

Abnormal Parachloromercuriphenylsulfonate-sensitive Cation Channel in the Erythrocytes of Hereditary Spherocytosis

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The erythrocytes of hereditary spherocytosis (HS) demonstrate an increased inward movement of sodium ions, an alteration which has been proposed as the primary defect leading to cell destruction. Parachloromercuriphenylsulfonate (PCMBS), an agent reacting with sulfhydryl groups of the membrane, increases the cation permeability of normal red cells, but does so to a much lesser extent in

the HS red cells. On the other hand, pronase that is specific for amino groups of the membrane increases cation permeability and decreases anion permeability equally in normal and HS red cells. It may be postulated that a decreased number of sulfhydryl sites or a mutation of proteins in the PCMBS-sensitive cation channels of the HS cell membrane may result in this hyposensitivity to PCMBS.

ALTHOUGH HEREDITARY SPHEROCYTOSIS (HS) is a relatively common hemolytic disorder, the molecular mechanism has not yet been elucidated. Increased passive permeability of HS red cells to sodium seems to be an accepted feature of a membrane abnormality.¹ Harris and Pranker² first observed sodium influx in the hereditary spherocytes from one patient, a phenomenon that was studied in detail by Bertles.³

The passive permeability of the red cell membrane to cations is lower than that to anions. The presence of "fixed charges" such as amino groups in the membrane contributes to the selectivity of ions. This hypothesis is reinforced by the finding that chemical and enzymatic modifiers such as 1-fluoro-2,4-dinitrobenzene (DNFB), pronase, and sulfhydryl reagents affect cation and anion movement. Though DNFB binds to a relatively wide variety of groups, its effect on permeability is considered to be largely via amino groups.⁴ This reagent penetrates relatively rapidly into the membrane. Pronase, which does not penetrate the ion barrier of the red blood cells, increases cation flux and decreases anion flux. The effects of the enzyme are qualitatively similar to those of DNFB, and the alteration of the density of charged protein amino groups is thought to be involved in its effect on passive ion permeability.⁵ Both parachloromercuriphenylsulfonate (PCMBS) and parachloromercuribenzoate (PCMB), which are specific for sulfhydryl groups, increase cation permeability, but the former is much more effective even though its rate of uptake is lower.⁶ In the present study, we have compared the effects of PCMBS and amino group-specific pronase on the passive cation permeability of normal and HS red cells.

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MATERIALS AND METHODS

Six cases of hereditary spherocytosis from six different families showing the characteristic hematologic features were studied. Two patients had been splenectomized. Hemoglobin electrophoresis revealed normal patterns. To eliminate the possibility of a specific enzyme deficiency in these patients, all enzymes of the glycolytic and shunt pathway were measured, and their activities were within normal limits. Control subjects were normal adult males of comparable age.

Venous blood was drawn into heparin, and the cells were washed three times. A medium composed of 150 mM NaCl, 4.8 mM Tris, pH 7.4, was used both for washing and incubation of red cell suspensions, while 20 mM of glucose and 0.14 mM of ouabain were routinely included in the final suspension. Ouabain was used to determine net passive cation flux.

Sodium influx was measured by the uptake of radioactivity into the cells incubated in the medium containing $^{22}\text{NaCl}$ essentially according to the methods of Glynn.⁷ The packed cells (500 μl) were suspended in isotonic NaCl-Tris (pH 7.4) in the presence or absence of PCMBs (0.02 mM) containing glucose 20 mM, ouabain 0.14 mM, and a trace of $^{22}\text{NaCl}$ to give a hematocrit of 5% in a total volume of 10 ml. The mixture was incubated at 37°C and 2 ml of suspension were removed every hour for 3 hr. The mixture was then centrifuged at 2000 rpm for 2 min. Potassium in the supernatant fluid was measured by flame photometry. The cells were washed three times with ice-cold isotonic NaCl-Tris, and ^{22}Na in the red cells was measured with a well-type scintillation counter. Red cells were preincubated with pronase (2 mg/ml) in isotonic NaCl-Tris at a hematocrit of 10% in a total volume of 5 ml at 37°C for 1 hr. The suspension was washed twice with ice-cold NaCl-Tris and cation permeabilities were measured as above.

RESULTS

In HS red cells, the effects of PCMBs on ^{22}Na uptake and K^+ efflux were much less pronounced than those in normal red cells (Figs. 1 and 2). The effects of pronase on cation (Figs. 1 and 2) permeability were almost identical for HS and normal red cells. ^{22}Na uptake and K^+ efflux were completely parallel in all the experiments.

DISCUSSION

In the HS red cell, the basic defect responsible for the hemolytic process is localized intracellularly, probably within the membrane, thus making the biochemical and biophysical characteristics of the erythrocyte important to the understanding of the hemolytic process. Excessive sodium influx and increased sodium turnover occur in erythrocytes of patients with HS before and after splenectomy. Although Jacob has reported that only sodium flux is increased in HS red cells,¹ the alterations in sodium uptake and potassium efflux have been parallel either in the presence or absence of PCMBs in our experiments. Considering the high specificity of PCMBs for sulfhydryl groups and the low sensitivity of passive cation flux in HS red cells to the reagent (Figs. 1 and 2), we find the observation compatible with the assumption that the concentration of sulfhydryl groups which are involved in the permeability of cations is disturbed in the HS red cells. Electrophoretic analyses of membrane proteins from HS erythrocytes did not reveal a gross abnormality.⁸ The increased activity of Na, K-ATPase reported previously is considered to be a compensatory action to maintain a normal intracellular cation composition.

Both PCMB and PCMBs increase cation permeability, but the former is much less effective, even though its rate of uptake is much higher.⁶ PCMBs, with its sulfonic acid group, is completely ionized and would not be expected

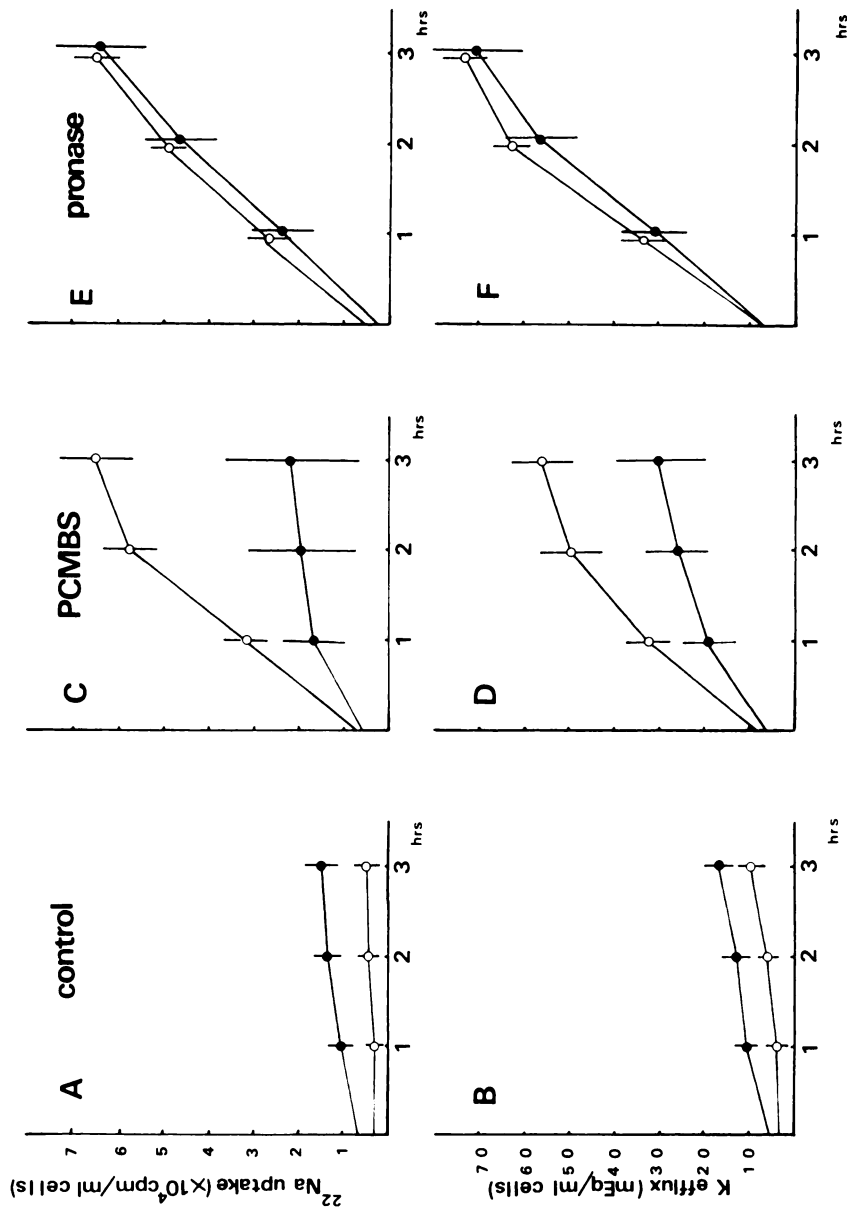


Fig. 1. Effects of PCMBs and pronase on cation permeabilities of normal (○—○) and hereditary spherocytic red cells (●—●). (A,B) Sodium uptake and potassium efflux without the modifier. (C,D) PCMBs (0.02 mM) added to each suspension. (E,F) Preincubation with pronase (2 mg/ml) for 1 hr.

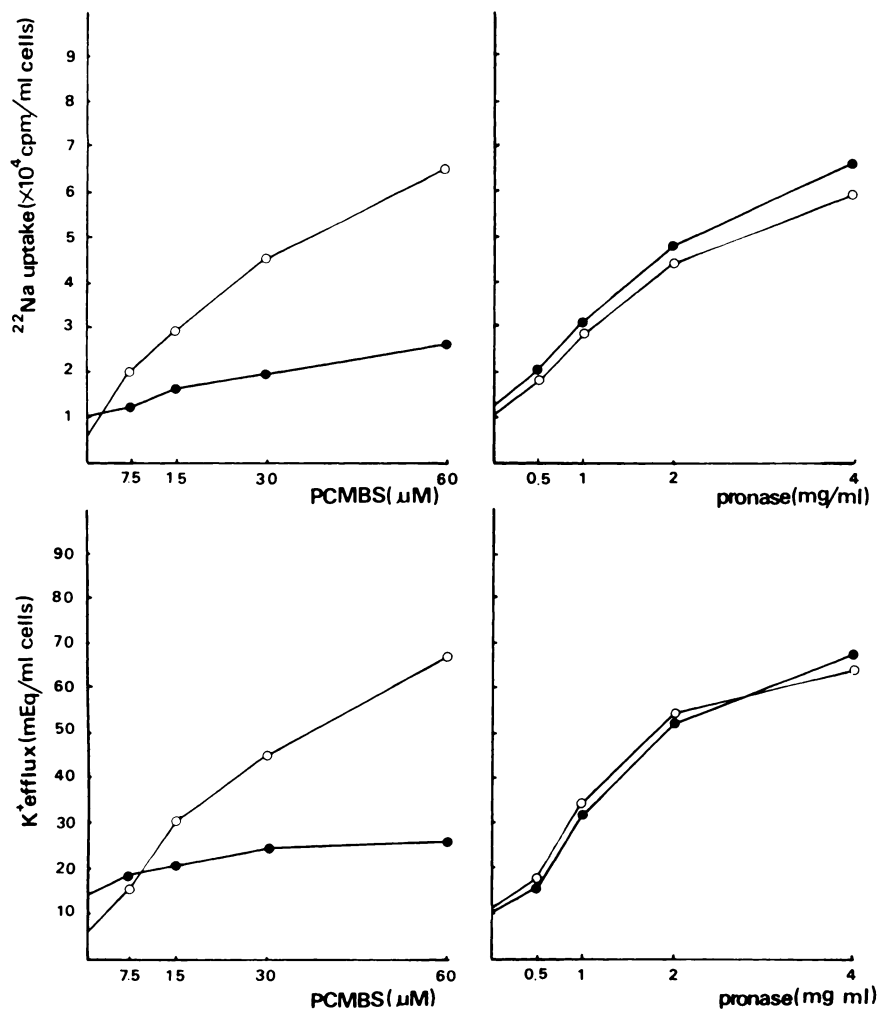


Fig. 2. Effects of varying concentrations of PCMBS and pronase on cation permeabilities. Incubation for 2 hr. Normal (○—○); a case of hereditary spherocytosis (●—●).

to partition into the lipid of the membrane.⁶ The PCMBS-sensitive cation channels are presumably protein in character and are known to comprise approximately one-fifth of the total sulfhydryl sites present in the membrane.⁹

In HS red cells, the number of PCMBS-sensitive sulfhydryl sites may be decreased or a mutation in the protein of the PCMBS-sensitive channels may result in hyposensitivity to PCMBS. The defect would render the cell hyperpermeable to cations. The nature of the hyposensitivity of HS erythrocytes to PCMBS is a subject for future study.

Differences in the behavior of the agents acting on membrane permeability can be attributed in part to the differences in chemical specificity, and in part to the topographic specificity. A first population of amino groups controlling anion permeability is superficially located and is accessible to nonpenetrating

reagents such as 4-acetoamide-4'-isothiocyanate-stilbene-2,2'-disulfonic acid (SITS),⁹ or 4,4'-diisothiocyano-2,2'-stilbenedisulfonate (DIDS).¹⁰ A second population of amino groups controlling cation permeability is more internal, accessible only to penetrating agents such as DNFB. Pronase causes an increase in cation permeability and a decrease in anion permeability.⁵ The interaction of the agent with charged amino groups (first and second populations) leads to a concomitant decrease in the number of positively charged amino groups in the membrane, and their decrease reduces the electrical charge barrier to cation movement, thus allowing more rapid movement down the electrochemical gradient. It also decreases the passive anion movement because its magnitude is, for reasons of electronegativity, proportional to the number of positively charged groups in the membrane. It should be noted that no significant difference was found in the pronase-sensitive cation channels between normal and HS red cells. It may be concluded from the present results that the sulfhydryl-related cation permeation channels appear to be topographically different from amino group-regulated passive permeation routes.

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