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EFFECT OF LITHIUM ON SYNAPTOSOMAL BRAIN ENZYMES

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Abstract—During 7 days of lithium administration to rats, the levels of adenylate cyclase bound to brain synaptosomes decreased, while those of acetylcholinesterase increased. Both enzyme levels returned to their initial values after 1 month of treatment. Monoamine oxidase, which is bound to a mitochondrial membrane, was not affected by lithium treatment. Arrhenius plots of the brain synaptosomal enzymes (Na + K)ATPase and acetylcholinesterase from rats treated with LiCl for 7 or 30 days showed a lower transition temperature. Also, when these synaptosomal enzymes were exposed to such exogenous agents as detergents or alcohol, the reaction of the enzymes obtained from lithium treated rats was different than that of control rats. These effects disappeared after ceasing lithium administration for one week. These data indicate changes in the structure of brain membranes after lithium administration.

Administration of lithium salts is used as a treatment for recurrent endogenous affective disorders [1]. Although it has been pointed out that lithium can block potassium channels [2], and that it might also compete for calcium, no comprehensive and experimentally testable hypothesis has thus far been advanced to clarify the biochemical mechanisms underlying the 'mood normalizing' effects of lithium salts.

We have reported recently that lithium administration reduced brain (Na + K)ATPase activity to about one-half in about 7 days and that the enzyme activity was restored to normal levels after longer periods of treatment [3]. The mechanism for this biphasic effect of lithium on this membrane enzyme remains to be clarified. One possibility is that lithium may alter enzyme activity by interacting directly with the enzyme or by competing with the Na^+ and/or K^+ ions. Alternatively, since (Na + K)ATPase is embedded in the neural membrane [4], it may alter activity indirectly by interacting with the lipid portions or other components of the membrane, thus disturbing the microenvironment in which the (Na + K)ATPase is located. Changes in membrane characteristics have been demonstrated to alter ATPase activity [5].

We therefore reasoned that an examination of other membrane-bound enzymes, e.g. adenylate cyclase, acetylcholinesterase and monoamine oxidase, after short and long-term lithium treatment could serve two purposes. The first would be to check if the biphasic effect of lithium on (Na + K)ATPase is specific for this enzyme or if it also affects other cell membrane enzymes. The second purpose would be to study the effect of lithium on the membrane by using such exogenous agents as detergents and alcohols, which are known to have profound effects on the biophysical and biochemical properties of membranes. We also tested enzyme activities in several temperature ranges to detect possible changes in lithium-membrane characteristics, as evidenced by changes in the slope of the Arrhenius plots of enzyme activity [6].

MATERIALS AND METHODS

Chemicals. Lithium chloride and other reagents were of the best quality available and were purchased from either Sigma Chemical Co. or from Merck Chemical Co. [^{3}H]adenosine-3′,5′-cyclic phosphate (cAMP) (23 Ci/mmole) was from the Radiochemical Centre Ltd., Amersham, U.K.

Male Wistar rats weighing 150–200 g were used. Lithium chloride (850 mg/l) was administered to the rats as their sole drinking fluid *ad libitum*. The amount of food and fluid consumed by lithium treated rats was the same as for normal rats, and the animals lost no weight under lithium treatment. The plasma levels of lithium were around 1 mM (which is the therapeutic level in humans).

Enzymatic measurements. After different periods of lithium treatment, rats were killed by decapitation. Blood collected from the carcass (using heparin as anticoagulant) was centrifuged and the packed cells were lysed with 10 vol. of cold distilled water. The hemolysate was used for acetylcholinesterase determination. Brains were quickly removed and homogenized in 9 vol. of cold 0.1 M glycyl–glycine buffer (containing 1% Lubrol-WX) with a Super Dispox Tissumizer at full speed for 50 sec, with cooling by immersion in an ice bath.

Adenylate cyclase was determined in the homogenate by measuring the amount of cAMP formed from ATP by the cAMP binding assay of Gilman [7] following the procedure of Johnson and Sutherland [8]. Acetylcholinesterase was assayed in the

homogenate and in the synaptosomal preparation according to the method of Ellman *et al.* [9] in which acetylthiocholine iodide was used as substrate. Monoamine oxidase activity was assayed by the method of Weissbach *et al.* [10] and (Na + K)ATPase activity was assayed as described previously [3]. Lubrol WX and alcohol, when required, were added to the assay mixture before the initiation of the enzyme activity with Tris–ATP.

Normal and lithium treated rats were sacrificed by decapitation, and brains were quickly removed. The cerebellum and brain stem were discarded and the remaining brain areas were weighed and then homogenized in 5 vol. ice-cold 0.32 M sucrose. Synaptosomes were prepared according to the method of Cotman [11]. The synaptosome-containing fraction was recovered from the interphase of the 6 and 13% Ficoll solution and was diluted (4-fold) with 0.05 M Tris–HCl pH 7.0. Synaptosomes were sedimented by centrifugation at 35,000 *g* for 35 min and resuspended in 0.05 M Tris–HCl pH 7.0. This suspension was frozen (−40°C) overnight, thawed and used for the assay of (Na + K)ATPase and acetylocholinesterase activities.

Arrhenius plots were fitted by eye.

Protein was determined by the method of Lowry *et al.* [12] or by the biuret method [13]; bovine serum albumin was used as the standard. Lithium was determined in plasma by atomic absorption (Zeis FMD-4).

RESULTS

Results in Fig. 1 show the effect on brain adenylate cyclase activity following lithium administration. The levels of enzyme decreased progressively reaching a minimum after 1 week of lithium treatment; these levels returned to the initial values after 1 month of treatment. On the other hand, acetylcholinesterase, another enzyme bound to the brain membrane, showed a slight increase in activity after 7 days of lithium treatment, which also returned to initial values after 30 days. We obtained similar results when testing acetylcholinesterase from erythrocytes (Fig. 2).

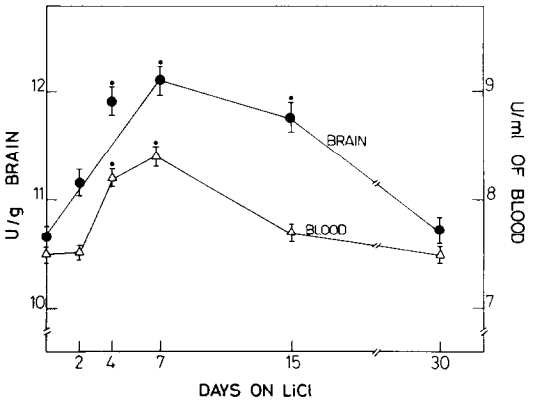

Fig. 2. Effect of LiCl administration on rat brain acetylcholinesterase. Rats were given LiCl in their drinking water. They were killed at the indicated intervals and the activity measured in brain homogenates and in blood. Each point represents the average from 6–8 animals ± S.D. The activity is expressed in μmoles/l·min × 10^{-4}/g brain; μmoles/l·min × 10^{-4}/ml blood. *P < 0.05 (Student's *t*-test).

We also studied the effect of lithium on monoamine oxidase, an enzyme bound to the outer mitochondrial membrane [14]. As shown in Fig. 3, a small increase in enzyme activity was initially observed but it did not reach the level of significance. No further changes were observed during the 30-day period of lithium administration. In view of these findings, it seemed of interest to study possible changes in membrane characteristics following different periods of lithium treatment. Since changes in the environment of a membrane-bound enzyme can be evidenced by changes in the slope of the Arrhenius plot of enzymatic activity [6], we measured enzyme activity at different temperatures.

Figure 4 shows that acetylcholinesterase activity of membranes from control rats increased with increasing temperature of the incubation medium.

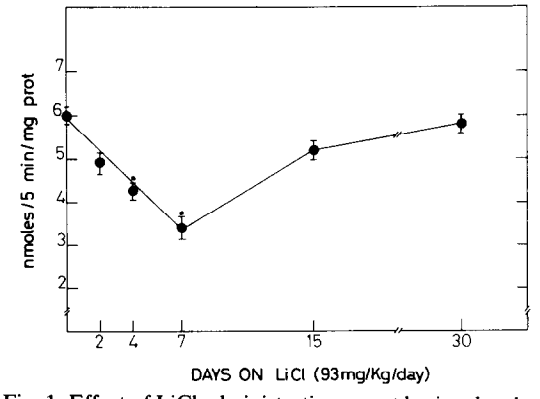

Fig. 1. Effect of LiCl administration on rat brain adenylate cyclase. Rats were given LiCl in their drinking water. They were killed at the indicated intervals and the activity measured in brain homogenates. Each point represents the average from 8–10 animals ± S.D. *P values were < 0.01.

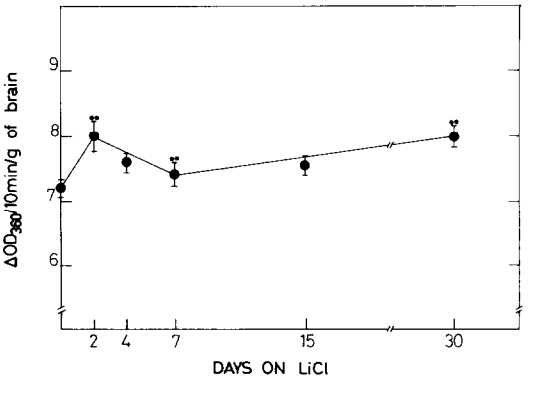

Fig. 3. Effect of LiCl administration on rat brain monoamine oxidase. Rats were given LiCl in their drinking water. They were killed at the indicated intervals and the activity measured in brain homogenates. Each point represents the average from 6–8 animals ± S.D. **N.S., no significant difference from control values.

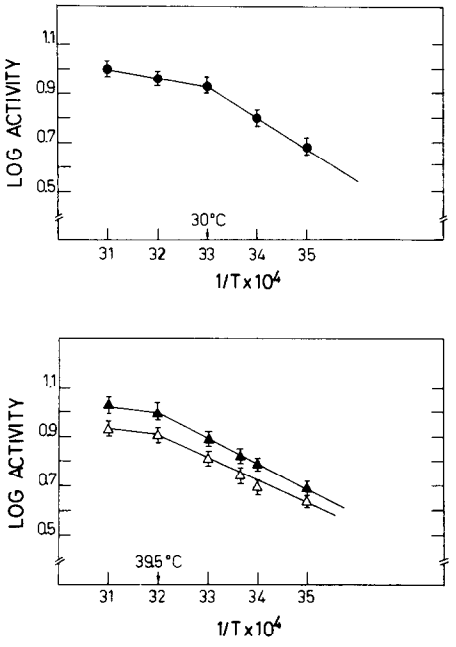

Fig. 4. Arrhenius plot of acetylcholinesterase activity of synaptosomal membranes from control and from lithium treated rats (7 and 30 days). The activity values are obtained from initial rate of reaction (40 μg of protein/assay). Each point represents the average value of 5–6 separate experiments ± S.D. (●) Control animals; (△) 7 day lithium treated rats; (▲) 30 day lithium treated rats.

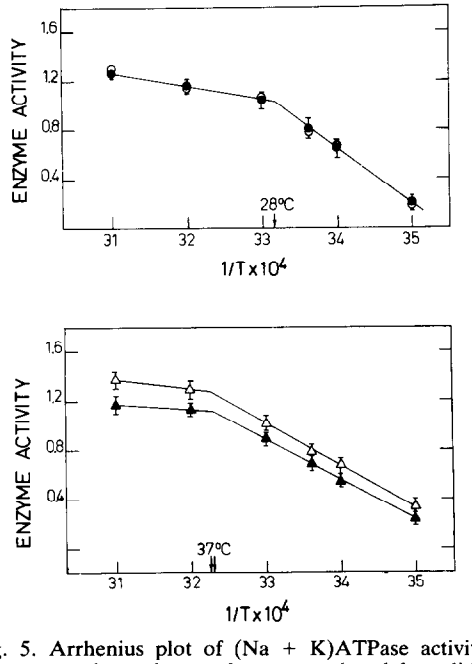

Fig. 5. Arrhenius plot of (Na + K)ATPase activity of synaptosomal membranes from control and from lithium treated rats (7 and 30 days). The activity values are obtained from initial rate of reaction, and are expressed in μmoles P_i/hr/50 g protein. Each point represents the average value of 5–6 separate experiments ± S.D. (●) Control animals; (▲) 7 day lithium treated rats; (△) 30 day lithium treated rats; (○) 7 day lithium treated rats and 7 days with water.

A biphasic Arrhenius plot is observed with a discontinuity of the plot at 30°. However, when the synaptosomal membranes were derived from brain of rats which had been treated with LiCl for 7 or 30 days, the transition temperature shifted to 39.5°. A similar observation was made when the temperature dependence of (Na + K)ATPase was studied. For this enzyme a break in the Arrhenius plot was observed at 28° when the preparation was obtained from control animals. When the enzyme derived from rats treated with LiCl for 7 or 30 days, the transition temperature shifted to 37°. The temperature dependency of the ATPase activity was almost identical for control rats and for rats which had been treated for 7 days with lithium and then kept 1 week without lithium. This indicates that the effect of lithium is fully reversible.

From the comparison of (Na + K)ATPase and acetylcholinesterase it can be concluded that the first enzyme is more sensitive to temperature changes than the latter. The steeper slope of the plot for (Na + K)ATPase suggests that this enzyme is more affected by the physical state of the membrane microenvironment and that it requires a higher energy activation than acetylcholinesterase.

Detergents can stimulate membrane-bound enzymes. They are thought to function by removing lipids which hinder the activity of such enzymes as ATPase, and thereby expose more enzyme sites. Figures 6 and 7 show that Lubrol WX inhibited the (Na + K)ATPase and acetylcholinesterase of membranes from control animals. However, when these membranes were derived from brains of rats treated with lithium for 7 or 30 days, the response of these two enzymes to varied concentrations of Lubrol was quite different. In the case of (Na + K)ATPase, as shown in Fig. 6, the enzyme activity increased with increased Lubrol WX concentrations. However, acetylcholinesterase, showed no significant change with increasing concentrations of Lubrol (Fig. 7). These effects of Lubrol were fully reversed one week after ceasing lithium administration.

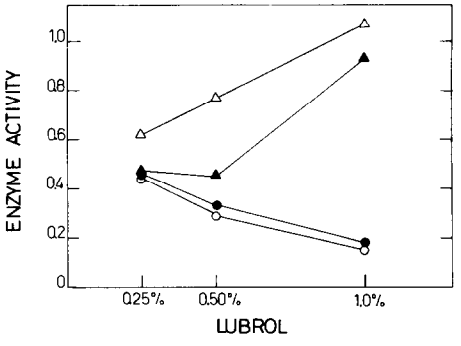

Fig. 6. Effect of Lubrol WX on synaptosomal (Na + K)ATPase from control and lithium treated rats (7 and 30 days). Synaptosomal protein (50 μg protein/assay) was added to a medium containing 0.25–1% Lubrol WX and preincubated for 5 min at 37°. The reaction was initiated by the addition of ATP. The activity is expressed in μmoles P_i/10 min/50 μg protein. Each point represents the mean of 5 experiments ± S.D. (●) Control animals; (▲) 7 day lithium treated rats; (△) 30 day lithium treated rats; (○) 7 day lithium treated rats and 7 days with water.

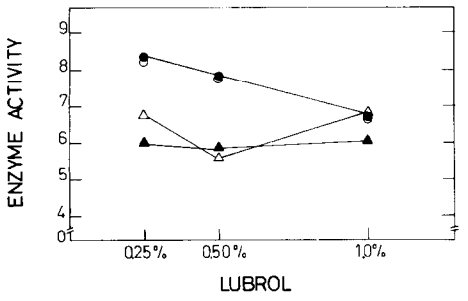

Fig. 7. Effect of Lubrol WX on synaptosomal acetylcholinesterase from control and lithium treated rats (7 and 30 days). Synaptosomal protein (40 μg/assay) was added to a medium containing 0.25–1% Lubrol WX and preincubated for 5 min at 30°. The reaction was initiated by the addition of acetylthiocholine. The activity is expressed in OD 412/10 min/mg protein. Each point represents the mean of 5 experiments ± S.D. (●) Control animals; (▲) 7 days lithium treated rats; (△) 30 days lithium treated rats; (○) 7 day lithium treated rats and 7 days with water.

It is known that ethanol, at a high dose, causes a disorganization of neural membrane structure, and since (Na + K)ATPase is integrated in the neural membrane, ethanol should alter enzyme activity [15]. As shown in Fig. 8, the enzyme from brain of control animals was inhibited by ethanol; however, less inhibition of enzyme activity occurred when the enzyme was derived from rat brains treated with lithium for 7 and 30 days.

DISCUSSION

It has been demonstrated that changes in the microenvironment of membrane-bound enzymes can have a significant effect on enzyme activity [15]. It is also generally considered that modifications in the physical state of membrane lipids may give rise to alterations of membrane function either by changing the protein conformation or the lipid–lipid or lipid–protein interactions. In this and previous studies [3], we have shown that enzymes bound to the synaptosomal membranes such as (Na + K)ATPase [16], adenylate cyclase [17] and acetylcholinesterase [18] react differently after short and long-term lithium administration. This effect of lithium seems to be specific for the synaptosomal membranes since enzymes bound to other membranes, such as monoamine oxidase which is bound to the mitochondrial membrane, are not affected significantly by lithium treatment.

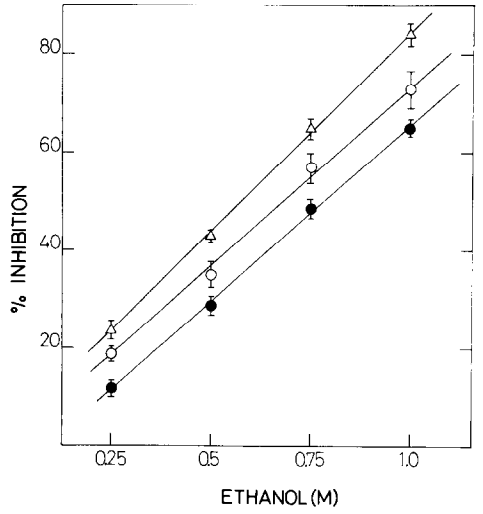

Fig. 8. Inhibitory effect of ethanol on (Na + K)ATPase activity in synaptosomes from control rats (△); from 7-day lithium treated rats (●) and from 30-day lithium treated rats (○). Each point represents the mean ± S.D. of 5 experiments.

Acetylcholinesterase and especially (Na + K)-ATPase are probably embedded in the lipid bilayer or vesicles of cell-free preparations [19]. Detergents have been shown to stimulate and produce a peak activation near a critical micelle concentration, and also to expose more enzyme sites by removing hindering lipids from the ATPase [20, 21]. In this study, we have shown that Lubrol WX interacts differently with (Na + K)ATPase and acetylcholinesterase obtained from control and from lithium treated rat brain membranes. Also, treatment with lithium changes the transition temperature in the biphasic Arrhenius plots obtained for these two enzymes. These results are indicative of microscopic changes in the relationship between these membrane proteins and their associated lipids, induced by lithium. The decreased inhibitory power of ethanol on the (Na + K)ATPase from lithium-treated rats is consistent with this idea. We cannot at present explain why lithium can induce such changes in the lipid–protein interactions.

The poorer solubility of the Li^+ salts than Na^+ salts of fatty acids has been invoked to explain membrane changes during lithium treatment and to account for the therapeutic efficiency of this ion in manic depressive psychosis [22]. The observed changes in enzyme activity with temperature, Lubrol and ethanol are all consistent with the lipid components of the membrane being in a more ordered state. It is not clear if formation of poorly-soluble lithium salts of fatty acids within the membrane, as postulated by Monnier [22], could be involved in inducing a more rigid structure of the membrane.

We have also shown that brain membranes go through an adaptation period of 7 days and finally stabilize after longer periods of treatment. In humans, the interval between the initiation of lithium treatment and therapeutic response is often 7 to 10 days. This time lag could possibly reflect the time needed for cell membrane adaptation [23].

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