This work was aided by grant from National Institutes of Health (E-1459). One of us (H.O.) was a postdoctoral trainee on National Institutes of Health Training Grant (2G-686).

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Received December 28th, 1962

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PN 1219

Apparent uncoupling of the Na^+ and K^+ activation of the human erythrocyte membrane adenosine triphosphatase

Human erythrocyte membrane contains a Mg^{2+}-dependent ATPase activity which can be stimulated by the presence of Na^+ and K^+ (refs. 1, 2). Many properties of this enzymic activity and those of the active transport of cations in intact erythrocytes and "reconstituted ghosts" have been correlated[1–5]. It has been established, for example, that cardiac glycosides at the same concentrations which inhibit the pump flux of cations can also inhibit the increase in ATPase activity due to the presence of Na^+ and K^+. Thus there is sufficient evidence indicating that this enzyme is intimately involved in the process of active transport of Na^+ and K^+ across the erythrocyte membrane.

A common feature of the enzyme preparations reported previously, on which some of the proposed models for active transport have been based[3], is the need for the simultaneous presence of Na^+ and K^+ for the increase in the activity of the enzyme. The ATPase of the ghosts and fragmented ghosts, prepared by POST *et al.*[1], was not activated by either Na^+ or K^+ alone, while several ghost preparations reported by DUNHAM AND GLYNN[2] were slightly activated by K^+ but not at all by Na^+. This communication presents preliminary data on the preparation and properties of an ATPase associated with erythrocyte-membrane fragments which can be activated by either Na^+ or K^+ alone.

Ghosts were prepared from washed erythrocytes by hemolysis in water and repeated washing in dilute Tris buffer according to POST *et al.*[1]. The properties of these ghosts were similar to those of the preparations reported by DUNHAM AND GLYNN[2]. Slight activation of the ATPase was observed with K^+. Sodium alone did not stimulate the activity. A suspension of ghosts in $5 \cdot 10^{-4}$ M Tris buffer (pH 7.4) was treated with ultrasonic waves generated by a Branson Model S-75 Sonifier for

15 sec. The mixture was centrifuged at 18 000 × *g* for 1 h. The sediment was thoroughly washed with dilute Tris buffer. Examination of a suspension of this sediment under a phase microscope revealed that the ghosts had been broken into small fragments. The effect of various concentrations of Na^+ and K^+ alone on the ATPase activity of this preparation are shown in Fig. 1.

Although the degree of activation obtained by either ion varied in different preparations, the following generalizations can be made: (1) At equal concentrations K^+ always activates more than Na^+. (2) The lowest concentration at which either ion produces its maximal effect is approximately 50 mM. (3) Half-maximal activation by K^+ is obtained at a concentration of 3 mM and by Na^+ at 7 mM. (4) As the concentration of either ion is increased over 100 mM, a progressive decrease from maximal activation is obtained.

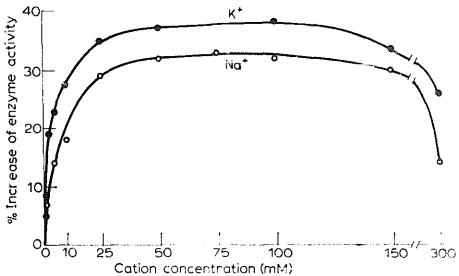

Fig. 1. Effect of varying Na^+ and K^+ concentration on the ATPase activity of a preparation of human erythrocyte membranes treated with ultrasonic waves. Experimental procedure was the same as described for Table I. Activity of the enzyme in the absence of Na^+ and K^+ was 0.88 μmole Pi/h/ml of enzyme.

The effect of the simultaneous presence of Na^+ and K^+ on the ATPase activity is complex and will be dealt with in detail in later publications. Briefly, depending on the ratio of the concentrations of the two ions, either an increase or a decrease in the activity due to a single ion can be obtained. Table I shows examples of situations from two typical preparations where the presence of the two ions increases the ATPase activity beyond the maximum increase which could have been obtained by either ion alone.

The effect of ouabain on the ATPase activity under various conditions is also included in Table I. Examination of the data reveals the following points about the action of ouabain: (1) At the concentrations used ouabain has no effect on the ATPase activity in the absence of Na^+ and K^+. (2) The activation produced by either Na^+ or K^+ alone is not affected by ouabain. (3) When both Na^+ and K^+ are present in the system, ouabain decreases the ATPase activity to the level which would have obtained if only that ion were present which would, at the concentration used, have given the greater activation.

The data presented here suggest the possibility that although a membrane ATPase may be involved in the pump mechanism for the influx of K^+ and the efflux

of Na^+, the linkage of these two processes may not be a property of the ATPase *per se*. It is also suggested that the inhibitory effect of the low concentrations of cardiac glycosides on the active transport of monovalent cations may be due to their action on the coupling mechanism.

TABLE I

EFFECT OF Na^+, K^+, AND OUABAIN ON ATPASE ACTIVITY OF ERYTHROCYTE MEMBRANES TREATED WITH ULTRASONIC WAVES

The system contained 5 μmoles of Tris–ATP, 5 μmoles of Mg^{2+}, 100 μmoles of Tris–HCl (pH 7.4), 1 ml of enzyme suspension, and the indicated amounts of NaCl, KCl, and ouabain. Final volume was 2.5 ml. Enzyme activity per unit dry weight was quite variable in different preparations. Suspensions were therefore made up to obtain the same range of activity per unit volume. The reaction mixture without the added Na^+ and K^+ contained less than 0.1 mM of either cation, as ascertained by flame photometry. The mixture was incubated at 37° for 1 h, then deproteinized by the addition of 1.5 ml of 8% $HClO_4$. Phosphate was determined according to FISKE AND SUBBAROW[6].

Preparation	*mM* Na^+	*mM* K^+	10^{-3} *M Ouabain*	*ATPase activity* μ*moles Pi/h/ml enzyme*
1	0	0	–	1.26
	0	0	+	1.26
	75	0	–	1.60
	75	0	+	1.60
	0	25	–	1.71
	0	25	+	1.72
	75	25	–	2.18
	75	25	+	1.71
2	0	0	–	1.01
	25	0	–	1.32
	0	0.5	–	1.05
	25	0.5	–	1.57
	25	0.5	+	1.32

This study as well as the postdoctoral fellowship of one of us (A.A.), and the predoctoral fellowship of the other (J.C.F.) was supported by U.S. Public Health Service Research Training Grant (2G-99).

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Received October 6th, 1962