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LITHIUM-INDUCED CHANGES IN SODIUM–LITHIUM COUNTERTRANSPORT

BARBARA E. EHRLICH* and JARED M. DIAMOND†
Department of Physiology, University of California Medical Center, Los Angeles, CA 90024, U.S.A.
and
LAWRENCE GOSENFELD
Department of Psychiatry, University of California Medical Center, and Brentwood Veterans Administration Hospital, Los Angeles, CA 90024, U.S.A.

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Abstract—The effect of chronic lithium (Li^+) administration on Li^+–Na^+ countertransport across the erythrocyte membrane has been studied in manic patients commencing Li^+ therapy. While Li^+ influx and Li^+ leak efflux remained unchanged, Li^+ countertransport efflux was inhibited by about 50 per cent. This inhibition was detectable within 1–4 days and was maximal in 4–7 days. The kinetic basis of the inhibition involved a 3-fold increase in the Michaelis–Menten parameter K_m, without change in the parameter V_{max}. In contrast, the kinetic basis of the genetically determined interindividual variation in countertransport described previously [B. E. Ehrlich and J. M. Diamond, *J. memb. Biol.* **52**, 187 (1980)] involves changes in V_{max} but not in K_m. We conclude that the effect on countertransport does not depend on changes in intracellular Na^+ concentration, intracellular choline concentration, or pH; it may depend on a change in the countertransport protein.

Lithium (Li^+) is similar in its chemical and physical properties to Na^+ and K^+, the major alkali cations in nature. Not surprisingly, the numerous biological actions of Li^+ depend on its ability to affect Na^+-dependent or K^+-dependent biological processes [1]. These actions have gained added interest through the serendipidous discovery that Li^+ is the most effective agent for treating the manic phase of manic-depressive illness. Although neither the basic defect in the illness nor the mechanism of Li^+ therapy is known, several pharmacological effects of chronic Li^+ administration that may be relevant to its therapeutic mechanism have been identified. These include: in rats, reduction of choline transport across the blood–brain barrier [2] and of tryptophan transport in synaptosomes [3]; and, in humans, increase in intracellular choline concentration of erythrocytes [4] and decrease in Na^+–Li^+ countertransport in erythrocytes [5–7].

The latter effect is of particular interest because the erythrocyte shares transport mechanisms with many other cells, and because the countertransport mechanism maintains the Li^+ concentration gradient between the erythrocyte interior and plasma. This mechanism involves a one-for-one exchange of Na^+ for Li^+ across the erythrocyte membrane, driven by the sum of the concentration gradients of these two ions. Since plasma [Na^+] is much higher than intracellular [Na^+], this Na^+ gradient drives a net efflux of Li^+ and maintains [Li^+] lower in the erythrocyte than in plasma [8–11]. Previous studies established that the rate of countertransport is reduced 20–50 percent by Li^+ therapy itself [5–7]. This reduction was detectable at the earliest time examined after initiation of Li^+ therapy, 2 or 3 days [5, 6]. The inhibition reached a maximum within 7 days, was maintained as long as the Li^+ dose was maintained, and disappeared when Li^+ therapy was discontinued [5].

The purpose of this paper is to describe the kinetic mechanism, and to examine more closely the time course, of the effect of Li^+ on Na^+–Li^+ countertransport in the human erythrocyte.

METHODS

This study is based on eight adult male subjects treated at the Affective Disorders Clinic of the Brentwood Veterans Administration Hospital. All subjects were diagnosed as manic-depressive by the Research Diagnostic Criteria of Spitzer and Endicott and were manic or hypomanic on admission to the ward, except that subject G was diagnosed as paranoid schizophrenic. Subject F was an outpatient, while the others were inpatients on the closed ward. None had taken Li^+ previously. For the duration of the study, the Li^+ dose was 900 mg Li_2CO_3/day (300 mg, three times a day). Other medications being taken concurrently were sinequan (subject E), nitroglycerine (subject H), and none (subjects A, B, C, D, F, and G). All procedures were approved by the UCLA and Brentwood Veterans Administration Hospital Human Subject Protection Committees.

Blood samples, obtained by venepuncture into heparinized tubes, were drawn prior to Li^+ therapy and up to three times within the week after commencement of Li^+ therapy. The samples were centrifuged, the plasma and buffy boat were removed,

* Present address: Department of Physiology, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461, U.S.A.
† Author to whom all correspondence should be sent.

and the erythrocytes (RBC) were washed three times in buffered choline–saline solution (140 mM choline Cl, 4 mM KCl, 10 mM glucose, and 15 mM HEPES*–Tris buffer at pH 7.4; buffered NaCl–saline solution was similar except that it contained 140 mM NaCl rather than choline Cl). For patients on Li^+ therapy, plasma Li^+ concentrations ranged from 0.52 to 1.22 mM, and erythrocyte intracellular concentrations were up to 0.82 mM. Li^+ and Na^+ concentrations were measured with a Varian flameless absorption spectrophotometer as described previously [12].

To load RBC with several different intracellular concentrations of Li^+ (abbreviated $[Li^+]_i$), we divided the RBC samples into three or four aliquots and suspended them in NaCl–saline solutions in which 20–140 mM Na^+ had been replaced with Li^+. After these suspensions had been incubated for 1 hr at 37°, the RBC were washed three times in choline saline to remove extracellular Li^+. The resulting values of $[Li^+]_i$ ranged from 0.42 to 4.78 mM.

To measure Li^+ efflux, we added Li^+-loaded RBC to test tubes containing either Na^+ saline or choline saline (both Li^+-free) prewarmed to 37°, to achieve a final hematocrit of 5–10%. Aliquots of this suspension were withdrawn immediately and 1 hr later for determinations of $[Li^+]_i$ and $[Li^+]_e$ (the latter means extracellular Li^+ concentration). This time interval is a suitable one for measuring the unidirectional Li^+ flux [11]. The increase in $[Li^+]_e$ over this time for Na^+ saline incubations yielded the total Li^+ efflux (countertransport efflux plus the so-called leak efflux, i.e. efflux thought to be by diffusion through membrane channels); for choline saline incubations it yielded the leak efflux alone. The difference between the total and the leak efflux determinations was taken as the efflux via the Na^+–Li^+ countertransport mechanism.

* HEPES = 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.

RESULTS

The rate of Na^+–Li^+ countertransport in RBC varies considerably among individuals, but shows little variation with time when measured repeatedly under the same conditions in RBC from the same healthy individual (coefficient of variation, 4%: Ref. 13; B. E. Ehrlich and J. M. Diamond, unpublished observation). Hence our experimental procedure for studying Li^+ inhibition of countertransport used each subject as his own control. In an initial series of four subjects, we measured countertransport at one $[Li^+]_i$ value, immediately prior to initiation of Li^+ therapy and then once at 3–5 days after initiation of therapy. Whereas Li^+ influx and Li^+ leak efflux were unaffected by Li^+ therapy (data not shown), Li^+ countertransport efflux was considerably reduced from pre-treatment values after 3–5 days of Li^+ therapy in all four subjects (data in Table 1). To take account of differences (significant in the experiments with two subjects, negligible in the other two experiments) between the loading $[Li^+]_i$ value for efflux determinations at day 0 and at days 3–5, we calculated the percent reduction in countertransport efflux

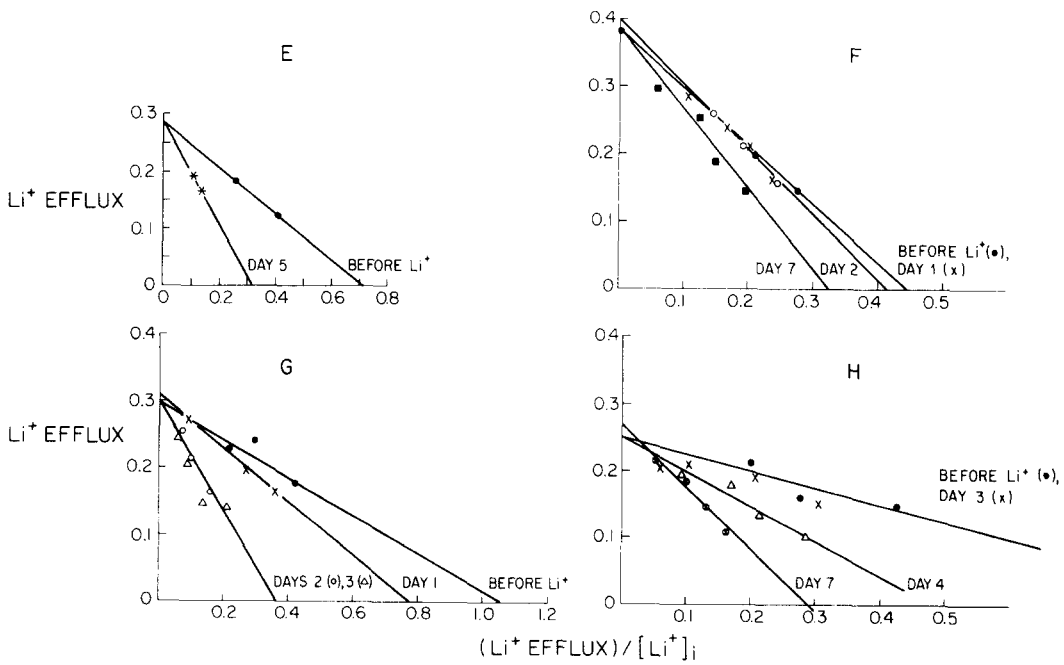

Fig. 1. Kinetic analysis of the effect of Li^+ treatment on Na^+–Li^+ countertransport. Li^+ efflux via the countertransport mechanism was measured several times before and during the first week of Li^+ treatment, at several $[Li^+]_i$ values. Each of the four panels represents a different subject; each line and set of symbols represents a different day from the start of Li^+ treatment; and each point represents a different $[Li^+]_i$ value. Ordinate, Li^+ efflux in units of mmoles (liter RBC)$^{-1}$(hr)$^{-1}$. The slope and y-intercept of straight lines fitted through the points for each day are, respectively, the Michaelis–Menten parameters K_m and V_{max} listed in Table 2.

on the assumption that Li^+ efflux varies linearly with $[Li^+]_i$. Although the countertransport efflux has saturable kinetics, this assumption is a useful first approximation for the low range of $[Li^+]_i$ levels used in this experiment (see Fig. 2 of Ref. 11). Calculated in this way, the reduction of countertransport in the four subjects was by 50 ± 18 percent (mean ± S.D.; range, 23–67 percent).

In a second series of four subjects, we measured efflux immediately prior to initiation of Li^+ therapy and up to three further times within the first week of Li^+ therapy, to determine the time course for Li^+ inhibition of countertransport. Measurements were confined to the first week because the cell-to-plasma ratio $[Li^+]_i/[Li^+]_e$ for the erythrocyte stabilizes within this period. At each time, we measured efflux in RBC preloaded to two to four different $[Li^+]_i$ levels, to permit calculations of kinetic parameters. Since Na^+–Li^+ countertransport is well described by Michaelis–Menten kinetics [14], we plotted the results using the Eadie–Hofstee form of the Michaelis–Menten equation:

$$V = -K_m V/[Li^+]_i + V_{max} \quad (1)$$

where V is the Li^+ efflux via the countertransport mechanism, K_m is the apparent affinity of Li^+ for the transport site, and V_{max} is the maximum rate of Li^+ transport. Panels E–H of Fig. 1 depict the resulting graphs of V against $V/[Li^+]$ for each of the four subjects at each time with straight lines fitted by linear regression analysis. Table 2 presents, for each subject at each time, the Michaelis–Menten kinetic parameters K_m and V_{max}, determined acording to equation 1 as the slopes and y-intercepts, respectively, of Fig. 1, E–H. Statistical significance of the change in the slopes was evaluated using a two-tailed t-test.

In all four subjects, the slope (K_m) increased 1.5- to 4-fold with Li^+ therapy, while the y-intercept (V_{max}) remained unchanged (Table 2). As for the time course of the change in K_m, it was detectable by day 1 in subject G, by day 2 but not by day 1 in subject F, and by day 4 but not by day 3 in subject H. On the average, K_m on the last day studied, 3–7 days after initiation of Li^+ therapy, was 3.0 ± 1.1 (mean ± S.D.; range 1.4–3.9) times the pre-therapy value.

We compared the results of our two series of experiments as follows. To extract a magnitude of countertransport inhibition from the second series of experiments under conditions comparable to the first series, we took the percent reduction in countertransport on the last day studied, at a $[Li^+]_i$ value near 1 mM. The resulting reduction in the four subjects was by 51 ± 18 percent at 3–7 days (range,

Table 1. Inhibition of countertransport by Li^+ treatment*

Subject	Days of treatment	$[Li^+]_i$	Efflux	% Reduction
A	0	0.33	0.12	65
	5	1.34	0.17	
B	0	0.93	0.20	23
	3	0.97	0.16	
C	0	0.53	0.34	45
	4	0.60	0.21	
D	0	0.34	0.15	62
	4	0.12	0.02	

* Li^+ efflux via the Na^+–Li^+ countertransport mechanism of RBC was measured before (day 0) and 3–5 days after initiation of Li^+ treatments in four subjects (A–D). $[Li^+]_i$: loading intracellular Li^+ concentration, in mmoles (liter RBC)$^{-1}$. Efflux: Li^+ countertransport efflux, in mmoles (liter RBC)$^{-1}$(hr)$^{-1}$. % Reduction: percent reduction in efflux corrected for differing $[Li^+]_i$ values; i.e. $100[(eff_X/Li_X) - (eff_0/Li_0)]/(eff_0/Li_0)$, where eff_X and eff_0 are efflux at days 3–5 and at day 0, respectively, and where Li_X and Li_0 are the $[Li^+]_i$ values at days 3–5 and at day 0 respectively.

Table 2. Changes in kinetic parameters of countertransport with Li^+ treatment*

	E		F†		G‡		H§	
Day	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
0	0.28	0.28	0.85	0.37	0.27	0.30	0.26	0.25
1	—	—	0.88	0.38	0.41	0.31	—	—
2	—	—	1.00	0.40	1.01	0.32	—	—
3	—	—	—	—	0.80	0.28	0.24	0.24
4	—	—	—	—	—	—	0.48	0.25
5	1.10	0.28	—	—	—	—	—	—
7	—	—	1.20	0.38	—	—	0.97	0.27
$[Na^+]_i$	8.4 ± 2.2 (3)		8.6 ± 0.5 (4)		9.3 ± 1.3 (4)		8.9 ± 1.7 (4)	

* K_m and V_{max} values for Li^+ efflux via the countertransport mechanism were calculated as the slope and y-intercept, respectively, of panels E–H of Fig. 1, for each of four subjects (E–H), at several times before and during the first week of Li^+ treatment. $[Na^+]_i$ values are the mean ± S.D. for all determinations between days 0 and 7. Units: for K_m and $[Na^+]_i$, mmoles (liter RBC)$^{-1}$; for V_{max}, mmoles (liter RBC)$^{-1}$(hr)$^{-1}$. Note that K_m increased during treatment,while V_{max} did not change.

† No significant change in K_m for day 0 vs 1 or 2; significant change ($P < 0.08$) in K_m for day 7.

‡ No significant change in K_m for day 0 vs 1; significant change ($P < 0.03$) in K_m for day 0 vs 2 or 3.

§ No significant change in K_m for day 0 vs 3; significant change ($P < 0.04$) in K_m for day 0 vs 4 and 7.

29–69 percent) compared to the reduction of 50 ± 18 percent at 3–5 days (range, 23–67 percent) already discussed for the first four subjects and shown in Table 1. To extract estimated kinetic parameters from the first series, we assumed a pre-therapy K_m of 0.4 mM (see Ref. 11: this value varies little among individuals not on Li^+ therapy). The pre-therapy efflux value was then used to calculate (by means of equation 3 of Ref. 11) a V_{max} value for that subject, and this V_{max} value was then combined with the efflux value during Li^+ therapy to yield K_m during Li^+ therapy, since the second series of experiments indicated that K_m but not V_{max} changed during therapy. The resulting estimated K_m for the four subjects was 3.3 ± 1.3 times (mean ± S.D.; range, 1.8–4.8) the pre-treatment value, compared to the increase of 3.0 ± 1.1 times already discussed for the second four subjects (Table 2). Thus, the results from the two sets of subjects are in good agreement.

Erythrocyte intracellular $[Na^+]$ levels were measured for the second series of four subjects (Table 2). The values did not change with time after initiation of Li^+ therapy; they remained within the normal range for untreated subjects.

DISCUSSION

This paper yields two main conclusions. First, it takes about 1–4 days for Li^+ inhibition of countertransport to become detectable, and about 4–7 days to become maximal. This time scale is similar to the time scale for buildup of plasma and erythrocyte intracellular Li^+ concentrations following intitation of Li^+ therapy. Hence, it is possible that the rate-limiting step for inhibition of countertransport is the buildup of these concentrations and that the inhibitory process itself is rapid. Our results, however, do not preclude the possibility that the inhibitory process itself takes a day or so to develop.

Second, inhibition involves an increase in K_m without a change in V_{max}. The three most obvious suggestions for the mechanism of this change in K_m can be ruled out: (a) Since Na^+ and Li^+ compete for the countertransport mechanism, an increase in $[Na^+]_i$ in the erythrocyte would raise the measured K_m for Li^+. But we found that $[Na^+]_i$ did not change during Li^+ therapy from pre-therapy values. (b) Perhaps the increased intracellular choline levels that accompany Li^+ therapy [4] inhibit Li^+ influx. The decrease in Li^+ efflux and increase in intracellular choline follow similar time courses at the start of Li^+ therapy (compare our Table 2 with Table 2 of Ref. 4). But the time courses on termination of Li^+ treatment are totally different: Li^+ transport returns to pre-treatment values within 14 days [6], whereas choline levels and choline transport return to pre-treatment values only after 3 months, the time needed to generate a whole new population of RBC [15]. (c) The K_m for Li^+ transport varies with pH [16]. But our experiments involved no variation in pH: pH was maintained at 7.4 in all *in vitro* experiments, and plasma pH does not change *in vivo* during Li^+ therapy.

The change in K_m but not in V_{max} during Li^+ therapy suggests some modification of the protein responsible for countertransport, without a change in the number of copies of the protein per cell. A speculative suggestion for the mechanism is modification of the protein by phosphorylation, acetylation, or methylation [17]. Li^+-induced changes in phosphorylation have been reported for small molecules [18, 19], though we are not aware of similar reports for proteins.

In addition to the Li^+-induced changes in RBC countertransport described in this paper, RBC countertransport also varies among individuals and among species [16], and this variation is probably genetic [20]. That the Li^+-induced variation is distinct from the interindividual variation was suggested by a comparison of countertransport in a pair of monozygotic twins when both were on Li^+ therapy, or when only one was on Li^+ therapy [7]. The present paper establishes this distinctness more clearly, by showing that the two types of variation have different kinetic mechanisms. Interindividual and species differences in countertransport involve differences in V_{max} [16], whereas the Li^+-induced variation is shown here to involve differences in K_m.

Finally, it is interesting to compare the Li^+-induced suppression of Na^+–Li^+ countertransport and of choline transport in the RBC. Li^+ therapy reduces RBC choline fluxes by up to 90 percent [15] and increases RBC intracellular choline several-fold [4, 21], despite no change in plasma choline levels. It was an attractive hypothesis that choline and Li^+ might share the same transport mechanism. The present paper makes this hypothesis unlikely: Li^+ therapy alters the K_m but not the V_{max} for Li^+ countertransport, while it alters the V_{max} but not the K_m for choline transport [15]. In addition, countertransport returns to pre-treatment values within 2 weeks after termination of Li^+ therapy, while choline transport remains irreversibly impaired throughout the lifetime of the RBC population. Hence, choline and Li^+ transport probably occur via different mechanisms.

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