ON THE INTERACTIONS OF Na^+, K^+, Mg^{++}, AND ATP WITH THE Na^+ PLUS K^+ ACTIVATED ATPase FROM RAT BRAIN.

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Sigmoidal curves have been repeatedly observed when (Na^+ plus K^+) ATPase activity is plotted against Na^+ concentration in the presence of high concentrations of K^+, (Skou, 1957; Post et al., 1960; Taylor, 1963). Even when the sigmoidal nature of the Na^+ and K^+ activation curves is not apparent, the upward-bending Lineweaver-Burk plots derived from these experiments show that the interactions of Na^+ and K^+ with the ATPase are not of the simple Michaelis-Menten type.

In the last few years a number of enzymes have been described which clearly fail to conform to simple Michaelis-Menten kinetics. Interestingly, most of these are subject to allosteric feed-back inhibition, (Monod et al., 1963; Gerhart and Pardee, 1963; Scarano, 1964; Sanwal et al., 1963; Viñuela et al., 1963; Cohen and Patte, 1963). It has been shown, for several of these, that interactions with substrate or allosteric inhibitor can be described by the Hill equation with $1 < |n| \leq 2$, (Monod et al., 1963; Scarano, 1964; Sanwal et al., 1963; Viñuela et al., 1963):

$$\log \left(\frac{V_m}{V} - 1 \right) = \log \text{"K"} - (n)\log(S) \qquad (1)$$

Thus when $\log \left(\frac{V_m}{V} - 1 \right)$ is plotted against $\log(S)$ a straight line is obtained with slope equal to -n. It has been suggested thar for $|n| > 1$ there is a cooperative interaction between two or more binding sites for the same ligand (Monod et al., 1963). In this paper it will be

shown that the interactions of K^+, Na^+, Mg^{++}, and ATP with rat brain (Na^+ plus K^+) ATPase can be described by the Hill equation with $1 < |n| < 2$. It will also be shown that the enzyme is protected from irreversible chlorpromazine inactivation by either Na^+ or K^+, and that this protective interaction can be described by the Hill equation with $1 < |n| < 2$.

METHODS

(Na^+ plus K^+)ATPase was prepared from rat brain and assayed essentially according to Skou (1962, 1963 a). ATPase activated by Mg alone can be reduced to less than 1o % of the total ATPase activity by heating the Skou enzyme at $4o^o$ for 30 minutes in 1M sodium acetate pH 5.0. Due to aggregation at this pH the enzyme is easily centrifuged down in 15 minutes at 27,000 xg. The pellet is then resuspended in Skou's storage medium (Skou, 1962). The resulting Na plus K ATPase has kinetic properties very similar, if not identical, to those of the original Skou enzyme.

The Skou enzyme contains about 15 or 2o % ATPase which is activated by Mg alone. This enzyme was used in all the experiments described in this paper

(Na^+ plus K^+) ATPase activity was calculated in all cases by subtracting the activity in the absence of added Na^+ or K^+ from the total ATPase activity.

In the chlorpromazine inactivation experiments, enzyme was preincubated for 30 minutes at 37^o C in a solution containing 0.01 M tris-HCl, pH 7.6; 40 µg/ml chlorpromazine; and various NaCl or KCl concentrations. After preincubation sufficient NaCl and KCl were added to bring their final concentrations to 100 mM and 20 mM, respectively. Enzyme activity was then assayed in the usual manner.

The apparent affinity constants, "K", were taken from the Hill plots, and are defined as the concentrations required to produce 5o % of maximum stimulated activity.

RESULTS

Hill interactions of Na^+, K^+, Mg^{++}, and ATP with (Na^+ plus K^+) ATPase.

Fig 1, shows typical Hill plots for the interactions

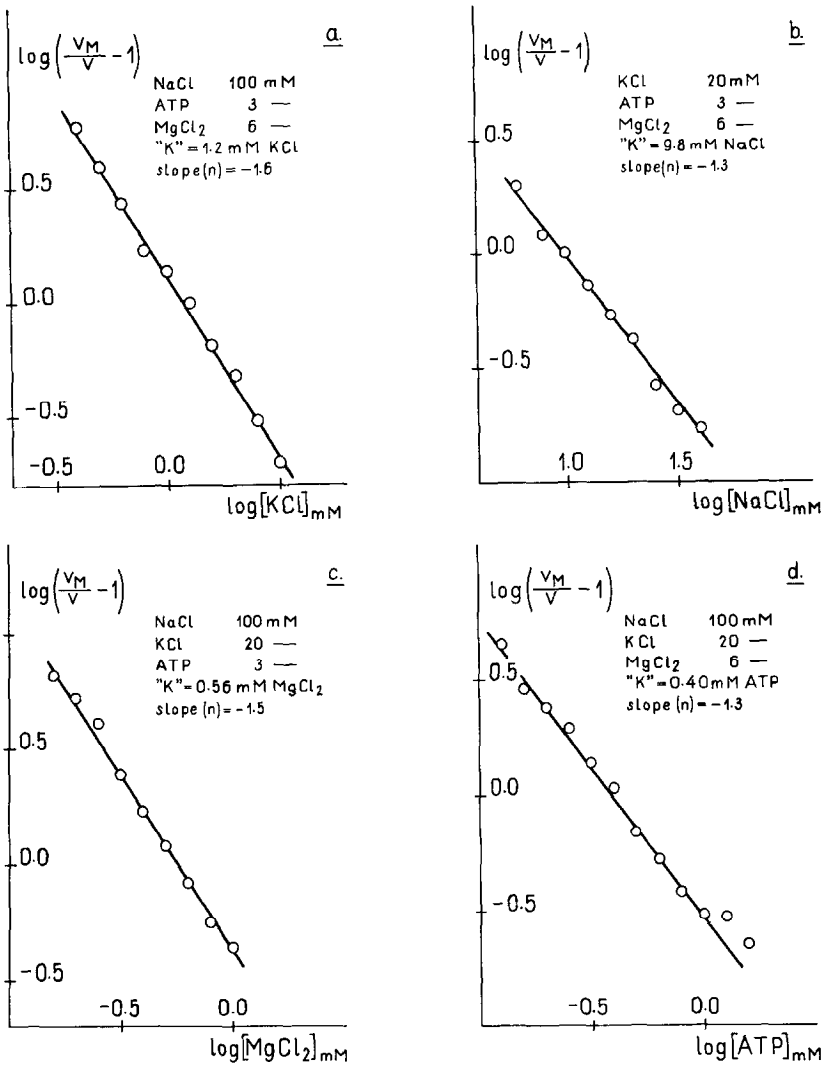

Fig. 1. Hill plots of ATPase activity expressed as a function of: K^+(a), Na^+(b), Mg^{++}(c), and ATP (d) concentrations.

of ATPase with Na^+, K^+, Mg^{++}, and ATP. All substances other than the one being varied were held constant at optimum concentrations. The slopes (n) and the apparent affinity constants ("K") were taken directly from the Hill plots. Slope values varied somewhat from one experiment to the next but in all cases lay between 1.3 and 2.0. The concentrations of Na^+, K^+, Mg^{++}, and ATP required to produce 50 %

of maximum stimulated activity were 9.8 mM, 1.2 mM, 0.56 mM and 0.40 mM, respectively.

Protection of ATPase from irreversible chlorpromazine inactivation by Na^+ or K^+.

Chlorpromazine is known to be an inhibitor of (Na^+ plus K^+) ATPase, (Skou, 1963). Preliminary experiments showed that chlorpromazine at 40 μg/ml irreversibly inhibits (Na^+ plus K^+) ATPase about 90 % when enzyme and inhibitor are preincubated together for 30 minutes at 37° C in the absence of Na^+ and K^+. If either Na^+ (200 mM) or K^+ (40 mM) is present during the preincubation the enzyme is partially protected from chlorpromazine inactivation. Maximum protective effect is obtained with either ion alone.

An experiment was carried out to determine the concentrations of Na^+ and K^+ required to give 50 % of maximum protection against chlorpromazine. It was found that the protective interactions of both Na^+ and K^+ with the ATPase are described by the Hill equation with $1 < |n| < 2$, (fig. 2) and are thus similar to the activation interactions. The concentrations of Na^+ and K^+ giving 50 % of maximum protection were 19 mM and 2.1 mM respectively. The ratio of the apparent affinity constants for Na^+ and K^+ is

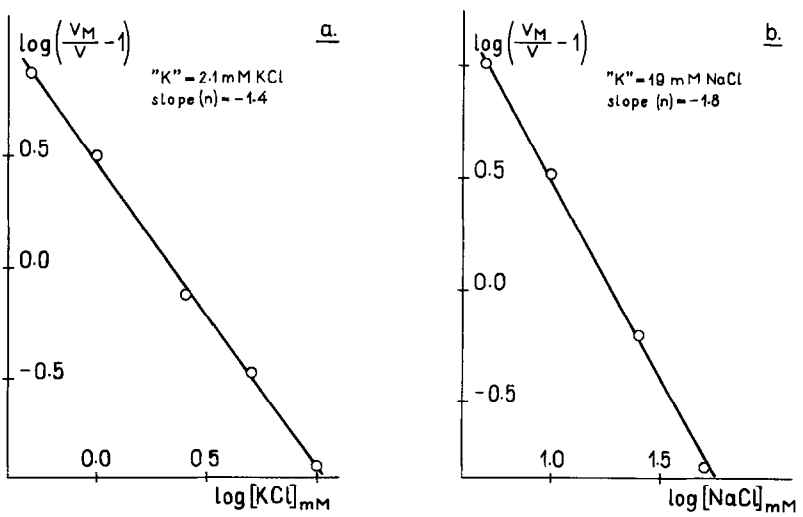

Fig. 2. Hill plots of ATPase activity as a function of K^+ (a) and Na^+ (b) concentration in medium during preincubation with chlorpromazine.

about the same for both activation and protection. The two types of interaction differ, however, in that either Na^+ or K^+ alone protects, while the simultaneous presence of both ions is required for activation.

Preliminary experiments indicate that similar protection is obtained against denaturation by 3M urea.

DISCUSSION

It has been asumed in the present work that 1) the (Na^+ plus K^+) ATPase preparations used are contaminated by other ATPases activated by Mg alone, and 2) the(Na^+plus K^+) ATPase is completely inactive in the absence of Na^+ or K^+. The assumptions are probably correct in view of the work of Nakao et al. (1963) who were able to isolate two ATPases from erythrocyte membranes, only one of which is inhibited by ouabain, a specific inhibitor of(Na^+plus K^+) ATPase. Rendi and Uhr (1964) have prepared a (Na^+ plus K^+) ATPase from calf kidney which contained less than 1 % ATPase activated by Mg^{++} alone. Our own results indicate that the Mg^{++} activated ATPases can be selectively inactivated by heating at pH 5.0 in the presence of Na^+.

The interactions of the (Na^+ plus K^+) ATPase with Na^+, K^+, Mg^{++}, and ATP are all described by the Hill equation with $1 < |n| < 2$. This finding suggests the existence of two or more cooperatively interacting binding sites for each ligand.

Although Hill coefficients greater than one indicate cooperative interactions between two or more binding sites, it is not possible to determine the exact number. The Hill equation only approximates a theoretically meaningfull function or functions which describe cooperative interactions. Hill coefficients are also known to be influenced by a number of non-specific agents such as ionic strength (Rossi-Fanelli, 1963), pH, heat, and inhibitors (Gerhart and Pardee, 1962).

Na^+ and K^+ should be regarded as allosteric activators of (Na^+plus K^+) ATPase. The fact that either Na^+ or K^+ alone will protect the enzyme from inactivation by chlorpromazine or urea strongly suggests that the binding of these ions alone induces conformational changes in the enzyme, and

that their binding sites are different from those occupied by ATP.

Sen and Post (1964) have reported that, in the intact human erythrocyte, about three Na^+ are transported outward, and two K^+ inward, for each ATP hydrolysed by the (Na^+ plus K^+) ATPase. This finding also supports the idea that there are several binding sites for both Na^+ and K^+ on an single active transport enzyme molecule, which is presumably identical with the (Na^+ plus K^+) ATPase.

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