Effect of Saliva from Cystic Fibrosis Patients and from Normal Subjects on Red Blood Cell Sodium Transport

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Summary

Saliva, whether taken from patients with cystic fibrosis or from normal subjects, caused an increase in red blood cell Na⁺ efflux (in the presence or absence of ouabain) of 19-29% as compared with non-saliva controls. However, there was no significant difference between the effects of cystic fibrosis saliva and normal saliva.

Speculation

Saliva, and possibly other body fluids as well, contain substances which influence Na⁺ transport across cell membranes, and which are, as yet, uncharacterized. Further investigation of such substances may be relevant to the transport abnormality of cystic fibrosis.

The raised Na⁺ concentrations of sweat and saliva in patients with cystic fibrosis (CF) are an important diagnostic feature of this disease (2), and may be closely related to its pathogenetic basis. The abnormality may be due to a humoral factor in CF secretions which inhibits Na⁺ transport, as suggested by the finding that Na⁺ reabsorption from the rat parotid duct is inhibited when CF saliva or sweat is introduced into the duct (7). A similar inhibition of Na⁺ reabsorption was found when CF secretions were introduced into sweat glands of normal humans *in vivo* (5).

The effects of CF secretions on Na⁺ transport have also been investigated using red blood cell systems. Taussig *et al.* (9) found no difference in the Na⁺ efflux or influx of normal red cells exposed to CF saliva as compared with normal saliva, although comparison with non-saliva control medium was not included. Duffy *et al.* (3), however, found that saliva, whether from normal subjects or CF patients, accelerated Na⁺ influx in normal red cells, but found no difference between CF patients and control subjects.

It is necessary to consider, however, that Na⁺ transport across the red cell membrane is mediated by several distinct processes, which include passive permeation and possibly more than one type of active transport (4). It is not known which, if any, of these processes will be affected by a humoral factor in CF saliva. Accordingly, it is not immediately obvious which parameter of Na⁺ transport should be measured in looking for its effect on the red cell.

For guidance as to which is an appropriate parameter to test, it is reasonable to refer to the Na⁺ transport abnormalities which have been found in the red cells of CF patients themselves. Balfe *et al.* (1) found that ouabain-sensitive and ouabain-insensitive Na⁺ efflux rates were reduced in CF red cells. Lapey *et al.* (6) found a reduction in ouabain-insensitive Na⁺ efflux in red cells from males and postpubertal females with CF, but did not find abnormal ouabain-sensitive efflux.

These results suggest that ouabain-insensitive Na⁺ efflux may reflect the influence of a CF factor more closely than either the total Na⁺ efflux or the Na⁺ influx, measured in previous studies. We have given particular attention to the ouabain-insensitive efflux, after a preliminary study of CF red cells to confirm that this parameter is an appropriate choice.

METHODS

Normal subjects were Caucasians of either sex, aged 20-34 years, with no personal or family history of CF, who were not taking any medication. The CF group was comprised of Caucasian patients of either sex, aged 16-32 years, with clinical and radiologic findings and sweat tests typical of CF; all had received treatment, including pancreatic supplements and antibiotics. The informed consent of all subjects was obtained.

MEASUREMENT OF NA⁺ EFFLUX

Fresh heparinized venous blood was centrifuged at 3000 × g for 5 min, plasma and buffy coat were aspirated, and the red cells were washed three times with isotonic NaCl. The red cells were preincubated for 3 hr at 37° in a solution containing NaCl 120 mM, KCl 10 mM, dextrose 28 mM, and Tris-Cl buffer 20 mM (pH 7.4 at 37°), to which 25 μCi ²²Na⁺ had been added. After preincubation, the cells were washed six times with isotonic NaCl and added to incubation medium prewarmed to 37° at a hematocrit of approximately 2%. The incubation medium contained NaCl 120 mM, KCl 10 mM, dextrose 11.1 mM, Tris-Cl buffer 20 mM (pH 7.4 at 37°), and ouabain, where indicated, 0.1 mM.

At 0 and 60 min, samples were centrifuged for 3 min at 3000 × g and a sample of supernatant fluid was weighed and taken for determination of radioactivity and of optical density at 420 nm. At some time during the incubation, a weighed sample of cell suspension was taken for determination of radioactivity and of optical density at 420 nm after hemolysis by dilution in distilled water. A Panax 160 crystal scintillation counter was used for measurement of radioactivity, and a Unicam SP600 spectrophotometer for optical density determination.

Efflux experiments were carried out on CF red cells and control red cells simultaneously, and measurements were made in duplicate. Sodium efflux was expressed as a rate constant, k, using the equation (8):

$$kt = -\ln \frac{1 - (Na_s^*/Na_h^*)}{1 - \text{fractional hemolysis}}$$

where Na_s^{*} = the activity of the supernatant in counts ml⁻¹ min⁻¹, Na_h^{*} = the activity of the hemolyzed sample, and t is the incubation time. This formulation is accurate at low hematocrit (<0.3%). However, for the present experimental conditions, the error incurred at 2% hematocrit is small (2-3%).

MEASUREMENT OF Na^+ INFLUX

After washing as described above, the red cells were suspended in incubation medium as used for efflux experiments, except that KCl, 7.5 mM, instead of ouabain, was used, at a hematocrit of about 10%. Then 2.5 μCi $^{22}\text{Na}^+$ were added and the suspension was incubated at 37° for 1 hr. At the end of the incubation period, the suspension was centrifuged and 50 μl supernatant fluid were taken for counting. The cells were washed three times with isotonic MgCl_2 to remove external $^{22}\text{Na}^+$ and then resuspended at a hematocrit of about 30%. The hematocrit was measured in triplicate. Next 1.0 ml of the red cell suspension was transferred to counting tubes. The influx rate was expressed as the proportion of supernatant counts which entered the cells per hour.

PREPARATION OF SALIVA

Mixed mouth saliva was collected in polythene containers and used within 6 hr. The saliva was centrifuged to remove particulate material and the supernatant was adjusted to isotonicity either by dialysis or by adding NaCl. KCl, dextrose, and Tris-Cl buffer (pH 7.4) were then added as isotonic solutions to give concentrations of 10 mM, 11.1 mM, and 20 mM, respectively. Ouabain was added where indicated at 0.1 mM.

Control media (without saliva) were prepared from aliquots of isotonic NaCl by similar additions. The samples were then used as incubation media for measurement of Na^+ efflux for normal red cells.

RESULTS

CF red cells were found to show a significant decrease in the ouabain-insensitive fraction of Na^+ efflux (Table 1). Total Na^+ efflux and ouabain-sensitive Na^+ efflux were not significantly different from normal. Influx of Na^+ appeared to be normal.

Table 2 shows that saliva, whether from normal subjects or from CF patients, produced an increase of Na^+ transport in red cells, as compared with the non-saliva control medium. Both ouabain-insensitive and total Na^+ efflux were affected; there was, however, no significant difference between the effects of normal saliva and of CF saliva. When the red cells were washed after brief exposure to saliva (normal or CF), ouabain-insensitive efflux was normal, indicating that the salivary constituent

responsible for the transport effect was not firmly bound to the cell.

DISCUSSION

The results shown in Table 1 confirm previous reports that ouabain-insensitive Na^+ efflux is reduced in CF red cells. No significant difference was found between males and females in this study. Since all patients in this series were postpubertal, the observation (6) that prepubertal females fail to show the transport abnormality was not tested. Although the observed transport abnormality may be due to the disease process itself, the possibility that it may merely reflect therapy (e.g., drug intake) has not been excluded.

In view of the normal Na^+ content of CF red cells (1, 6), it has been pointed out that reduced efflux should be balanced by a reduced Na^+ influx (1). The expected reduction in influx, however, is small in relation to overall influx, and would be relatively difficult to demonstrate in the presence of random variation. Presumably for the same reason, the total Na^+ efflux is not significantly abnormal, even though a small component of efflux (the ouabain-insensitive fraction) is consistently reduced. There is, however, no satisfactory explanation for the inconsistencies between various studies regarding ouabain-sensitive efflux.

From these results, the ouabain-insensitive fraction of Na^+ efflux appears to be more satisfactory than other parameters as a marker of CF abnormality in red cells. Accordingly, this parameter was measured in normal red cells exposed to CF saliva and to normal saliva. The effect on total Na^+ efflux was also measured for comparison with previous reports.

Table 2 shows that saliva (normal or CF) alters Na^+ transport rate. This nonspecific effect of saliva appears to be similar to the nonspecific increase found for Na^+ influx by Duffy *et al.* (3). It is due to a nondialyzable component of saliva, the nature of which is obscure. The effect could not be reproduced by addition of bovine serum albumen (5%, two experiments), human immunoglobulin (2.5%, two experiments), or bradykinin (15 mg/liter, two experiments) to control media.

It is unlikely that contamination from the dialysis membrane could be responsible, since four samples of saliva which were prepared without dialysis in this series gave similar results to those which were dialyzed; and because control experiments in which incubation medium was exposed to dialysis differed by only 5% from the effect of incubation medium which had not been dialyzed. Furthermore, the suggestion that the transport effect is intrinsic to saliva is apparently strengthened by the discovery of a similar effect (21–26% increase) when normal red cells were tested with autologous blood serum in the same way (two experiments).

It remained possible that the "nonspecific" effect of saliva on Na^+ transport, with its relatively large size and wide random variation, might be obscuring a "specific" effect of CF saliva. A simple attempt to separate these possibilities was made. It was evident that the transport-accelerating effect of saliva on red cells was easily removed by washing. The behavior of the CF factor, however, as described (5, 7), implies that it is firmly

Table 1. Na^+ transport of normal and CF red cells¹

	Normal	CF
Ouabain-insensitive efflux	0.084 ± 0.014 (10)	0.055 ± 0.022 ² (10)
Ouabain-sensitive efflux	0.245 ± 0.073 (10)	0.247 ± 0.057 (10)
Total efflux	0.329 ± 0.068 (10)	0.302 ± 0.053 (10)
Influx	0.0108 ± 0.0012 (12)	0.0101 ± 0.0014 (12)

¹ Data are expressed as rate constants (per hour), means ± SD, with number of determinations shown in parentheses.

² Significantly different from normal group ($P < 0.01$).

Table 2. Na^+ efflux of normal red cells in normal saliva and in CF saliva¹

	Normal saliva			CF saliva		
	Mean ± SD	(n)	P	Mean ± SD	(n)	P
Ouabain-insensitive Na^+ efflux	1.19 ± 0.20	(12)	<0.01	1.29 ± 0.27	(7)	<0.05
Total Na^+ efflux	1.20 ± 0.08	(10)	<0.01	1.28 ± 0.21	(7)	<0.02
Ouabain-insensitive Na^+ efflux (after washing)	1.02 ± 0.10	(8)		1.02 ± 0.07	(6)	

¹ Data are expressed as the ratio efflux in saliva/efflux in non-saliva control. The salivary sample and non-saliva control were matched for inorganic constituents and glucose (see "Methods") and red cells from the same blood sample were used in both media simultaneously. The saliva groups for which P values are quoted were significantly different from non-saliva controls. There was no significant difference between the effects of CF saliva groups and normal saliva groups.