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Lithium and Rubidium Interactions with Sodium- and Potassium-Dependent Adenosine Triphosphatase: A Molecular Basis for the Pharmacological Actions of these Ions

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SUMMARY

TOBIN, THOMAS, AKERA, TAI, HAN, C. S., AND BRODY, THEODORE M.: A molecular basis for the pharmacological actions of lithium and rubidium on sodium- and potassium dependent adenosine triphosphatase. *Mol. Pharmacol.* **10,** 501–508 (1974).

Although lithium ion substitutes poorly for potassium ion in the reaction cycle of (Na^+ + K^+)-ATPase (ATP phosphohydrolase, EC 3.6.1.3), it consistently activated this enzyme in the presence of sodium and potassium. In contrast, rubidium ion, a much more effective substitute for potassium than lithium, was generally inhibitory in the presence of sodium and potassium. Li^+ did not appear to activate the enzyme by stimulating its phosphorylation from ATP. Rather, Na^+ was required for Li^+ to stimulate ATPase activity, and Li^+ directly (though weakly) stimulated dephosphorylation. Under conditions such that increasing concentrations of K^+ and Rb^+ inhibited turnover of the enzyme, Li^+ stimulated its activity above values observed with K^+ or Rb^+. It appears that Li^+ stimulates the turnover of the ATPase by triggering its dephosphorylation and dissociating rapidly from the dephospho-enzyme, thus allowing the enzyme to rephosphorylate readily. With Rb^+ the stability of the dephospho-enzyme·rubidium complex hinders subsequent rephosphorylation of the enzyme and thus inhibits its turnover. Because Li^+ and Rb^+ respectively stimulate and inhibit (Na^+ + K^+)-ATPase relative to its activity in the presence of Na^+ and K^+, they may hyperpolarize or depolarize nerve cells in corresponding fashion. It is suggested that these actions of lithium and rubidium on the turnover of (Na^+ + K^+)-ATPase may be involved in their pharmacological actions.

INTRODUCTION

Although lithium ion is effective in both the prophylaxis and treatment of manic-depressive illness, the molecular basis of its pharmacological actions remains unclear (1).

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Because lithium treatment has been shown to reduce brain sodium levels (2), it seemed appropriate to investigate the interactions of lithium ion with other alkali metal cations important in the functioning of the central nervous system.

The (Na^+ + K^+)-ATPase (ATP phosphohydrolase, EC 3.6.1.3), or the Na^+ pump, is the biochemical basis of active cation transport in mammalian tissues (3). Since this system sharply discriminates among sodium, potassium, and lithium ions (4), it consti-

tutes a potential biochemical point of interaction for the monovalent cations, the more so since this discrimination is apparently much sharper than that of the action potential mechanism (5). However, studies on the interactions of monovalent cations with $(Na^+ + K^+)$-ATPase have tended to concentrate on the actions of Na^+ and K^+ (6, 7), although the interaction of rubidium ion with this enzyme has been investigated in some detail (8). Previous work with lithium ion has shown that it is a poor activator of the enzyme system, substituting for potassium ion with about one-eighth the apparent affinity and one-quarter the efficacy of potassium ion (7). There are, however, reports which suggest that lithium will substitute for Na^+ in the phosphorylation step of this enzyme (9) and that, like Na^+, it stimulates ATP hydrolysis in red blood cells (10).

Because lithium is a poor substitute for K^+ in the reaction mechanism of this enzyme, a common assumption concerning the action of Li^+ on $(Na^+ + K^+)$-ATPase has been that it acts to inhibit the enzyme *in vivo* (11, 12). However, the results presented here suggest that Li^+ stimulates the activity of $(Na^+ + K^+)$-ATPase *in vitro* by stimulating its dephosphorylation and dissociating rapidly from the enzyme to allow its rephosphorylation. In contrast, Rb^+ inhibits the turnover of the enzyme by forming a relatively stable rubidium-enzyme complex and slowing its subsequent rephosphorylation. It is suggested that the pharmacological actions of lithium and rubidium ions may be related to their actions on the rate of turnover of the sodium pump.

MATERIALS AND METHODS

Rat brain $(Na^+ + K^+)$-ATPase was prepared by the method of Akera and Brody (13) for the initial experiments. In the experiments subsequent to Fig. 3 "low-sodium" enzymes were required, and so all enzymes were washed twice after treatment with sodium iodide by alternate centrifugation at $10^6 \times g$ and resuspension in 10 mM Tris–1 mM EDTA and 2 mM $MgCl_2$. Tris-buffered tetrahydro-EDTA was used throughout the enzyme preparation rather than disodium EDTA as described by Akera and Brody (13). When the enzymes were prepared in this way little activation by lithium alone was observed (see RESULTS), and such low-sodium enzymes were used in the remaining experiments. The protein concentration of these enzymes was estimated by the method of Lowry *et al.* (14).

Phosphorylation of nerve cell membranes and estimation of their ATPase activity were performed as described by Tobin *et al.* (15). Modification of the $(Na^+ + K^+)$-ATPase activity assay from that described by Tobin *et al.* (15) is detailed in the appropriate figure legends. The $(Na^+ + K^+)$-ATPase activity of the membranes varied between 150 and 301 μmoles of P_i per milligram of protein per hour at 37°, and more than 90% of the activity was ouabain-sensitive. The lithium chloride used in these experiments was Mallinckrodt analytical grade and contained less than 1 part in 10,000 of K^+. Unless otherwise noted, all experimental points are the means of values obtained with four separate enzyme preparations ± standard errors. To allow comparison of data obtained on different enzymes, an appropriate value was set at 100% in each case and other values were expressed as a percentage of this (15). Statistical significance was calculated by means of a paired *t*-test, the criterion for significance being $p \times 0.05$.

RESULTS

A common assumption made concerning the interaction of Li^+ with $(Na^+ + K^+)$-ATPase is that this ion inhibits the activity of the enzyme *in vivo* (11, 12). Figure 1 shows typical data which suggest this action of lithium. In this experiment the activity of the enzyme was assayed in the presence of different concentrations of Na^+ and K^+ (or Li^+ or Rb^+). While Rb^+ was a relatively effective substitute for K^+, Li^+ was not effective, particularly at the lower concentrations attained *in vivo*. On the basis of these data it is tempting to assume that when Li^+ or Rb^+ is added in the presence of Na^+ and K^+, as occurs *in vivo*, their primary action will be inhibitory, since they may be expected to interfere with the actions of the more effective potassium ion.

Figure 2 shows the effect of Li^+ on $(Na^+ + K^+)$-ATP assay in the presence of different concentrations of Na^+ and K^+. Under all

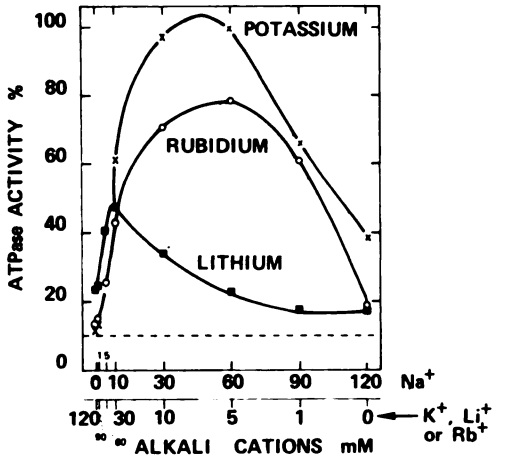

FIG. 1. *Effects of sodium and potassium, lithium, or rubidium ions on rat brain $(Na^+ + K^+)$-ATPase activity*

Rat brain $(Na^+ + K^+)$-ATPase was incubated with the indicated concentrations of monovalent cations in the presence of 5 mM MgATP at 37° for 10 min. The concentration of Na^+ was increased from left to right, and the concentrations of the other monovalent cations, from right to left. ×——×, ATPase activity in the presence of Na^+ and K^+; ○——○, in the presence of Na^+ and Rb^+; ■——■, in the presence of Na^+ and Li^+. Activity is plotted as a percentage of that in the presence of 60 mM Na^+ and 5 mM K^+, which was about 217 μmoles of P_i per milligram of protein per hour. ATPase activity in the presence of 250 μM ouabain is indicated by the dashed line. The points are single experimental determinations.

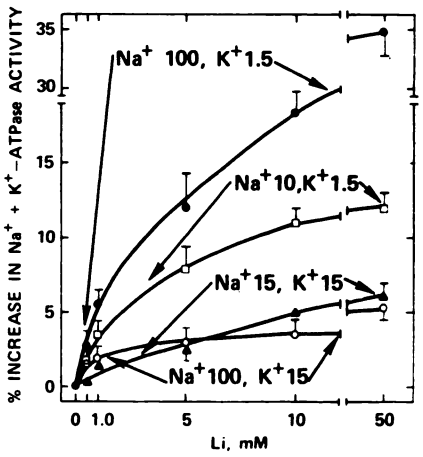

FIG. 2. *Effects of lithium ion on $(Na^+ + K^+)$-ATPase activity in the presence of sodium and potassium*

Rat brain $(Na^+ + K^+)$-ATPase was incubated with 5 mM MgATP at 37° for 10 min in the presence of the indicated concentrations of monovalent cations. Activity in the presence of Na^+ and K^+ was set at 100%, and the changes produced by the indicated concentrations of Li^+ are plotted as percentage increases in activity. ○——○, activity in the presence of 100 mM Na^+ and 15 mM K^+; ▲——▲, 15 mM Na^+ and 15 mM K^+; □——□, 10 mM Na^+ and 1.5 mM K^+, ●——●, 100 mM Na^+ and 1.5 mM K^+. Activity in the presence of 100 mM Na^+ and 15 mM K^+ averaged 263 ± 21 μmoles of P_i per milligram of protein per hour.

conditions tested Li^+ acted to increase the activity of the $(Na^+ + K^+)$-ATPase. As might be expected, this effect was greatest when the concentration of K^+ in the system was low. No inhibitory actions of up to 100 mM Li^+ were apparent, even in the presence of 100 mM Na^+ and 15 mM K^+, the optimal cation concentrations for activation of this enzyme. These observations raise the possibility that the action of Li^+ *in vivo* is to stimulate the $(Na^+ + K^+)$-ATPase.

In contrast to the stimulatory action of Li^+, Rb^+ was inhibitory to the $(Na^+ + K^+)$-ATPase except when the Na^+ concentration was high and the K^+ concentration low (Fig. 3). This finding with Rb^+ was not unexpected, since it has been shown to bind to the E_2 form of $(Na^+ + K^+)$-ATPase and only slowly dissociate from it (8). Since the dissociation of K^+ or its congeners from the E_2 form of the $(Na^+ + K^+)$-ATPase is considered to be the rate-limiting step in the reaction sequence of this enzyme (8), it was not surprising that Rb^+ acted to inhibit this enzyme, for Rb^+ forms one of the most stable ion-enzyme complexes of all the monovalent cations.

The mechanism by which Li^+ stimulates the $(Na^+ + K^+)$-ATPase was further studied. Because there are reports that Li^+ can increase the steady-state levels of the phospho-enzyme, that Li^+ alone stimulates ATP hydrolysis in the presence of Mg^{2+} (7) (Fig. 1), and that Li^+ mimics the action of Na^+ on the reaction sequence of this enzyme (10), its effects on the phosphorylation of $(Na^+ + K^+)$-ATPase from [γ-^{32}P]ATP were investigated. No effects of up to 50 mM Li^+ on the steady-state level of the phospho-enzyme formed in the presence of 1 mM

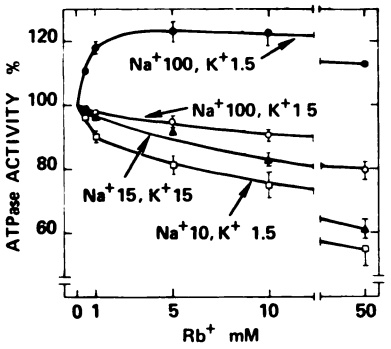

FIG. 3. *Effect of rubidium ion on* $(Na^+ + K^+)$-*ATPase activity in the presence of sodium and potassium*

Duplicate of Fig. 2, except that Rb^+ was substituted for Li^+. The ionic conditions and symbols are as in Fig. 2, and $(Na^+ + K^+)$-ATPase activity in the absence of rubidium averaged 248 ± 13.5 μmoles of P_i per milligram of protein per hour.

Mg^{2+} and 0.05 mM [γ-^{32}P]ATP were observed, suggesting that Li^+ has no effect on the steady-state labeling of the enzyme from [γ-^{32}P]ATP.

Lack of an effect of Li^+ on the steady-state levels of the phospho-enzyme did not rule out acceleration by lithium of both the phosphorylation and dephosphorylation steps, which would result in increased turnover of the enzyme without any change in the steady-state levels of the phosphorylated form. To test this possibility, rat brain enzyme was incubated with various concentrations of Li^+, alone and in the presence of Na^+ and K^+. In these experiments no activation of the enzyme by Li^+ or $Li^+ + K^+$ was seen if care was taken exclude Na^+ from the reaction system (see MATERIALS AND METHODS). However, the addition of Na^+ to the reaction system allowed activation by Li^+, suggesting that the latter only stimulates $(Na^+ + K^+)$-ATPase after the system has interacted with Na^+.

Figure 4 shows the effects of Li^+ and Rb^+ on the dephosphorylation step of the $(Na^+ + K^+)$-ATPase enzyme system. These experiments were performed at 0° because the rate of the dephosphorylation step is too fast to be monitored at 37° by techniques currently available (15). Although Li^+ stimulated the dephosphorylation step of this enzyme, it was much less effective than K^+. This action of Li^+ is consistent with the data of Fig. 5, which show that Na^+ is required for Li^+ to stimulate the activity of the enzyme. However, in contrast to the relatively poor action of Li^+ on the dephosphorylation step, Rb^+ was more effective than K^+ in stimulating dephosphorylation of $(Na^+ + K^+)$-ATPase.

Stimulation by Li^+ of the dephosphorylation step in the reaction cycle is not sufficient to explain its stimulation of $(Na^+ + K^+)$-ATPase activity in the presence of optimal concentrations of Na^+ and K^+. For example, Rb^+ was more effective than K^+ in triggering the dephosphorylation step, yet its action when added in the presence of Na^+ and K^+ was generally inhibitory. The mechanism of the Li^+ stimulation and Rb^+ inhibition of the enzyme probably depends on differences in stability of the lithium, potassium, and rubidium ion·enzyme complexes. In experiments on the stability of these complexes in kidney enzymes at 0°, Post and co-workers (8) showed that the lithium·enzyme complex is relatively labile while the rubidium·enzyme complex is apparently the most stable of all the monovalent cation·enzyme complexes. Thus, while all these agents act to stimulate the dephosphorylation step of $(Na + K^+)$-ATPase, the relative stability of the cation·enzyme complexes results in rubidium inhibiting turnover of the enzyme relative to turnover in the presence of potassium, while lithium stimulates it. This hypothesis requires that the stability of the rat brain lithium·enzyme complex be less than that of the potassium·enzyme complex, so that lithium can dissociate more rapidly from the enzyme and thus allow rapid rephosphorylation and increased turnover of the enzyme.

Although it is possible to measure the stability of these ion·enzyme complexes directly at 0° (8), it is not currently possible at more physiological temperatures. Therefore the experimental approach was indirect. In experiments to compare the stability of the potassium, lithium, and rubidium ion·enzyme complexes, their abilities both to stimulate and to inhibit enzyme activity were compared. The experiments are based on recent data of Post *et al.* (8), which show that potassium forms a stable complex with the E_2 form of the enzyme and that this

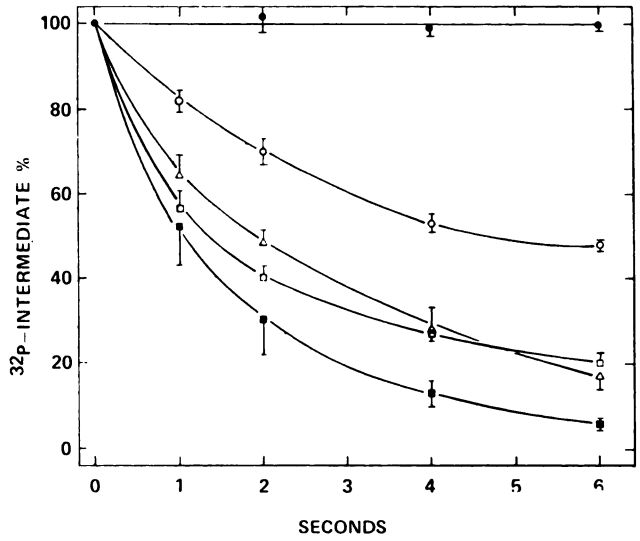

FIG. 4. *Effects of lithium or rubidium ion on rate of dephosphorylation of* $(Na^+ + K^+)$*-ATPase*
About 200 μg of rat brain enzyme were incubated with 15 mM NaCl, 5 mM $MgCl_2$, and 50 mM Tris-HCl, pH 7.4, at 0°. Five seconds before indicated zero time 0.05 mM [γ-^{32}P]ATP was added to the system. ●——●, time course of the labeling of the membranes from [γ-^{32}P]ATP; ○——○, labeling when 1 mM unlabeled ATP was added to the system at zero time; △——△, labeling when 0.05 mM K^+ was added with the unlabeled ATP. The other symbols show labeling after 1 mM ATP and 5.0 mM Li^+ (□——□) or after 1 mM ATP and 0.05 mM Rb^+ (■——■). Labeling is plotted as a percentage of that at zero time in the absence of additions, which averaged 640 ± 69.4 pmoles of ^{32}P per milligram of protein. Labeling in the presence of 100 mM K^+ was deducted as background.

complex inhibits enzyme turnover under certain circumstances. Thus, if lithium is less effective than potassium or rubidium in complexing with the ATPase, it should be less effective in inhibiting turnover. Figure 5 shows such a study performed in the presence of 1 μM ATP at 23°, where the stability of the potassium·enzyme complex renders K^+ inhibitory to the turnover of the enzyme (8). Under these conditions Rb^+ is an even more effective inhibitor than K^+ consistent with the concept that the rubidium·enzyme complex is more stable than the potassium·enzyme complex. Li^+, however, did not inhibit but markedly activated the enzyme. These observations with Li^+ are consistent with the concept that it acts to trigger the dephosphorylation step in the $(Na^+ + K^+)$-ATPase reaction and then dissociates much more rapidly than either K^+ or Rb^+ to allow rephosphorylation of the enzyme. These findings are also in good agreement with the observations of Hegyvary and Post (4), who have shown that Li^+ is unique among the monovalent cations in that it does not displace ATP from $(Na^+ + K^+)$-ATPase or mimic the action of Na^+ in antagonizing the displacement of ATP from this enzyme by K^+.

If Li^+ and Rb^+ produce their pharmacological effects by respectively stimulating and inhibiting $(Na^+ + K^+)$-ATPase, they should produce these effects *in vitro* under conditions which approximate the state *in vivo*. Figure 6 shows that in the presence of the cation and nucleotide concentrations which activate the enzyme *in vivo*, Li^+ stimulates and Rb^+ both stimulates and inhibits its activity. In this study the concentration of Na^+ was 40 mM and that of ATP was 2 mM, the approximate concentrations of these ligands in brain cells (16). The K^+ concentration was varied from 0 to 4 mM, and Li^+ and Rb^+ were 3 and 2 mM, respectively. Under these conditions Li^+ stimulated the activity of the enzyme by up to 50%, consistent with previous data. The action of Rb^+ was biphasic, however, stimulating

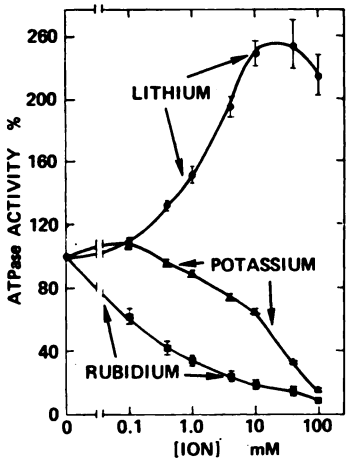

FIG. 5. *Effects of potassium, rubidium, and lithium on (Na^+)-ATPase activity at low ATP concentrations*

About 20 μg of rat brain enzyme were incubated with 10 mM NaCl, 1 mM $MgCl_2$, and 50 mM Tris-HCl, pH 7.4, in a final reaction volume of 1 ml, with the indicated concentrations of K^+, Li^+, or Rb^+ at 22°. The ATPase reaction was started by adding 1 μM ATP containing tracer amounts of [γ-^{32}P]ATP and stopped 30 sec later by the addition of 0.5 ml of 1.2 M $HClO_4$. The $^{32}P_i$ was then extracted into 1.5 ml of butyl acetate and counted in a liquid scintillation spectrometer. Na^+-ATPase activity was calculated by deducting ATPase activity observed in the presence of 250 μM ouabain. ●——●, ATPase activity in the presence of Li^+; ▲——▲, in the presence of K^+; ■——■, in the presence of Rb^+. Na^+-ATPase activity in the absence of other added monovalent cations is plotted as 100% ATPase activity and averaged about 25 nmoles of P_i per milligram of protein per minute.

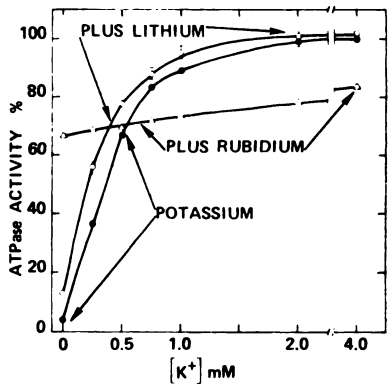

FIG. 6. *Effects of lithium and rubidium ion on ATPase activity under "physiological" conditions*

Forty micrograms of rat brain enzyme were incubated with 40 mM NaCl, 2 mM ATP, 5 mM $MgCl_2$, and 50 mM Tris-HCl, pH 7.4, for 5 min at 37° in the presence of 3 mM Li^+ and 2 mM Rb^+. K^+ concentration was varied as indicated on the horizontal axis. ●——●, activity in the presence of the indicated concentrations of K^+; ○——○, in the presence of K^+ plus 3 mM Li^+; △——△, in the presence of K^+ plus 2 mM Rb^+. Activity is plotted as a percentage of that in the presence of 40 mM Na^+ and 4 mM K^+, which averaged 137 ± 3.1 μmoles of P_i per milligram of protein per hour. Li^+ significantly increased ATPase activity ($p < 0.05$) at all concentrations of K^+ less than 2 mM.

enzyme activity below 0.5 mM K^+ and inhibiting it above this concentration. Since the concentration of K^+ is greater than 0.5 M at the extracellular potassium-activating site of the enzyme *in vivo*, these data are consistent with the concept that Li^+ and Rb^+ may act *in vivo* to stimulate and inhibit (Na^+ + K^+)-ATPase, respectively.

DISCUSSION

As reported by other investigators, Li^+ in the presence of Na^+ and ATP is a poor activator of (Na^+ + K^+)-ATPase and in some of the early experiments of Skou (7) it was clearly the weakest activator of all the monovalent cations. Despite its poor effect in the presence of Na^+, Li^+ consistently stimulated the activity of the enzyme above the level seen in the presence of Na^+ and K^+. This effect was observed even in the presence of 100 mM Na^+ and 15 mM K^+, cation concentrations at which most mammalian (Na^+ + K^+)-ATPases are considered fully activated, and also at low concentrations of ATP, where K^+ or Rb^+ was inhibitory. Even more remarkable is the fact that some activation was observed at concentrations up to 100 mM Li^+. These observations are in good agreement with those recently reported by Gutman *et al.* (17), who observed that Li^+ was able to activate renal medullary (Na^+ + K^+)-ATPase to 60% more than the level observed in the presence of Na^+ and K^+.

Li^+ did not appear to stimulate the ATPase by any action on the phosphorylation step. The steady-state level of phosphoenzyme formed in the presence of Mg^{2+},

[γ-^{32}P]ATP, and residual Na^+ was not altered by the presence of 50 mM Li^+ in the system. Similarly, Li^+ alone or together with K^+ was essentially unable to stimulate any ATPase activity, as might be expected if lithium stimulated the phosphorylation of this enzyme. Na^+ was required for Li^+ to stimulate ATPase activity, suggesting that the action of Li^+ on this enzyme occurs after the enzyme has interacted with Na^+. These results all suggest that the principal action of Li^+ is on the phospho-enzyme, and Fig. 4 shows directly the action of Li^+ in accelerating dephosphorylation of $(Na^+ + K^+)$-ATPase.

The results of Fig. 5 show that Li^+ is a more effective activator of the $(Na^+ + K^+)$-ATPase than either K^+ or Rb^+ under conditions in which the inhibitory actions of these ions would be maximal; Li^+ was virtually unable to inhibit the enzyme. These results emphasize the major difference bebetween lithium and the other ions which activate this enzyme, and provide a framework within which the stimulatory actions of lithium can be interpreted. The inability of Li^+ in forming inhibitory complexes with the $(Na^+ + K^+)$-ATPase is apparently the reason why this ion consistently stimulates $(Na^+ + K^+)$-ATPase, apparently being unable to form a stable lithium$\cdot E_2$ complex. Because essentially no inhibition of the ATPase by Li^+ was observed under the many ionic conditions tested, it seems reasonable to conclude that Li^+ will not act to inhibit the enzyme *in vivo*.

These very basic differences in the interaction of Li^+ and Rb^+ with $(Na^+ + K^+)$-ATPase may be related to their pharmacological actions. Rb^+ is concentrated 15-fold (18) inside brain cells, like K^+, and presumably substitutes electrophysiologically for K^+ both inside and outside the nerve cell. However, Rb^+ inside the nerve cell will inhibit the turnover of the sodium pump more than a similar concentration of K^+. Therefore in the presence of Rb^+ the sodium pump will turn over more slowly than in a normal cell, and somewhat less sodium and potassium (or rubidium) will be pumped by rubidium-loaded cells. Thus rubidium-treated cells will maintain a slightly lower membrane potential than normal cells and, other things being equal, will be more responsive to depolarizing influences. In this way the behavioral effects of Rb^+, which acts to increase locomotor activity (19) and aggression and produces electroencephalographic activation in monkeys (20) and rats (21), may be dependent on the fact that it dissociates somewhat less readily from the $(Na^+ + K^+)$-ATPase than does K^+.

In contrast to Rb^+, Li^+ may be expected to stimulate the $(Na^+ + K^+)$-ATPase *in vivo* and produce increased activity of the sodium pump. A simple hypothesis to explain the pharmacological actions of Li^+ would be to assume that it is transported like K^+ and substitutes electrophysiologically for K^+. In this way its lack of inhibitory actions on the pump would allow more rapid pumping by the sodium pump and hyperpolarization of nerve cells. Such hyperpolarized cells would be more resistant to depolarizing influences, and the resultant membrane stabilization might explain the pharmacological actions of lithium, whose therapeutic usefulness is in controlling the manic phase of manic-depressive psychosis.

This simple hypothesis is consistent with the observation that lithium therapy in rats reduces brain sodium levels and produces transient natriuresis (2), as would be expected if sodium pumping were increased in the presence of lithium. Against this hypothesis is the small size of the stimulation by lithium, especially at the higher concentrations of K^+ observed in Fig. 6. There may, however, be differences in the lithium sensitivity of $(Na^+ + K^+)$-ATPase in various portions of the central nervous system, as observed by Gutman *et al.* (17) in the kidney, and there also appears to be evidence that while lithium can activate this pump, it is not transported by it (22). Thus, as well as activating the sodium pump, Li^+ may increase its electrogenicity and in this way significantly increase the membrane potential of and electrically stabilize brain cells.

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