Journal of the Arkansas Academy of Science

Volume 43 Article 12

1989

7Li NMR of Normal Human Erythrocytes

Rao P. Gullapalli
University of Arkansas at Little Rock

Roger M. Hawk
University of Arkansas at Little Rock

Richard A. Komoroski
University of Arkansas for Medical Sciences

^{7}Li NMR OF NORMAL HUMAN ERYTHROCYTES

R.P. GULLAPALLI,[1] R.M. HAWK,[1] and R.A. KOMOROSKI[2]
[1]Department of Electronics and Instrumentation
University of Arkansas at Little Rock
and
[2]University of Arkansas for Medical Sciences
Departments of Radiology and Pathology
Little Rock, AR

ABSTRACT

Lithium has been known to be an effective medication for people with bipolar disorder. The mechanisms of action of lithium in the brain is not very well understood. NMR spectroscopy and imaging are effective both in determining lithium levels in tissue and brain. We have monitored lithium levels in red blood cells. We have been able to separate intra- and extracellular compartments of lithium using shift reagents, thereby obtaining T^1's of both the compartments. Lithium uptake as a function of hematocrit was monitored weekly over a 3 week period. The time constant of 50 mM lithium uptake at 25 °C and 85% hematocrit was found to be 16.5 hrs. The time constant of 1.8 mM lithium uptake at 37 °C and 45% hematocrit was found to be 11.6 hrs. Experiments on the visibility of the quadrupolar nuclei indicate that it is only 74-90% visible and the visibility decreased with decreasing concentrations.

INTRODUCTION

Lithium salts have been used with considerable success in treating both depressive and manic recurrences of bipolar illnesses. Despite much research, little is known concerning the mechanism of action (Stern and Lydiard, 1987). We and others have detected *in vivo* ^{7}Li NMR signals from the brains of both rats and humans on lithium therapy (Komoroski *et al.*, 1989; Renshaw and Wicklund, 1988). It is important to know the intra- to extracellular ratio in the brain and, hence, the origin of the *in vivo* ^{7}Li NMR signals, because the presumed site of action is intracellular in the brain. A simple model where intra- to extracellular ratios of lithium have been determined and lithium transport studied is the red blood cell (Pettegrew *et al.*, 1987; Espanol, *et al.*, 1987; Hughes *et al.*, 1988; Mota de Freitas *et al.*, 1988). Apparently conflicting results concerning the ^{7}Li NMR visibility of intracellular lithium have been reported (Pettegrew *et al.*, 1987; Hughes *et al.*, 1988). We report studies of lithium transport across erythrocyte membranes, spin-lattice relaxation times, and NMR visibility at low concentrations of lithium.

MATERIALS AND METHODS

Lithium-7 spectra were acquired at 116.8 MHz on a General Electric GN-300 FT NMR spectrometer using 10 or 20 mm O.D. sample tubes. Spin-lattice relaxation times (T_1) were measured using the inversion-recovery technique. Dysprosium tripolyphosphate was the extracellular shift reagent. A typical spectrum showing intra and extra peak separation is shown in Figure 1 after incubating red blood cells with lithium for 24 hours. One pint of fresh venous blood was drawn into citrate dextrose anticoagulant (CPDA-1) and typically was used in 1-2 days. Blood obtained from the UAMS Blood Bank was examined up to one month later. Erythrocytes were spun down and washed in a buffer containing 150 mM NaCl according to published methods (Espanol *et al.*, 1987).

RESULTS AND DISCUSSION

We obtained a ^{7}Li T_1 of 5.7 s for intracellular lithium at 25 °C, a value comparable to that previously reported (Espanol *et al.*, 1987). For packed erythrocytes at 25 °C, the extracellular T_1 was 6.5 s, substantially shorter than the 16.5 s reported previously at 13% hematocrit (Espanol *et al.*, 1987). At 37 °C the intra- and extracellular T_1 values are increased to 6.5 and 8.2 s, respectively. This latter value is reduced from the expected value of about 22 s, which we found for pure NMR buffer (see Table 1). The two-component behavior of the T_1 curves will not be a suitable method for distinguishing these compartments in the human brain *in vivo* because the intra- and extracellular T_1s are not greatly different.

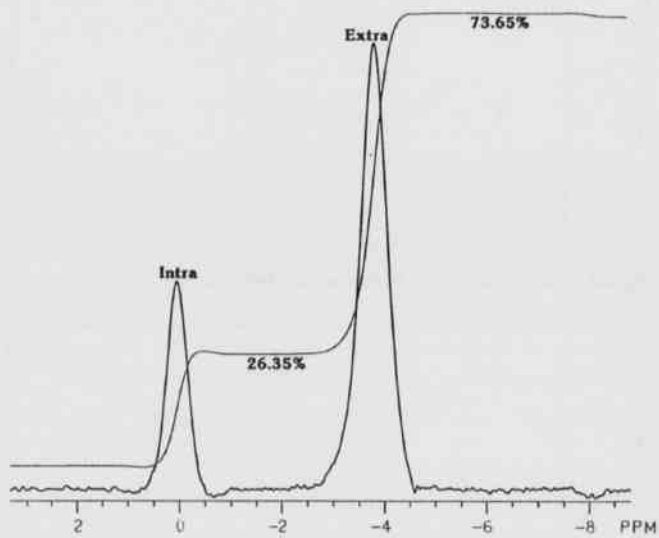

Figure 1. Intra- and Extra-Cellular Components Separated by Dysprosium Tripolyphosphate

Table 1. Spin Lattice (T_1) Relaxation Times of Various Solutions.

@25°C	
1M LiCl	13.0 secs.
3.5 mM LiCl	13.0 secs.
Extracellular Li (RBC)	6.5 secs.
Intracellular Li (RBC)	5.7 secs.
@37°C	
1M LiCl	23.0 secs.
50 mM LiCl	20.3 secs.
NMR Buffer	22.0 secs.
Extracellular Li (RBC)	8.2 secs.
Intracellular Li (RBC)	6.5 secs.

As expected, the percentage of intracellular lithium decreases with decreasing hematocrit. The percentage of intracellular lithium increased substantially (100 - 200%) with aging from one to three weeks (see Figure 2). We have measured the kinetics of lithium transport into the cells for two situations. At 50 mM lithium (25 °C) and about 85% hematocrit (packed cells), we obtained a time constant of 16.5 hours, close to the 14.7 hours found by Pettegrew *et al.*, (1987). We first detected lithium about one-half hour into the experiment (see Figure 3). The kinetic data for 1.8 mM lithium at a level of 45% hemotocrit and 37 °C are shown in Figure 4. These conditions closely approximate those expected clinically. The time constant was 11.6 hours. In this case, intracellular lithium was first observed 1.5 hours into the experiment.

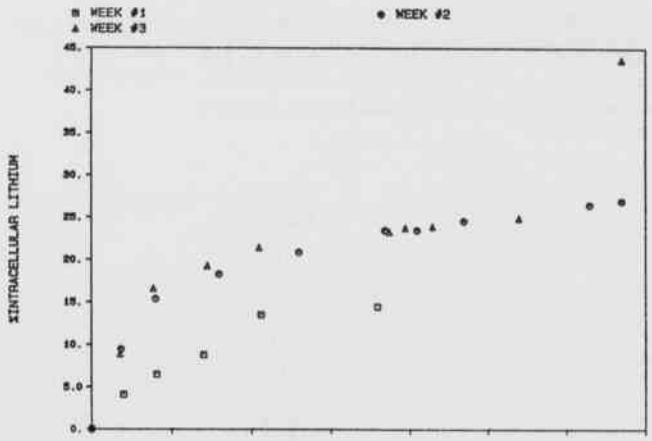

Figure 2. Li Uptake as a Function of Hematocrit

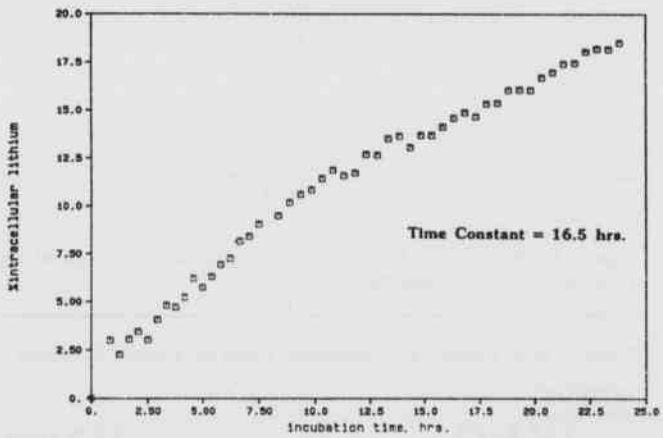

Figure 3. Kinetics of Li (50 mM) Transport in Packed Red Blood Cells at 25 °C

Spin-3/2 nuclei can exhibit reduced "NMR visibility" in motionally restricted environments (Bull, 1972). Such is common for ^{23}Na but, in this regard, little is known about ^{7}Li. Pettegrew *et al.* (1987) reported 98% visibility for the total signal from packed erythrocytes with about 33% intracellular lithium at 50 mM LiCl, whereas Hughes *et al.* (1988) reported full extracellular but reduced intracellular visibility at 40 mM LiCl. This reduced visibility is attributed to the binding of lithium to cell membrane and other cellular components.

We have examined the visibility of ^{7}Li at several concentrations for packed red cells. At 40 mM, we found a total visibility of 88-90%, whereas at 1 to 10 mM, visibility was reduced to 74 to 84% (see Table 2). Our experiments do not permit separate determination of intra- and extracellular visibility. These results suggest that ^{7}Li signals detection *in vivo* suffer from reduced visibility of one or both components.

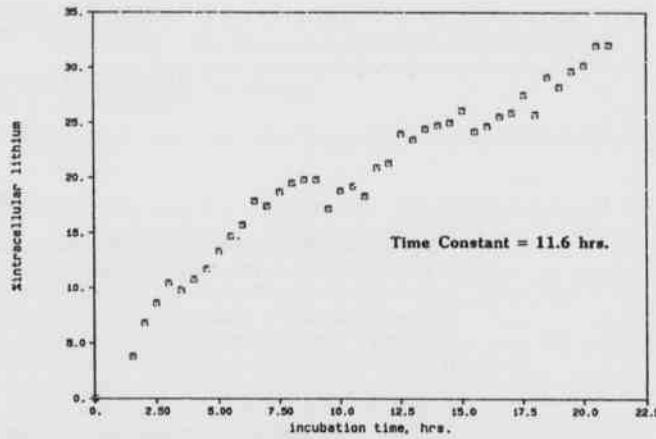

Figure 4. Kinetics of Li (1.8 mM) Transport in Red Blood Cells (45% Hematocrit) at 37 °C

Table 2. NMR 'Visibility' of Lithium in Erythrocytes

Conc. of LiCL	% Total Visibility	% Intracellular Visibility *
40 mM	87.5	74.0
10 mM	80.0	70.0
5 mM	78.0	66.0
1 mM	74.0	63.0

* assuming all the invisibility is due to intracellular compartment.

ACKNOWLEDGMENT

We thank the Whitaker Foundation for a grant in support of this work.

LITERATURE CITED

BULL, T.E. 1972. Nuclear magnetic relaxation of spin-3/2 nuclei involved in chemical exchange. J. Magn. Reson. 8:344-353.

ESPANOL, M.C. and D. MOTA DE FREITAS. 1987. ^{7}Li NMR studies of lithium transport in human erythrocytes. Inorg. Chem. 26:4356-4359.

HUGHES, M.S., K.J. FLAVELL, and N.J. BIRCH. 1988. Transport of lithium into human erythrocytes as studied by ^{7}Li nuclear magnetic resonance and atomic absorption spectroscopy. Biochem. Soc. Trans. 16:827-828.

KOMOROSKI, R.A., J. NEWTON, C. KARSON, E. WALKER, D. CARDWELL, and S. RAMAPRASAD. 1989. *In vivo* NMR spectroscopy of psychoactive drugs in humans. Magn. Reson. Imaging. 7, 1:32.

MOTA DE FREITAS, D., M.C. ESPANOL, R. RAMASAMY, J. SILBERBERG, and W. DORAS. 1988. Measurement of lithium transport rates in human erythrocytes of manic depressive patients and normal controls by ^{7}Li NMR. Abstracts, 7th Annual Meeting, Society of Magnetic Resonance in Medicine. pp. 506.

PETTEGREW, J.W., J.F.M. POST, K. PANCHALINGAM, G. WITHERS, and D.E. WOESSNER. 1987. ^{7}Li NMR study of normal human erythrocytes. J. Magn. Reson. 71:504-519.

RENSHAW, P.F. and S. WICKLUND. 1988. *In-vivo* measurement of lithium in humans by nuclear magnetic resonance spectroscopy. Biol. Psychiatry. 23:465.

STERN, T.A. and R.B. LYDIARD. 1987. Lithium therapy revisited. Psychiatric Med. 4:39.