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MECHANISMS BY WHICH Li^+ STIMULATES THE $(Na^+ + K^+)$-DEPENDENT ATPase

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SUMMARY

The addition of LiCl stimulated the $(Na^+ + K^+)$-dependent ATPase activity of a rat brain enzyme preparation. Stimulation was greatest in high Na^+/low K^+ media and at low Mg · ATP concentrations. Apparent affinities for Li^+ were estimated at the α-sites (moderate-affinity sites for K^+ demonstrable in terms of activation of the associated K^+-dependent phosphatase reaction), at the β-sites (high-affinity sites for K^+ demonstrable in terms of activation of the overall ATPase reaction), and at the Na^+ sites for activation. The relative efficacy of Li^+ was estimated in terms of the apparent maximal velocity of the phosphatase and ATPase reactions when Li^+ was substituted for K^+, and also in terms of the relative effect of Li^+ on the apparent K_M for Mg · ATP. With these data, and previously determined values for the apparent affinities of K^+ and Na^+ at these same sites, quantitative kinetic models for the stimulation were examined. A composite model is required in which Li^+ stimulates by relieving inhibition due to K^+ and Na^+ (i) by competing with K^+ for the α-sites on the enzyme through which K^+ decreases the apparent affinity for Mg · ATP and (ii) by competing with Na^+ at low-affinity inhibitory sites, which may represent the external sites at which Na^+ is discharged by the membrane Na^+/K^+ pump that this enzyme represents. Both these sites of action for Li^+ would thus lie, in vivo, on the cell exterior.

INTRODUCTION

Although Li^+ is a poor substitute for K^+ as an activator of the $(Na^+ + K^+)$-dependent ATPase (ATP phosphohydrolase, EC 3.6.1.3) and ineffective as a substitute for Na^+, Gutman et al. [1] and Tobin et al. [2] have recently reported that Li^+ can stimulate ATPase activity in the presence of Na^+ and K^+, even with saturating concentrations of K^+. This seemingly paradoxical effect of Li^+ raises a number of questions about the characteristics of the cation sites of the enzyme and how occupancy of these sites affects both enzymatic activity and membrane transport of Na^+ and K^+, for this enzyme represents the biochemical basis of the cellular Na^+/K^+ pump [3, 4]. Beyond these issues, the interaction of Li^+ with the enzyme (and pump)

may also bear on the pharmacological role of Li^+, which is an important therapeutic agent in the treatment of mania [5].

To account for the stimulation of the enzyme by Li^+, Tobin et al. [2] proposed that Li^+ displaced K^+ from the K^+-discharge sites (those sites from which, in vivo, K^+ is released by the pump into the cytoplasm). An alternative mechanism has been suggested [6] in which Li^+ displaces K^+ from α-sites [7], moderate-affinity sites for K^+ that lie, in vivo, on the external membrane surface and through which K^+ decreases the apparent affinity for the substrate, Mg · ATP [6–9]. Although both of these proposals may be supported by plausible qualitative arguments, a true discrimination between these, or yet other, formulations must depend on quantitative tests.

This paper describes attempts to account for stimulation of the enzyme by Li^+ in terms of specific kinetic models, using parameters for cation affinities consistent with current and previous evaluations of their affinities at specific, definable sites. These studies expose shortcomings in both previous proposals. An examination of the data and the kinetic mechanisms suggests a further site of interaction between Li^+ and the enzyme, to relieve inhibition by Na^+. A composite model, in which Li^+ stimulates the enzyme by competing both at inhibitory sites for Na^+ and at inhibitory α-sites for K^+, provides reasonable quantitative agreement with the experimental observations.

METHODS

The $(Na^+ + K^+)$-dependent ATPase was obtained from a rat brain microsomal preparation by treatment with deoxycholate and then NaI, as previously described [10].

$(Na^+ + K^+)$-dependent ATPase activity was measured in terms of the production of P_i, as previously described [10]. The standard medium contained 30 mM Tris/histidine/HCl (pH 7.8), 3 mM $MgCl_2$, 3 mM ATP (as the Tris salt), 90 mM NaCl, 10 mM KCl, and the enzyme preparation (0.1 mg protein/ml.) Incubation was for 4–8 min at 37 °C; activity was linear with time during these periods. Activity in the absence of Na^+ and K^+ was measured concurrently; such activity averaged only a few percent of the $(Na^+ + K^+)$-dependent ATPase activity [10], and was subtracted from the total activity in the presence of Na^+ and K^+ to give the $(Na^+ + K^+)$-dependent activity. Because of variations in the absolute activity of different enzyme preparations, enzyme velocities are expressed relative to the $(Na^+ + K^+)$-dependent ATPase activity of a concurrent control incubation in the standard medium, defined as 1.0. K^+-dependent phosphatase activity was measured in terms of the production of *p*-nitrophenol after incubation with *p*-nitrophenylphosphate, as previously described [11]. The standard medium contained 30 mM Tris/histidine/HCl (pH 7.8), 3 mM $MgCl_2$, 3 mM *p*-nitrophenylphosphate (as the Tris salt), 20 mM KCl, and the enzyme preparation (0.1 mg protein/ml). Incubation was for 8–15 min at 37 °C; activity in the absence of added KCl was measured concurrently; such activity averaged only a few per cent of the K^+-dependent phosphatase activity under optimal conditions [11], and was subtracted from the total activity in the presence of KCl to give the K^+-dependent activity. As with the ATPase, velocities are expressed relative to the K^+-dependent phosphatase activity of a concurrent control incubation in the standard medium, defined as 1.0. For both activities the data presented represent the averages of five or more experiments performed in duplicate or triplicate.

RESULTS AND DISCUSSION

Stimulation by Li^+ and occupancy of α-sites

The addition of LiCl, at concentrations from 10 to 50 mM, increased ($Na^+ + K^+$)-dependent ATPase activity in the presence of low or high ("saturating") concentrations of KCl and low or high concentrations of NaCl (Figs 1 and 2), in accord with the observations of Tobin et al. [2]. At first glance, the stimulation by Li^+ at high K^+ concentrations would seem to be the most peculiar feature, for although Li^+ can substitute for K^+ it has a lower efficacy (i.e. the maximal velocity with Li^+ is less than that with K^+) and would thus be expected to decrease activity, if anything, when added with "saturating" levels of K^+.

A possible explanation for this apparent paradox is suggested by the relationship between the magnitude of stimulation and the concentration of Mg · ATP: as the Mg · ATP concentration is reduced toward its K_M value the stimulation by Li^+ is increased (Fig. 1). Previous studies have described antagonistic effects of K^+ on the apparent K_M for Mg · ATP [10]. Moreover, a quantitative model [6] is consistent with the binding of Mg · ATP to the substrate sites being incompatible with the binding of K^+ to α-sites on the enzyme. These α-sites have a moderate affinity for K^+ and are demonstrable in terms of the K^+-dependent phosphatase activity [7, 9] that is associated with the overall ($Na^+ + K^+$)-dependent ATPase reaction [4, 11]. The

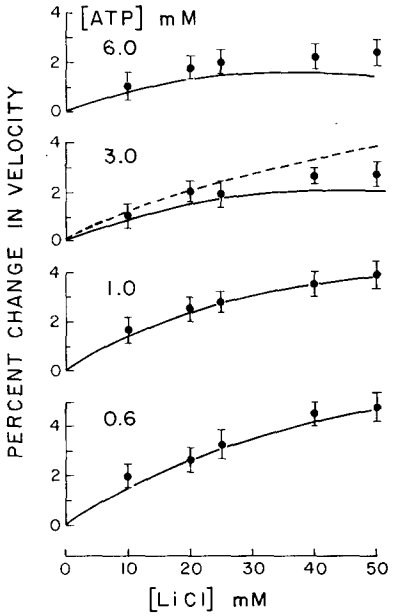

Fig. 1. Stimulation of ($Na^+ + K^+$)-dependent ATPase activity by LiCl. Enzymatic activity was determined in the presence of 90 mM NaCl and 10 mM KCl in the standard medium (see Methods) but with the concentrations of ATP shown; the $MgCl_2$ concentration was 3 mM except in incubations with 6 mM ATP when it was increased to 6 mM. The percentage increases in initial velocity resulting from the additions of the LiCl concentration indicated are presented, with the standard errors of the mean. The solid lines represent Eqn G using the kinetic parameters of Table 1, while the dashed line represents Eqn I.

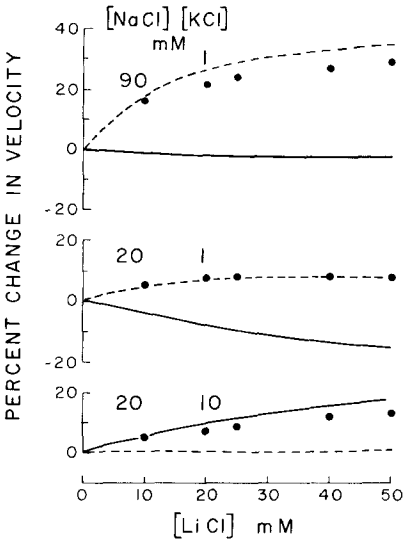

Fig. 2. Stimulation by LiCl in the presence of low concentrations of KCl or of NaCl. Experiments were performed as in Fig. 1, except that in all cases 3 mM ATP was present, and the concentrations of NaCl and KCl were as shown. The percentage increases in initial velocity resulting from the additions of LiCl are presented as in Fig. 1; here the standard errors were of the same magnitude as the data symbols and are not shown. The solid and dashed lines again represent Eqns G and I, respectively, using the parameters of Table I.

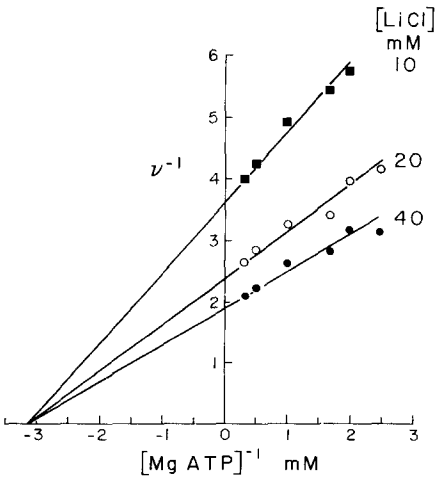

Fig. 3. Effect of LiCl on the apparent K_M for Mg · ATP. Initial velocities were determined from incubations in the presence of 90 mM NaCl, the concentrations of LiCl indicated, and with the ATP and $MgCl_2$ concentrations varied as shown, so that the concentration of $MgCl_2$ always exceeded that of ATP by 0.5 mM. Data are presented in terms of a double-reciprocal plot of velocity against the calculated Mg · ATP concentration [13].

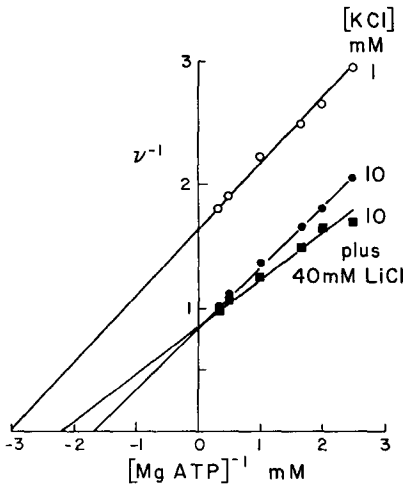

Fig. 4. Effect of KCl on the apparent K_M for Mg · ATP. Initial velocities were determined, as in Fig. 3, from incubations in the presence of 90 mM NaCl and 1 mM KCl (○), or 10 mM KCl (●) or 10 mM KCl plus 40 mM LiCl (■).

α-sites are readily distinguishable, by a variety of criteria, from the β-sites through which K^+ activates the ATPase reaction [7].

A plausible explanation might then involve Li^+ stimulating the ATPase by displacing K^+ from the α-sites and thus effectively lowering the apparent K_M for Mg · ATP. As an initial test of this proposal the effect of Li^+ on the apparent K_M for Mg · ATP was examined in experiments in which Li^+ was substituted for K^+ (Fig. 3): no appreciable change in the apparent K_M was seen as the Li^+ concentration was increased. By contrast, the apparent K_M for Mg · ATP increased with increasing KCl concentrations (Fig. 4), as previously demonstrated [6, 10]. Furthermore, addition of Li^+ together with 10 mM K^+ decreased the apparent K_M for Mg · ATP compared to that with K^+ alone (Fig. 4): from 0.60 mM in the absence of LiCl to 0.57 mM, 0.52 and 0.45 mM in the presence of 10, 20 and 40 mM LiCl, respectively. (With both K^+ and Li^+ the apparent maximal velocity at infinite Mg · ATP increased with the concentration of cation, as would be expected with successively higher levels of an activator of the ATPase reaction.)

If, as proposed earlier [7], K^+ binding to the α-sites is mutually incompatible with Mg · ATP binding to the substrate sites, then the apparent K_M, K'_M, is given by [6]:

$$K'_M = K_M \left\{ 1 + \left[\frac{[K^+]}{K_1 \left(1 + \frac{[Na^+]}{K_2} \right)} \right]^{n_1} \right\} \qquad \text{(A)}$$

where K_1 is the dissociation constant for K^+ from the inhibitory α-sites and n_1 is the index of cooperativity between the α-sites when occupied by K^+. Since Na^+ antagonizes the effects of K^+ on K'_M in a competitive fashion without itself affecting K'_M [6], a competitive inhibitory factor toward K^+ binding is included, in which K_2 is the

dissociation constant for Na^+ from the α-sites. To include Li^+ as a competitor toward K^+ at the α-sites a formulation in terms of two simultaneously acting competitors, I and I', is required:

$$v = \frac{V}{1 + \frac{K_M}{[S]}\left(1 + \frac{[I]}{K_i} + \frac{[I']}{K_i'}\right)} \tag{B}$$

Incorporating this expression into Eqn A, with K_3 representing the dissociation constant for Li^+ at the α-sites, gives:

$$K_M' = K_M \left\{1 + \left[\frac{[K^+]}{K_1\left(1 + \frac{[Na^+]}{K_2} + \frac{[Li^+]}{K_3}\right)}\right]^{n_1}\right\} \tag{C}$$

K_3 may be evaluated by examining the concentration of Li^+ for half-maximal activation ($K_{0.5}$) of the K^+-dependent phosphatase reaction (Fig. 5), since it is through the α-sites that the phosphatase reaction is activated [7, 9, 11]. The $K_{0.5}$ for Li^+ increased with the Mg^{2+} concentration, just as the $K_{0.5}$ for K^+ varies with Mg^{2+} in a competitive fashion [7, 11]. In assaying the ATPase reaction the concentrations of free Mg^{2+} are routinely quite low due to chelation by ATP; however, in assaying the phosphatase reaction the concentrations of free Mg^{2+} are routinely far greater

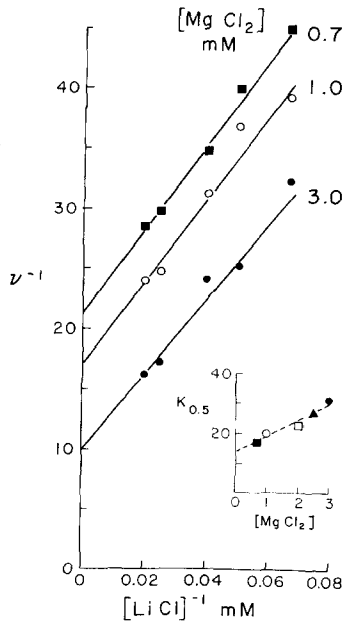

Fig. 5. Li^+-dependent nitrophenylphosphatase activity. Phosphatase activity was determined in the standard medium (see Methods), but with the concentrations of $MgCl_2$ shown and with LiCl substituted for KCl. Data are presented in terms of double-reciprocal plots. In the inset, the $K_{0.5}$ for LiCl from these experiments is plotted against the $MgCl_2$ concentration; in addition, the values from experiments with 2.0 (□) and 2.5 (▲) mM $MgCl_2$ are also plotted.

TABLE I

KINETIC CONSTANTS

Values were selected as described in the text, and are used to generate the lines of Figs 1 and 2, using Eqns G and I. Units for the K values are mM, maximal velocities are in arbitrary units and n values are dimensionless.

Constant	Value
K_M	0.32
K_1	1.0
K_2	6.0
K_3	14
K_4	0.22
K_5	40
K_6	7.0
K_7	100
K_8	1.0
K_9	5.0
V	1.0
V_{Li}	0.75
n_1	1.4
n_2	1.4
n_3	1.0

because of the poor chelating ability of the substrate [11]. Consequently, an estimate for the dissociation constant for Li^+, K_3, that is more pertinent to the ATPase reaction may be approached by extrapolating the change in $K_{0.5}$ to zero Mg^{2+} concentration (Fig. 5, inset). This value, 14 mM, may then be substituted in Eqn C, together with values for K_1, K_2 and n_1 in accord with previous determinations [6–9], as listed in Table I. This formulation assumes that occupancy of the α-sites by Li^+ has, like occupancy by Na^+, little effect on enzymatic properties: this is consistent with the failure of Li^+ to alter the K_M for Mg · ATP (Fig. 3) and the poor efficacy of Li^+ as an activator of the phosphatase reaction (Fig. 5: maximal velocity with Li^+ is about a tenth that with K^+).

Actions of Li^+ at the β-sites

Although the interactions described above might seem to account for stimulation by Li^+ in the presence of high K^+ concentrations, a further consideration must be introduced before testing this model quantitatively. Li^+ should also compete with K^+ at the β-sites, through which the overall ATPase reaction is activated. These β-sites are high-affinity sites for K^+ that become demonstrable only after enzyme phosphorylation [7–9, 11]. The velocity of the ATPase should be proportional to the occupancy of these sites by K^+:

$$v \propto \frac{V}{1+\left[\frac{K_4}{[K^+]}\left(1+\frac{[Na^+]}{K_5}\right)\right]^{n_2}} \qquad (D)$$

where K_4 is the dissociation constant for K^+ at the β-sites and n_2 is the index of cooperativity between the β-sites when occupied by K^+. As at the α-sites, Na^+ acts as

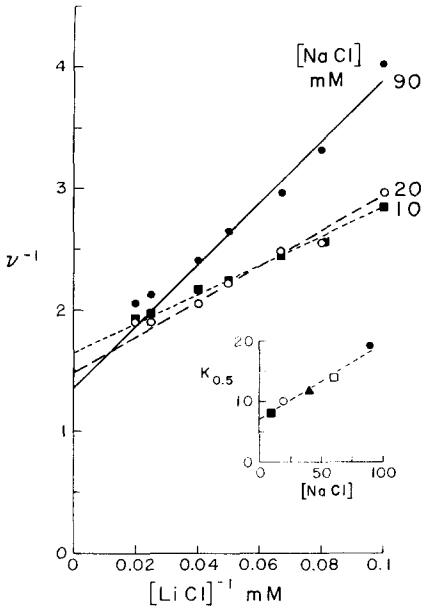

Fig. 6. Activation of the ATPase reaction by LiCl. ATPase activity was determined in the presence of 3 mM $MgCl_2$, 3 mM ATP, and the concentrations of LiCl shown, at fixed levels of NaCl: 90 mM (●), 20 mM (○) and 10 mM (■). In the inset the $K_{0.5}$ for LiCl from these experiments is plotted against the NaCl concentration; the values from experiments with 40 (▲) and 60 (□) mM NaCl are also plotted.

an antagonist toward K^+ at these β-sites [9, 12]; this is represented here in terms of competitive inhibition, with K_5 the dissociation constant for Na^+ at the β-sites. K_4, K_5 and n_2 have been evaluated in terms of the $K_{0.5}$ for K^+ at various concentrations of Na^+ [9, 12], and values in accord with these experiments are listed in Table I.

Li^+ can substitute for K^+ in activating the ATPase reaction, and its dissociation constant at the β-sites may be approached in terms of the $K_{0.5}$ for this reaction (Fig. 6). As has been seen with K^+, there is apparent competition between Li^+ and Na^+: the $K_{0.5}$ for Li^+ increased with the Na^+ concentration. By extrapolation (Fig. 6, inset) the $K_{0.5}$ for Li^+ at zero Na^+ concentration would be about 7 mM (Table I).

The maximal velocity with Li^+ was no greater than 0.75 that with K^+ (Fig. 6), and may be less. The generous estimate is made assuming that the deviation from linearity seen at higher Li^+ concentrations is largely due to competition with Na^+ at the Na^+ sites (see below). On this basis ATPase activity in the absence of K^+ would be proportional to the Li^+ concentration, in an analogy to Eqn D:

$$v \propto \frac{V_{Li}}{1+\left[\frac{K_6}{[Li^+]}\left(1+\frac{[Na^+]}{K_5}\right)\right]^{n_3}} \qquad \text{(E)}$$

where V_{Li} is the relative maximal velocity with Li^+ as a substitute for K^+, K_6 is the dissociation constant for Li^+ at the Li^+ sites and n_3 is the index of cooperativity between the β-sites when occupied by Li^+ (in keeping with the straight lines drawn in Fig. 6, n_3 is assumed to be 1.0).

In the presence of K^+, Li^+ and Na^+, the expression relating the total velocity as the sum of the velocity for each activator at the β-sites (K^+ and Li^+) is:

$$v \propto \frac{V}{1+\left[\frac{K_4}{[K^+]}\left(1+\frac{[Na^+]}{K_5}+\frac{[Li^+]}{K_6}\right)\right]^{n_2}} + \frac{V_{Li}}{1+\left[\frac{K_6}{[Li^+]}\left(1+\frac{[Na^+]}{K_5}+\frac{[K^+]}{K_4}\right)\right]^{n_3}} \quad \text{(F)}$$

The extent of competition between Na^+ and Li^+ at the Na^+ sites, through which Na^+ activates the ATPase reaction, must also be considered. K^+ appears to be a significant competitor toward Na^+ at the Na^+ sites, as shown by determinations of the $K_{0.5}$ for Na^+ at various K^+ concentrations [12]: the apparent dissociation constant for Na^+ is 2 mM while the K_i for K^+ is about 10 mM [9]. Analogous experiments, measuring the $K_{0.5}$ for Na^+ at various Li^+ concentrations, are consistent with mutual competition at the Na^+ sites, as well as at the β-sites (Figs 6 and 7). The plot of $K_{0.5}$ against Li^+ concentration (Fig. 7, inset) is, however, quite shallow, and it can be estimated that the K_i for Li^+ is greater than 100 mM. Consequently, in these experiments in which Na^+ concentrations (20–90 mM) are far greater than the apparent dissociation constant for Na^+ at the Na^+ sites, while the Li^+ concentrations (10–50 mM) are far lower than the K_i, effects of Li^+ at the activating Na^+ sites may be safely assumed to exert a quantitatively minor effect, and will be ignored here.

Evaluation of the model

For the model being described, the velocity of the ATPase reaction in the presence of Na^+, K^+ and Li^+ would thus be proportional both to factors affecting the K_M for Mg · ATP and to occupancy of the activating β-sites by K^+ or Li^+; as a first approximation, Eqns C and F may be combined:

$$v \propto \left\{\frac{1}{1+\frac{K_M}{[Mg\cdot ATP]}\left[1+\left(\frac{[K^+]}{K_1\left(1+\frac{[Na^+]}{K_2}+\frac{[Li^+]}{K_3}\right)}\right)^{n_1}\right]}\right\} \quad \text{(G)}$$

$$\cdot \left\{\frac{V}{1+\left[\frac{K_4}{[K^+]}\left(1+\frac{[Na^+]}{K_5}+\frac{[Li^+]}{K_6}\right)\right]^{n_2}} + \frac{V_{Li}}{1+\left[\frac{K_6}{[Li^+]}\left(1+\frac{[Na^+]}{K_5}+\frac{[K^+]}{K_4}\right)\right]^{n_3}}\right\}$$

Substituting the kinetic parameters of Table I for Mg · ATP, Na^+ and K^+ taken from previous determinations [6–9, 12] and for Li^+, as evaluated above, gives rise to the solid lines of Figs 1 and 2. Reasonable agreement obtains at high K^+ concentrations and with varying concentrations of Mg · ATP (Fig. 1; Fig. 2, bottom). But at low K^+ concentrations (Fig. 2, top and middle) this model is clearly inadequate. Eqn G cannot be made to fit the stimulation by Li^+ in the presence of low concentrations of K^+ using any plausible values of the kinetic parameters. The uncertainties in the values for the kinetic parameters from such studies may be as high as 25 % of the values presented in Table I [6–8, 12]. Nevertheless, no appreciable improvement in

fitting the equation to the observed data of Fig. 2 can be achieved by varying the parameters of Table I within such limits.

The difficulty with this formulation lies in the displacement by Li^+ of the more effective activating cation, K^+. At low K^+ concentrations Li^+ will markedly decrease that fraction of the total enzyme binding K^+ at the β-sites, and since the substitution of Li^+ for K^+ results in a lower velocity (cf. apparent maximal velocity with K^+ and Li^+), little or no increase in total enzymatic activity is found even though more β-sites may be filled by Li^+ plus K^+ than by K^+ alone. Therefore, although Eqn G describes certain aspects of the stimulation by Li^+, notably that in the presence of low Mg · ATP and high K^+ concentrations, other mechanism(s) must operate as well.

Alternative formulations

The possible sites by which cations interact with the enzyme include (i) moderate-affinity α-sites for K^+, (ii) high-affinity β-sites for K^+, (iii) low-affinity discharge sites for K^+ [6, 9], which, unlike the α-and β-sites, in vivo face the interior of the cell, (iv) high-affinity activating sites for Na^+, which would also face in vivo the interior, (v) low-affinity discharge sites for Na^+, across the permeability barrier from the activating Na^+ sites and (vi) activating divalent cation sites [13]. Of these, stimulation by Li^+ through occupancy of (i) the α-sites and (ii) the β-sites has been considered in Eqn G. Occupancy by Li^+ of (iv) the activating sites for Na^+ is inhibitory (Fig. 7), and displacement of divalent cations from (vi) their activating sites would also not be expected to stimulate. This leaves (iii) and (v), the discharge sites for K^+ and Na^+, respectively.

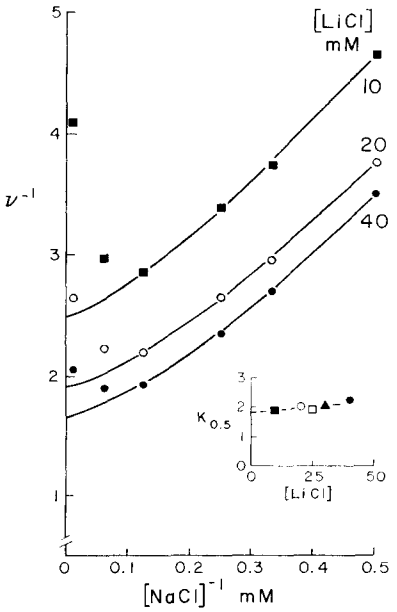

Fig. 7. Effect of LiCl on the activation of the ATPase reaction by NaCl. Experiments were performed as in Fig. 6 except that NaCl was varied at fixed levels of LiCl: 40 mM (●), 20 mM (○) and 10 mM (■). In the inset, the $K_{0.5}$ for NaCl from these experiments is plotted against the LiCl concentration; the values from experiments with 25 (□) and 30 (▲) mM LiCl are also plotted.

Tobin et al. [2] proposed that Li^+ stimulated the ATPase because of its ability to dissociate from the K^+-discharge sites more rapidly than could K^+. Li^+, when substituted for K^+, would not be expected to traverse the total pump mechanism of the enzyme (acceptance sites to discharge sites) faster than K^+, for the maximal velocity with Li^+ is clearly less than that with K^+. Thus, rather than being better able to traverse the pump, if Li^+ is to act at the discharge sites to speed release of cations it must do so by approaching these sites from the medium. If it is proposed that stimulation by Li^+ is due to its dissociating from the discharge sites more readily than K^+ would [2, 14], then Li^+ must bind to the discharge sites rapidly, effectively displacing the transported K^+, and then dissociate still more rapidly from these sites than would K^+ itself; this seems rather implausible.

However, a stronger argument exists against Li^+ stimulating the ATPase and the pump by acting at the discharge sites for K^+: if K^+ binding to the discharge sites does inhibit the ATPase, then a greater relative stimulation by an antagonist such as Li^+ would occur at high concentrations of K^+ compared to that at low concentrations. In fact, the opposite is seen (Figs 1 and 2). Given an inhibitor (here K^+) and an antagonist to this inhibitor (here Li^+), it can be demonstrated that for a given level of antagonist the relative stimulation (percentage increase in velocity) is greater at high concentrations of inhibitor than it is at low concentrations of inhibitor.

Li^+ stimulation by relieving inhibition due to Na^+

Of the likely sites listed above at which Li^+ might stimulate the ATPase there thus remains only (v) the discharge sites for Na^+, sites at which occupancy by Na^+ would be expected to inhibit: in the functioning of the pump, Na^+ must be released with each catalytic cycle. Examination of the stimulation by Li^+ suggests a plausible model in terms of these discharge sites: whereas occupancy by Na^+ is inhibitory, Na^+ might be displaced by K^+ or Li^+ (i.e. the monovalent cations would be mutually competitive as at the other cation sites) to relieve the inhibition. Thus stimulation due to Li^+ would be greater at low than at high K^+ concentrations. Moreover, following the argument developed above, the relative effect of Li^+ as an antagonist to the inhibitor Na^+ would be greater at high Na^+ concentrations than at low. These predictions are in accord with the data (Figs 1 and 2).

Occupancy by Na^+ of such inhibitory sites could be described in terms of a noncompetitive inhibition:

$$v = \frac{V}{\left(1+\frac{K_M}{[S]}\right)\left(1+\frac{[I]}{K_i}\right)} \tag{H}$$

With such inhibition antagonized by K^+ and Li^+, the enzymatic activity would then be proportional to the occupancy of these sites:

$$v \propto \frac{1}{1+\left[\frac{[Na^+]}{K_7\left(1+\frac{[K^+]}{K_8}+\frac{[Li^+]}{K_9}\right)}\right]} \tag{I}$$

where K_7, K_8 and K_9 are the dissociation constants for Na^+, K^+ and Li^+, respectively.

Although no firm values for the dissocation constants for Na^+, K^+ and Li^+ at the Na^+-discharge sites are available, a plausible value for Na^+, in accord with the requirement that Na^+ be released in vivo into the high-Na^+ extracellular fluid, would be of the order of 100 mM, at least. Correspondingly, the stimulation at low K^+ concentrations can be described fairly well with dissociation constants for K^+ of 1 mM and for Li^+ of 5 mM (Table I): the dashed lines of Figs 1 and 2 result from substituting these values into Eqn I. An exhaustive examination of various values for K_7, K_8 and K_9 to provide optimal fit to the data has not, however, been undertaken; nevertheless, a reasonable simulation of the effects of Li^+ at low concentrations of K^+ is seen for both high and low Na^+ concentrations*. To account for the stimulations under all the conditions examined, high and low Mg · ATP concentrations, high and low Na^+ and K^+ concentrations, both Eqns G and I are required, the stimulation resulting from the composite of these two mechanisms. Thus, although Eqn I describes the stimulation by Li^+ in the presence of 10 mM KCl, 90 mM NaCl and 3 mM Mg · ATP essentially as well as does Eqn G (Fig. 1), Eqn I cannot describe the response to variations in the Mg · ATP concentration; conversely, Eqn G, while incorporating expressions relating to the Mg · ATP concentration, does not satisfy the data at low KCl concentrations (Fig. 2).

CONCLUSIONS

Stimulation of the $(Na^+ + K^+)$-dependent ATPase by LiCl may be accounted for by a composite kinetic model in which Li^+ relieves inhibition by competing with K^+ at the α-sites and with Na^+ at the Na^+-discharge sites: reasonable quantitative agreement obtains with plausible values for the kinetic parameters. This formulation also proposes certain properties for the Na^+-discharge sites of the membrane Na^+/K^+ pump, sites that previously have been approached in terms of Na^+-Na^+ exchange processes [15] and that may also be reflected in low-affinity external Na^+ sites that modify ouabain binding [16]. In addition, the data support formulations [6–9] in terms of α- and β-sites for K^+, through which activation of specific enzymatic reactions and inhibition of others can occur. Albers and associates [17–19] have also described two classes of K^+ site on the enzyme in terms of a heterotropic relaxation model; although certain implications differ between the models, the general properties of the two classes of K^+ site are similar.

Beaugé [20] has recently reported that, in the absence of K^+, the addition of Li^+ to the external bathing medium can activate the efflux of Na^+ from muscle cells, an efflux attributed to the Na^+/K^+ pump. The maximal velocity of Na^+ efflux with Li^+ as the external activating cation was less than that with K^+; nevertheless, in the presence of low concentrations of K^+ marked stimulation by Li^+ occurred. These observations are thus in general accord with the above models, as well as in terms of Li^+ stimulating the ATPase through sites that lie, in vivo, on the external membrane surface. On the other hand, Partridge and Thomas [21] found no evidence for a stimulation of the Na^+/K^+ pump after injection of Li^+ into nerve cells.

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* Moreover, with these values for K_7 and K_8, only 2 % of such external sites would be occupied by Na^+ in the presence of 135 mM Na^+ and 5 mM K^+, in keeping with an efficient functioning of the membrane pump.

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