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AN IRREVERSIBLE EFFECT OF LITHIUM ADMINISTRATION TO PATIENTS

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1 Lithium administration to patients leads to a pronounced inhibition (about 90%) of the choline transport system in erythrocytes. The transport system does not recover when ghosts are prepared from the erythrocytes, thereby removing intracellular as well as extracellular lithium.

2 When a patient is taken off lithium, the choline transport in erythrocytes recovers only very slowly over a period of three months, i.e. at about the same rate at which the erythrocytes that had been exposed to lithium are replaced by new cells.

3 It is concluded that therapeutic concentrations of lithium produce an irreversible inhibition of the choline transport system in human erythrocytes.

Introduction

It has recently been shown that the administration of lithium to patients reduces choline uptake into erythrocytes to around 10% of the normal value (Lee, Lingsch, Lyle & Martin, 1974). A very surprising feature of this inhibition is that it is apparently completely independent of the presence of lithium in the external medium. The activity of the choline transport system in erythrocytes from patients on lithium could not be restored to normal by extensive washing and incubation of the cells in lithium-free buffers. There are other observations suggesting that lithium may produce effects that are not readily reversible. The lithium treatment of rats has effects on transport systems in synaptosomes (Baldessarini & York, 1970) and on renal adenyl cyclase (Geisler, Wraae & Olesen, 1972) that are not dependent on the presence of lithium in the incubation media during the *in vitro* measurement of transport or enzyme activity. In both of these papers the authors point out that their results are compatible with an effect of lithium that is not readily reversible, but no efforts were made to remove the lithium that might be trapped in the vesicles that form during tissue homogenization. Baldessarini & York (1970) found in fact that their synaptic vesicles still contain 0.07 mEq/l of lithium. It is therefore conceivable that the persistence of the effects described is the result of the presence of small concentrations of lithium in the vesicles. These results suggest that the therapeutic use of lithium may produce an irreversible inhibition of certain transport processes in man. We have therefore determined whether inhibition of the choline uptake into red cells that is associated with lithium treatment is truly irreversible or a consequence of the presence of lithium inside the cells.

Methods

Erythrocytes from fresh heparinized blood of healthy volunteers and patients were obtained and washed as described previously (Martin, 1972). The patients studied were treated at Fulbourn Hospital, Cambridge for manic depressive disease and the erythrocytes were obtained when blood was taken at the hospital to determine plasma lithium levels.

Choline influx

The procedures for measurement of the influx of [^{14}C]-choline and for calculation of the influx of choline into erythrocytes have been described previously (Martin, 1972; Lee *et al.*, 1974). The rate of choline influx was determined after the cells had been incubated for 10 min at 37°C in a buffer containing 1 μM choline.

Choline efflux from released ghosts

To prepare the ghosts, erythrocytes were washed four times with about five volumes of a solution containing (mM): KCl 105, $MgCl_2$ 30, Tris (pH 7.7 at 25°C) 10, disodium edetate (EDTA) 0.2. After the last wash the cells were packed for 10 min at 1500 *g* and squirted into 70 volumes of stirred ice cold lysing solution containing $MgCl_2$ 2, Tris 10, EDTA 0.2 and [^{14}C]-choline and unlabelled choline to give a concentration of 10 μM.

After the addition of the cells the suspension was allowed to stand for about 5 minutes. Sufficient 3 M KCl was added to restore the tonicity to 320 ideal osmolar and the suspension was incubated at 37°C for 90 min; in some experiments, described in the text, sufficient 2 M LiCl to give 4mM LiCl was added after 30 min of incubation. The ghosts were then washed

and the efflux of radioactive choline was followed by taking aliquots of the suspension at 5, 15, 25, 35 and 60 min and measuring the radioactivity in the supernatant; the rate constant for the efflux of choline was calculated from a graph on which the fraction of radioactivity remaining in the ghosts was plotted on a log scale against time (Martin, 1971).

The efflux of choline was always measured in the absence as well as in the presence of 1 mM hemicholinium-3 (HC-3) in the external medium. The HC-3-sensitive efflux was calculated by subtracting the rate constant found in the presence of HC-3 from the rate constant found in its absence.

Lithium determinations

All lithium concentrations given in this paper were determined with a Unicam SP 90 Atomic Absorption Spectrophotometer and standards varying from 2 μM to 2 mM LiCl.

Materials

The sources for the chemicals used were the same as described previously (Martin, 1972). The [methyl-^{14}C]-choline chloride (The Radiochemical Centre, Amersham) had a specific activity of 40–60 mCi/mmol.

Results

The experiments summarized in Table 1 were designed to obtain some direct evidence concerning the relationship between intracellular lithium levels and the pronounced inhibition of choline transport that is observed after several weeks of lithium administration (Lee *et al.*, 1974). Erythrocytes from healthy individuals were incubated for 90 min in buffers containing 4 mM or 8 mM LiCl, resulting in intracellular lithium concentrations similar to those found in patients treated with lithium. Choline uptake from lithium-free buffers was measured after the cells had been carefully washed. The results indicate that the extent to which the influx is inhibited is apparently not related to the concentration of lithium in the intracellular water. The *in vitro* exposure of normal cells to lithium produced about 25% inhibition, irrespective of whether the cells were incubated in 4 mM or 8 mM LiCl. Choline influx into erythrocytes from patients on lithium was only about 15% of the normal value, confirming previous observations (Lee *et al.*, 1974) and suggesting that this second phase of pronounced inhibition is in some way the consequence of prolonged *in vivo* exposure to lithium.

The experiments to be described were designed to examine the effects of removing intracellular lithium as far as possible. Since the lithium-induced inhibition of choline transport is not dependent on the continued presence of lithium in the external medium, or on the concentration of lithium in the intracellular water, washed erythrocytes from patients and healthy individuals were lysed and resealed as described in the section on methods, thus obtaining resealed ghosts that contain [^{14}C]-choline and lithium at only about 1 to 2% of the concentration found in the intact cells. Measurements of the lithium concentrations in the supernatant after the ghosts had been spun down confirmed that virtually all the lithium present in the intact cells had been released.

Unlike intact erythrocytes, resealed ghosts have a significant leak permeability to choline, i.e. some radioactive choline will leave the ghosts even when the carrier mediated choline transport is completely blocked by the presence of 1 mM HC-3 in the external medium (Martin, 1971). The efflux of choline from

Table 1 Choline influx into erythrocytes from patients on lithium and healthy individuals

Origin of blood No. of experiments	*Incubation medium (90 min, 37°C)*	*Intracellular lithium (mEq / cells^{-1})*	*Choline influx (nmol / cells^{-1} min^{-1})*
Patients (11)	Na buffer	0.364 ± 0.053	6.5 ± 1.0
	4 mM Li	0.378 ± 0.061	6.9 ± 1.0
Healthy individuals (12)	Na buffer	—	44 ± 5.0
	4 mM Li	0.218 ± 0.012	33 ± 4.3
Healthy individuals (4)	Na buffer	—	45 ± 5.3
	8 mM Li	0.402 ± 0.021	31 ± 4.6

Aliquots of erythrocyte suspensions were incubated in Na buffer (150 mM NaCl, 10 mM Tris HCl) and 4 mM or 8 mM Li (LiCl added to the Na buffer) as indicated. The concentrations of intracellular lithium were determined after the 90 min incubation period and after washing the cells several times with a lithium free buffer; the haematocrit was determined by measuring the optical density of a suitably diluted aliquot at 540 nm.

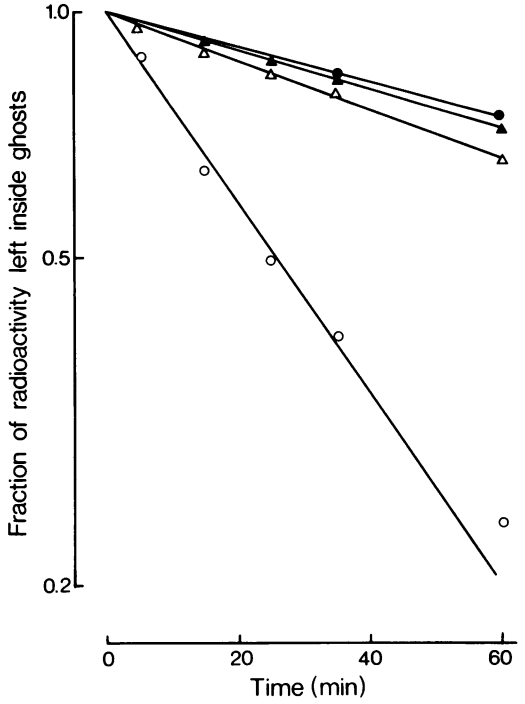

Figure 1 The efflux of [^{14}C]-choline from ghosts prepared from normal erythrocytes (○, ●), and erythrocytes from patients on lithium (△, ▲). The efflux was measured with (●, ▲) and without (○, △) 1 mM hemicholinium-3 in the external medium.

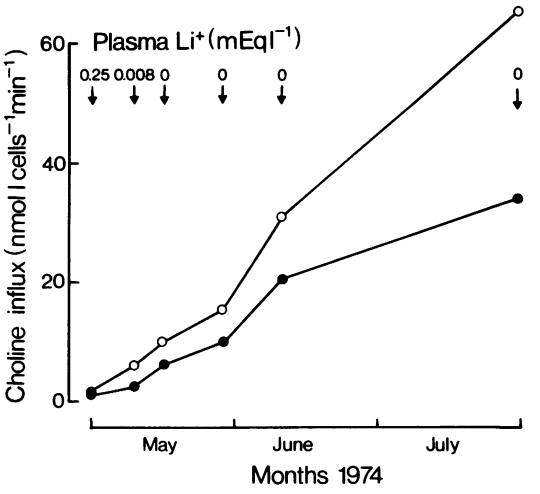

Figure 2 The recovery of choline transport in the erythrocytes of a patient who was taken off lithium on May 1st, 1974. Choline influx was measured after the erythrocytes had been incubated for 90 min in a lithium-free buffer (○) or a buffer containing 4 mM Li (●); the plasma lithium levels were determined using the plasma from the same blood sample.

resealed ghosts is therefore always measured in the presence as well as in the absence of external HC-3; the carrier mediated flux is the difference between the total efflux and the HC-3-insensitive flux. The experimental data shown in Figure 1 were obtained and analysed as described in the section on methods. It is clear that for ghosts prepared from a patient's erythrocytes the rate constant for total choline efflux is only 6.3×10^{-3} min^{-1} and almost the same as that found in the presence of HC-3 (5.02×10^{-3} min^{-1}), indicating that there is very little carrier-mediated choline efflux (1.28×10^{-3} min^{-1}). In contrast to this the data obtained with ghosts from normal blood give a rate constant of 22.1×10^{-3} min^{-1} for the carrier-mediated choline efflux; the HC-3-insensitive fluxes, i.e. the leak permeability to choline is almost identical for the two types of ghosts. Essentially similar results were obtained in all the experiments measuring the efflux of choline from resealed ghosts prepared from the blood of patients on lithium (Table 2). If this inhibition of carrier-mediated choline flux is compared with the reduction of choline influx into the intact cells of the same patients (Table 1) it appears that the removal of intracellular lithium has no effect on the pronounced inhibition of choline transport produced by the administration of lithium. The results of Table 2 also confirm the previous observation (Lee *et al.*, 1974) that there is no obvious correlation between the inhibition of choline transport and the concentrations of lithium in the patient's plasma.

Further evidence indicating that the lithium effect described here is apparently irreversible comes from a study of the recovery of choline transport in a patient taken off lithium (Figure 2). Eight days after the administration of lithium had been stopped the plasma concentration had fallen to about 8 μEq l^{-1} and after a further week no lithium could be detected in the patient's plasma or erythrocytes. In contrast to this the choline transport system in the patient's erythrocytes recovered much more slowly over a period of several weeks; assuming that the life time of human erythrocytes is around 120 days the time course of this recovery might suggest that choline transport returns to normal at the same rate at which old erythrocytes are destroyed and replaced by new ones. When the patient's blood was available for examination for the last time, 3 months after he had been taken off lithium, the value of choline influx was higher than the average found in the normal population but still within normal limits (Askari, 1966; Lee *et al.*, 1974).

As choline transport recovers, *in vitro* exposure of erythrocytes to lithium becomes increasingly effective in producing an inhibition of choline influx (Figure 2). This is again consistent with the idea that the recovery

is in fact the result of a gradual replacement of erythrocytes with an irreversibly inhibited choline transport system by normal, newly formed erythrocytes. It is known that *in vitro* exposure to low concentrations of lithium reduces choline influx to between 50 and 70% of the control value in normal cells but has virtually no effect on cells that have already been exposed to lithium *in vivo* (Martin, 1974; Lee *et al.*, 1974).

This evidence suggests very strongly that the pronounced inhibition of choline transport in the erythrocytes of patients treated with lithium is a truly irreversible effect.

Some preliminary experiments were carried out to see whether or not measurable amounts of lithium remain adsorbed to the cell membrane when ghosts are prepared from erythrocytes of patients treated with lithium. After lysing the erythrocytes the membranes were concentrated by centrifugation to about 5% (w/w) and the lithium concentration of this suspension compared with that of the membrane free supernatant. No difference was found; in some experiments the erythrocytes were lysed in 35 volumes of lysing medium, in other experiments in 200 volumes. This allows one to calculate an upper limit for the amount of lithium adsorbed to the membrane. Unfortunately, since the concentration of the membrane suspension used was only 5% (it is difficult to use a significantly higher concentration for technical reasons) and since the lower limit of the spectrophotometer's sensitivity was about 1–2 μM, the value for this upper limit comes to 20–40 μmol of lithium per kg membrane. This is a rather high value; the possibility that significant amounts of lithium remain adsorbed to the cell membrane is therefore not ruled out by these experiments.

Discussion

The purpose of this paper is to show that the administration of lithium to patients can inhibit a physiological function such as choline transport in erythrocytes extensively and irreversibly; the transport system recovers only at the rate at which the affected cells are replaced by new cells.

Baldessarini & York (1970) and Geisler *et al.* (1972) also report lithium effects that persist after the ion has been largely removed. Baldessarini & York (1970) do not discuss possible mechanisms for this

Table 2 Choline efflux from resealed ghosts prepared from erythrocytes of patients on lithium and healthy individuals

Patient	*Plasma lithium (mEq l^{-1})*	*Rate constant for choline efflux, k (10^{-3} min^{-1})*
A.F. (M. 53y)	1.09	0.8
M.L. (F. 42y)	1.24	5.5
B.L. (M. 25y)	0.77	1.5
R.P. (M. 39y)	0.25	0
J.R. (F. 50y)	1.35	4.1
J.R.	1.27	5.7
D.R. (F. 53y)	0.88	1.4
D.R.	0.80	3.7
D.R.	—	0
R.S. (F. 36y)	0.78	2.0
R.S.	0.67	0
Patients, HC-3-sensitive efflux (mean ± s.e. mean)		2.24 ± 0.65
22 controls, HC-3-sensitive efflux (mean ± s.e. mean)		22.34 ± 2.09
Patients, HC-3-insensitive efflux (mean ± s.e. mean)		4.78 ± 0.38
22 controls, HC-3-insensitive efflux (mean ± s.e. mean)		5.02 ± 0.47

Blood was obtained on 11 separate occasions from a total of 7 patients. Plasma lithium levels were determined and resealed ghosts prepared from the erythrocytes. The rate constant listed for each individual experiment is that of the hemicholinium-3 (HC-3)-sensitive choline efflux. The controls are identical experiments with ghosts prepared from the erythrocytes of healthy individuals.

unexpected persistence, possibly because their experiments do not rule out the possibility that lithium trapped inside the synaptic vesicles might be responsible for it. In the experiments of Geisler *et al.* (1972) there is no indication of trapped lithium and to explain the persistence of the inhibition of adenyl cyclase it has been suggested 'that lithium causes an alteration of some intermediary which itself affects adenyl cyclase' (MacNeil & Jenner, 1975). Nothing is known about the relationship of the rate at which these effects do reverse and the rate at which the affected structures are replaced. The results presented in this paper appear to be the most unequivocal evidence that therapeutic concentrations of lithium can produce an irreversible effect.

The fact that the therapeutic and toxic effects of lithium appear to be reversible does not entitle one to conclude that the basis for these effects must be processes that are reversible on the molecular level. Erythrocytes are very unusual in that their membrane proteins are not continuously removed and replaced. It is now generally accepted that all tissues made up of nucleated cells have a rapid turnover of their membrane proteins (Schimke, 1975). It is therefore conceivable that lithium effects that are irreversible on the molecular level do not appear as such *in vivo* because the structures affected are replaced fairly rapidly.

It is clearly very difficult to envisage how a monovalent cation like lithium, at a concentration of 2 mM, produces this irreversible inhibition of choline transport. Since hexose transport as well as active and passive sodium movement in erythrocytes from patients on lithium are not affected (Lee *et al.*, 1974), one should first consider the possibility that the inhibition of choline transport is a specific effect.

Virtually all biological systems influenced by lithium are physiologically either activated or inhibited by Na^+, K^+, Mg^{2+} or Ca^{2+} and one might therefore expect that the primary cause for any lithium effect is, in the end, the replacement of another cation. There is no indication that choline transport in erythrocytes requires any monovalent or divalent cation; the only site on the transport system known to have an affinity for monovalent cations, including lithium, is the actual transport site (Martin, 1972). It therefore seems reasonable to assume that lithium attacks the transport system by binding to this site. Evidence supporting this assumption is the observation that *in vitro* extracellular choline very effectively protects the carrier against inhibition by lithium (Martin, 1974).

To the erythrocytes this inhibition of choline transport will probably be of no consequence since these cells do not metabolize choline; the choline transport system in their cell membrane appears to be merely a vestige. The relevant question is whether or not a similar inhibition of choline transport occurs in other tissues, especially the central nervous system.

It may seem that an answer to this question could easily be obtained by studying the effects of lithium administration to rats on the various choline transport systems. However, preliminary experiments in our laboratory have shown that the choline transport system in the erythrocytes of rats and rabbits is not affected by the prolonged administration of lithium to these animals. Similarly, while choline transport in human erythrocytes is inhibited by incubation of the cells with lithium *in vitro* (Martin, 1974), this effect cannot be obtained with erythrocytes from rats or rabbits. This suggests that the lithium effect on choline transport described in this paper might be species specific.

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