Competition Between Li^+ and Mg^{2+} for Red Blood Cell Membrane Phospholipids: A ^{31}P, ^7Li, and ^6Li Nuclear Magnetic Resonance Study

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ABSTRACT: The mode of action of the lithium ion (Li^+) in the treatment of manic depression or bipolar illness is still under investigation, although this inorganic drug has been in clinical use for 50 yr. Several research reports have provided evidence for Li^+/Mg^{2+} competition in biomolecules. We carried out this study to characterize the interactions of Li^+ and Mg^{2+} with red blood cell (RBC) membrane components to see whether Li^+/Mg^{2+} competition occurs. ^{31}P nuclear magnetic resonance chemical shift measurements of the phospholipids extracted from the RBC membranes indicated that the anionic phospholipids, phosphatidylserine and phosphatidylinositol, bind Li^+ and Mg^{2+} most strongly. From ^6Li relaxation measurements, the Li^+ binding constant to the phospholipid extract was found to be 45 ± 5 M^{-1}. Thus, these studies showed that the phospholipids play a major role in metal ion binding. ^7Li spin-lattice relaxation measurements conducted on unsealed and cytoskeleton-depleted RBC membrane in the presence of magnesium indicated that the removal of the cytoskeleton increases lithium binding to the more exposed anionic phospholipids (357 ± 24 M^{-1}) when compared to lithium binding in the unsealed RBC membrane (221 ± 21 M^{-1}). Therefore, it can be seen that the cytoskeleton does not play a major role in Li^+ binding or in Li^+/Mg^{2+} competition.

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To test the Li^+/Mg^{2+} competition hypothesis for the pharmacological action of lithium, investigators have used small biomolecules or biomembranes as model systems (1–3). In the human body, blood transports Li^+ to the central nervous system; therefore, it is important to understand the interaction of Li^+ with the red blood cells (RBC) and their components. Rong *et al*. (3), using ^7Li nuclear magnetic resonance (NMR) relaxation measurements (T_1 and T_2), observed that the interactions of Li^+ with the RBC components ATP, 2,3-bisphosphoglycerate, spectrin, and Hb in different oxygenation forms were very weak. The RBC membrane did provide, however, most of the high-affinity Li^+ binding sites. The erythrocyte membrane displays very high mechanical stability and resilience, which comes from a partnership between the plasma membrane and an underlying meshwork called the membrane cytoskeleton. The major constituent of the membrane cytoskeleton that provides the infrastructure is spectrin, which binds indirectly to the RBC membrane *via* interactions with protein 4.1 and ankyrin (2).

In previous NMR studies based on Li^+ binding to agar gels (2) or on Na^+ binding to human RBC membranes measured by double-quantum experiments (4), investigators speculated that the cytoskeletal proteins provide binding sites for alkali metal ions in human RBC membranes. An NMR study with purified spectrin, however, did not show evidence of Li^+ binding to the major component of the cytoskeleton (3). In this study, we tested the contribution of the spectrin–actin network toward Li^+/Mg^{2+} competition by conducting ^7Li NMR relaxation measurements with unsealed and cytoskeleton-depleted RBC membrane suspensions.

The major class of lipids present in the RBC membrane is that of phospholipids. The most common in the RBC membrane are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), and sphingomyelin (SM). It is interesting to note that both of the anionic phospholipids, PI and PS, are found only in the inner leaflet of the RBC membrane (5,6). The intrinsic binding constants for interactions between some alkali and alkaline earth metal ions and PS have been reported (7). Evidence for Li^+ interactions with PS-containing liposomes was previously obtained from ^7Li and ^2H NMR relaxation data (8–10). Measurements conducted on inside-out and right-side-out vesicle suspensions clearly indicated that the inner leaflet of the RBC membrane provided the major Li^+ binding site (3). Significant differences between T_1 and T_2 values were also observed for suspensions of phospholipids extracted from the RBC membrane, suggesting that phospholipids, and not the proteins in or anchored to the membrane, provided the major Li^+ binding sites (3). Therefore, it is believed that the anionic

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Abbreviations: AA, atomic absorption; NMR, nuclear magnetic resonance; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEPLAS, plasmalogen PE; PI, phosphatidylinositol; PS, phosphatidylserine; RBC, red blood cell; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SM, sphingomyelin; T_1, spin-lattice relaxation time; T_2, spin-spin relaxation time.