KINETICS OF ($Na^+ + K^+$)-STIMULATED ATPase OF RABBIT KIDNEY MICROSOMES

A. L. GREEN and C. B. TAYLOR

Smith Kline and French Research Institute,
Welwyn Garden City, Herts, England.

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Skou (1957) showed that hydrolysis of ATP by microsomes of crab nerve is activated by Mg^{++} and stimulated by Na^+ and K^+. Similar enzyme systems are widely distributed and appear to be intimately concerned in active cation transport (Post et al., 1960). The general pattern of stimulation by Na^+ and K^+ is now well known (Skou, 1961), but there has been little attempt to carry out more than a partial kinetic analysis in order to test proposed mechanisms. This report summarizes a more complete kinetic analysis.

Microsomes of rabbit kidney (Skou, 1962) were used. ATPase activity was estimated as previously described (Taylor, 1963).

Na^+ and K^+ alone stimulate ATPase only slightly, if at all, but when present together stimulate markedly. The pattern of stimulation is illustrated by the families of curves obtained when ATPase is measured over a range of concentrations of one ion at various concentrations of the other (Fig. 1). Na^+ also inhibits when its concentration is very high relative to that of K^+ (Fig. 2).

This pattern may be qualitatively accounted for if stimulation is brought about by the existence in the system of two binding sites, one for Na^+ and one for K^+ (Skou, 1961). Maximal stimulation would

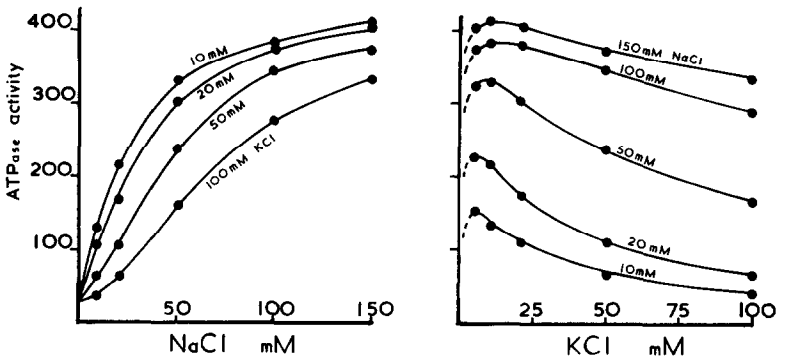

Fig. 1. The effect of sodium or potassium on ATPase activity at constant concentrations of the other cation. The medium contained 2.5mM ATP, 5.0mM $MgCl_2$, 30mM tris:HCl buffer (pH 7.5) in addition to the various concentrations of NaCl and KCl indicated. ATPase activity is expressed as μmoles P_i/mg N / h.

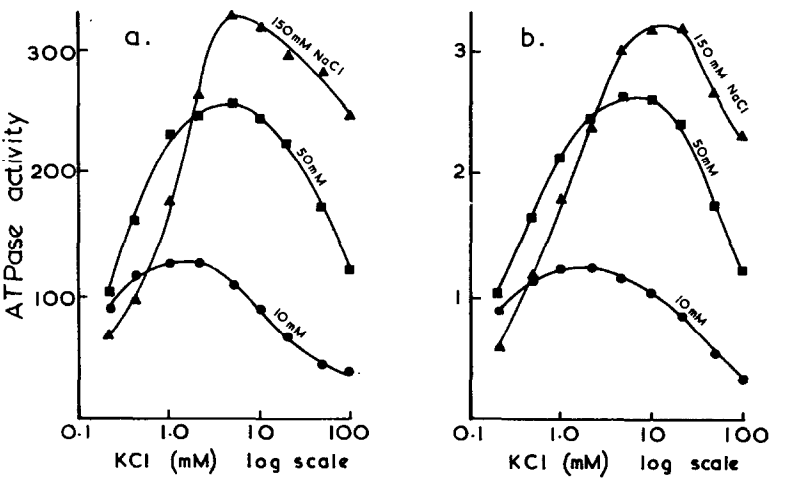

Fig. 2. The effect of potassium on ATPase activity at constant concentrations of sodium. a. experimental results - ATPase activity expressed as μmoles P_i/mg N / h. ; b. theoretical curves, calculated from equation (1) - ATPase activity expressed in arbitrary units.

only occur when both sites are appropriately occupied. Furthermore, at high concentrations both ions inhibit to an extent inversely related to the concentration of the other ion. This suggests that there is competitive inhibition by K^+ at the Na^+-site and by Na^+ at

the K^+-site. The recent experiments of Charnock and Post (1963), with radioactive ATP, suggest that hydrolysis occurs in the following two steps. In the first, which is activated by Na^+, an intermediate phosphoprotein is formed; in the second, which is activated by K^+, this phosphoprotein is hydrolyzed to inorganic phosphate. Provided that the ATP concentration is sufficient to saturate the enzyme, such a two-step reaction would, during its early stages, reach a steady state, where the rates of formation and hydrolysis of the intermediate phosphoenzyme (EP) would be equal, i.e. $k_1[ENa]=k_2[EPK]$ where k_1 and k_2 are the rate constants for the two steps, ENa is a Na^+-enzyme complex with dissociation constant K_1 (=[E][Na]/[ENa]), and EPK is a K^+-phosphoenzyme complex with dissociation constant K_3 (=[EP][K]/[EPK]). The rate of inorganic phosphate liberation at the steady state would then be given by:-

$$v = k_2[EPK] = \frac{k_1 k_2 a b E_t}{(k_1+k_2)ab + k_1 K_3 a + k_2 K_1 b + \frac{k_1 K_3}{K_4} a^2 + \frac{k_2 K_1}{K_2} b^2} \qquad (1)$$

where a and b are the Na^+ and K^+ concentrations, K_2 is the dissociation constant for the binding of K^+ instead of Na^+ to the free enzyme and K_4 is the dissociation constant for the binding of Na^+ instead of K^+ to the phosphoenzyme and E_t is the total concentration of enzyme plus phosphoenzyme.

From the Figures, it is clear that maximal activity is reached at much lower concentrations of K^+ than Na^+, and that at high concentrations, inhibition is much greater by K^+ than by Na^+. If equation (1) is to reproduce this pattern, the coefficients of b and b^2 must be much greater than those of a and a^2, i.e.

$k_2K_1 \gg k_1K_3$ and $k_2K_1/K_2 \gg k_1K_3/K_4$. These requirements may be satisfied if the binding constants K_1-K_4 are all of the same magnitude and if the rate constant (k_2) for dephosphorylation is much greater than the apparent rate constant (k_1) for phosphorylation. The possibility that $K_3 \ll K_1$ seems less likely but cannot be excluded on kinetic grounds alone.

It has not been possible to derive values for all the constants by rigorous application of equation (1) to the experimental results. However, as shown in Fig. 2, (1) with the following values inserted, $K_1 = K_2 = 20$mM, $K_3 = K_4 = 40$mM and $k_1 = 160k_2$, gives fair quantitative fit.

The imperfect fit may in part be due to errors in the values assigned to the constants. However, the sigmoidal nature of the Na^+ stimulation curves at high K^+ concentrations cannot be reproduced by equation (1). It is possible that when the Na^+ concentration is low and the K^+ concentration high, the steady state may not have been reached at the time when the reaction rates were measured. This would lead to experimental rates lower than those calculated from (1).

Although the observed kinetics are consistent with the assumed mechanism, a word of warning must be added about using this agreement as evidence that this particular mechanism is the only permissible one. Firstly, as pointed out above, the kinetics cannot distinguish between the situation in which the binding constants are approximately equal but $k_2 \gg k_1$, and that in which $k_1 \sim k_2$ but $K_3 \ll K_1$. Secondly, and more seriously, it is possible to obtain similar agreement between experimental and calculated activities even if it is assumed that the rate-limiting step is not one of two sequential steps, both depending upon the relative concentrations

of Na^+ and K^+, but a single stage involving two sites, one requiring Na^+ (dissociation constant K_1) and blocked by K^+ (K_2) and the other requiring K^+ (K_3) and blocked by Na^+ (K_4). The rate of hydrolysis under these conditions can be shown, as for example by the type of analysis given by Laidler and Socquet (1950), to be:-

$$v = \frac{k\,E_t}{\left[\frac{K_1}{a}\left(1+\frac{b}{K_2}\right)+1\right]\left[\frac{K_3}{b}\left(1+\frac{a}{K_4}\right)+1\right]} \qquad (2)$$

$$= \frac{k\,ab\,E_t}{K_1K_3+\left(1+\frac{K_1K_3}{K_2K_4}\right)ab+\left(K_3+\frac{K_1K_3}{K_4}\right)a+\left(K_1+\frac{K_1K_3}{K_2}\right)b+\frac{K_3}{K_4}a^2+\frac{K_1}{K_2}b^2} \qquad (3)$$

Equation (3) differs in form from (1) only by the presence of the constant term K_1K_3 in the denominator. At high concentrations of Na^+ and K^+, or both, (3) will give theoretical curves identical in appearance to those given by (1). Numerical values for the constants K_1 - K_4 in (3) may be obtained by equating coefficients of a, a^2, ab, b and b^2 in (1) and (3). These values are $K_1=K_2=K_4=20$mM and K_3 = 0.175mM. Thus, K_1K_3 would be very small and at almost all concentrations of Na^+ and K^+ studied, equations (1) and (3) will give virtually identical rates. Thus the kinetic pattern alone does not distinguish between a one-stage and a two-stage reaction, although the evidence of Charnock and Post (1963) strongly favours the latter possibility.

The fact that a satisfactory account of the kinetics of the two-stage mechanism can be given assuming approximately equal values for K_1 - K_4 suggests that there may not, in fact, be two cation sites. If there were only one site with approximately equal affinities for Na^+ and K^+, the same result would be found

provided that phosphorylation only occurs when the site is occupied by Na^+ and hydrolysis only when it is occupied by K^+. This possibility may be of importance in considering the mechanism of sodium transport.

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