INHIBITION OF HUMAN RED CELL NaK-ATPase BY MAGNESIUM AND POTASSIUM

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SUMMARY: The inhibition of NaK-ATPase (EC 3.6.1.3) from human red cells by Mg^{2+} is markedly dependent on the relative concentrations of Na^+ and K^+. Inhibition increases with increasing K^+ and decreases with increasing Na^+. The inhibition appears to be a combined effect of Mg^{2+} and K^+ at sites distinct from the sites at which these cations activate the enzyme. The kinetics of activation of the enzyme by Na^+ with inhibitory levels of Mg^{2+} and K^+ are biphasic, indicating both low and high affinity Na^+ sites. At noninhibitory levels of Mg^{2+} and K^+ only high affinity Na^+ sites are seen. The results are inconsistent with any model in which Mg^{2+} and K^+ compete with Na^+ at a single site. A kinetic model is proposed to explain the mechanism of inhibition by Mg^{2+} and K^+.

INTRODUCTION: Magnesium ion has a biphasic effect on NaK-ATPase; activity as a function of Mg^{2+} concentration passes through a maximum at a Mg^{2+}/ATP ratio of about 1.0 (1-3). Since Mg^{2+} is required for activity, the mechanism of activation by Mg^{2+} has been studied in some detail (4); but the mechanism of inhibition at higher Mg^{2+} has received little attention.

Previous workers suggested that Mg^{2+} inhibits, at least in part, by competing with Na^+ or K^+ at their respective sites (5-6), but this point does not appear to have been studied systematically. In preliminary experiments we found that inhibition of NaK-ATPase from human red cells by Mg^{2+} was strongly dependent on the relative concentrations of Na^+ and K^+. This report concerns the influence of these cations on inhibition of the enzyme by Mg^{2+}.

METHODS: Red cell membranes were prepared from freshly outdated human blood according to standard procedures (7). The protein content of each preparation was measured by the method of Lowry et al. (8). Activity was assayed by measuring the production of inorganic phosphate (Pi) according to a modification of the method of Fiske and SubbaRow (9) as described previously (7). The following conditions were common to all experiments: ATP, 2.0 mM; Tris·HCl (pH 7.4), 63 mM; and Tris·EGTA[1], 0.25 mM. The incubation temperature was 38°. Other conditions are described in the text.

All activities reported represent NaK-ATPase. In each case Mg-ATPase, measured in the presence of ouabain (0.5 mM), was subtracted from activity in the presence of Na^+ and K^+ to obtain the NaK-dependent ATPase activity. Activity is expressed as μmoles Pi/mg protein/hr. All figures represent the average of two or more experiments run in duplicate.

[1]Ethyleneglycol-bis(β-aminoethyl ether)N,N'-tetraacetic acid.

RESULTS AND DISCUSSION: Inhibition of human red cell NaK-ATPase is strongly dependent on the relative concentrations of Na^+ and K^+. Figure 1 (curve A) shows that with 160 mM Na^+ and 8 mM K^+ a Mg^{2+}/ATP ratio of about 1.0 is optimal, and inhibition at higher Mg^{2+} is gradual. This result is consistent with the findings of others (1-3), who used high Na^+, low K^+ media for assay. However, when K^+ was increased to 25 mM with 160 mM Na^+ (curve B), or with 30 mM Na^+ (curve D), the optimum was sharper, inhibition was evident at lower Mg^{2+} and was more pronounced. With 30 mM Na^+ inhibition was nearly complete with 6 mM Mg^{2+}. At the same Na^+ concentration (30 mM), however, Mg^{2+} had virtually no inhibitory effect when K^+ was reduced to 0.5 mM (curve C). This concentration of K^+ is half-saturating with respect to activation in the presence of 30 mM Na^+. It thus appears that for Mg^{2+} to inhibit, K^+ must bind to a site in

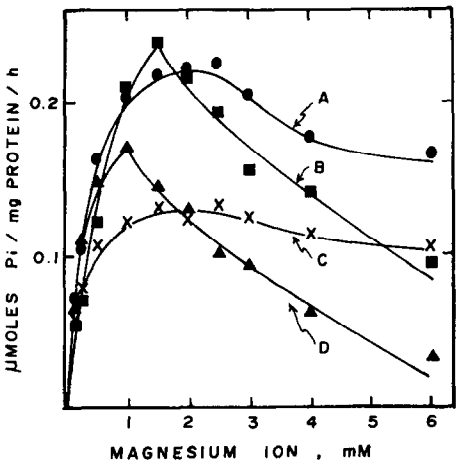

Fig. 1. NaK-ATPase activity as a function of Mg^{2+} concentration with varying monovalent cation concentrations. Curve A - 160 mM Na^+, 8 mM K^+; curve B - 160 mM Na^+, 25 mM K^+; curve C - 30 mM Na^+, 0.5 mM; curve D - 30 mM Na^+, 25 mM K^+.

addition to the site at which it activates; and higher K^+ concentrations are required.

Figure 2 shows more clearly the effect of varying the Na/K ratio in the presence of 2.5 mM Mg^{2+}. In this experiment the concentrations of Na^+ and K^+ were varied reciprocally to maintain a constant ionic strength. When Na^+ increased with saturating K^+, activity increased as Na^+ sites were occupied and reached a plateau through which the enzyme appeared to be saturated with respect to both cations. However, a second increment of activity appeared abruptly when the Na/K ratio exceeded about 5. In this portion of the curve the relative change in K^+ concentration greatly exceeds that in Na^+. It thus appeared likely that the additional activity resulted from the dissociation of K^+ from a site at which it can inhibit with excess Mg^{2+}. Clearly, Na^+ could compete with K^+ at this site, and this possibility was tested in the experiment shown in fig. 3. Here activity is plotted as a function of Na^+ with 1.0 mM Mg^{2+} (noninhibitory) and 3.0 mM Mg^{2+} (inhibitory). The K^+ concentration (6 mM) was within the range over which the second increment of activity appeared in fig. 2. At the lower Mg^{2+} concentration, activation by Na^+ obeyed Michaelis-

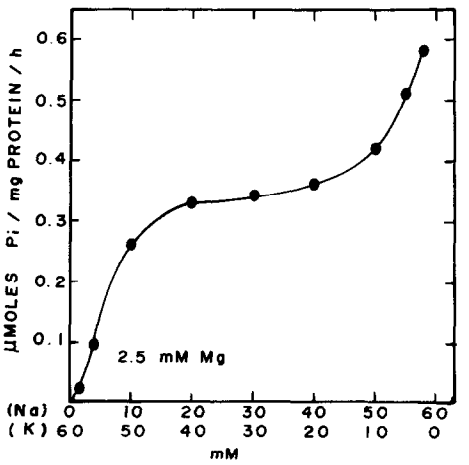

Fig. 2. NaK-ATPase activity as a function of Na/K ratio. Other conditions are described in METHODS.

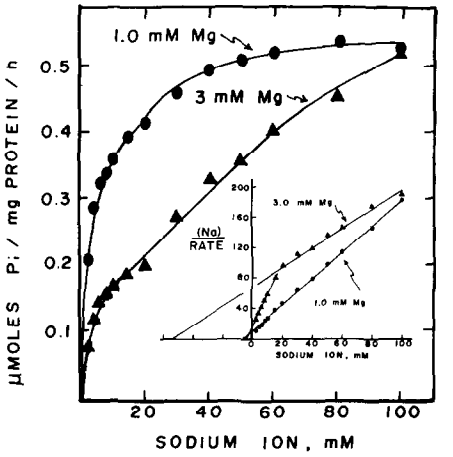

Fig. 3. NaK-ATPase activity as a function of Na^+ concentration at two Mg^{2+} concentrations. The K^+ concentration was 6 mM. The inset is a modified Lineweaver-Burke plot of the same data.

Menton kinetics with an apparent Km of about 7 mM (see inset). With 3.0 mM Mg^{2+}, however, activation by Na^+ was distinctly biphasic, suggesting two classes of sites for Na^+ with widely different affinities (see inset). The low affinity Na^+ sites were evident only with inhibitory levels of Mg^{2+} and relatively low K^+. In fig. 2, the low affinity sites are those on the right; sites which are inhibitory when occupied by K^+ (with excess Mg^{2+}), but not when occupied by Na^+. Na^+ can displace K^+ from these sites only when the Na/K ratio is sufficiently high.

Figure 4 A shows the pattern of activation by K^+ at 30 mM Na^+ and at two Mg^{2+} concentrations, one inhibitory (3 mM) and one noninhibitory (0.5 mM). At the lower Mg^{2+}, activation by K^+ appeared to follow typical saturation kinetics At the higher Mg^{2+}, however, activity peaked sharply at 1.0 mM K^+. This was followed by inhibition up to 10 mM K^+; then activity was independent of K^+ concentration. Figure 4 B shows a similar experiment at two fixed levels of Na^+ and with 3.0 mM Mg^{2+}. Na^+ delayed the onset of inhibition, but as the K^+ concentration increased the two curves merged.

Present results suggest that inhibition of red cell NaK-ATPase by Mg^{2+} is

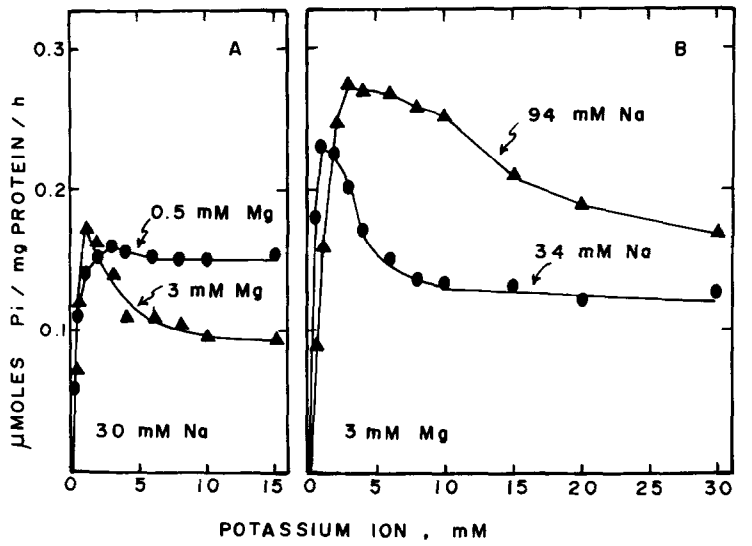

Fig. 4. NaK-ATPase activity as a function of K^+ concentration. A - 30 mM Na^+ and two Mg^{2+} concentrations; B - two Na^+ concentrations and 3 mM Mg^{2+}.

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a combined effect of Mg^{2+} and K^+ at separate sites. One consequence of this inhibitory interaction is the appearance of a new class of low affinity Na^+ sites whose occupancy can at least partially overcome the inhibition (fig. 3). The co-existance of two classes of Na^+ sites appears to be incompatible with any model for inhibition by K^+ and Mg^{2+} which involves competition at a single Na^+ site. The results could be explained by postulating two populations of enzyme, only one of which is susceptible to displacement of Na^+ by K^+ and Mg^{2+}. This could account for the fact that inhibition by K^+ at an inhibitory level of Mg^{2+} does not go to completion as K^+ increases (fig. 4 A), but leaves the problem of explaining why inhibition by Mg^{2+} at high K^+ is complete (fig. 1).

The following tentative model could account for the results without requiring two populations of enzyme. The assumptions are: 1) K^+ must combine with a regulatory site before Mg^{2+} can combine with its site to form an inactive complex, and K^+ bound to this site has not effect on activity at low Mg^{2+}; 2) Na^+ can also combine with the K^+-site; this is the low affinity Na^+-site. The complex with Na^+ is fully active but has a low affinity for Mg^{2+}.

According to this scheme, inhibition by K^+ is not complete, because as long as Mg^{2+} is less than saturating, the amount of inactive complex will be limited by the availability of Mg^{2+}. With saturating Mg^{2+}, inhibition will go to completion. The low affinity Na^+ sites are evident only when Mg^{2+} and K^+ are inhibitory because only then would the combination of Na^+ with this site have any observable kinetic effect on the overall reaction, i.e. to displace Mg^{2+} secondary to displacing K^+.

The results described could be unique to NaK-ATPase from red cells, but it appears that Wang and Lindenmayer (10) obtained similar results with NaK-ATPase from sheep kidney. They state that Mg^{2+} inhibits in the presence of high K^+.

These results suggest that intracellular Mg^{2+}, at moderate inhibitory levels, could regulate the Na,K pump in red cells. With normal high intracellular K^+, the pump would be partially inhibited, but this inhibition could be abruptly overcome when the Na/K ratio exceeded a critical threshold. In this way a reserve pump activity could be available when needed, to protect the cell from colloid osmotic lysis.

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REFERENCES

1. Dunham, E.T. and Glynn, I.M. (1961) J. Physiol. (London), 156, 274-293.
2. Schwartz, A., Laseter, A.H., and Kraintz, L. (1963) J. Cell. Comp. Physiol., 62, 193-203.
3. Wheeler, K.P. and Whittam, R. (1962) Biochem. J., 85, 495-507.
4. Glynn, I.M. and Karlish, S.J.D. (1975) Physiol. Rev., 37, 13-54.
5. Wilkes, A.B. and Bader, H. (1969) Pharmacol. Res. Comm., 1, 211-217.
6. Robinson, J.D. (1972) Biochim. Biophys. Acta, 266, 97-102.
7. Bond, G.H. and Green, J.W. (1971) Biochim. Biophys. Acta, 241, 393-398.
8. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem., 193, 265-275.
9. Fiske, C.H. and SubbaRow, Y. (1925) J. Biol. Chem., 66, 375-400.
10. Yang, T. and Lindenmeyer, G.E. (1975) Biophys. J. (Abstracts), 15, 244a.