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Change of synaptic membrane lipid composition and fluidity by chronic administration of lithium

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The effect of chronic administration of lithium salts on the lipid composition and physical properties of the synaptosomal plasma membrane was examined in rat brain. The effect of lithium treatment has been studied on the fluorescence polarization of synaptosomal plasma membrane and artificial lipid vesicles and on the lipid composition of the membranes. Fluorescence polarization of lipophilic probes was used to study membrane lipid structure. Steady-state polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH), a probe of the hydrophobic core, was significantly lower in plasma membranes from lithium-treated animals. Altered DPH polarization was due to a decrease in the order parameter of the probe. The lithium-treatment also changed the fluorescence of 1-anilino-8-naphthalene sulfonate (ANS), a probe that binds to the polar head group of the phospholipids and to proteins on the membrane surface. Synaptic plasma membranes from treated rats presented no significant changes on the cholesterol-to-phospholipid ratio, although the phospholipid class distribution was altered and the membrane phospholipid unsaturation increased. In summary, the neural plasma membranes became disorder after chronic lithium administration at therapeutic levels. This structural change may be due to changes in plasma membrane phospholipid distribution and to the degree of unsaturation of phospholipid fatty acids.

Introduction

The maintenance of membrane fluidity within narrow limits is presumably a prerequisite for proper functioning of a cell. Lipids play a key role in determining the membrane fluidity, and changes in lipid composition have been reported to alter important cellular functions [1–4]. For nerve membranes, changes in the dynamic properties have been associated with the mechanisms of action of some neuroactive drugs [5].

The term 'lipid fluidity' as applied to model bilayers and natural membranes is used throughout this report to express the relative motional freedom of the lipid molecules or substituents thereof and it was assessed by the estimation of steady-state fluorescence anisotropy of fluorescence probe molecules.

Lithium is used extensively in the therapy of affective disorders [6]. It inhibits and prevents episodes of manic excitement and to a lesser de-

Abbreviations: DPH, 1,6-diphenyl-1,3,5-hexatriene; ANS, 1-anilino-8-naphthalene sulfonate; P, steady-state fluorescence polarization; r_s, steady-state fluorescence anisotropy; r_o, maximum limiting fluorescence anisotropy; PE, phosphatidylethanolamine; PS, phosphatidylserine; PC, phosphatidylcholine; PI, phosphatidylinositol; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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gree inhibits and prevents episodes of depression in manic-depressive psychosis. However, its mode of action is still unknown. Although lithium ions have been reported to act in several different ways, most of the hypotheses concerning the mechanisms of the therapeutic action of lithium salts are associated with alterations in amine neurotransmission [7–12]. The ability of lithium to affect the phosphatidylinositol system which is stimulated by biogenic amine neurotransmitters, and hence the protein kinase C activity, has led to the speculation that interference with this cycle may mediate the therapeutic actions of lithium [13–15].

It is worth noting that most of the lithium effects described in the literature involve a great variety of membrane-bound activities i.e., carriers and receptors [1]. Recently we have shown that the systematic administration of lithium modulates the tryptophan transport in plasma-membrane vesicles isolated from rat brain by maintaining a more negative membrane potential inside the vesicles and by changing the properties of the synaptosomal plasma membranes [16,17]. This prompted us to investigate the effects of the chronic administration of lithium salts on the fluidity of the neural plasma membranes and the chemical determinants of this fluidity.

Experimental procedures

Lithium administration and lithium analysis

Lithium was administered to 300–320 g male Wistar rats in the drinking water (1 mg/ml LiCl). Serum lithium was measured by flame photometry (Perkin-Elmer model 372) in carotid blood obtained at the time of killing. For brain tissue, aliquots were weighed, homogenized in 3% perchloric acid, and centrifuged; the lithium content was determined in the acid extract.

Preparation of synaptosomes and synaptic plasma membrane vesicles

Rat forebrain synaptosomes were prepared from a washed crude mitochondrial pellet essentially by the method of Booth and Clark [18] modified in our laboratory [19]. In the final step of the procedure, the P_2 fraction was resuspended in 5 ml of a medium containing 0.32 M sucrose/1 mM K^+-EGTA/10 mM Tris-HCl (pH 7.4) (isolation medium), and layered at the top of a discontinuous Ficoll gradient consisting of 3 ml of 7.5% (w/v) Ficoll in isolation medium on 3.5 ml of 13% (w/v) Ficoll in isolation medium. Tubes were placed in a Beckman-swing-out rotor (SW 40 Ti) and centrifuged at $98\,000 \times g$ for 30 min. The synaptosomal fraction was then sucked out after carefully removing the myelin layer, diluted 10-fold with the isolation medium and centrifuged at $15\,000 \times g$ for 10 min. Synaptosomes were kept in an ice bath either as pellets or resuspended with 0.32 M sucrose at 40–60 mg protein/ml until utilization.

Synaptic plasma membranes were prepared and stored as described previously [20,21]. After osmotic disruption of the synaptosomes, the suspension was centrifuged at $27\,000 \times g$ for 20 min, and the pellet resuspended in 10 mol of a 284 mosM medium (pH 7.4) containing 120 mM NaCl/22 mM potassium phosphate. Finally, the suspension was centrifuged at $27\,000 \times g$ for 15 min and the pellet resuspended in the former medium to a protein concentration of 15–25 mg/ml. Aliquots were frozen in liquid nitrogen, and stored at $-70\,^{\circ}C$. When required, aliquots were quickly thawed out at $37\,^{\circ}C$. Under these conditions, membrane vesicles were functional for at least 1 month.

Membrane lipid vesicle preparation

Total lipid fractions from the plasma membrane vesicles were prepared by a nonaqueous extraction procedure [22]. Vesicles were prepared from total lipids by first drying the extracts to a thin film under nitrogen, resuspending in buffered saline (5 mM Tris-Hepes/150 mM potassium gluconate/1 mM magnesium sulfate (pH 7.4)) and finally placing the samples in an ultrasonic desintegrator (150 watt Measuring and Scientific Equipment) for 8 min using 30 s operation intervals in order to avoid excess heat. In addition, the tube was immersed in an ice water mixture under nitrogen. Vesicles formed under these conditions are mostly in single-layered liposomal rather than micellar form [23]. Vesicles were used for fluorescence polarization studies immediately after preparation.

Membrane lipid and protein composition

Lipid phosphorus measurements were carried

out by the method of Rouser and Fleischer [24]. Polar lipids were fractioned by two-dimensional thin-layer chromatography on HPTL chromatoplates (Merck, Darmstadt, F.R.G.). Lipid samples (500 μg) were developed in the first direction with chloroform/methanol/water/ammonia (65:35:2:4, by vol.) dried under nitrogen, and then developed in the second direction with chloroform/methanol/water/acetic acid (75:25:3:7, by vol.). Lipid spots were visualized exposing the plate to iodine vapour.

Transmethylation of fractioned lipid classes was performed with a boron trifluoride/methanol reagent (Merck). 0.9 ml of benzene, 1 ml of methanol and 1 ml of boron trifluoride/methanol reagent was added to the scraped spots from silica gel plates containing the lipid samples. The tube was closed under N_2 and kept at 110 °C for 90 min. Fatty acid methyl esters were extracted in a water-hexane system and dryed under N_2 in small conical tubes.

Free and sterified cholesterol was separated on silica gel H plates in *n*-hexane/ethyl ether/acetic acid (70:30:1, by vol) as described [25], and cholesterol content was determined by the method of Zlatkis and Zak [26]. Fatty acid analysis was performed by gas-liquid chromatography (Hewlett-Packard HP-5790) using a fused silica capillary column. Peak areas were analyzed by using an automatic integrator (HP-3390A). Methyl pentadecanoate was used as internal standard. Identification of individual peaks was performed by using gas-liquid chromatography combined with mass spectrometry (HP 5985) and an integrator computer (HP 7906).

Membrane proteins were assayed by the method of Lowry et al. [27].

Fluorescence and anisotropy measurements

1,6-Diphenyl-1,3,5-hexatriene (DPH) was introduced into the synaptic plasma membranes essentially according to the method of Shinitzky and Barenholz [28] and Shinitzky and Inbar [29]. The membranes (10 μg of protein/ml) or the liposomes (10 μg lipid/ml) were incubated for 20 min at 37 °C in 5 mM Hepes-Tris/150 mM potassium gluconate/1 mM magnesium sulphate (pH 7.4), containing 1.3 μM DPH, giving a phospholipid-to-probe molar ratio of about 300. A sample without DPH was prepared as a blank. Steady-state fluorescence polarization of the equilibrated samples was measured using a RRS 1000 Shoeffel Instruments Corp. fluorescence spectrometer equipped with polarizers in both exitation and emission beams. Excitation and emission wavelengths were set at 365 and 425 nm with band widths of 2 and 5 nm, respectively. With the excitation beam vertically polarized, the fluorescence intensity was measured with the emission polarizer in the parallel position first ($I_{\parallel}$) and in the perpendicular position ($I_{\perp}$) later. P was calculated according to $P = (I_{\parallel} - I_{\parallel F}) - (I_{\perp} - I_{\perp F})/(I_{\parallel} - I_{\parallel F}) + (I_{\perp} - I_{\perp F})$, where $I_{\perp F}$ and $I_{\parallel F}$ are the fluorescence intensities measured with membranes or liposomes without the probe. To minimize reversible deactivation of the probe, all experiments were conducted in the dark. Fluorescence anisotropy was calculated according to $r_s = 2P/(3 - P)$. The anisotropy parameter, $[(r_o/r_s) - 1]^{-1}$, was calculated by using 0.362 as the maximal limiting anisotropy (r_o) of DPH [28]. Increased anisotropy represents increased restriction of the probe's motion and has been shown in membrane systems to reflect increased 'microviscosity' (or decreased 'fluidity') in the core of the lipid bilayer [30]. The order parameter (S) was calculated from the steady-state fluorescence anisotropy [31,32]. The structural component of anisotropy, r_∞ is equal to $(4r = /3) - 0.1$. The order parameter (S) can be calculated from $S = (r_\infty/r_o)^{0.5}$.

Analysis of the data

Statistical analysis was performed by Student's *t*-test for non-paired samples and compared with the control group. P values of 0.05 or less were taken as significant, and the results expressed as the means ± S.E.

Results

After 4 weeks of treatment with lithium salts by ad libitum intake in the drinking water, the plasma levels of Li^+, as determined by flame photometry, were 0.72 ± 0.11 mequiv./liter (mean ± S.E. of ten animals), equivalent to the therapeutic levels usually found in humans. LiCl-treated animals showed a light poliuria but did not differ significantly from controls in weight gain and exhibited no

TABLE I

FLUORESCENCE POLARIZATION OF DPH INCORPORATED INTO SYNAPTIC PLASMA MEMBRANE IN CONTROLS AND LITHIUM-TREATED RATS

P values are the means ± S.E. of triplicate determinations performed in samples from ten different experiments.

	Fluorescence polarization (*P*)	
	25 °C	37 °C
Controls	0.35 ± 0.01	0.29 ± 0.01
Li^+-treated animals	0.28 ± 0.01 [a]	0.24 ± 0.01 [a]

[a] Significantly different from control ($P < 0.001$).

signs of lithium intoxication. The levels of lithium in the brain (1.05 ± 0.17 mequiv./g wet weight) were always higher than those in plasma.

The fluidity of the synaptosomal plasma membranes prepared from both controls and animals treated for 4 weeks, was compared by using steady-state fluorescence polarization. In plasma membrane fractions, a significant decrease in the polarization of fluorescence of DPH was obtained in treated animals (Table I). The fluorescence polarization values of plasma membranes labeled with diphenylhexatriene indicate that the hydrophobic core of the membrane (the domain occupied by this probe) is more fluid in the case of lithium-treated rats than in controls.

To further characterize changes induced by lithium in the physical properties of the membranes, the temperature dependence of DPH fluorescence polarization was determined in synaptosomal plasma membrane (Fig. 1A) and in lipid vesicles obtained from both and lithium-treated animals (Fig. 1B). The fluorescence polarization term was calculated from *P* and the results were plotted as $\ln [(r_o/r) - 1)]^{-1}$ vs. $1/T$. In all cases computer analysis of the data revealed no break points, indicating the absence of a phase transition in the temperature range covered by the experiments (10–45 °C). As shown in Fig. 1, values of the fluorescence anisotropy were significantly lower, i.e., the fluidity was enhanced in membranes from lithium-treated animals as compared to the controls at each temperature tested. The lipid composition of the membranes has to be involved in the temperature response of the membrane viscosity, since the same pattern of values was obtained for lipid vesicles. On the other hand,

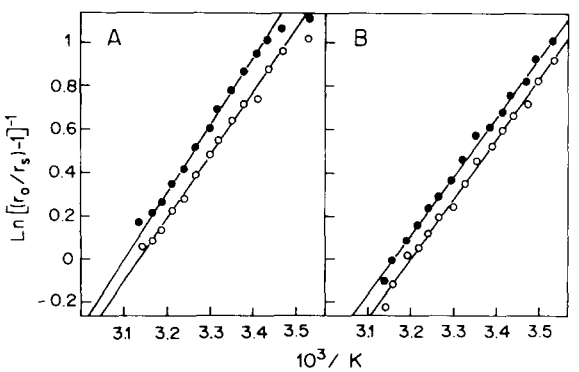

Fig. 1. Effect of temperature on DPH anisotropy parameter in synaptic plasma membranes (A) and lipid vesicles (B). Polarization values were converted to the anisotropy parameter, $[(r_o/r)-1]^{-1}$, as described in the text; ●, control plasma membrane; ○, plasma membrane from lithium-treated rats.

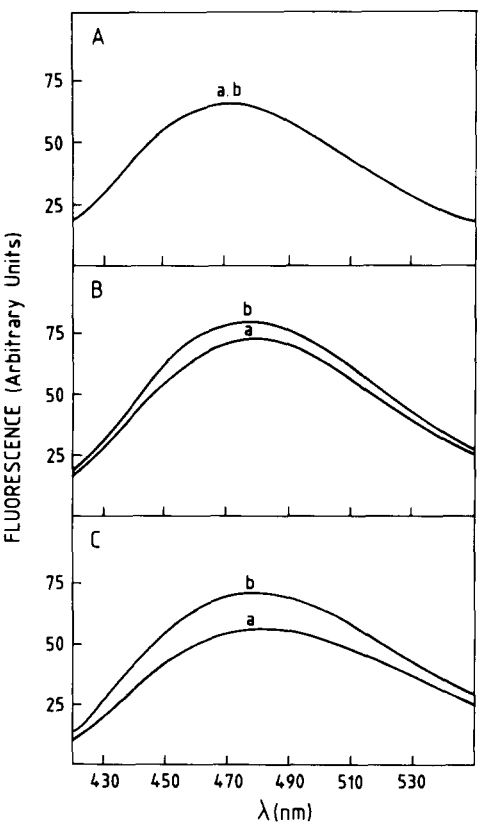

Fig. 2. Fluorescence emission spectra of ANS in the presence of synaptosomal plasma membranes from control (a) and lithium-treated rats (b). λ (excitation), 365 nm. Protein concentration, 50 μg/ml. ANS concentration: A, 25 μM; B, 100 μM; C, 150 μM.

the other parameter of DPH in synaptic plasma membranes decreased from 0.75 to 0.66 after lithium treatment.

The binding of the anionic fluorescent probe ANS is determined both by the membrane surface charge [33] and the degree of lipid disorder [34]. Since membrane lipids and membrane proteins are practically the only components of cell surface structures known to possess high affinity for ANS, these are the most important candidates for ANS binding. As the number of protein binding sites for ANS on the membrane is lower than the number of lipid binding sites but their affinity for ANS is greater [35], depending on the ANS concentration, the probe would (at low concentrations) bind first to the proteins, and only subsequently to the lipids [35,36]. The results in Fig. 2 show different fluorescence spectra of ANS in the presence of synaptosomal plasma membranes from (a) control or (b) lithium-treated animals and depending on the concentration of the probe. Our experiments show that lipid vesicles obtained with lipids extracted from lithium-treated animals can accommodate a greater amount of probe than those obtained from controls. In both cases the process showed saturation kinetics (data not shown).

The lipid composition of plasma membranes from synaptosomes of control and lithium-treated rats was compared. As shown in Table II, the lipid/protein ratio was higher in treated animals. The synaptosomal plasma membrane content of total phospholipid relative to protein was enhanced by 20% after lithium treatment and the total cholesterol content relative to protein increased as well. As a result, the molar ratio of cholesterol to phospholipid in the plasma membrane of the synaptosomes was unaltered by long-term lithium treatment. The cholesterol and phospholipid contents of the synaptosomal plasma membrane from control animals were adequate to previously reported data [37,38].

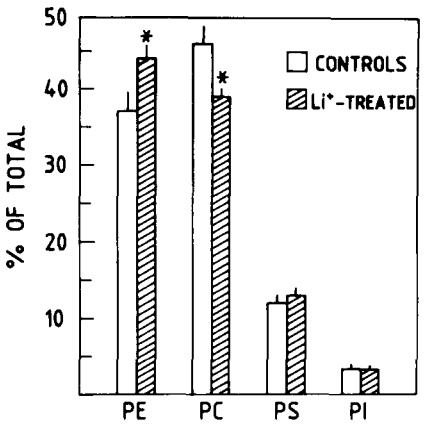

Fig. 3. Phospholipid composition of synaptic plasma membranes from rat brain. 100% corresponds to 674 ± 82 nmol/mg of protein (controls) and 792 ± 90 nmol/mg of proteins (lithium-treated animals). Values are the means ± S.E. of six triplicate experiments. The symbol * indicates that data are significantly different from control data ($P < 0.05$).

Phospholipid polar headgroup species composition was determined after separation by two-dimensional thin-layer chromatography (Fig. 3). Phosphatidylethanolamine (PE) and phosphatidylserine (PS) increased, by 30 and 7%, respectively, after lithium treatment; phosphatidylcholine (PC) decreased by 28%, whereas phosphatidylinositol (PI) did not change after treatment.

TABLE II

PHOSPHOLIPID AND CHOLESTEROL CONTENTS OF SYNAPTIC PLASMA MEMBRANE FROM CONTROLS AND LITHIUM-TREATED RATS

Total lipid and proteins were estimated gravimetrically and by the Lowry's procedure, respectively. Data are the means and S.E. of ten triplicate experiments.

	Lipid/protein (w/w)	Cholesterol (nmol/mg protein)	Cholesterol esters (nmol/mg protein)	Phospholipids (nmol/mg protein)	Cholesterol/ phospholipid (molar ratio)
Controls	0.856 ± 0.07	584 ± 97	95 ± 3	674 ± 82	0.880 ± 0.008
Li^+-treated rats	0.925 ± 0.10	668 ± 56	98 ± 2	792 ± 90	0.871 ± 0.01

TABLE III

FATTY ACID COMPOSITION OF PHOSPHOLIPID CLASSES

Values are means ± S.E. (weight percent) for five duplicate experiments. tr., trace, n.d., not detected.

Fatty acid	PE		PS		PI		PC	
	controls	Li^+-treated animals	controls	Li^+-treated animals	controls	Li^+-treated animals	controls	Li^+-treated animals
14:0	1.30 ± 0.3	1.18 ± 0.4	0.75 ± 0.1	0.59 ± 0.2	1.79 ± 0.5	1.50 ± 0.3	0.52 ± 0.1	0.47 ± 0.1
14:1	0.49 ± 0.1	0.66 ± 0.1	0.77 ± 0.4	0.28 ± 0.1	0.59 ± 0.3	0.63 ± 0.2	0.38 ± 0.1	0.57 ± 0.1 [c]
16:0	12.87 ± 1.3	10.77 ± 1.4 [c]	7.80 ± 1.6	6.93 ± 1.1	21.19 ± 2.9	18.91 ± 2.1 [c]	56.69 ± 2.3	56.66 ± 2.2
16:1	0.82 ± 0.1	0.99 ± 0.3	0.74 ± 0.4	0.53 ± 0.1	1.20 ± 0.1	2.03 ± 0.1 [a]	0.85 ± 0.1	0.69 ± 0.1
17:0	3.85 ± 1.1	1.85 ± 0.2 [a]	0.61 ± 0.1	0.51 ± 0.1	3.03 ± 0.1	1.45 ± 0.2 [a]	0.52 ± 0.1	0.46 ± 0.1
18:0	41.85 ± 1.1	40.89 ± 4.2	51.92 ± 4.8	48.85 ± 2.4	46.94 ± 4.0	47.66 ± 3.9	19.62 ± 1.2	18.13 ± 1.6
18:1	13.05 ± 0.8	13.90 ± 0.7	15.78 ± 1.3	18.29 ± 0.9 [b]	9.91 ± 1.1	14.61 ± 2.7 [c]	15.77 ± 1.5	17.04 ± 1.6
18:2 + 18:3	2.18 ± 0.2	2.30 ± 0.4	5.14 ± 1.3	6.07 ± 1.9	1.48 ± 0.2	1.73 ± 0.2	3.13 ± 0.4	4.55 ± 0.3 [a]
20:0	0.82 ± 0.1	0.66 ± 0.1 [b]	0.51 ± 0.2	0.80 ± 0.3	2.38 ± 0.4	1.89 ± 0.4	0.67 ± 0.3	n.d.
20:4	6.51 ± 1.6	8.29 ± 1.6 [b]	0.48 ± 0.2	0.49 ± 0.2	3.37 ± 1.5	1.05 ± 0.9 [b]	0.68 ± 0.1	1.08 ± 0.2 [b]
22:0	tr.	n.d.	1.19 ± 0.1	1.06 ± 0.2	2.35 ± 0.3	1.38 ± 0.2 [a]	0.54 ± 0.1	n.d.
22:1	2.06 ± 0.8	1.67 ± 0.4	1.64 ± 0.1	1.65 ± 0.3	3.28 ± 1.4	3.79 ± 0.6	n.d.	n.d.
22:5	3.45 ± 0.5	3.81 ± 0.6	0.47 ± 0.1	1.16 ± 0.5 [b]	n.d.	n.d.	n.d.	n.d.
22:6	10.82 ± 0.9	13.19 ± 1.6 [b]	13.07 ± 3.6	13.02 ± 3.8	2.45 ± 0.1	4.56 ± 0.3 [a]	0.37 ± 0.2	0.37 ± 0.2

[a] Significantly, different from control ($P < 0.001$); [b] ($P < 0.01$); [c] ($P < 0.05$).

The fatty acid composition of the major phospholipid classes of synaptosomal plasma membranes is presented in Table III. Some saturated fatty acids showed a significant decrease in PE and PI after treatment. In PE of all subjects, a significative decrease was seen in 16:0, 17:0 and 20:0, whereas in PI, 17:0 and 22:0 decreased significantly. On the other hand, lithium treatment for 4 weeks induced a generalized increase in the unsaturated fatty acid content of all phospholipids specially in 20:4 and 22:6 in PE, 18:2 + 3 and 20:4 in PC, 18:1 and 22:5 in PS, and 16:1 and 22:6 in PI.

Table IV shows an extract of the above data summarized as three indices: the average chain length, the ratio of saturated to unsaturated fatty acids (saturation ratio), and an unsaturation index that gives a relative estimate of the number of

TABLE IV

SUMMARY OF THE FATTY ACID COMPOSITION OF THE SYNAPTOSOMAL PLASMA MEMBRANE FROM CONTROL AND LITHIUM-TREATED RATS

The unsaturation index was calculated as sum of weight % multiplied by number of olefinic bounds for each fatty acid in the mixture.

	PE		PS		PI		PC	
	controls	Li^+-treated animals	controls	Li^+-treated animals	controls	Li^+-treated animals	controls	Li^+-treated animals
Average chain length	18.2 ± 0.3	18.3 ± 0.5	18.5 ± 0.4	18.5 ± 0.2	17.8 ± 0.4	17.7 ± 0.1	16.8 ± 0.05	16.9 ± 0.1
Ratio saturated to unsaturated fatty acids	1.58 ± 0.09	1.50 ± 0.32	1.70 ± 0.21	1.40 ± 0.22	3.10 ± 0.77	2.70 ± 0.67	4.06 ± 0.87	2.99 ± 0.19 [b]
Unsaturation index	1.27 ± 0.10	1.39 ± 0.25	1.14 ± 0.22	1.18 ± 0.25	0.48 ± 0.11	0.88 ± 0.09 [a]	0.28 ± 0.04	0.37 ± 0.04

[a] Significantly different from control ($P < 0.001$); [b] ($P < 0.05$).

olefinic bonds in each phospholipid class. After lithium treatment, there was an unmistakable trend towards increased fatty acid unsaturation, and hence, the unsaturation index was increased, in some cases significantly, a change which was expected to increase lipid fluidity.

Discussion

The foregoing results show that lithium salts administration at therapeutic levels alter the lipid composition and fluidity of nerve terminal membranes.

Fluorescence polarization measurement using probes that interact with different domains of the membrane has been proved to be an efficient method of determining the structure and function of biological membranes. Fluorescence polarization of DPH reflects the bulk properties of membranes, since this probe appears to partition equally well into liquid-cristalline and gel-phase lipid regions [39]. Thus, DPH is assumed to be aligned with the phospholipid acyl chains [40]. The data presented indicate that lithium treatment caused a decrease in the order parameter of DPH in synaptic plasma membranes. The order parameter of DPH is a measure of the degree to which molecular packing of membrane lipids hinders the rotation of the probe. The fluorescence polarization data at physiological temperature obtained with this probe indicate a more fluid environment within the membrane core of plasma membranes from treated animals than control rats.

A number of plasma membrane proteins whose activities are influenced by lipid fluidity respond to the presence of lithium [41]. Na^+–H^+ exchange has been recently demonstrated to be modulated by the physical properties of the membrane [42], and carrier-mediated metabolite transport, including neurotransmitters in nerve terminals, has been shown to be influenced by the lipid fluidity [43–45]. In our previous studies [16] we found that chronic lithium treatment to rats increases significantly the transport of tryptophan across synaptic membranes, induces more negative membrane potentials into the synaptosomes and causes changes in the Arrhenius plots, decreasing the transition temperatures for the tryptophan transport system in treated animals [17]. P values obtained with lipid vesicles indicate that lipid components are responsible for the observed differences in lipid fluidity between control and lithium-treated rat membranes.

The fluorescence probe ANS binds noncovalently to membrane lipids as well as to proteins, and is extremely sensitive to changes in the probe environment. ANS is submerged in the direction of the fatty acid alkyl chains so that the center of the aromatic ring of ANS lies approximately at the level of the phosphatidylcholine carbonyl group [46], and it has been demonstrated that the probe binds more effectively to membranes containing unsaturated phospholipids [34]. As ANS reflects charge and packing effects [47], our results demonstrate that at ANS concentrations far above the saturation of the protein-binding sites, the fluorescence of membrane-bound ANS increases with higher concentrations of the probe in lithium-treated animals; thus, it appears that lithium treatment increases the disorder of the membranes. On the other hand, membrane proteins do not seem to play an important role in this fluidizing effect induced by lithium treatment, since the same behavior is observed for both plasma membranes and lipid vesicles.

An insight into the lipid composition for synaptic membranes from both control and lithium-treated animals indicates that lithium treatment increases the lipid content of the membrane and the lipid/protein ratio. However, the cholesterol/phospholipid ratio does not change significantly after treatment. These results suggest that the membrane cholesterol content plays no important role in the alteration of the physical properties of lithium-treated synaptosomal plasma membranes. Thus, the polar head group and fatty acid composition of plasma membrane phospholipid were measured to identify biochemical changes associated with altered membrane dynamic properties. Our results show that lithium treatment induces changes in phospholipid class distribution. The increase in PE and PS content while PC content decreases (the more unsaturated phospholipid classes, the less unsaturated phospholipid), may contribute to the change in the membrane properties mentioned above. But the most interesting results come from the changes observed in fatty acid composition after lithium treatment. Positive

correlations between fluorescence polarization and the ratios of saturated/unsaturated fatty acids in the major phospholipid have been shown previously in different membranes, and it is currently assumed that changes in degree of unsaturation affect membrane fluidity.

Although lithium is widely used for the treatment of mania, its effects on the central nervous system are poorly understood. It is worth noting that many of the effects of lithium described in the literature involve a great variety of membrane-bound activities (i.e., carriers, receptors and enzymes). Although little is known about the regulation of enzymes involved in the synthesis of polyunsaturated fatty acids in the brain, it is tempting to speculate that the observed alterations of the lipid phase of the membrane after lithium treatment could be related to a change in the substrate selectivity or affinity of the key enzymes, such as phospholipases and/or transacylases, involved in the distribution of unsaturated fatty acids in the different phospholipid classes.

On the other hand, there must be mechanisms as direct interaction of lithium salts at the interface between the lipids and proteins of the membrane surface; otherwise, electrostatic interactions of lithium with some acidic phospholipids essential for certain membrane-bound protein activities, cannot be excluded. Thus, it can be suggested that the therapeutic actions of lithium take place through alterations in the composition and/or the dynamic properties of the neural membranes.

In summary, the foregoing evidence supports the conclusion that after 4 weeks of lithium treatment up to therapeutic levels, the increased membraned phospholipid unsaturation, induced by increasing the degree of acyl chain unsaturation, and the altered phospholipid class distribution, contribute to increase the lipid fluidity of the synaptic plasma membrane as assessed by fluorescence polarization measurements.

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