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Effects of lithium on the human erythrocyte membrane and molecular models

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Abstract

The mechanism whereby lithium carbonate controls manic episodes and possibly influences affective disorders is not yet known. There is evidence, however, that lithium alters sodium transport and may interfere with ion exchange mechanisms and nerve conduction. For these reasons it was thought of interest to study its perturbing effects upon membrane structures. The effects of lithium carbonate (Li^+) on the human erythrocyte membrane and molecular models have been investigated. The molecular models consisted in bilayers of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE), representing classes of phospholipids located in the outer and inner monolayers of the erythrocyte membrane, respectively. This report presents the following evidence that Li^+ interacts with cell membranes: a) X-ray diffraction indicated that Li^+ induced structural perturbation of the polar head group and of the hydrophobic acyl regions of DMPC and DMPE; b) experiments performed on DMPC large unilamellar vesicles (LUV) by fluorescence spectroscopy also showed that Li^+ interacted with the lipid polar groups and hydrophobic acyl chains, and c) in scanning electron microscopy (SEM) studies on intact human erythrocytes the formation of echinocytes was observed, effect that might be due to the insertion of Li^+ in the outer monolayer of the red cell membrane.

Keywords: Lithium; Erythrocyte membrane; Phospholipid bilayer

1. Introduction

Bipolar affective disorder is a serious and enduring mental health problem, which is associated with a high mortality rate worldwide [1]. In spite of a narrow safety margin, lithium salts are widely used in the treatment and prophylaxis [2,3], particularly in the form of Li_2CO_3 [4,5]. The mechanism whereby lithium controls manic episodes and possibly influences affective disorders is not yet known. There is evidence, however, that lithium alters sodium transport and may interfere with ion exchange mechanisms and nerve conduction [6]. Lithium ions are rapidly absorbed from gastrointestinal tract, and plasma lithium peaks are reached 2 to 4 h after administration. Toxicity associated with lithium treatment is highly prevalent as 75 to 90% of patients have signs or symptoms of toxicity during their treatment [7]. The occurrence of toxicity is related to the serum concentration of lithium. Mild toxicity appears at levels up to 2.5 mM, and life-threatening effect is manifest at levels above 3.5 mM [8,9]; however, patients with concentrations as high as 6 mM [10], and even 9.6 mM [11] lithium serum levels following acute intoxication have been reported. The more common side effect involves the central nervous system [4]; chronic lithium treatment affects some signal transduction mechanisms such as cAMP, cGMP, Gi protein, and protein kinase C [3].

Lithium affects ion transport and cell membrane potential by competing with sodium and potassium; these effects may alter neuronal function [12]. Lithium is transported across cell membranes by an exchange diffusion process referred to as Na^+–Li^+ countertransport, so called because sodium, at opposite with the sodium pump, is moved toward the intracellular compartment. This transport mechanism appears to be active in the membranes of cells from many types of tissues and to play a significant role

Abbreviations: SEM, scanning electron microscopy; IUM, isolated unsealed human erythrocyte membrane; LUV, large unilamenar vesicles; *r*, fluorescence anisotropy; GP, fluorescence general polarization; DPH, 1,6-diphenyl-1,3,5-hexatriene; Laurdan, 6-dodecanoyl-2-dimethylaminonaphthalene; DMPC, dimyristoylphosphatidylcholine; DMPE, dimyristoylphosphatidylethanolamine.

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in the biological disposition of clinically administered lithium [13]. Transmembrane ion movement *via* Na^+–Li^+ countertransport is impeded during lithium treatment because of reduced affinity of the intracellular transport sites for lithium [14]. In fact, lithium is a small ion with virtually no protein binding [8]. Lithium exchange activity has been extensively studied in human red blood cells [14–16]. However, the structural effects of lithium on the human erythrocyte membrane have scarcely been reported.

In the course of *in vitro* systems search for the toxicity screening of chemicals, different cellular models have been applied to examine their adverse effects in isolated organs. This article describes the interaction of lithium carbonate (Li^+) with the human erythrocyte membrane as well as lipids of model membranes. The cell membrane is a diffusion barrier that protects the cell interior; therefore, its structure and functions are susceptible to alterations as a consequence of interactions with foreign species. Erythrocytes were chosen because although less specialized than many other cell membranes, they carry on enough functions in common with them such as active and passive transport, and the production of ionic and electric gradients, to be considered representative of the plasma membrane in general. With the aim to better understand the molecular mechanisms of the interaction of Li^+ with cell membranes we have utilized three well-established models. They regard intact human erythrocytes and molecular models of the erythrocyte membrane. The latter consisted of multibilayers of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE), representative of phospholipid classes located in the outer and inner monolayers of the human erythrocyte membrane, respectively [17,18], and large unilamellar vesicles (LUV) of DMPC. The capacity of Li^+ to perturb the multibilayer structures of DMPC and DMPE was determined by X-ray diffraction, while DMPC LUV were studied by fluorescence spectroscopy. Intact human erythrocytes incubated with this compound were observed by scanning electron microscopy. These systems and techniques have been used in our laboratories to determine the interaction with and the membrane-perturbing effects of other metal ions such as Al^{3+} [19], Pb^{2+} [20], Cd^{2+} [21], Au^{3+} [22], Ti^{4+} [23], Fe^{2+}, and Fe^{3+} [24].

2. Materials and methods

2.1. X-ray diffraction studies of phospholipid multilayers

The capacity of Li^+ to perturb the structures of DMPC and DMPE multilayers was evaluated by X-ray diffraction. Synthetic DMPC (lot 140PC-224, MW 677.9), DMPE (lot 140PC-230, MW 635.9) from Avanti Polar Lipids, AL, USA), and Li_2CO_3 (p.a., Merck, Darmstadt, Germany) were used without further purification. About 2 mg of each phospholipid was introduced into 1.5 mm diameter special glass capillaries, which were then filled with 200 μl of (a) distilled water and (b) aqueous solutions of Li_2CO_3 in a range of concentrations (0.1 mM to 10 mM). The specimens were X-ray diffracted after 1 h incubation at 37 °C and 60 °C with DMPC and DMPE, respectively, in flat plate cameras. Specimen-to-film distances were 8 and 14 cm, standardized by sprinkling calcite powder on the capillary surface. Ni-filtered CuKα radiation from a Bruker Kristalloflex 760 (Karlsruhe, Germany) X-ray generator was used. The relative reflection intensities were obtained in a MBraun PSD-50M linear position-sensitive detector system (Garching, Germany); no correction factors were applied. The experiments were performed at 19±1 °C, which is below the main phase transition temperature of both DMPC and DMPE. Each experiment was repeated three times, and in case of doubts additional experiments were carried out.

2.2. Fluorescence measurements of DMPC large unilamellar vesicles (LUV)

The influence of Li^+ on the physical properties of DMPC LUV was examined by fluorescence spectroscopy using DPH (1,6-diphenyl-1,3,5-hexatriene) and laurdan (6-dodecanoyl-2-dimethylaminonaphtalene) (Molecular Probe, Eugene, OR, USA) fluorescent probes. DPH is widely used as a probe for the hydrophobic regions of the phospholipid bilayers because of its favorable spectral properties. Its steady-state fluorescence anisotropy measurements were used to investigate the structural properties of DMPC LUV, as it provides a measure of the rotational diffusion of the fluorophore, restricted within a certain region such as a cone, due to the lipid acyl chain packing order. Laurdan, an amphiphilic probe, has a high sensitivity of excitation and emission spectra to the physical state of membranes. With the fluorescent moiety within a shallow position in the bilayer, laurdan fluorescence spectral shifts provide information of its molecular dynamic properties at the level of the phospholipid polar headgroups. The quantification of the laurdan fluorescence shifts was effected using the general polarization GP concept [25].

DMPC LUV suspended in water were prepared by extrusion of frozen and thawed multilamellar liposome suspensions (final lipid concentration 0.4 mM) through two stacked polycarbonate filters of 400 nm pore size (Nucleopore, Corning Costar Corp., MA, USA) under nitrogen pressure at 37 °C, which is above the lipid phase transition temperature. DPH and laurdan were incorporated into DMPC LUV by addition of 2 μl/ml aliquots of 0.5 mM solutions of the probe in dimethylformamide and ethanol respectively in order to obtain final analytical concentrations of 2 μM, incubating them at 37 °C for 60 min. Fluorescence spectra and anisotropy measurements were performed on a K-2 steady-state and time-resolved spectrofluorometer (ISS Inc., Champaign, IL, USA) interfaced to computer, using the corresponding ISS software for data collection and analysis. Measurements of LUV suspensions were made at 18 °C and 37 °C using 5 mm path-length square quartz cuvettes. Sample temperature was controlled by an external bath circulator (Cole-Parmer, Chicago, IL, USA) and monitored before and after each measurement using an Omega digital thermometer (Omega Engineering Inc., Stanford, CT, USA). Anisotropy measurements were made in the L configuration using Glan Thompson prism polarizers (I.S.S.) in both exciting and emitting beams. The emission was measured using a WG-420 Schott high-pass filter (Schott WG-420, Mainz, Germany) with