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Identification of Li^+ binding sites and the effect of Li^+ treatment on phospholipid composition in human neuroblastoma cells: A ^7Li and ^{31}P NMR study

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Abstract

Li^+ binding in subcellular fractions of human neuroblastoma SH-SY5Y cells was investigated using ^7Li NMR spin–lattice (T_1) and spin–spin (T_2) relaxation measurements, as the T_1/T_2 ratio is a sensitive parameter of Li^+ binding. The majority of Li^+ binding occurred in the plasma membrane, microsomes, and nuclear membrane fractions as demonstrated by the Li^+ binding constants and the values of the T_1/T_2 ratios, which were drastically larger than those observed in the cytosol, nuclei, and mitochondria. We also investigated by ^{31}P NMR spectroscopy the effects of chronic Li^+ treatment for 4–6 weeks on the phospholipid composition of the plasma membrane and the cell homogenate and found that the levels of phosphatidylinositol and phosphatidylserine were significantly increased and decreased, respectively, in both fractions. From these observations, we propose that Li^+ binding occurs predominantly to membrane domains, and that chronic Li^+ treatment alters the phospholipid composition at these membrane sites. These findings support those from clinical studies that have indicated that Li^+ treatment of bipolar patients results in irregularities in Li^+ binding and phospholipid metabolism. Implications of our observations on putative mechanisms of Li^+ action, including the cell membrane abnormality, the inositol depletion and the G-protein hypotheses, are discussed.
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1. Introduction

Lithium has been used for the treatment of bipolar disorder for more than 50 years without knowledge of its exact mechanism of action [1,2]. An improved understanding of the molecular mechanism of the therapeutic action of Li^+ requires the investigation of the binding of Li^+ to the different subcellular fractions of the nerve cell and ascertaining the effect that chronic Li^+ treatment has on the Li^+ binding sites.

Previous studies on red blood cells (RBCs) used as a model cell line have addressed the question of where Li^+ binds in these cells [3–5]. In a ^7Li NMR relaxation study, the major Li^+ binding site in RBCs was determined to be the plasma membrane. In contrast, other RBC components such as hemoglobin, spectrin, 2,3-bisphosphoglycerate, and ATP were found to contribute only minimally toward Li^+ binding [5]. It was further determined that the inner leaflet of the

Abbreviations: PC, phosphatidylcholine; PC_{AA}, β-acyl-γ-alkylphosphatidylcholine; PE, phosphatidylethanolamine; PE_P, phosphatidylethanolamine plasmogen; PI, phosphatidylinositol; PS, phosphatidylserine; RBC, red blood cell; τ_c, rotational correlation time; SM, sphingomyelin; T_1, spin–lattice relaxation time; T_2, spin–spin relaxation time

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plasma membrane contributed more extensively to Li^+ binding than did the outer leaflet, due to the presence of more anionic phospholipids in the inner leaflet [5]. In a more recent study, it was demonstrated qualitatively that Li^+ was immobilized more in intact human neuroblastoma SH-SY5Y cells, a nerve cell model, than in intact RBCs [6]. Comparable Li^+ binding was, however, observed in suspensions of RBC and neuroblastoma plasma membranes [6]. This observation suggests that additional Li^+ binding sites accounted for the greater extent of Li^+ immobilization in neuroblastoma cells than in RBCs.

Membrane abnormalities have been suggested to occur in bipolar illness, based on observations of irregular Li^+-transport rates and phospholipid membrane composition in RBCs from Li^+-treated bipolar patients [7]. In particular, it has been found that rates of Na^+–Li^+ exchange in RBCs were significantly lower in Li^+-treated bipolar patients than in normal individuals [8,9]. Three weeks after discontinuation of Li^+ treatment, however, the Na^+–Li^+ exchange rates in these same patients returned to normal values, indicating an effect of Li^+ treatment [10,11]. Abnormal phospholipid composition has been observed in the RBC plasma membranes of Li^+-treated bipolar patients [9], which may explain that the decrease in Na^+/Li^+ transport rates results from altered interactions between metal ions and membrane proteins or lipids. Li^+ has been demonstrated to have varying binding affinities to different phospholipids isolated from RBC plasma membranes [12]. Specifically, Li^+ was shown to have the highest binding affinity to phosphatidyl serine (PS), followed by phosphatidyl inositol (PI). Sphingomyelin (SM), phosphatidyl ethanolamine (PE), β-acyl-γ-alkylphosphatidylcholine (PC_{AA}), and phosphatidyl choline (PC) were shown to have lower binding affinities to Li^+ than PS or PI. In contrast to the case of RBCs, the Na^+–Ca^{2+} exchanger, and, to a smaller extent, the voltage-sensitive Na^+ channel and the Na^+–Li^+ exchanger, are the main Li^+ transport pathways in neuronal cells [6,13]. Unlike in RBCs, in neuronal cells from bipolar patients, however, the effects of Li^+ on Na^+–Ca^{2+} exchange and on Na^+–Li^+ exchange, as well as on voltage-sensitive Na^+ transport rates, are unknown. Because the presumed site of Li^+ action in bipolar illness is neuronal tissue, the relationship between the membrane abnormality hypothesis in neuronal tissue and the pharmacologic action of Li^+ warrants further exploration.

To further our understanding of the applicability of this membrane abnormality hypothesis to neuronal cells, we examined two aspects of Li^+ action in human neuroblastoma SH-SY5Y cells: (1) the identification of major intracellular site(s) of Li^+ binding and (2) the effect of chronic Li^+ treatment on these binding sites. Using differential centrifugation techniques, we isolated subcellular fractions from human neuroblastoma SH-SY5Y cells. We used the ratio of measured ^7Li NMR spin–lattice (T_1) to spin–spin (T_2) relaxation rates, which is a sensitive measure of molecular mobility [3,4], to determine Li^+ binding characteristics in different subcellular fractions. Because phospholipids are the major determinants of Li^+ binding to membrane fractions, phospholipid concentrations were determined by ^{31}P NMR spectroscopy in the cell homogenates and in the major Li^+ binding site, the plasma membrane, without prior Li^+ incubation and under chronic Li^+ incubation conditions (2.5 mM and 5.0 mM Li^+ over 4 to 6 weeks). We have also interpreted our observations on Li^+ binding sites and the effects of Li^+ incubation on the phospholipid composition of the plasma membrane in the SH-SY5Y nerve cell model in terms of putative mechanisms of lithium action.

2. Materials and methods

2.1. Materials

The human neuroblastoma SH-SY5Y cell line was provided by Dr. E. Stubbs, Jr. (Department of Neurology, Loyola University Medical Center). The Bradford dye for the protein assay was purchased from Bio-Rad Laboratories (Hercules, CA). Pure phospholipids were purchased from Avanti Polar Lipids (Alabaster, AL), and Nunclon TripleFlask flasks were from Fisher Scientific (Pittsburgh, PA). All other biochemicals and inorganic salts were purchased from Sigma Chemical Company (St. Louis, MO).

2.2. Preparation of human neuroblastoma SH-SY5Y cell fractions

Human neuroblastoma SH-SY5Y cells were grown for 4–6 weeks with 0, 2.5, or 5.0 mM LiCl supplemented in the growth medium and harvested as previously described [14]. We confirmed that the cells were viable in the absence and presence of up to 5.0 mM Li^+ (>90%) using a Trypan Blue test. The viability of neuroblastoma SH-SY5Y cells is unaltered after chronic Li^+ incubation [15]. For each fractionation method, the mixture of protease inhibitors consisted of 1.0 mM phenylmethylsulfonyl fluoride, 0.1 μM pepstatin, and 50.0 μM leupeptin [16]. All buffers were kept on ice during preparation, washing, and storage. Each final pellet was washed twice with 5.0 mM Tris–HCl (pH 7.3) buffer for plasma and nuclear membranes and microsomes or with 20 mM Tris–Cl (pH 7.4) and 145 mM tetramethylammonium chloride buffer for the intact organelles (nuclei and mitochondria). Membranes and intact organelles were stored at −80 °C for less than 2 weeks before use.

The cell homogenate was prepared by lysing of harvested cells in a 5.0 mM Tris–Cl buffer (pH 7.3). The cytosol and cytosol-free fractions were further purified from the cell homogenate fraction by centrifugation (100,000×g/60 min/4 °C), in which the resulting supernatant and pellet were used for the preparation of cytosol and cytosol-free fractions, respectively. In each case, the isolated fraction was washed

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