat duct.

First, in CF glands both Cl reabsorption and X^- secretion appear to be inhibited. A direct explanation for simultaneous inhibition of two species transported in opposite directions is a defective exchange. A defect in a Cl/ X^- exchange would decrease Cl absorption and limit the supply of luminal X^- . In CF, even partial Na reabsorptive would further reduce luminal X^- by neutralization with H secreted into the duct via a Na/H exchange (Fig. 5). The concentration of X^- would rise only when the load of precursor fluid X^- saturated ductal H secretion at high sweat rates (Fig. 4).

Second, in normal glands the concentrations of Cl and X^- both decrease as sweat rate falls (Fig. 2 and 4). If a Cl/ X^- exchange is a mechanism of ductal Cl absorption and X^- secretion, lower luminal Cl concentrations at lower sweat rates would create less favorable transductal Cl gradients and reduce X^- secretion. Lower ductal X^- secretion would allow Na/H exchange to consume increasing fractions of precursor fluid X^- entering the duct and decrease the final sweat X^- concentration at lower sweat rates as observed.

SPECIAL NOTES

First, it may be of interest clinically that CF sweat glands can, if the sweat rate is low enough, reduce Na concentrations to well within the range of normal values (Fig. 1). This finding clearly

Fig. 4. Concentration of organic anions as a function of % maximal rate. The concentration of organic anions and/or HCO_3^- (X^-) was calculated as the difference between the sum of Na and K concentrations and the Cl concentrations: $X^- = Na + K - Cl$. In contrast to Na and Cl, the concentration of this anion is generally substantially lower in CF sweat (●) than in normal sweat (○). Curves fitted as in Figure 1.

Fig. 5. Possible components of NaCl reabsorption in normal and cystic fibrosis sweat ducts. In both normal and CF ducts, it is postulated that Na^+/K^+ transport at the basal membrane creates a high intracellular K and low intracellular Na concentration. Low intracellular Na favors the entry of Na from the lumen which is exchanged for H across the apical membrane. The secreted H consumes luminal HCO_3^- which favors additional HCO_3^- entry into the duct lumen. If the secretion of HCO_3^- is coupled to the reabsorption of Cl , the net result is that when NaCl is absorbed, H_2CO_3 is formed in the lumen. The H_2CO_3 can be dissipated by conversion to CO_2 and H_2O . In the CF duct, Na^+/K^+ transport and Na^+/H exchange may be normal, but the $\text{Cl}^-/\text{HCO}_3^-$ exchange may be defective. The defect results in the consumption of larger fractions of HCO_3^- in the precursor fluid consumed by secreted H . Consequently, the anion deficit is lower and the Na concentration tends to approach the Cl concentration in CF sweat. In the model, HCO_3^- is used to illustrate the role of X^- used in the text.

dictates that a relatively high sweat rate must be assured if false negatives are to be avoided in the use of the sweat test (10) in diagnosis. As low sweat rates we assume that other pathways may allow absorption of Cl and dissipation of ductal H so that Na can reach these low levels or that the anion exchange retains sufficient function to permit NaCl reabsorption at these low secretory loads.

Second, the present results and interpretations confirm and may explain the observation of Slegers (28) that the ratio of Na to Cl concentrations in CF sweat is always lower than the same ratio in normal sweat. The possibility that a Na/H exchange is normal while a Cl/X^- exchange may be defective in CF ducts predicts as observed that CF glands should reabsorb more Na than Cl . Thus, in CF glands the Na/Cl ratio tends to be lower relative to normal glands, which reabsorbed Na and Cl almost equally.

Third, it has been known for some time that HCO_3^- secretion in the pancreas in CF individuals is severely diminished (11). If pancreatic HCO_3^- secretion required an anion exchange, this observation may fit well with the present suggestion of diminished X^- secretion in the CF sweat gland. The proposed abnormal anion exchange may relate to an underlying genetic defect common to sweat glands, pancreas, and other exocrine glands affected in CF.

OTHER FINDINGS

Our conclusions are in conflict with those of Kaiser and Drack (15). These investigators reported that HCO_3^- in CF sweat is higher, not lower as found here, and concluded that a Na/H exchange in CF glands is defective. Since the highest values of X^- found in any sample in the present study was slightly more than 40 meq/liter in normal sweat, it is difficult to explain the high value of about 80 meq/liter in CF sweat reported by these workers. The HCO_3^- determinations of Kaiser and Drack (15) were calculated from the Henderson-Hasselbach equations after equilibrating sweat samples with predetermined partial pressures of CO_2 and measuring the equilibrium pH. This method assumes, (1) that the CO_2 of luminal fluid is in equilibrium with CO_2 in the extracellular fluid, which may not be valid (29) and (2) that samples being measured are completely equilibrated at a given PCO_2 . If either of these conditions fails, an exponential error in calculated HCO_3^- concentration results.

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30. Alternatively, we might imagine that for some reason CF glands elaborate a HCO_3^- depleted precursor fluid. But this possibility seems unlikely because (1) at very high sweat rates X^- in CF sweat approaches normal concentrations (Fig. 4) and (2) maximal secretory rates are approximately the same for CF and normal glands; if the CF secretory coil secreted fluid deficient in HCO_3^- , we might expect the CF sweat rate to be lower than normal.
A reduced X^- might also be observed if CF sweat contains a relatively higher concentration of a cation such as NH_4^+ , which is also "invisible" by x-ray analysis as applied here. However, a decrease in X^- as a function of increased NH_4^+ , for example, would also support a defective Cl/X^- exchange. In the presence of such a defect, luminal H should rise, thereby favoring ionic tapping of a diffusible uncharged species (NH_3) as an impermeable ion (NH_4^+) in the duct lumen. The significance of this possibility requires further investigation.
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