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erythrocytes sodium transport

Abnormal Erythrocyte Sodium Transport in Cystic Fibrosis of the Pancreas

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Extract

Sodium content and various components of sodium outflux were measured in erythrocytes from 26 normal subjects, 25 patients with cystic fibrosis of the pancreas (CFP), and 20 parents of patients with CFP.

Ouabain-insensitive, ethacrynic acid-sensitive fractional sodium outflux was diminished in erythrocytes from males with CFP (0.027 *versus* 0.051 for normal males) and from postpubertal females with CFP (0.012 *versus* 0.023 for normal adult females). This same component was normal in erythrocytes from prepubertal females with CFP (0.052 *versus* 0.046 for normal prepubertal females). Fractional sodium outflux in erythrocytes from obligate heterozygotes of either sex did not differ significantly from that of their normal counterparts.

Speculation

None of the components of sodium outflux reported here can be used as a genetic marker for CFP. The abnormalities of sodium outflux in patients with CFP are not related directly to the basic defect but instead represent a secondary feature of the disease.

Introduction

Elevated electrolyte concentrations in exocrine secretions from patients with cystic fibrosis of the pancreas (CFP) have been recognized for many years [8]. Recently, abnormalities have been demonstrated in non-exocrine tissue from patients with CFP. Unusual staining characteristics have been observed in cultured skin fibroblasts from patients with CFP and their relatives [4], and abnormal erythrocyte sodium transport has been reported in patients with CFP and obligate heterozygotes [1]. If the postulated defect of sodium transport in the sweat and salivary ducts in CFP [9] represents the fundamental abnormality in this disease state, further study of the previously reported abnormality of erythrocyte sodium transport may help clarify the pathogenetic defect in CFP. To this end, we have measured various components of sodium outflux and sodium content in erythrocytes from normal controls, obligate heterozygotes for CFP, and patients with CFP.

Methods

Subjects

Controls were 21 normal adults (10 males, 11 females) 18–30 years of age and 5 normal premenarche females, 10–11 years of age. Heterozygotes were 20 parents of patients with CFP (10 males, 10 females), 27–60 years of age. Control subjects and heterozygotes were taking no medications. Patients were 25 children and young adults with CFP (11 males, 14 females), 5–27

years of age. Diagnosis was based upon typical history and abnormal sweat electrolytes using the pilocarpine iontophoresis technique [12]. All patients were receiving vitamins and pancreatic enzyme supplements, and 18 were receiving antibiotics. No patients were acutely ill when studied. Informed consent in accordance with the provisions set forth in the Declaration of Helsinki was obtained from all subjects.

Procedure

Fresh heparinized venous blood was centrifuged at 3000 × *g* for 5 min, plasma and buffy coat were aspirated, and erythrocytes were washed three times with 150 mM NaCl by alternate resuspension and centrifugation to remove all traces of plasma. The cells were preincubated at 37° for 3 hr in a low potassium Ringer's solution (K = 1.0 mmole/liter) containing ^{24}Na (4 mCi/mg Na) [26]. At the end of preincubation, the cells were washed four times with chilled 150 mM LiCl. Erythrocytes were then added to prewarmed 37° incubation solutions at an hematocrit less than 4%.

At 0, 20, 40, and 60 min, samples were poured into chilled (4°) tubes and centrifuged for 3 min at 3000 × *g*, and a sample of the supernatant was taken for determination of radioactivity. At some time during the incubation, a sample of cells plus supernatant was also taken for determination of radioactivity.

Radioactivity was measured with a liquid scintillation counter [27] or a crystal scintillation counter [28]. Bray's solution [2] or a solution composed of 15 parts toluene, 5 parts Triton X-100 [29] and 1 part Liquifluor [30] were used for liquid scintillation counting. Counts were over 10,000 cpm whenever possible and were corrected for decay.

The standard incubation solution contained 146 mM NaCl, 4 mM KCl, 1 mM K_2HPO_4, 25 mM Tris buffer (pH 7.4), and 11 mM glucose. All solutions were adjusted to pH 7.4. When the concentration of potassium or sodium was altered, it was replaced with an equivalent amount of LiCl. Ouabain and ethacrynic acid [31] were added at concentrations of 0.1 mM and 1 mM, respectively.

Erythrocyte sodium content was determined after the method of Sachs and Welt [17]. At the end of the preincubation, hemoglobin and hematocrit were determined on a cell suspension. Cells were washed four times with 150 mM LiCl, lysed, and diluted to 100 ml. Sodium and hemoglobin were determined on the hemolysate. Hemoglobin was determined by the cyanmethemoglobin method [5]. Hematocrit was measured after centrifugation for 3 min on a microhematocrit centrifuge [32]. Sodium was measured on a flame photometer [33].

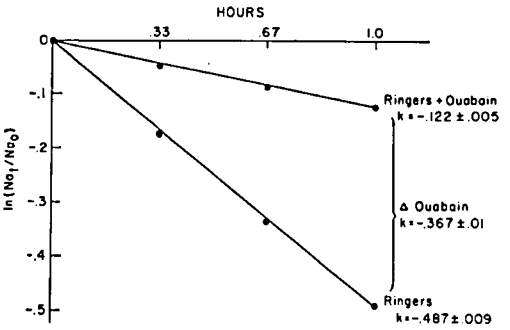

Fig. 1. Data from a representative outflux experiment, in which erythrocytes from a normal adult male were incubated with and without ouabain. The "least squares" slope of each line, derived by plotting $\ln(Na_t/Na_0)$ as a function of time, represents the fractional outflux (k) in that particular medium. The difference between the two slopes is the change in fractional outflux brought about by adding ouabain (0.1 mM) to the medium.

Calculations

Fractional sodium outflux (k) was calculated from the following equation:

$$\ln(Na_t/Na_0) = -kt$$

t = time

Na_t and Na_0 = radioactivity contained in a given volume of cells at time t and zero, respectively.

k is the "least squares" slope of the line derived by plotting $\ln(Na_t/Na_0)$ as a function of t (Fig. 1).

This calculation depends on the assumption that erythrocytes and their incubation medium can be treated as a two-compartment system and that the change in erythrocyte sodium content is the algebraic sum of two first order reactions [11]. Since the cells were incubated at an hematocrit of less than 5%, the sodium content of the medium was approximately 250 times greater than that of the cells. Therefore, during the 1-hr incubation period, the amount of ^{24}Na which moves from medium to cells can be neglected.

Results

In this discussion, the following abbreviations will be used: O^+, ouabain-sensitive; O^-, ouabain-insensitive; O^-/E^+, ouabain-insensitive, ethacrynic acid-sensitive; O^-/E^-, ouabain-insensitive, ethacrynic acid-insensitive.

Table I summarizes the mean erythrocyte sodium content in patients with CFP, heterozygotes, and nor-

mal adults of both sexes. There were no significant differences in sodium content among any of these six groups. These values are greater than those reported by others using similar techniques [1]. This discrepancy is attributable to our use of a low potassium preincubation medium which increases sodium content [14].

Figure 1 illustrates the data from a representative outflux experiment, in which erythrocytes from a normal adult male were incubated with and without ouabain. The slope of each line represents the fractional sodium outflux in that particular medium, and the change in fractional outflux brought about by the addition of ouabain to the medium (Δ ouabain) is the difference between these two slopes.

The values for the various components of fractional sodium outflux are summarized in Table II. The O^-/E^+ and O^-/E^- components were significantly lower in erythrocytes from normal and heterozygote females compared with those from males from the same group. These two components were not significantly different when females with CFP were compared with males with CFP. Fractional sodium outflux in heterozygotes of either sex did not differ significantly from that of their normal counterparts. There was a significant reduction in both of the ouabain-insensitive components in males with CFP. In females with CFP none of the three components differed significantly from that of their normal adult counterparts.

In Table III, females with CFP have been divided into two groups, 10–15 and 16–27 years of age. None of the younger subjects had reached menarche; all of the older subjects were postmenarche. In older females with CFP, the O^-/E^+ component of fractional outflux was significantly less than that of normal adult females. This same component in younger females with CFP was significantly greater than that of their adult counterparts. In males with CFP, there was no significant difference for the O^-/E^+ component when older patients were compared with younger patients, and the mean value for each group was significantly lower than that for normal adult males.

In a separate study, five females with CFP, 5–13 years of age, were compared with five normal females, 10–11 years of age. Neither patients nor controls had reached menarche, and there were no significant differences between the two groups for any of the three fractional outflux components (Table IV). The procedure used for these studies differed from those discussed previously in that the potassium concentration in the preincubation medium was 6 mmoles/liter rather than 1. The values for the normal adult female group cannot be compared validly with those for the normal prepubescent female group since the observed differences might be due to an effect of the preincubation medium rather than age.

When the data presented in Tables II, III, and IV are calculated as outflux (mmoles/liter erythrocytes per hour) rather than fractional outflux, the significant

Table I. Erythrocyte sodium content

Subjects	Sodium, mmoles/liter erythrocytes
Normal male (10)	9.9 ± 1.8[1]
Normal female (11)	9.6 ± 1.4
Heterozygous male (10)	10.3 ± 2.3
Heterozygous female (10)	10.1 ± 1.6
Cystic male (11)	10.4 ± 1.8
Cystic female (11)	10.1 ± 2.1

[1] Data expressed as mean ± 1 SD. See text for experimental conditions.

Table II. Erythrocyte fractional sodium outflux[1]

Components[2]	Normal		Heterozygote		Cystic fibrosis	
	Male (10)[3]	Female (10)	Male (10)	Female (10)	Male (11)	Female (11)
O^+	0.374 ± 0.057[4]	0.365 ± 0.064	0.363 ± 0.086	0.351 ± 0.060	0.368 ± 0.068	0.340 ± 0.054
O^-/E^+	0.051 ± 0.023	0.023 ± 0.014[5]	0.052 ± 0.025	0.033 ± 0.012[5]	0.027 ± 0.017[6]	0.028 ± 0.023
O^-/E^-	0.078 ± 0.014	0.054 ± 0.012[5]	0.070 ± 0.017	0.055 ± 0.012[5]	0.054 ± 0.009[6]	0.052 ± 0.006
Total	0.503 ± 0.058	0.442 ± 0.074	0.485 ± 0.104	0.439 ± 0.068	0.450 ± 0.081	0.420 ± 0.056

[1] Fractional outflux per hour.
[2] O^+: ouabain-sensitive; O^-/E^+: ouabain-insensitive, ethacrynic acid-sensitive; O^-/E^-: ouabain-insensitive, ethacrynic acid-insensitive.
[3] Numbers in parentheses indicate numbers of subjects.
[4] Data expressed as mean ± 1 SD.
[5] Significantly less than male counterparts, $P < 0.02$.
[6] Significantly less than normal sex-matched group, $P < 0.01$.

Table III. Fractional sodium outflux in females[1]

Components[2]	Adult normal (11)[3]	CFP: after menarche (7)	CFP: before menarche (4)
O^+	0.365 ± 0.064[4]	0.348 ± 0.051	0.325 ± 0.062
O^-/E^+	0.023 ± 0.014	0.012 ± 0.005[5]	0.057 ± 0.003[6]
O^-/E^-	0.054 ± 0.012	0.050 ± 0.006	0.054 ± 0.003
Total	0.442 ± 0.074	0.411 ± 0.053	0.436 ± 0.006

[1] Fractional outflux per hour.
[2] O^+: ouabain-sensitive; O^-/E^+: ouabain-insensitive, ethacrynic acid-sensitive; O^-/E^-: ouabain-insensitive, ethacrynic acid-insensitive.
[3] Number in parentheses indicates number of subjects.
[4] Data expressed as mean ± 1 SD.
[5] Significantly less than normal, $P < 0.01$.
[6] Significantly greater than normal, $P < 0.001$.

Table IV. Fractional sodium outflux in females before menarche[1]

Components[2]	Cystic fibrosis (5)[3]	Normal (5)
O^+	0.316 ± 0.073[4]	0.315 ± 0.025
O^-/E^+	0.052 ± 0.013	0.046 ± 0.011
O^-/E^-	0.061 ± 0.009	0.060 ± 0.013
Total	0.429 ± 0.083	0.422 ± 0.041

[1] Fractional outflux per hour.
[2] O^+: ouabain-sensitive; O^-/E^+: ouabain-insensitive, ethacrynic acid-sensitive; O^-/E^-: ouabain-insensitive, ethacrynic acid-insensitive.
[3] Number in parentheses indicates number of subjects.
[4] Data expressed as mean ± 1 SD.

differences for the O^-/E^+ and O^-/E^- components do not change.

Discussion

The ouabain-insensitive components of sodium outflux are poorly characterized and represent a small fraction of the total outflux. Figure 2 illustrates the relative magnitude of the effects of ouabain and ethacrynic acid on erythrocyte sodium outflux. Ouabain inhibits approximately 75% of total outflux. Of this 75%, one-half depends on the presence of potassium in the external medium. The potassium-dependent, ouabain-sensitive component is thought to represent an energy-dependent, sodium-for-potassium exchange mechanism [13], while the potassium-independent, ouabain-sensitive component is thought to represent a sodium-for-sodium exchange mechanism [11].

Ethacrynic acid inhibits one-half of total sodium outflux. Of this one-half, 80% is also inhibited by ouabain (O^+/E^+); however, in the presence of ouabain, ethacrynic acid has an additional effect (O^-/E^+) which represents about 10% of total outflux. There is some question whether this component is a sodium "pump" [15], or sodium-for-sodium exchange [16].

After the addition of both ouabain and ethacrynic acid, 15% of total outflux (O^-/E^-) remains, and this residual outward movement of sodium is poorly characterized.

Our results disagree with those of Balfe *et al.* [1], who described significantly decreased O^+, O^-/E^+, and O^-/E^- outflux in patients with CFP and decreased O^-/E^+ outflux in heterozygotes.

There are several possible explanations for the discrepancies between the two studies. Variation about the mean value for a particular group of subjects comprises both inter- and intraindividual variation. Determinations of fractional sodium outflux in the same individual on multiple occasions gave good reproducibility (range = 0.46–0.51/hr). Fractional outflux among different adult controls, however, had a range of 0.39–0.58 in males, and 0.33–0.58 in females. Balfe *et al.* [1] matched each patient with a control and expressed the value for the patient as a fraction of the paired control value. This maneuver, which assumes that the major source of variation is intraindividual rather than interindividual, might produce apparently significant differences when none would be detected by comparing patients as a group with controls as a group.

The differences in erythrocyte sodium outflux between males and females could also account for the discrepancy. For example, a preponderance of females among heterozygotes and of males among controls might produce decreased values for O^-/E^+ outflux related to sex rather than to CFP.

In subsequent studies, Welt [22] has found no significant difference for the O^-/E^+ component when normal adult females were compared with normal adult

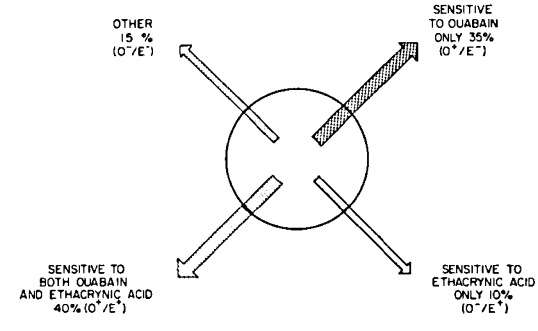

Fig. 2. The relative magnitude of the decrease in erythrocyte sodium outflux produced by adding ouabain (0.1 mM) or ethacrynic acid (1.0 mM) to the external medium.

males, and in a preliminary comparison of heterozygote females as a group with normal females as a group there was a lower value for the O^-/E^+ component in heterozygote females.

A methodological difference which might account for the difference between our data and those of Balfe *et al.* [1] is that they used glycylglycine-$MgCO_3$ buffer while the incubation solutions used for the present studies contained Tris buffer. Others [16, 23] have demonstrated differences in erythrocyte sodium outflux depending on whether or not magnesium was present in the incubation medium.

It is possible that differences in levels of progesterone or of other sex-related hormones could explain the transport differences between erythrocytes from males and those from females. DeVenuto *et al.* [6] showed that stored erythrocytes from males had less osmotic resistance and greater spontaneous hemolysis than did erythrocytes from females, and that these differences could be minimized by the addition of progesterone to blood from male donors. Furthermore, during storage of male erythrocytes there were distinct changes in the chemical composition of the erythrocyte membrane, while in stored female cells there was little or no change [7]. These findings suggested that progesterone may interact with the erythrocyte membrane and prevent changes in its permeability. If future studies reveal an effect of progesterone on erythrocyte sodium transport, this variable will have to be considered when comparing various adult females. In collecting the data reported in this paper, no attempt was made to study adult female subjects during the same phase of their menstrual cycle.

We have found no abnormality of sodium transport in erythrocytes from obligate heterozygotes for CFP; therefore, none of the components which we have studied can be used as a genetic marker for this disease. Furthermore, since heterozygotes and younger females with CFP have normal erythrocyte sodium outflux, the differences that we have described are probably not related directly to the basic defect, but instead represent a secondary feature of the disease.

Welt *et al.* [25] observed an elevated erythrocyte sodium content, reduced ouabain-sensitive sodium outflux, and reduced ouabain-sensitive ATPase activity in certain patients with uremia, metastatic carcinoma, or severe third degree burns. The patients who had these abnormalities tended to be the most severely affected by their particular disease. It is unlikely that we are observing the same phenomenon ("the sick cell"), since in our patients with CFP erythrocyte sodium content and ouabain-sensitive fractional outflux were normal. Furthermore, we were unable to correlate severity of disease with the magnitude of the change in fractional sodium outflux. Several of the younger females with normal outflux had the most severe chronic pulmonary disease, while several older males and females with minimal pulmonary involvement had very low O^-/E^+ values.

It is possible that the reduced ouabain-insensitive sodium outflux in erythrocytes of patients with CFP and the high sodium concentration in sweat of patients with CFP reflect a similar abnormality. The sweat defect presumably results from faulty reabsorption of sodium in the duct [3, 10, 18], and measurements of the electrical potential difference between duct and interstitium suggest that a ouabain-insensitive mechanism is involved [19, 20].

In the human erythrocyte, ouabain-insensitive mechanisms account for 25% of sodium outflux (Fig. 2). Of the ouabain-insensitive fraction, that portion which was sensitive to ethacrynic acid is energy-dependent [15]. Unlike ouabain-sensitive sodium outflux, this component (O^-/E^+) did not directly utilize ATP as its energy source [24] and was not affected when potassium was removed from the external medium [15]. Because O^-/E^+ depends on the presence of external sodium, it has been postulated to operate by sodium-for-sodium exchange [16]. Sodium-for-sodium exchange, however, is generally considered not to depend on cellular-energy production [21]. Further definition of the metabolic requirements of the O^-/E^+ component and precise delineation of the transport mechanism for this component may provide insight into the erythrocyte and sweat gland defects in CFP, and perhaps the underlying abnormality as well.

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