Volume 244, number 1, 223–226 FEB 06806 February 1989

Competition between Li^+ and Mg^{2+} for ATP in human erythrocytes

A ^{31}P NMR and optical spectroscopy study

Ravichandran Ramasamy and Duarte Mota de Freitas

Department of Chemistry, Loyola University of Chicago, 6525 N. Sheridan Road, Chicago, IL 60626, USA

Received 12 December 1988; revised version received 19 December 1988

We have investigated the influence of Li^+ on free intracellular Mg^{2+} concentration in human erythrocytes by ^{31}P NMR and optical absorbance spectroscopies. In red cells loaded with 3 mM intracellular Li^+, the chemical shift separation between the α- and β-phosphate resonances of $MgATP^{2-}$ was approx. 0.9 ppm larger than that observed in Li^+-free red cells. By analyzing the interaction of each red cell component with Mg^{2+} and Li^+, we found that Mg^{2+} is displaced in part from $MgATP^{2-}$ upon addition of Li^+ and that the released Mg^{2+} is bound to the red cell membrane causing an overall decrease in free intracellular Mg^{2+} concentration.

ATP; Li^+; Mg^{2+}; Competition; NMR, ^{31}P-

1. INTRODUCTION

Mg^{2+} is an essential component of all living systems, playing a crucial role in a multitude of biochemical processes occurring within a cell. The intracellular concentration of free Mg^{2+} is of fundamental importance, since this ion is known to regulate the activity of various enzymes involved in macromolecular synthesis, glycolysis, respiration and membrane transport processes.

Lithium salts are preferred drugs in the treatment and maintenance of both manic and depressive episodes of bipolar patients [1]. Lithium has also been used in a variety of other psychiatric and medical conditions, including treatment of low white blood cell count resulting from cancer chemotherapy and conditions caused by the Herpes simplex virus [1]. Despite the important pharmacological action of lithium, the mechanism(s) for its biological action remain(s) uncertain. However, there are several hypotheses. One is the competition between Li^+ and Mg^{2+} for biomolecules which is based on the existence of a diagonal relationship between Li^+ and Mg^{2+}. In particular, it has been shown that biological ligands with a set of 3 oxygens and 1 nitrogen coordination sites would bind Li^+ rather than Na^+, K^+, or Ca^{2+}, and moreover, would still be able to compete for one-quarter of the Mg^{2+}-binding sites [2].

The distribution of Mg^{2+} is known to be altered after Li^+ administration to rats [3]. However, some researchers have found a reduction in brain Mg^{2+} levels [4], while others have reported an increase [5,6]. In bipolar patients receiving Li^+ therapy, some studies have shown an increase in serum Mg^{2+} levels [7], although other reports have either shown a decrease [8] or no change in serum Mg^{2+} levels [9,10]. It is not clear from the above investigations if the variability is due to the difficulty in obtaining accurate Mg^{2+} concentrations by atomic absorption methods. Here, we investigated the influence of Li^+ on the free intracellular Mg^{2+} concentration in human red blood cells (RBCs) by ^{31}P NMR and optical absorbance spectroscopies.

2. MATERIALS AND METHODS

Freshly drawn packed RBCs from normal healthy volunteers were supplied by Life Source, Chicago. Experiments were per-

Correspondence address: D.M. de Freitas, Department of Chemistry, Loyola University of Chicago, 6525 N. Sheridan Road, Chicago, IL 60626, USA

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

formed after 48 h of drawing blood to minimize fluctuations in free Mg^{2+} concentration in RBCs due to storage [11]. LiCl, $MgCl_2$, Tris-Cl and antipyrylazo-III were obtained from Aldrich while ATP (Na^+ form), and Hepes were purchased from Sigma. A23187 was from Boehringer Mannheim. All experiments were performed at 37°C and pH 7.2. pH measurements were carried out with an Orion pH meter. The osmolarity of all RBC samples was measured with a Wescor vapor pressure osmometer and found to be 300 ± 5 mosM. ^{31}P NMR spectra were recorded on a Varian VXR-300 NMR spectrometer equipped with a 10 mm probe and a variable-temperature unit. The operating frequency for the ^{31}P nucleus was 121.4 MHz. 45° pulses followed by an acquisition time of 1.5 s were used to acquire 2800 transients for each sample.

LiCl loading of RBCs was achieved by incubating the cells in a medium containing 40 mM LiCl, 100 mM NaCl, 5 mM KCl, 10 mM glucose, and 20 mM Hepes (pH 7.2) at 37°C for 12 h. Using these loading conditions, no Li^+-induced hydrolysis of ATP in RBCs was detected by ^{31}P NMR. Mg^{2+} levels were also measured in suspension media of control samples and Li^+-loaded samples. The RBC Li^+-loading procedure outlined above does not induce Mg^{2+} leak, since the Mg^{2+} concentration, measured by optical absorbance using antipyrylazo-III [12], was the same in suspension media and Li^+-loaded RBC and Li^+-free control samples. The intracellular pH in Li^+-loaded and Li^+-free RBCs was monitored by measuring the separation between the P_i and P_α resonances. The pH was found to be 7.2 ± 0.1 and thus, Li^+ loading of RBC had no significant effect on intracellular pH. Mg^{2+} loading and depletion of RBCs was achieved by incubating the cells in suspension media containing 20 mM $MgCl_2$ or 20 mM EDTA, respectively, in the presence of the Mg^{2+} ionophore, A23187 [13]. Before recording NMR spectra, Li^+-loaded, Mg^{2+}-saturated and Mg^{2+}-depleted RBCs were packed by centrifuging at 2000 × g for 5 min. They were then washed 3 times and suspended in the same medium containing 140 mM KCl, 10 mM glucose, and 10 mM Tris-Cl, pH 7.2. Use of the above medium minimizes the loss of intracellular Li^+ and Mg^{2+} via the Na^+-Li^+ countertransport and the Na^+-stimulated Mg^{2+}-transport pathways [14], respectively. Resealed RBC ghosts were prepared and loaded with antipyrylazo-III according to Yingst and Hoffman [12]. Optical measurements were carried out at a fixed wavelength (600 nm) with an IBM 9420 Vis/UV spectrophotometer. Mg^{2+} complexation to the dye causes a large change in the optical absorbance at 600 nm, the Mg^{2+} complex having an extinction coefficient of 24 ± 0.2 $M^{-1}\cdot cm^{-1}$ [15]. However, the Li^+-dye complex causes a relatively small change in the absorbance at 600 nm and its extinction coefficient (1.8 ± 0.2 $M^{-1}\cdot cm^{-1}$) must be taken into account in calculating free Mg^{2+} concentrations. Since Mg^{2+} has a higher affinity for ATP than Li^+, it was assumed for the purpose of correcting the optical data in table 1 that 3 mM Li^+ was free to complex to the dye. Thus, the values shown in the last column of table 1 are low estimates of free [Mg^{2+}].

3. RESULTS AND DISCUSSION

Fig.1 displays the effect of the presence and absence of Li^+ on ^{31}P NMR resonances of ATP in

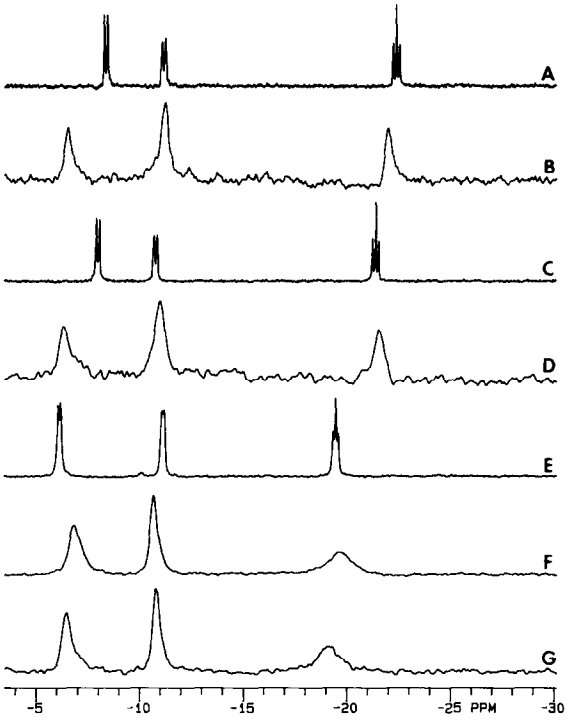

Fig.1. ^{31}P NMR (121.4 MHz, 37°C) spectra of ATP under the following conditions: (A) 5 mM ATP in the Na^+ form. (B) RBC + 20 mM EDTA + 40 mg/l ionophore A23187 (Mg^{2+}-depleted cells). (C) 5 mM ATP + 5 mM LiCl. (D) Li^+-loaded RBC + 20 mM EDTA + 40 mg/l A23187 (Li^+-loaded-Mg^{2+}-depleted cells). (E) 5 mM ATP + 20 mM $MgCl_2$. (F) RBC + 20 mM $MgCl_2$ + 40 mg/l A23187 (Mg^{2+}-saturated cells). (G) Li^+-loaded RBC + 20 mM $MgCl_2$ + 40 mg/l A23187 (Li^+-loaded-Mg^{2+}-saturated cells). Following the incubation procedures indicated above, RBCs used in spectra B, D, F, and G were resuspended in the same medium containing 140 mM KCl, 10 mM glucose, and 10 mM Tris-Cl, pH 7.2. Line broadening of 5 Hz was used in all spectra. In sample D, Li^+ was loaded to Mg^{2+}-depleted RBC (EDTA would otherwise interfere with Li^+ loading), while in sample F Li^+ was loaded before saturating with Mg^{2+}. From left to right, the ^{31}P NMR signals are due to the γ-, α- and β-phosphate resonances of ATP. The exchange broadening of the γ and β resonances of ATP present in F and G is due to incomplete saturation of RBC with Mg^{2+}.

Mg^{2+}-depleted and saturated RBCs. The chemical shift separation between the α- and β-phosphate resonances of ATP ($\delta_{\alpha\beta}$) in Li^+-loaded-Mg^{2+}-depleted RBCs (fig.1D) is smaller than the values observed in free ATP solutions or Mg^{2+}-depleted cells (fig.1A,B, respectively). However, the value of $\delta_{\alpha\beta}$ for Li^+-loaded-Mg^{2+}-depleted cells resembles that observed in

Li^+-saturated ATP solutions (cf. fig.1C and D) indicating that Li^+ loading has a significant effect on $\delta_{\alpha\beta}$ measured in Mg^{2+}-depleted RBCs. By contrast, $\delta_{\alpha\beta}$ is approximately the same in Mg^{2+}-saturated cells in either the presence or absence of Li^+ (fig.1E–G). Mg^{2+}, because of its higher charge, has a higher affinity than Li^+ for ATP [2,16]. Because of their relative affinities for ATP, the competition between Li^+ and Mg^{2+} in RBCs is better felt in Mg^{2+}-depleted than in Mg^{2+}-saturated cells.

Fig.2 shows the effect of Li^+ loading on the $\delta_{\alpha\beta}$ separation of ATP in RBCs containing normal intracellular Mg^{2+} levels. $\delta_{\alpha\beta}$ for Li^+-free RBCs was 8.54 ± 0.04 ppm (n = 14) while for Li^+-loaded RBCs it was 9.46 ± 0.06 ppm (n = 14). While the intracellular Mg^{2+} levels were manipulated in fig.1 by addition of an ionophore, those in fig.2 represent normal intracellular Mg^{2+} levels and yet, at an intracellular concentration of 3 mM Li^+, competition between the two metal ions occurs. Estimation of the ratio of free ATP to total ATP can be obtained from $\delta_{\alpha\beta}$ [13]. This separation is greater for free ATP (fig.1A) than for $MgATP^{2-}$ (fig.1E), and for intermediate degrees of complexation, the observed separation represents a weighted average, since Mg^{2+} exchanges rapidly between ATP molecules and solution on the NMR time scale. If Li^+ were to displace Mg^{2+} from ATP one would predict an increase in the ratio of free to Mg^{2+}-bound ATP and an increase in $\delta_{\alpha\beta}$, as observed.

We quantitated intracellular free Mg^{2+} concentrations using a combined ^{31}P NMR and optical absorbance spectroscopic approach as shown in table 1. Calibration graphs ($r > 0.98$) were constructed, in the presence and absence of Li^+, for samples A–C shown in table 1. These curves correlated $\delta_{\alpha\beta}$ obtained by ^{31}P NMR and intracellular free Mg^{2+} concentration obtained by optical spectroscopy both measured in antipyrylazo-III-loaded, resealed RBC ghost samples. Using these calibration graphs, the intracellular free Mg^{2+} concentrations in control RBCs and Li^+-loaded RBCs were obtained (sample D). It was found that the free intracellular Mg^{2+} concentration in RBCs decreases upon Li^+ loading. Model studies with ATP solutions containing the RBC components 2,3-diphosphoglycerate (DPG), and ADP (sample A) indicate that Li^+ displacement of Mg^{2+} from ATP could only account for a 0.3 ppm increase in $\delta_{\alpha\beta}$ as opposed to a 0.9 ppm increase observed in Li^+-loaded RBCs. Moreover, the free Mg^{2+} concentration increased in sample A and decreased in intact RBCs. However, the presence of the RBC membrane (samples B,C) is able to mimic the effects occurring in intact RBCs. Thus, the decrease in intracellular free Mg^{2+} concentrations in Li^+-loaded RBCs is mostly due to displacement of Mg^{2+} from $MgATP^{2-}$ and subsequent binding of Mg^{2+} to the RBC membrane.

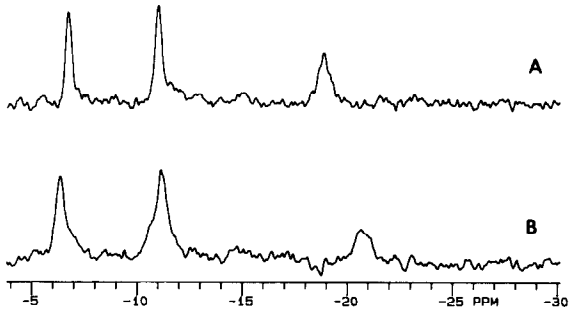

Fig.2. ^{31}P NMR spectra of ATP in (A) Li^+-free RBCs and (B) 3 mM Li^+-loaded RBCs at 37°C. The suspension medium for both samples contained 140 mM KCl, 10 mM glucose, and 10 mM Tris-Cl, pH 7.2. Line broadening of 5 Hz was used.

Similar observations of an increase in $\delta_{\alpha\beta}$ and a decrease in free intracellular Mg^{2+} concentration have been reported upon storage of RBCs [11,17]. These changes may also be the result of enhanced Mg^{2+} binding to RBC membranes in stored blood but that possibility remains to be tested. The equation of Gupta et al. [13] used to quantitate free intracellular Mg^{2+} concentration in intact RBCs needs to be modified in order to be applied to Li^+-loaded RBCs. The existing Gupta equation predicts a free Mg^{2+} concentration of 156 μM ($\delta_{\alpha\beta}$ = 8.82 ppm) for an isolated system containing 2 mM ATP, 3 mM LiCl and 2.4 mM $MgCl_2$ (sample A), as opposed to 360 μM directly measured by the dye method. This calculation clearly indicates that information about free intracellular Li^+ concentration, along with K_{LiATP} affinity constant, will have to be incorporated into the original Gupta equation in order to calculate free intracellular Mg^{2+} concentrations in Li^+-loaded RBCs. Obtaining the free Li^+ concentration directly in the presence of other cations such as Mg^{2+} and Na^+ may be technically difficult. However, the recent development of an Li^+-selective electrode shows

Table 1

Effect of Li^+ on free Mg^{2+} concentrations in model systems and in RBCs obtained by ^{31}P NMR[a] and optical absorbance[b] techniques

	No LiCl		With 3 mM LiCl	
	$\delta_{\alpha\beta}$ (ppm)[a]	$[Mg^{2+}]$ (μM)[b]	$\delta_{\alpha\beta}$ (ppm)[a]	$[Mg^{2+}]$ (μM)[b]
Sample A (n = 6) 2 mM ATP + 5.4 mM 2,3-DPG + 0.2 mM ADP + 2.4 mM $MgCl_2$	8.59 ± 0.06	324 ± 8	8.82 ± 0.02	356 ± 6
Sample B (n = 6) Resealed RBC membranes + 2 mM ATP + 2.4 mM $MgCl_2$	8.89 ± 0.04	386 ± 9	9.68 ± 0.06	288 ± 8
Sample C (n = 6) Resealed RBC membranes with 2 mM ATP + 2.4 mM $MgCl_2$ + 5.4 mM 2,3-DPG + 0.2 mM ADP	8.66 ± 0.08	336 ± 16	9.52 ± 0.06	246 ± 18
Sample D (n = 14) Intact RBC	8.54 ± 0.04	252 ± 12	9.46 ± 0.06	146 ± 14

some promise in this respect [18]. Moreover, mixed ternary complexes such as Li-ATP-Mg could be present in solution. Their presence and influence (if any) on free Mg^{2+} concentration must also be taken into account.

In summary, we have shown by two independent methods that Li^+ can partially displace Mg^{2+} from ATP in Li^+-loaded RBCs. Although the observations made here with RBCs may have no relevance to brain nerve cells, where the therapeutic action of lithium is presumably felt at lower intracellular Li^+ concentrations [1] than those employed in this study, they indicate however that a mechanism for the biological action of lithium involving competition between Li^+ and Mg^{2+} for biomolecules [2] is feasible. Moreover, our results also indicate that RBC membranes may act as an Mg^{2+} buffer [11,17].

Acknowledgements: Financial support from a Grant-in-Aid from the American Heart Association of Metropolitan Chicago and a BRSG grant is gratefully acknowledged by D.M.de F. The authors are grateful to Professors Carlos Geraldes (University of Coimbra, Portugal) and Richard Labotka (University of Illinois College of Medicine, Chicago, IL) for their helpful suggestions during the course of this project.

REFERENCES

[1] Jefferson, J.W., Greist, J.H. and Baudhuin, M. (1985) in: Lithium in Psychiatry (Bach, R.O. ed.) Lithium: Current Applications in Science and Medicine and Technology, pp.345–352, Wiley, New York.

[2] Frausto Da Silva, J.J.R. and Williams, R.J.P. (1976) Nature 263, 237–239.

[3] Birch, N.J. and Fenner, F.A. (1973) Br. J. Pharmacol. 47, 586–594.

[4] Bond, P.A., Brooks, B.A. and Judd, A. (1975) Br. J. Pharmacol. 53, 235–239.

[5] King, L.J., Carl, J.L. and Archer, E.G. (1969) J. Pharmacol. Exp. Ther. 168, 163–170.

[6] Essman, W.B. (1975) Lancet 2, 547.

[7] Aronoff, M.S., Evens, R.G. and Durell, J. (1971) J. Psychiatr. Res. 8, 139–159.

[8] Frizel, D.A., Coppen, A. and Marks, V. (1969) Br. J. Psychiatry 115, 1375–1377.

[9] Dunner, D.L., Meltzer, H.L. and Schreiner, H.C. (1975) Acta Psychiatry Scand. 51, 104–109.

[10] Pavlinic, D., Langer, R. and Lenhard, L. (1979) Biol. Psychiatry 14, 657–661.

[11] Brock, J.L., Wenz, B. and Gupta, R.K. (1985) Blood 65, 1526–1530.

[12] Yingst, D.R. and Hoffman, J.F. (1984) J. Gen. Physiol. 83, 1–17.

[13] Gupta, R.K., Benovic, J.L. and Rose, Z.B. (1978) J. Biol. Chem. 253, 6172–6176.

[14] Feray, J.-C. and Garay, D. (1986) Biochim. Biophys. Acta 856, 76–84.

[15] Gupta, R.K., Gupta, P., Yushok, W.D. and Rose, Z.B. (1983) Biochem. Biophys. Res. Commun. 117, 210–216.

[16] Martin, R.B. and Mariam, Y.H. (1979) in: Interactions Between Metal Ions and Nucleic Bases, Nucleosides, and Nucleotides in Solution (Sigel, H. ed.) Metal Ions in Biological Systems, vol.8, pp.58–124, Dekker, New York.

[17] Brock, J.L., Wenz, B. and Gupta, R.K. (1986) Biochim. Biophys. Acta 928, 8–12.

[18] Xie, R.Y. and Christian, G.D. (1986) Anal. Chem. 58, 1806–1810.