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EFFECTS OF CHRONIC DIETARY LITHIUM ON ACTIVITY AND REGULATION OF (Na^+,K^+)-ADENOSINE TRIPHOSPHATASE IN RAT BRAIN

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Abstract—Chronic dietary administration of lithium appeared to reduce (Na^+,K^+)-adenosine triphosphatase in rat brain by at least two mechanisms. Selective inhibition of the enzyme form with high affinity for ouabain occurred in hippocampus. Nonselective inhibition, manifested by decreased V_{max} for activation by Na^+, occurred in hippocampus, cortex and corpus striatum. There was no effect on Na^+ affinity. Because lithium administration decreased shock-elicited fighting behavior in the presence of moderate tissue lithium levels and in the absence of changes in weight or gross appearance or behavior, the effects on adenosine triphosphatase were pharmacologic rather than toxic.

The mechanisms of the behavioral effects of lithium ions have not been established, though lithium effects on many neuroeffector systems have been studied, and changes have been found in most of them [1–3]. Due to the physical properties of lithium ions [4, 5], the effects could result from interactions with the fluxes of cations that are involved in the activity of nerve cells, rather than from effects on a specific neurotransmitter.

Lithium ions share some physical properties with Na^+, K^+ and Mg^{2+} [4, 5], each of which is an effector of (Na^+,K^+)-ATPase [6], the enzymatic basis of coupled Na^+ efflux and K^+ influx [7]. *In vitro*, Li^+ has been shown to interact with a K^+ site [8, 9], and possibly with some Na^+ effects [10]. Li^+ can stimulate [11, 12] or inhibit [6] (Na^+,K^+)-ATPase, depending on ionic conditions.

(Na^+,K^+)-ATPase is involved in several phases of the regulation of nerve cell activity including maintenance and re-establishment of the resting membrane potential [13] and uptake of neurotransmitters and of other metabolites [14]. It has been proposed that (Na^+,K^+)-ATPase is involved in the actions of catecholamines [15], opiates [16] and thyroid hormone [17]. The possibility that (Na^+,K^+)-ATPase may have specific regulatory roles in brain has been strengthened by reports of an apparent nerve-specific form of (Na^+,K^+)-ATPase [18] and of endogenous (Na^+,K^+)-ATPase inhibitors in brain [19, 20].

In previous studies of the effects of acute and chronic administration of Li^+ in rats, decreases in (Na^+,K^+)-ATPase activity have been shown in a few brain regions [21–24]. These experiments were not performed under conditions where regulatory changes in (Na^+,K^+)-ATPase would be evident. Behavioral effects of Li^+ were also not demonstrated. Increases in activity corresponding to those in red blood cells of some human subjects treated with Li^+ [25] have not been found in experimental animals.

In the present study, the effects of chronic dietary lithium treatment on the regulation of (Na^+,K^+)-ATPase in rat brain were examined. This treatment regimen produced behavioral effects (reduction in shock-elicited fighting [26]) without weight loss or other evidence of toxicity. In order to circumvent the problems of the large endogenous phosphate pool and competition for ATP utilization [27], effects on cation regulation of the K^+-*p*-nitrophenylphosphatase associated with (Na^+,K^+)-ATPase[28] were examined, as well as effects on (Na^+,K^+)-ATPase activity *per se*. Ouabain inhibition curves were used to determine whether lithium treatment preferentially affects one of the two proposed molecular forms of (Na^+,K^+)-ATPase, which differ in their affinities for ouabain [18].

METHODS

Animals and diets. Albino male Sprague–Dawley rats weighing about 200 g were given a diet containing lithium and supplemental Na^+ and K^+ [29]. Control rats received the same diet without Li^+. The amount of Li^+ added was 20 m-equiv./kg dry diet for the first week, 40 m-equiv./kg dry diet for the second week, and 60 m-equiv./kg dry diet thereafter. After 5 weeks this resulted in lithium concentrations of 0.71 ± 0.07 (S.D.) mM in plasma and 0.60 ± 0.12 m-equiv./kg in corpus striatum. Lithium and control groups did not differ in weight or in gross behavior; there were fourteen rats in each group.

Shock-elicited fighting. Pairs of rats were tested for shock-elicited fighting in a $30 \times 28 \times 14$ cm cage in a sound-attenuated room. Thirty shocks of 1.5 mA intensity and 1-sec duration were delivered at 7-sec intervals. The shock-elicited fighting frequency was the percentage of shocks followed by fighting behavior [26].

Tissue preparation. After decapitation without anesthesia, brains were removed and dissected over ice. Cerebral hemispheres, corpus striatum, hippocampus and brainstem were dissected using gross landmarks [30]. Tissue was homogenized in 10–

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20 vol. of 0.25 M sucrose containing 0.05 M imidazole (pH 7.2) and 2 mM Tris EDTA. The homogenates were centrifuged at 700 *g* for 10 min, the precipitate was washed with homogenization medium, and the supernatant fractions were combined. The combined supernatant fractions were centrifuged at 17,000 *g* for 15 min, washed twice, suspended in homogenization medium, and stored at −70°. Protein was determined by the method of Lowry *et al.* [31].

(Na^+,K^+)-ATPase assay. About 10 μg of crude synaptosomes were incubated in 60 μl medium containing 0.05 M Tris–HCl (pH 7.5), 5 mM $MgCl_2$ and 5 mM Tris ATP. (Na^+,K^+)-ATPase was determined by addition of 100 mM NaCl and 20 mM KCl. After 10 min of incubation, the reaction was stopped with 8% trichloroacetic acid, and inorganic phosphate was determined by the method of Chen *et al.* [32].

K^+-p-nitrophenylphosphatase assay. About 10 μg of tissue was incubated in 60 μl medium containing 10 mM Tris *p*-nitrophenylphosphate, 5 mM $MgCl_2$, 0.05 M Tris–HCl (pH 7.5), and other ligands as described in the text. After 10 min of incubation, the reaction was terminated with 0.1 M NaOH, and *p*-nitrophenol was determined spectrophotometrically by measurement of absorption at 420 nm. Activity without K^+ was subtracted from that with K^+ to obtain K^+-dependent activity.

Ouabain inhibition. About 15 μg of tissue was preincubated for 60 min with 2 mM $MgCl_2$, 2 mM Tris phosphate (pH 7.2), 50 mM Tris–HCl (pH 7.5), and graded concentrations of ouabain, in a volume of 20 μl. Then 40 μl of additional medium containing Tris *p*-nitrophenylphosphate was added to give a final concentration of 10 mM *p*-nitrophenylphosphate, 5 mM $MgCl_2$, and zero or 25 mM KCl. The final P_i concentration, 0.67 mM, was well below the K_i for this system [28]. After 6 min of incubation, the reaction was stopped and *p*-nitrophenol production was measured as described above. Enzyme activity in the absence of K^+ did not vary as a function of ouabain concentration.

Equations and computer curve fitting. The equation for activation by low concentrations of Na^+ followed by inhibition by higher concentrations is:

$$v = V[(K_a/M)^m + 1]^{-1}\,[(M/K_i)^n + 1]^{-1}, \quad (1)$$

where v is the observed rate, V is the maximal rate, M is the Na^+ concentration, K_a is the concentration of Na^+ for half-maximal activation, K_i is the concentration of Na^+ for half-maximal inhibition, and m and n are Hill coefficients. This equation was used for Fig. 1 and Table 2. K_a, K_i, and the Hill coefficients did not change with lithium administration. The derivation of this equation and its applications to (Na^+,K^+)-ATPase kinetics are described in Refs. 28 and 33.

Inhibition by high and low concentrations of the same ligand was described by:

$$v = 1 - \{[VS/K_1 + S)] + [(1 - V)S/(K_2 + S)]\}, \quad (2)$$

where v is the observed reaction rate as a fraction of the maximal rate in the absence of inhibitor, V is the fraction of enzyme inhibited by the ligand S with half-maximal inhibition at concentration K_1, $(1 - V)$ is the fraction of enzyme inhibited with half-maximal ligand concentration K_2, and S is the concentration of inhibitor. This equation was used for Fig. 2 and Table 3.

For concentration curves, tissue was pooled from groups of three or four animals. The mean of four triplicate determinations for each experimental group was thus used for each point for curve fitting.

We used an iterative least-square routine, MLAB, for curve fittings [34, 35]. This routine finds optimal parameter values by repeated iterations until the root means squared error of the theoretical curve compared to the data is minimized. The standard error for each parameter is given by the routine along with a term, the 'dependency', which indicates the dependence of the parameter value on the values of other parameters. Ideally, parameters would be totally independent of each other, yielding a dependency of one; if parameters were dependent on each other, the dependency could be as high as 1,000,000, implying that an essentially infinite assortment of parameter values would provide an equally good fit. The simulations used here satisfied the criteria for low root means squared error and dependency.

RESULTS

Effect of chronic dietary lithium on shock-elicited fighting. Incidence of shock-elicited fighting was 42 ± 23 per cent (S.D.) in controls and 21 ± 17 per cent in lithium rats, a significant reduction by lithium treatment (two tailed *t*-test = 2.42, $P < 0.05$). This was similar to previous results [26].

Effect of lithium administration on (Na^+,K^+)-ATPase activity in crude synaptosomes. (Na^+,K^+)-ATPase activity in crude synaptosomes from four brain regions from control and lithium-treated rats is shown in Table 1. Activity was significantly lower in hippocampus from lithium-treated rats, but was not changed in the other regions studied.

Effect of lithium administration on regulation of (Na^+,K^+)-ATPase, converting the enzyme to its K^+-sensitive form, can be measured by Na^+ activation of the K^+-*p*-nitrophenylphosphatase associated with (Na^+,K^+)-ATPase in the presence of ATP and a low concentration of K^+ [28]. Activation by Na^+ is biphasic [28, 34]. Figure 1 shows the Na^+-activation curves for corpus striatum. The kinetic parameters are summarized in Table 2 [28]. Lithium administration consistently decreased the V_{max} for Na^+ activation without significant effects on apparent Na^+ affinity.

Table 1. Effect of lithium treatment on (Na^+,K^+)-ATPase activity in crude synaptosomes*

Region	Control	Lithium
Brain stem	0.127 ± 0.003	0.127 ± 0.003
Cortex	0.155 ± 0.005	0.157 ± 0.001
Hippocampus	0.147 ± 0.009	0.118 ± 0.005†
Corpus striatum	0.136 ± 0.001	0.134 ± 0.008

* Activity is expressed in μmoles P_i·(mg protein·min)$^{-1}$ ± S.E.M.

† Lithium different from control, $P < 0.05$.

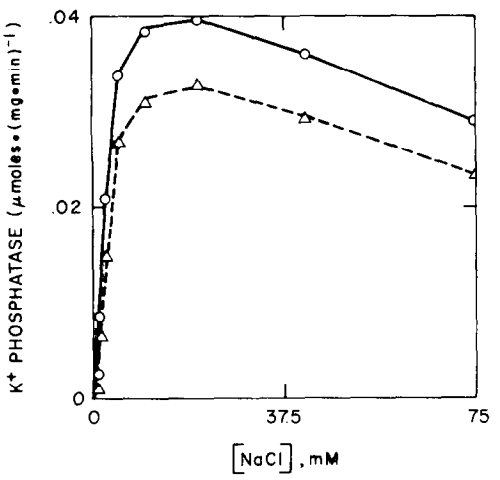

Fig. 1. K^+-*p*-nitrophenylphosphatase activity in crude synaptosomes from rat corpus striatum, as a function of Na^+ concentration. Activity is the difference between *p*-nitrophenol production in the presence and in the absence of 0.5 mM KCl. Conditions were as described in Methods, with the addition of 0.1 mM Tris ATP. The lines represent the least-squares fits to an equation giving apparent Na^+ affinities and maximal activation [28]: the parameters derived from the equation are in Table 2. The solid line is control; the dashed line, lithium.

Effects of lithium administration on ouabain inhibition. It has been shown that there are two molecular forms of (Na^+,K^+)-ATPase that differ in their affinities for ouabain as measured by inhibition of phosphorylation [18]. Figure 2 shows that, in the rat, these forms can be distinguished by ouabain inhibition of K^+-*p*-nitrophenylphosphatase. The biphasic inhibition has K_i values of about 5×10^{-8} M and 5×10^{-3} M, comparable to the affinities reported by Sweadner [18]. Table 3 shows the total amounts of enzyme activity with high and low ouabain affinity. Lithium administration selectively decreased the high-affinity component of ouabain inhibition in hippocampus and the low-affinity component in corpus striatum.

DISCUSSION

In studies of biochemical actions of lithium, it is important to provide evidence that the model for lithium administration used produces behavioral effects in the absence of toxicity. Previous studies of lithium effects on (Na^+,K^+)-ATPase activity, using a variety of tissue preparations and routes of administration, have revealed small decreases in activity in some brain regions [21–24]. Behavioral effects of lithium administration were not examined

Table 2. Kinetic parameters for Na^+ activation of K^+-*p*-nitrophenylphosphatase*

Region	V_{max}† Control	V_{max}† Lithium	K_a‡ Control	K_a‡ Lithium
Cortex	56.6 ± 2.4	49.2 ± 0.9§	3.10 ± 0.13	2.82 ± 0.07
Hippocampus	54.8 ± 4.4	39.4 ± 0.9§	3.24 ± 0.22	2.57 ± 0.69
Corpus striatum	42.5 ± 1.3	34.8 ± 1.9§	2.60 ± 0.12	2.84 ± 0.23

* Incubations were carried out as described in Methods, with 0.1 mM Tris ATP, ± 0.5 mM KCl, and 0, 0.2, 0.4, 0.8, 1.5, 3, 6, 12.5, 25 and 50 mM NaCl.
† In nmoles *p*-nitrophenol produced ·(mg protein·min)$^{-1}$ ± S.E.
‡ Na^+ concentration for half-maximal activation [28] in mM ± S.E.
§ Li^+ different from control, $P < 0.05$.

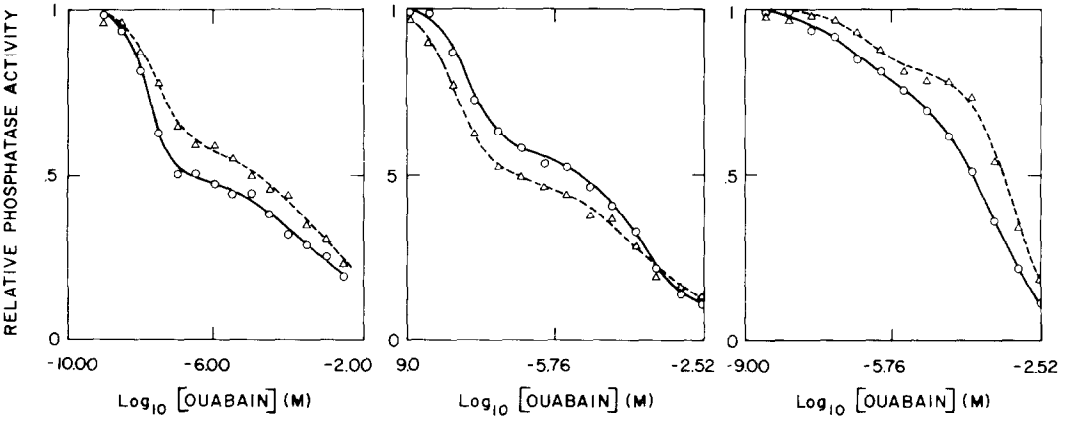

Fig. 2. K^+-*p*-nitrophenylphosphatase activity in crude synaptosomes as a function of ouabain concentration. Conditions were as described in Methods. The lines (solid = control, dashed = lithium) represent least-squares fits to an equation describing inhibition with two affinities by the same inhibitor; the parameters are given in Table 3. Left panel: hippocampus; middle panel: corpus striatum; right panel: cortex.

Table 3. K^+-*p*-nitrophenylphosphatase activity with high and low ouabain affinity*

Region		High affinity	Low affinity
Cortex	Control	4.30 ± 0.74	16.8 ± 2.75
	Lithium	3.15 ± 0.71	15.25 ± 3.32
Hippocampus	Control	10.03 ± 0.44	8.37 ± 0.37
	Lithium	6.66 ± 0.55†	8.14 ± 0.67
Corpus striatum	Control	5.57 ± 0.23	7.03 ± 0.29
	Lithium	6.07 ± 0.29	5.53 ± 0.28†

* Activity is in nmoles · (mg protein · min)$^{-1}$ ± S.E.
† Lithium different from control, $P < 0.05$.

in those studies, and toxic effects could not be distinguished from non-toxic effects. In the present study, chronic dietary lithium decreased shock-elicited fighting significantly in the presence of moderate brain and serum lithium concentrations and without weight loss or evidence of illness.

Other potential problems in investigations of pharmacological effects on (Na^+,K^+)-ATPase arise from the presence of a large endogenous phosphate pool and competition for ATP utilization [27]. The present study was an attempt to circumvent these problems by measuring hydrolysis of *p*-nitrophenylphosphate by (Na^+,K^+)-ATPase [28]. This activity was reduced more consistently by lithium administration than was production of inorganic phosphate from ATP.

The apparent Na^+ affinity for the Na^+-mediated transition of (Na^+,K^+)-ATPase to its K^+-sensitive form [28] was not changed by lithium (Table 2). One possible interpretation for the increased red blood cell Na^+ content and (Na^+,K^+)-ATPase activity that have been observed in humans treated with lithium [25] is a reduction in Na^+ affinity. These results rule out such a reduction, at least in the rat, and suggest increased Na^+ influx as an alternative explanation.

Inhibition of K^+-*p*-nitrophenylphosphatase by ouabain was used to determine whether inhibition by lithium was specific for either of the two molecular forms of (Na^+,K^+)-ATPase, which can be differentiated by affinity for cardiac glycosides, recently described by Sweadner [18]. In hippocampus, lithium selectively inhibited the form with high ouabain affinity, which may be specific for nerve tissue. The low-affinity form, which may occur in both nerve and non-nerve (such as glial) tissue, was inhibited selectively in corpus striatum. Affinity for ouabain could parallel that for endogenous inhibitors of (Na^+,K^+)-ATPase [19, 20], and distinguish functional subgroups of (Na^+,K^+)-ATPase molecules.

Direct effects of lithium on (Na^+,K^+)-ATPase may vary according to neural activity. In the presence of low extracellular K^+ concentrations, such as those following repeated nerve stimulation [36], lithium ions can stimulate (Na^+,K^+)-ATPase [11, 12], thereby possibly prolonging the relative refractory period of the cell [37] but potentially leading to a compensatory decrease in synthesis of (Na^+,K^+)-ATPase molecules [38]. With higher K^+, Li^+ inhibits (Na^+,K^+)-ATPase and the opposite effects could occur. Interactions with Mg^{2+}, which physically resembles Li^+ [4, 5], have also been proposed [39], though Li^+ does not appear to compete with Mg^{2+} *in vitro* [9].

The data reported here show that lithium administration, in a dose that produces behavioral effects without evidence of toxicity, affects (Na^+,K^+)-ATPase by at least two mechanisms. The first, reflected in the decreased V_{max} for Na^+ activation, is relatively widespread and appears not to be specifically limited to either of the putative forms of (Na^+,K^+)-ATPase. The second, reflected by the reduction in the form of (Na^+,K^+)-ATPase with high ouabain affinity, was limited to hippocampus among the regions studied here and may represent interactions with a specific neurotransmitter, such as norepinephrine [15].

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