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DOG KIDNEY (Na^+,K^+)-ATPase IS MORE SENSITIVE TO INHIBITION BY VANADATE THAN HUMAN RED CELL (Na^+,K^+)-ATPase

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(Na^+,K^+)-ATPase (EC 3.6.1.3) from kidney is more sensitive to inhibition by vanadate than red cell (Na^+,K^+)-ATPase. The difference appears to be in the apparent affinities of the two enzymes for K^+ and Na^+ at sites where K^+ promotes and Na^+ opposes vanadate binding. As a result of Na^+-K^+ competition at these sites, reversal of vanadate inhibition was accomplished at lower Na^+ concentrations in red cell than in kidney (Na^+,K^+)-ATPase. It is possible that vanadate could selectively regulate Na^+ transport in the kidney.

It is generally assumed that the kinetic properties of (Na^+,K^+)-ATPase are independent of the source of the enzyme or the method of preparation, and that tissue or species differences are referrable mainly to differences in specific activity [1]. However, exceptions to this generalization have been noted. For example, (Na^+,K^+)-ATPase from different tissues and from different species can exhibit marked variation in sensitivities to inhibition by cardiac glycosides [2]. Sweadner [3] reported the presence of two molecular forms of (Na^+,K^+)-ATPase in brain, localized in different cell populations. The two forms differ in their sulfhydryl reactivities and in their sensitivities to ouabain and to tryptic inactivation. Indirect evidence indicates that they also differ in their apparent affinities for K^+ at activation sites. Fukushima and Nakao [4] showed that (Na^+,K^+)-ATPase from brain and kidney differ in their apparent affinities for divalent cations.

The present study describes a difference between (Na^+,K^+)-ATPase from dog kidney and human red cells that is expressed as a greater sensitivity of the kidney enzyme to inhibition by vanadate. This difference appears to be due to the fact that the kidney enzyme has a lower affinity for Na^+ at sites where Na^+ antagonizes inhibition by vanadate.

In order to simplify the discussion of monovalent cation binding sites, we have made a distinction between activation and inhibitory sites. Activation sites (A-sites) are high-affinity sites at which Na^+ and K^+ activate (Na^+,K^+)-ATPase. There are separate A-sites for Na^+ and for K^+. Inhibitory sites (I-sites) are relatively low-affinity sites at which K^+ promotes and Na^+ opposes vanadate binding. There may be a single class of I-sites at which Na^+ competes with K^+ [5].

Kidney (Na^+,K^+)-ATPase was prepared as described by Post and Sen [6]. These preparations were 90–95% ouabain-sensitive with specific activities of approximately 100 μmol P_i/mg protein per h.

Red cell (Na^+,K^+)-ATPase was prepared from freshly outdated human blood as described previously [5]. The specific activity of red cell (Na^+,K^+)-ATPase was approximately 0.4 μmol P_i/mg protein per h.

(Na^+,K^+)-ATPase activity was assayed in terms of P_i production according to a modification of the method of Fiske and SubbaRow [7]. All assays were carried out at 38°C. Conditions common to all experi-

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ments were: 2 mM ATP (Sigma Chemical Co., St. Louis, MO; Grade II ATP as Na^+ or $Tris^+$ salt); 4 mM Mg^{2+}; 63 mM Tris-HCl (pH 7.4) and 0.25 mM Tris-EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid]. Other conditions are described in figure legends.

Mg^{2+}-ATPase activity was measured in the presence of 0.25 mM ouabain and without K^+. (Na^+,K^+)-ATPase activity was calculated by subtracting Mg^{2+}-ATPase activity from the activity in the presence of Na^+ and K^+.

The concentration of vanadate required for half-maximal inhibition ($K_{1/2}$ for vanadate) was calculated as described previously [5]. The $K_{1/2}$ for activation by Na^+ and K^+ at their respective A-sites was obtained from Hill plots. The $K_{1/2}$ for inhibition by K^+ at I-sites was calculated from the K^+-dependence of inhibition in the presence of vanadate (Fig. 1). Changes in the $K_{1/2}$ for inhibition by vanadate were taken as a measure of the extent of occupation of I-sites by K^+.

Table I compares the influence of Na^+ and K^+ on the $K_{1/2}$ for vanadate as an inhibitor of (Na^+,K^+)-ATPase from dog kidney and human red cells. $K_{1/2}$ values were not significantly different in the presence of 20 mM K^+ and 34 mM Na^+. With 4 mM K^+ and 34 mM Na^+, however, the $K_{1/2}$ for red cell (Na^+,K^+)-ATPase doubled, while that for kidney (Na^+,K^+)-ATPase was unchanged (Table I). Therefore, I-sites on kidney (Na^+,K^+)-ATPase remained saturated at 4 mM K^+ in the presence of 34 mM Na^+, whereas I-sites on the red cell enzyme did not.

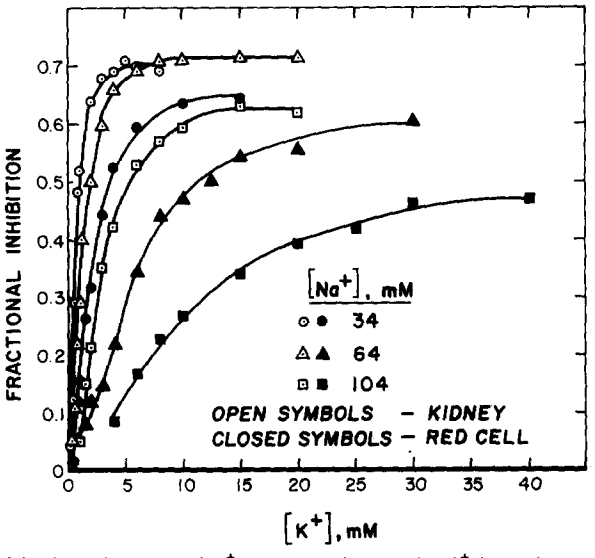

Fig. 1. Influence of Na^+ concentration on the K^+-dependence of fractional inhibition of kidney and red cell (Na^+,K^+)-ATPase in the presence of 0.5 μM vanadate. Fractional inhibition was calculated at each K^+ concentration relative to a corresponding control activity without vanadate. Curves represent the average of four or more separate experiments.

TABLE I

APPARENT $K_{1/2}$ VALUES FOR VANADATE OBTAINED WITH KIDNEY AND RED CELL (Na^+,K^+)-ATPase UNDER VARIOUS CONDITIONS

$K_{1/2}$ Values are presented as mean ± S.E. of three to six duplicate determinations.

Cation (mM)		Apparent $K_{1/2}$ for vanadate (μM)	
Na^+	K^+	Kidney ATPase	Red cell ATPase
34	20	0.21 ± 0.02	0.22 ± 0.03
84	20	0.32 ± 0.04	0.41 ± 0.03
124	20	0.48 ± 0.05	0.78 ± 0.06
34	4	0.22 ± 0.02	0.43 ± 0.05
84	4	0.52 ± 0.04	3.22 ± 0.25
124	4	1.19 ± 0.05	8.82 ± 0.42

Na^+ competes with K^+ at both A-sites [9] and I-sites [5]. Competition at I-sites resulted in an increase in the $K_{1/2}$ for vanadate as K^+ was displaced at higher Na^+ concentrations (Table I). For both enzymes, the increase was greater in the presence of 4 as compared to 20 mM K^+, as would be expected of a competitive interaction between Na^+ and K^+. Furthermore, the $K_{1/2}$ for vanadate increased more sharply as a function of Na^+ concentration in the case of red cell enzyme as compared to the kidney enzyme. This result can be explained if the affinity for K^+ is lower at I-sites on red cell (Na^+,K^+)-ATPase, or if the affinity for Na^+ is higher.

We examined the apparent affinities of I-sites for K^+ more directly in Fig. 1, where fractional inhibition is shown as a function of K^+ concentration. These curves depict the occupation of I-sites by K^+ and allow an estimate of $K_{1/2}$ values for K^+ at these sites. At each Na^+ concentration, the curve with kidney (Na^+,K^+)-ATPase fell to the left of the corresponding curve with red cell (Na^+,K^+)-ATPase. This observation shows that I-sites on kidney (Na^+,K^+)-ATPase have a

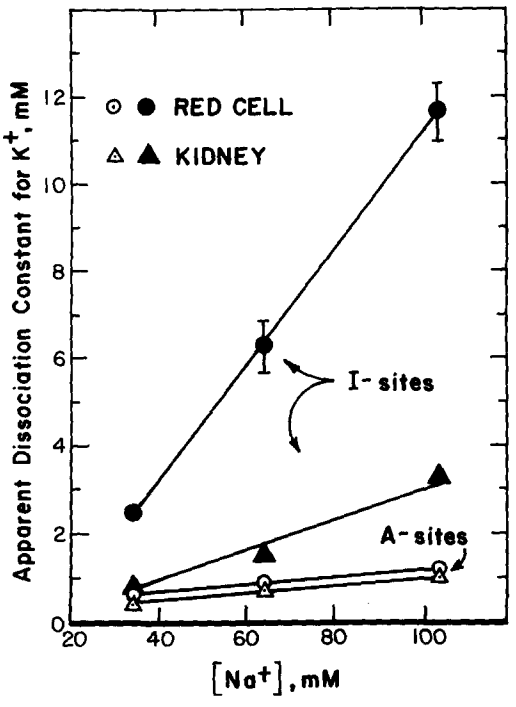

Fig. 2. Influence of Na^+ concentration on the apparent dissociation constant for K^+ at A-sites (no vanadate) and I-sites (0.5 μM vanadate) on kidney and red cell (Na^+,K^+)-ATPase. Each point is the mean ± S.E. of four or more separate experiments. Vertical bars were omitted where they were smaller than the symbol.

higher apparent affinity for K^+ than those on red cell (Na^+,K^+)-ATPase. Curves for both enzymes shifted to the right at higher Na^+ concentrations. This is a reflection of Na^+-K^+ competition at I-sites. At higher Na^+ concentrations, the curves approached lower maximal levels of inhibition. This may be a reflection of competition between Na^+ and Mg^{2+} [10].

Fig. 2 compares $K_{1/2}$ values for K^+ at A-sites and at I-sites. $K_{1/2}$ values for K^+ at A-sites on red cell (Na^+,K^+)-ATPase were higher than at A-sites on kidney (Na^+,K^+)-ATPase ($P < 0.05$). The fact that $K_{1/2}$ values for K^+ increased as Na^+ concentration increased, indicates that Na^+ competed most effectively with K^+ at I-sites on red cell (Na^+,K^+)-ATPase, less effectively at I-sites on kidney (Na^+,K^+)-ATPase, and least effectively at A-sites on both enzymes. Since all $K_{1/2}$ values converged at low Na^+ concentrations, the differences in $K_{1/2}$ values for K^+ must be due mainly to different apparent affinities for Na^+; i.e. I-sites on red cell (Na^+,K^+)-ATPase have a higher apparent affinity for Na^+ than any of the other sites considered in Fig. 1.

In the presence of vanadate, curves describing activity as a function of Na^+ concentration were biphasic (solid symbols on Fig. 3), as noted previously [5]. The initial portions of these curves reflect the occupation of A-sites by Na^+. The second portions reflect competition with K^+ at I-sites to reverse inhibition by vanadate [5]. It is evident from these curves, as well as those in Fig. 1, that I-sites on red cell (Na^+,K^+)-ATPase have a higher apparent affinity for Na^+ than those on kidney (Na^+,K^+)-ATPase.

Other differences in Na^+ affinities are also evident in the control curves in Fig. 3. The apparent $K_{1/2}$ values for activation by Na^+ at A-sites were 5.1 mM

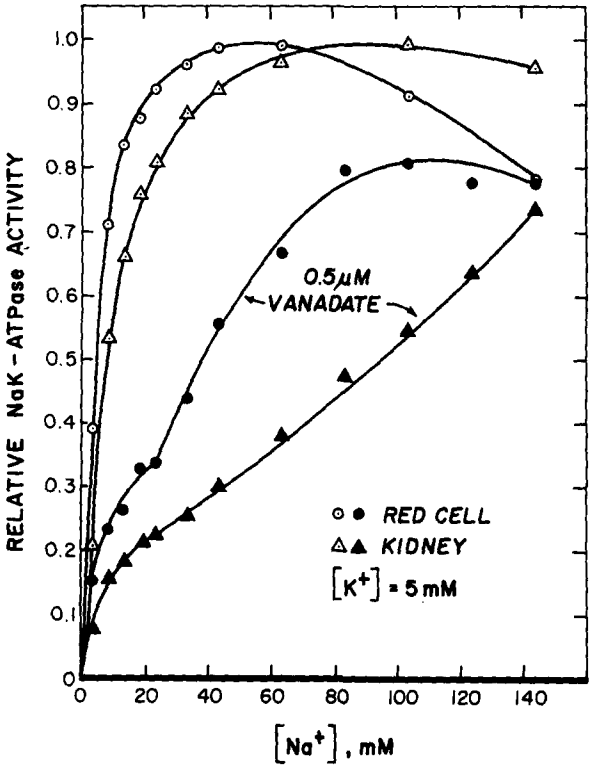

Fig. 3. Relative (Na^+,K^+)-ATPase activity as a function of Na^+ concentration in the presence and absence of vanadate (0.5 μM). Relative (Na^+,K^+)-ATPase activity at each point was calculated with respect to the maximum in control curves (open symbols, no vanadate). Each point represents the average of three separate experiments.

for red cell and 9.1 mM for kidney (Na^+,K^+)-ATPase. Furthermore, only the red cell (Na^+,K^+)-ATPase was inhibited at Na^+ concentrations above 60 mM. Therefore, an inhibitory site for Na^+ on (Na^+,K^+)-ATPase from red cells also had a higher affinity for Na^+. Thus, all sites on red cell (Na^+,K^+)-ATPase have higher apparent affinities for Na^+. It is the difference in apparent affinity at I-sites, specifically, that appears to explain the greater sensitivity of kidney (Na^+,K^+)-ATPase to inhibition by vanadate.

These appear to be three kinetically distinct sites for Na^+ on (Na^+,K^+)-ATPase: A-sites, I-sites and sites at which Na^+ inhibits (Na^+,K^+)-ATPase in the absence of vanadate. It is of interest that all three Na^+ sites on red cell (Na^+,K^+)-ATPase have uniformly higher affinities for Na^+ than those on kidney (Na^+,K^+)-ATPase. This finding suggests a relationship between these three sites. Cavieres and Ellory [11] suggested that three internal A-sites for Na^+ are converted to two external A-sites for K^+ as Na^+ is transported outward. The remaining external site is then a site at which external Na^+ can inhibit (Na^+,K^+)-ATPase. Therefore, two conformations of a single site can explain activation by internal Na^+ and inhibition by external Na^+. Since external Na^+ displaces external K^+ from I-sites [12], it seems possible that Na^+ might act at a third conformation of a common site to displace K^+ and reverse inhibition by vanadate.

The difference between human red cell and dog kidney (Na^+,K^+)-ATPase could reflect a tissue or species difference, or could be related to differences in the method of enzyme preparation. In one experiment we eliminated the urea treatment step in the preparation of kidney (Na^+,K^+)-ATPase and found that the response to vanadate was unaltered.

The fact that (Na^+,K^+)-ATPases from different sources can have different sensitivites to inhibition suggests that vanadate has the potential to selectively modulate (Na^+,K^+)-ATPase in certain tissues. Post et al. [13] have shown that there is six times as much vanadate in kidney as in human red cells (0.66 vs. 0.11 nmol/g of tissue). Taken with the present observation that I-sites on kidney (Na^+,K^+)-ATPase are more sensitive to K^+, it seems possible that vanadate might selectively regulate (Na^+,K^+)-ATPase in the kidney.

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