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Extensive Surface Changes of Ehrlich Ascites Tumor Cells Induced by Intracellular Li^+

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Ehrlich ascites tumor cells took up Li^+ with alanine through an Na^+ dependent amino acid transport system. Cells that had taken up Li^+ with alanine had small swellings or blebs on their surface, whereas cells that had been incubated with 1.0 mM Li^+ alone showed no morphological changes. These results indicate that Li^+ incorporated into Ehrlich cells causes extensive surface changes. These changes may be due to disruption of the cytoskeleton.

Keywords——Ehrlich ascites tumor cell; lithium ion$^+$ transport; amino acid transport; cell surface; manic-depressive state; lithium

Introduction

Lithium, mostly as Li^+ carbonate, has been used in therapy of the manic-depressive state, although its margin of safety between therapeutic and toxic concentrations is rather narrow (for a review, see ref. 2) and its mechanism of action is unknown. The basis of the therapeutic effect of the cation may be its ability to mimic the function of other biologically important cations such as Na^+, K^+ and Mg^{2+}. Since these cations are related to membrane function, it seems likely that the effects of Li^+ are related at least in part to the cell membrane. The transport of Li^+ into erythrocytes, excitable cells and epithelial cells has been studied extensively,[2)] although little attention has been paid to the effect of the cation on the surface membrane. In this study, we found that Li^+ is co-transported with amino acids such as alanine into Ehrlich ascites tumor cells, and that the cation in the cells induced extensive morphological change of the cell surface membrane, possibly by affecting the cytoskelton. The change in the membrane was observed by both phase contrast and scanning electron microscopy. When added without amino acids, Li^+ did not induce detectable morphological change of the cells. The membrane change observed in this study may be important in understanding the therapeutic and toxic effects of Li^+.

Materials and Methods

Ehrlich ascites tumor cells were maintained in mice by transfer of about 2×10^5 cells in phosphate-buffered saline. The cells were harvested 7 to 9 d later and washed three times with 100 mM MOPS–Tris, pH 7.5, containing 1.0 mM $MgCl_2$ (buffer A). Transport of Li^+ was assayed with an Li^+-specific electrode developed recently.[3)] MOPS(3-*N*-morpholinopropanesulfonic acid) and LiCl were obtained from Sigma Chem. Co. and Wako Chem. Co., respectively.

Scanning electron microscopy was carried out as follows. Cells were placed on poly L-lysine-coated glass and prefixed with OsO_4 vapor for 20 min. They were then fixed with 2.0% glutaraldehyde in phosphate buffered saline for 1 h. The piece of glass with attached cells was gently shaken in phosphate buffered saline to remove excess cells, dehydrated in a graded series of ethanol, immersed in isoamyl acetate and dried by the critical point method with carbon dioxide as the transition fluid using a Hitachi HCP-1 critical point dryer. The cells were coated with Pt–Pd

using an Eiko ion coater (model LB-3) and observed in a JEOL-LSM-U3 scanning electron microscope.

Results and Discussion

Ehrlich ascites tumor cells were incubated with 1.0 mM LiCl and the uptake of Li^+ was observed immediately after addition of 40 mM alanine (Fig. 1a). The uptake reached a plateau within 3 min after addition of alanine, and the intracellular concentration of Li^+ was estimated as 5 mM, assuming that the binding of Li to the cell membrane was negligible and taking the internal volume as 1.8 μl/10^6 cells.[4] Thus the intracellular concentration of Li^+ was about 5 times that in the medium. This value is higher than the cell to plasma ratio of Li^+ of 0.2 to 1.1 of erythrocytes.[2,5] As shown in Fig. 1b, Na^+ was also taken up into Ehrlich cells with alanine: its rate of uptake and intracellular concentration were essentially similar to those of Li^+. We analyzed the transport of Li^+ or Na^+ with alanine or other amino acids extensively, and found that an Na^+ −dependent neutral amino acid transport system also uses Li^+ as a coupling ion. Detailed results, including results on the kinetics and specificity of the reaction will be published elsewhere.

The finding of co-transport of Li^+ with alanine enabled us to compare the effects of intra- and extracellular Li^+ on Ehrlich cells. Cells were incubated with 1.0 mM Li^+ and 40 mM alanine until the uptake of the cation reached a plateau as shown in Fig. 1, and then the morphology of the cells was examined by phase contrast microscopy. As shown in Fig. 2A, most cells that had taken up Li^+ with alanine had small swellings, or blebs, on their surface, whereas cells that had been incubated with 1.0 mM Li^+ alone showed no morphological changes (Fig. 2B). Cells that had taken up Na^+ with alanine also showed no morphological changes such as blebs. The formation of blebs was more striking when cells that had incorporated Li^+ with alanine were placed on a slide glass and covered with a thin glass (Fig. 2C), suggesting that the pressure due to the weight of the cover glass induced extensive change of the surface morphology. This morphological change was not observed in control cells incubated with 1.0 mM Li^+ alone (Fig. 2D), or with Na^+ and alanine (not shown). Even when the cells were incubated with 5, 20 or 50 mM LiCl (without alanine) for 15 min they showed no morphological change. These results suggest that the formation of blebs or small swellings was caused by the intracellular accumulation of Li^+ with alanine.

The morphological change of cells that had accumulated Li^+ was also examined by scanning electron microscopy. Most cells incubated with Li^+ alone had long thin microvilli distributed on their surface (Fig. 3A), like cells immediately after their isolation.[4] Cells that had been incubated with Li^+ and alanine (Fig. 3B) had fewer microvilli than control cells and

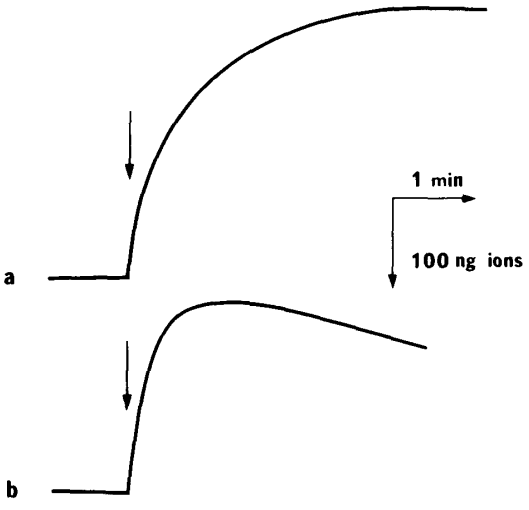

Fig. 1. Co-transport of Li^+ and Alanine into Ehrlich Cells

Samples of 5×10^7 cells were incubated for 14 min in 3.0 ml of buffer A containing 1 mM LiCl (a) or NaCl (b) at 37 C. The concentrations of Li^+ and Na^+ in the assay mixtures were monitored with Li^+- and Na^+-selective electrodes, respectively. At the times indicated by arrows, 80 μl of 1.5 M alanine was added to the cell suspension with rapid stirring. Upward deflection indicates uptake of Li^+ or Na^+ by the cells.

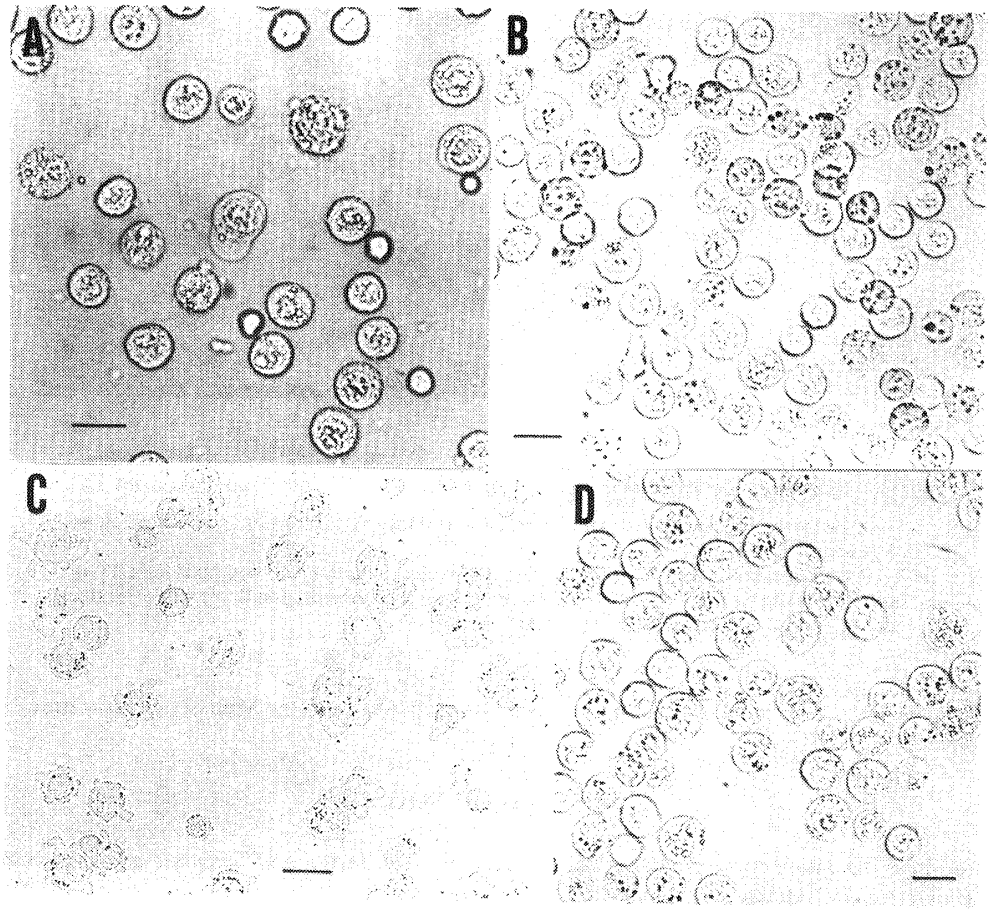

Fig. 2. Phase Contrast Micrographs of Ehlich Cells that Had Taken up Li^+

A, Ehrich cells incubated at 37 °C for 15 min with 1 mM with 1 mM LiCl in buffer A with 40 mM alanine. After 10 min, the cells were placed in a well (0.15 mm depth) and examined by phase contrast microscopy.

B, cells were incubated essentially as for (A) but without 40 mM alanine. They were examined as for (A).

C, cells were incubated with Li^+ and alanine as for (A). They were then placed on a glass slide and covered with a thin glass cover slip ($0.5 \times 18 \times 18$ mm, 40 mg/cm^2) before examination.

D, cells were incubated with Li^+ alone as for (B) and then examined as for (C).

Cells had taken up Li^+ (A and C) with alanine had small swellings or blebs on their surface. The scale bar represents 10 μm.

had smooth areas and blebs on their surface.

These results indicate that Li^+ incorporated into Ehrlich cells causes extensive morphological changes, including loss of microvilli. These changes may be due to disruption of the cytoskeleton, especially bundles of actin filaments in the microvilli.[6,8] It is also possible that Li^+ destroys the connection between actin filaments and the microvillus membrane, or the perpendicular network. The major function of these filaments appears to be to maintain the stiffness of the microvilli and the cell itself. This possibility would be consistent with the fact that cytochalasins, which are known to disrupt the cytoskeleton by binding to one end of actin filaments,[9,10] induce morphological changes such as zeiosis or blebbing in established cell lines[11-14] and Ehrlich cells.[15] It is striking that Li^+ has similar morphological effects to cytochalasins on Ehrlich cells, and these effects may be important in relation to its therapeutic and toxic effects. We are now planning experiments on the interesting problem of whether Li^+ has similar morphological effects on nerve cells *in vitro*.

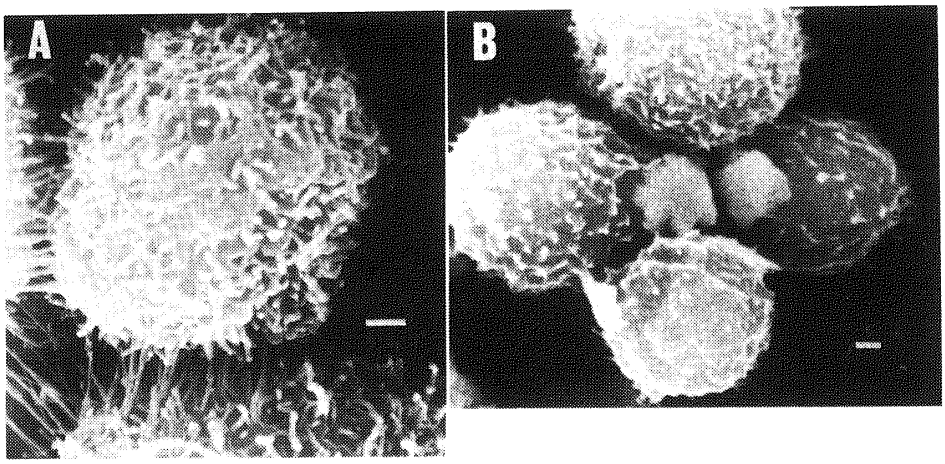

Fig. 3. Scanning Electron Micrographs of Ehrlich Cells That Had Taken up Li^+

A: A typical cell incubated with 1 mM LiCl in buffer A for 10 min as described in the legend to Fig. 2A.

B: Cells incubated with 1 mM LiCl and 40 mM alanine in buffer A as described in (A). Cells incubated with Li^+ and alanine (B) have smooth areas and blebs, and fewer microvilli than control cells (A). The scale bar represents 1 μm.

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References and Notes

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