Molecular and Cellular Biochemistry **198:** 129–133, 1999.
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Lithium induced changes in intracellular free magnesium concentration in isolated rat ventricular myocytes

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Received 25 June 1998; accepted 15 October 1998

Abstract

We have examined the effect of exposing isolated rat ventricular myocytes to lithium while measuring cytosolic free magnesium ($[Mg^{2+}]_i$) and calcium ($[Ca^{2+}]_i$) levels with the fluorescent, ion sensitive probes mag-fura-2 and fura-2. There was a significant rise in $[Mg^{2+}]_i$ after a 5 min exposure to a solution in which 50% of the sodium had been replaced by Li^+, but not when the sodium had been replaced by *bis*-dimethylammonium (BDA). However, there were significant increases in $[Ca^{2+}]_i$ when either Na^+ substitute was used. The possibility that Li^+, which enters the cells, interferes with the signal from mag-fura-2 was eliminated as Li^+ concentrations up to 10 mM had no effect on the dye's fluorescence signal. A possible explanation for these findings is that Li^+ displaces Mg^{2+} from intracellular binding sites. Having considered the binding constants for Mg^{2+} and Li^+ to ATP, we conclude that Li^+ can displace Mg^{2+} from Mg-ATP, thus causing a rise in $[Mg^{2+}]_i$. This work has implications for other studies where Li^+ is used as a Na^+ substitute. (Mol Cell Biochem **198**: 129–133, 1999)

Key words: lithium, magnesium, rat heart, cardiac myocytes, fluorescent ion probes, fura-2, mag-fura-2

Introduction

Lithium has been used for 50 years in the treatment of mental depressive illnesses such as the mood swings which occur in manic depression. Its mechanism of action is still unclear, although several models have been suggested, such as inhibition of inositol monophosphatase, an important pathway for signal transduction (e.g. see [1]). This effect may be due in part to the known interactions of Li^+ and Mg^{2+}. Physically, there is a diagonal relationship between the two metals in the periodic table, thus Li^+ and Mg^{2+} form complexes and compounds with similar physico-chemical properties [2]. This observation is important since it has been suggested that Li^+ can compete for the binding sites on ATP normally occupied by Mg^{2+} [3, 4]. Moreover, Mg^{2+} is a cofactor for many cellular enzymes such as the sodium pump, phosphodiesterases, G-protein complexes, and adenyl cyclase. Competitive interaction between Li^+ and Mg^{2+} at such sites may lead to alteration of normal cellular responses, since Li^+ (1 mM) inhibits brain G-protein function, an effect reversed by increasing the Mg^{2+} concentration [5]. Li^+ also antagonises Mg^{2+} activation of the forskolin-stimulated catalytic subunit of adenyl cyclase in rat brain [6]. Thus it is possible that Li^+ can suppress central second messenger systems by displacing Mg^{2+}.

Interactions of Li^+ and Mg^{2+} have also been reported at the cellular level: in rats maintained for 3 months on a diet with added Li^+, where a mean plasma Li^+ level of 0.5 mM was achieved, there was a decrease in total synaptosome Mg^{2+} content [7]. In the same study, this effect was mimicked *in vitro* by incubation of control preparations with Li^+ (1 mM). Thus it seems clear that interactions between Mg^{2+} and Li^+ can exist. Consequently, we have studied the effect of Li^+ on intracellular free Mg^{2+} ($[Mg^{2+}]_i$) in an excitable cell model, the isolated rat ventricular myocyte, using the

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fluorescent, ion-sensitive probe mag-fura-2. This is an appropriate model in which to investigate the actions of Li^+, since cardiac effects such as arrhythmias and ECG changes occur in patients suffering from Li^+ toxicity [8]. Cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) was also measured using fura-2 to examine the possibility that changes in $[Mg^{2+}]_i$ are secondary to changes in $[Ca^{2+}]_i$ or that changes in $[Ca^{2+}]_i$ might interfere with mag-fura-2 fluorescence.

Materials and methods

Solutions

The normal Tyrode solution contained (mM): NaCl, 134; KCl, 6; HEPES, 10; $CaCl_2$, 1; $MgCl_2$, 1 and glucose, 10. Tyrode pH was adjusted to 7.4 at 37°C with NaOH. For solutions which contained 50% of the normal sodium concentration, NaCl was replaced with either *bis*-dimethylammonium-Cl ([9]; BDA, Phase Separations, UK) or LiCl on an equimolar basis to give 70 mM Na^+/70 mM BDA or Li^+. The buffer used for calibration of mag-fura-2 contained (mM): KCl, 136; NaCl, 10; HEPES, 10; glucose, 10; mag-fura-2 free acid, 0.005; the pH was adjusted to 7.4 at 37°C with KOH. Contaminant calcium was chelated with ethyleneglycol-*bis*(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA; 0.5 mM). The composition of the St. Thomas' cardioplegic solution has been described elsewhere [10].

Cell isolation and dye-loading

Isolated ventricular myocytes were prepared based on the method of Lee and Levi [11] with modifications [12]. Briefly, male Sprague-Dawley rats (100–300 g) were anaesthetised with sodium pentobarbitone and then heparinised (1,000 IU). The heart was removed as rapidly as possible into cardioplegic solution, and transferred to the laboratory for preparation of isolated myocytes. Cells were counted on a haemocytometer, rod-shaped cells being classed as 'viable' ventricular myocytes. Myocytes were loaded with fura-2 or mag-fura-2 by incubating the cells with 5 µM fura-2 acetoxymethyl ester (Sigma), or mag-fura-2 acetoxymethyl ester (Molecular Probes, Oregon, USA), together with 0.025% pluronic F127 (Molecular Probes) for 30 min at room temperature [13]

Microspectrofluorimetry

Measurements were made at 37°C using a perspex perfusion chamber, with a glass coverslip as base, mounted on an inverted microscope as previously described [12]. Solutions perfusing the cells were gassed with 100% O_2. Before an experiment, cells loaded with dye were placed into the chamber, and allowed to settle and equilibrate for 15–20 min prior to beginning the experiment. A microscope-based spectrophotometer (Cairn Research, Sittingboume, UK) was used to record changes in $[Mg^{2+}]_i$ and $[Ca^{2+}]_i$, recording the output signal at a rate of 0.25 Hz. Background readings were taken from cell-free areas of the coverslip, and these values subtracted from the raw data before calculation of the ratio of light emitted following stimulation at 340/380 mn [14].

Calibration of mag-fura-2 and fura-2

Mag-fura-2 was calibrated as follows: various standard concentrations of $[Mg^{2+}]$ ranging from 0.1–5 mM were made up by adding $MgCl_2$ to the calibration buffer containing mag-fura-2 free acid (5 µM). The zero and saturation standards were made up of the same buffer, to which ethylene-diaminetetraacetic acid (EDTA, 0.5 mM) or 2 mM $CaCl_2$ were added respectively (the affinity of the dye for Ca^{2+} is greater than for Mg^{2+}). The background fluorescence was estimated from a solution of buffer with EGTA, but no fluorescent probe. Background was subtracted from all readings before ratios, R_{min}, R_{max}, and B (defined below) were calculated. The K_d was calculated from a Hill plot of $[Mg^{2+}]$ vs. $(R\text{-}R_{min})/(R_{max}\text{-}R)$, where R is the 340/380 ratio at a given Mg^{2+} concentration, R_{min} is the ratio in the absence of Mg^{2+} or Ca^{2+}, and R_{max} is the ratio obtained when the dye is saturated with bound ion (Ca^{2+} in this case). Using this plot, the gradient of a line fitted by linear regression corresponds to $1/(K_d \times B)$, where B is the ratio of the 380 signal in the absence of Mg^{2+} or Ca^{2+}, to the 380 signal in the presence of saturating amounts of Ca^{2+}. Since the gradient and B are known, the K_d can be calculated directly. This arrangement gave us R_{min}, R_{max}, and B values of 0.16, 4.29, and 51.0 respectively, with a K_d of 1.61 mM at 37°C. These values were used in the equation described by Grynkiewicz *et al.* ($[ion] = K_d.(R\text{-}R_{min})/(R_{max}\text{-}R)$. B; [14]) to calculate $[Mg^{2+}]_i$ from the ratios obtained from cells loaded with mag-fura-2. For the experiment comparing the Li^+ standard curve with Mg^{2+} using mag-fura-2, the solutions used were as above except either $LiCl_2$ or $MgCl_2$ was added to give standards in the range 0.1–10 mM and the solutions (3 ml) examined in a Cairn cuvette system using the same data collection parameters.

The calibration for Ca^{2+} was carried out as described for Mg^{2+}, except commercially available Ca^{2+} standards (Calbuf-1, WPI, Stevenage, UK) were used, with fura-2 free acid added at a final concentration of 5 µM. This calibration gave us R_{min}, R_{max}, and B values of 0.2, 10.4, and 20.7 respectively, with a K_d of 294 nM at 37°C. The $[Ca^{2+}]_i$ from the cells loaded with fura-2 was calculated as above. Under these conditions, the quoted apparent Mg-dissociation constant (K_{Mg}) for fura-2 is approximately 5.6 mM [14].

Modelling of cytoplasmic Mg buffering

A simple model assumes that ATP is the only Mg^{2+} ligand in the cytoplasm and that Li^+ competes with Mg^{2+} for binding. The binding of Li^+ to ATP has been described in several ways assuming that a single Li^+ ion binds to form Li_2ATP, or that two ions bind to form Li_2ATP, or that both species are present [15]. The latter model describes binding data best and is used here. The values for dissociation constants from [15] are: K_{LiATP} = 1.15 mM; K_{Li2ATP} = 91 mM^2, K_{MgATP} = 0.05 mM. Total cell ATP content was assumed to be 8.5 mM (calculated from data of [16]) in viable, isolated rat myocytes. Equations describing all possible binding reactions were written and solved simultaneously using Mathcad 7 (MathSoft, Cambridge, MA, USA). Total cell Mg was set at 9.1 mM to yield an initial $[Mg^{2+}]_i$ of 1 mM in the absence of Li^+. In a more realistic model, total cell Mg^{2+} was set at 15 mM for isolated rat myocytes (calculated from data of [17]). In order to obtain an initial $[Mg^{2+}]_i$ of 1 mM an additional Mg^{2+} ligand, X (15 mM), was assumed to be present in the cytoplasm. The dissociation constant K_{MgX} was assumed to be 1.5 mM and was chosen to represent the non-ATP Mg^{2+} ligands in the cytoplasm.

Analyses

Results are expressed as means ± S.E.M.; differences were examined by Student's paired *t*-test, a value of $p < 0.05$ being taken as statistically significant.

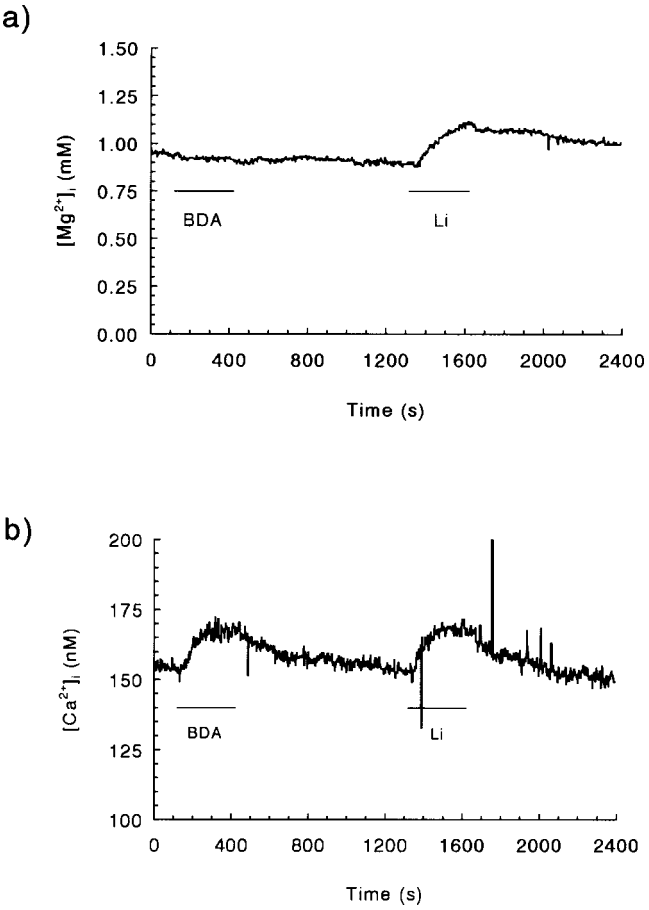

Fig. 1. Mean traces showing the effect of substituting 50% of the Na^+ in Tyrode with either BDA or Li^+ on (a) $[Mg^{2+}]_i$ (n = 4) and (b) $[Ca^{2+}]_i$ (n = 3) in isolated rat cardiac myocytes.

Results

When cells loaded with mag-fura-2 were superfused for 5 min with solutions containing 50% [Na], there was no change in $[Mg^{2+}]_i$ when BDA was used as the Na substitute (basal, 1.07 ± 0.36 mM, and 1.03 ± 0.33 mM after 5 min; not significant, p = 0.5; Fig. la, n = 4). However, when Li^+ was used as a substitute $[Mg^{2+}]_i$ rose from 0.99 ± 0.33 mM to 1.09 ± 0.34 mM an increase of 13.0 ± 3.1% ($p < 0.01$; n = 4) when results were expressed as the mean % rise relative to the pre-exposure value. Under similar conditions, mean $[Ca^{2+}]_i$ rose from a basal value of 155 ± 20 nM to 168 ± 18 nM, a rise of 9.0 ± 2.9% ($p < 0.05$, n = 3) when BDA was used as substitute, and returned to baseline following restoration of the Na^+ in the Tyrode (Fig. 1b, mean of 3 experiments). An almost identical $[Ca^{2+}]_i$ response was obtained using the same cells with Li^+ as substitute. $[Ca^{2+}]_i$ rose 9.5 ± 2.3% ($p < 0.02$, n = 3) from 153 ± 16 nM to 168 ± 17 nM. In order to exclude a direct effect of Li^+ on mag-fura-2, Li^+ standards were made up with mag-fura-2, and compared with the Mg^{2+} standard curve. The results (Fig. 2) show that $[Li^+]$ of up to 10 mM had no effect on the mag-fura-2 signal. One possible explanation for the results illustrated in Fig. 1 is that there was an increased Mg^{2+} influx from the extracellular medium in the Li^+-substituted solutions. Figure 3 shows that this was not the case, since the rise in $[Mg^{2+}]_i$ which occurred on exposure to Li^+ was maintained even in the absence of added external Mg^{2+}.

Discussion

Partial substitution of Na^+ in Tyrode with Li^+ caused a significant rise in $[Mg^{2+}]_i$ whereas substitution with BDA had no effect. On the other hand, small but significant increases in $[Ca^{2+}]_i$ were seen when Na was substituted by either agent. The rise in $[Ca^{2+}]_i$ is expected, since under conditions of low $[Na^+]_o$, altered Na^+/Ca^{2+} exchange will cause $[Ca^{2+}]_i$ to rise. It has been proposed that rises in $[Ca^{2+}]_i$ in isolated cardiac myocytes loaded with mag-fura-2 may be detected as rises

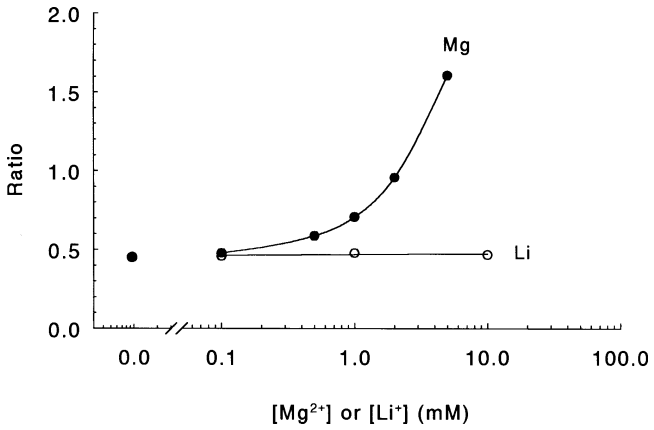

Fig. 2. The effect of increasing Mg^{2+} (closed circles) or [Li] (open circles) on the 340/380 ratio obtained with magfura-2.

in $[Mg^{2+}]_i$ due to (a) direct interference of Ca^{2+} with mag-fura-2 [18], or (b) displacement of Mg^{2+} from cellular binding sites by the increase in $[Ca^{2+}]_i$ [19]. Clearly, since the rises in $[Ca^{2+}]_i$ seen in our experiments were the same when either BDA or Li^+ was used as the Na^+ substitute, and since no rise in $[Mg^{2+}]_i$ was found when BDA was the Na^+ substitute, it is unlikely that the rise in $[Mg^{2+}]_i$ seen with Li^+ was due to interference by Ca^{2+}. This idea is confirmed by calculation. Using apparent dissociation constants for mag-fura-2 at 37°C reported by Hurley *et al*. [20], (K_{Ca} = 20 µM K_{Mg} = 2.25 mM), it can be shown that less than 1% of mag-fura-2 will be bound to Ca as $[Ca^{2+}]_i$ increases from 155–168 nM, whereas more than 30% will be bound to Mg at the normal $[Mg^{2+}]_i$ of mM. Such changes in Ca would not be easily detected by mag-fura-2. This is an important observation, since in some instances (e.g. [19]), rises in $[Mg^{2+}]_i$ have been attributed to concomitant changes in $[Ca^{2+}]_i$.

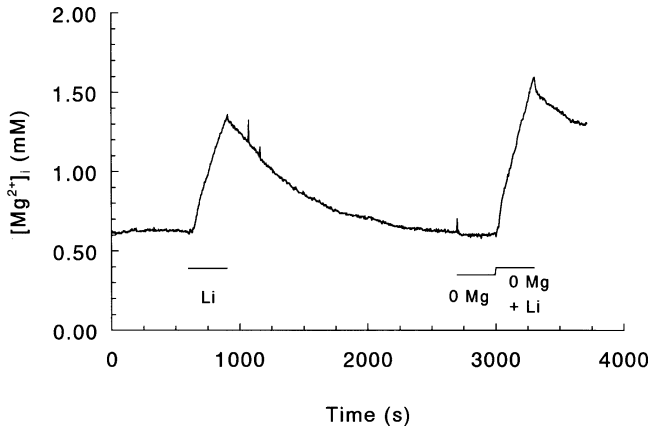

Fig. 3. The effect of removing external Mg^{2+} on the Li-induced rise in $[Mg^{2+}]_i$ (an example trace from one of four similar experiments).

We also considered whether Li^+ would affect mag-fura-2 directly. Lithium enters heart cells and neurones quickly, and leaves slowly [21–23]. When isolated Purkinje fibres were exposed to a Tyrode solution where 50% of the Na^+ was replaced by Li^+, the $[Li^+]_i$ was approximately 1 mM after 5 min [21, 22]. Absolute $[Li^+]_i$ in isolated heart cells in the present experiments is unknown, but if we assume that similar transport processes occur in the Purkinje fibre and ventricular myocyte, then it is anticipated that the $[Li^+]_i$ in our isolated cells should be in the order of 1 mM. Consequently, since a calibration solution containing 10 mM Li^+ had no effect on the fluorescence ratio of mag-fura-2, it is clear that under our experimental conditions, the rise in $[Mg^{2+}]_i$ we measured was not due to Li^+ interference with the fluorescent probe, and therefore represents a real rise in $[Mg^{2+}]_i$.

The source of the Mg^{2+} causing the rise in $[Mg^{2+}]_i$ is unknown at present. An obvious source is through reversed or reduced Na^+/Mg^{2+} exchange, where reduced $[Na]_o$ would favour Mg^{2+} entry and/or reduced efflux through this route. However, these changes in Mg^{2+} flux should be similar in both BDA and Li^+, and in addition, our recent work [12] suggests that this mechanism is ineffective when external $[Ca^{2+}]$ is normal, as is the case here. An alternative route for Mg^{2+} entry is through a voltage and verapamil-sensitive channel [12, 24]. The influx of Li^+ into cardiac cells causes a depolarisation (approximately 10 mV in Purkinje fibres [21, 22]), which in turn may open voltage-dependent channels, and allow Mg^{2+} entry down its electrochemical gradient. Again, our recent work suggests that entry through this route is also low when Ca is present in the medium [12]. Furthermore, since removal of external Mg^{2+} did not abolish the rise in $[Mg^{2+}]_i$ following exposure to Li^+ (Fig. 3), we conclude that the rise in $[Mg^{2+}]_i$ is not due to Mg^{2+} influx. Thus a more likely source is Mg^{2+} liberated from intracellular binding sites, particularly from ATP which is present at high concentrations in the cytoplasm. ATP and related nucleotides are important cellular ligands for Mg^{2+}. Li^+ competes with Mg^{2+} for binding sites on these compounds although with a much lower affinity than Mg^{2+} [15]. Using a highly simplified model of cytoplasmic Mg^{2+} binding which assumes that Mg^{2+} only binds to ATP (see Materials and methods), and with the estimated cellular $[Li^+]$ and [ATP] under our experimental conditions, a rise in $[Mg^{2+}]_i$ of about 0.16 mM is predicted. In support of this is the finding that ionized $[Mg^{2+}]$ increases when Li^+ is added to a mixture of ATP, Mg^{2+} and mag-fura-2 *in vitro* [15]. Using a more realistic model which assumes that there are additional Mg^{2+} ligands in the cytoplasm (see Materials and methods), the predicted change is smaller, about 0.05 mM. Thus the models predict changes in $[Mg^{2+}]_i$ of a similar magnitude to those observed experimentally, and it seems possible that Li^+ induces a rise in $[Mg^{2+}]_i$ in heart cells under physiological conditions by displacing Mg^{2+} from MgATP. Clearly, the observation that Li^+ can increase $[Mg^{2+}]_i$ may have important

implications for the clinical action of Li^+. Given that Li^+ can be cardiotoxic causing ECG changes similar to hyperkalaemia [9, 25], and Mg^{2+} is used therapeutically as an antiarrhythmic agent (e.g. [26]), the interactions between Mg^{2+} and Li^+ deserve study in much greater detail.

Acknowledgements

IFG was supported by a British Heart Foundation grant awarded to DE, PWF, and IFG.

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