Measurement of cation transport in vivo in healthy volunteers after the oral administration of lithium carbonate

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SUMMARY

1. We have measured cation transport in vivo in seven healthy volunteers under control conditions and after they had taken lithium carbonate for 21 days in doses which maintained the serum lithium concentration in the range 0.6–0.8 mmol/l.

2. We have measured cation transport in vivo after the administration of an oral load of rubidium chloride, and have found that, although intra-erythrocytic concentrations of rubidium were significantly lower 1 h after the administration of rubidium when the subjects were taking lithium, there was a significant increase in the rate of uptake of rubidium into the erythrocytes over the subsequent period of the test, suggesting a direct stimulation of sodium, potassium-activated adenosine triphosphatase by lithium.

3. Lithium administration did not affect the plasma concentration versus time profile of rubidium after the rubidium load, implying that the lithium-stimulated uptake of rubidium which occurs in erythrocytes does not necessarily occur in other cell types.

4. These results suggest that previous studies of cation transport using peripheral cells and assay systems in vitro do not necessarily reflect changes in cation transport in vivo in excitable tissues.

Key words: cation transport, erythrocytes, lithium.

Abbreviations: Na⁺,K⁺-ATPase, sodium, potassium-activated adenosine triphosphatase (EC 3.6.1.37).

INTRODUCTION

Lithium is widely used in the treatment of acute episodes of mania and in the prophylactic management of patients suffering from bipolar affective illness [1, 2], but its mode of action is unknown. One field of interest has been the investigation of the link between the therapeutic actions of lithium and the abnormalities of cation transport which have been described in patients with affective illness.

Early studies of the electrolyte status of patients suffering from depression [3] or mania [4] have shown a fall in the intracellular concentration of sodium on recovery from periods of illness. After these initial observations, studies in vitro of the sodium, potassium-activated adenosine triphosphatase (Na⁺,K⁺-ATPase, the sodium pump; EC 3.6.1.37) have shown an increase in enzyme activity measured in the erythrocyte membrane on recovery from periods of affective illness in both unipolar depressed patients [5–7] and patients with bipolar affective illness [8, 9]. Whilst these results are in line with the observed changes in electrolyte concentrations, they have not been universally confirmed. For example, some workers have detected no change in Na⁺,K⁺-ATPase activity in depressed patients [10], while others have shown increased activity during periods of illness in patients with both unipolar and bipolar illness [11, 12]. There is still no clear consensus.

The erythrocytes from patients treated with lithium salts have been shown to have increased sodium pump activity in vitro [13–16], but studies of this sort are unable to distinguish between a direct effect of lithium on cation transport and an indirect effect mediated by mechanisms which are secondary to the normalization of mood after the instigation of effective treatment. A single study of healthy volunteers given lithium salts for 14 days did not reveal any change in erythrocyte membrane Na⁺,K⁺-ATPase activity in vitro [15], supporting the latter explanation.

There are several possible reasons for the conflicting results reported by different workers in the field, including difficulties in the accurate clinical assessment of patients and the confounding effects of concurrent treatment with a variety of psychotropic drugs, but differences between the techniques for the measurement of Na⁺,K⁺-ATPase activity in vitro may also be important.

In an attempt to overcome the difficulties associated with measures in vitro, we have developed a technique...
which allows the assessment of cation transport in vivo [17]. This has already been used to demonstrate differences in sodium pump activity in patients suffering from hypertension [18] and chronic renal failure [17] and during treatment with digoxin [17, 19]. We have now used this technique in vivo to assess the effect of treatment with lithium salts upon cation transport in healthy volunteers.

The results of this study have been presented in preliminary abstract form elsewhere [20].

METHODS

Patients

We have studied seven healthy male volunteers aged between 25 and 35 years, all of whom were questioned before the study to exclude a personal or family history of hypertension, renal disease or affective illness. No volunteer took any other drug during the period of the study. Blood pressure was measured on at least three separate occasions, using an automated sphygmomanometer cuff (Dinomapp Critikon, Tampa, FL, U.S.A.) with repeated measurements over a period of 60 min with the subject resting. All the volunteers had resting blood pressures of less than 139/82 mmHg (18.5/11 Wa) measured in this way.

Treatment schedule

The subjects took lithium (lithium carbonate B.P.) orally, once daily in the evening for 21 days. The serum lithium concentration was measured after 3 days and then weekly during the period of administration. Lithium doses were adjusted in order to maintain serum concentrations in the range 0.4–0.8 mmol/l (daily maintenance dose range 600–1200 mg). Serum samples were always taken 12 h after the previous lithium dose for lithium estimation by flame photometry. The final dose of lithium was taken on the evening of day 21 and cation transport was assessed in vivo on the next day. The measurement of cation transport in vivo when the subjects were drug-free was made at least 6 months after lithium was discontinued. This interval between tests is necessary because of the slow rate of clearance of rubidium from the body after the administration of lithium chloride.

Measurement of cation transport

Rubidium loading in vivo. Cation transport was assessed in vivo by the oral administration of non-radioisotopic rubidium [17]. Rubidium chloride (British Drug Houses, Atherstone, Warwickshire, U.K.) was dissolved in tap water and given in a total dose of 8 mg/kg body weight in 240 ml of water, as eight separate aliquots given at 15 min intervals. Venous blood was taken once immediately before the first dose, and again at 1 h (t = 1) and 5 h (t = 5) after the last dose of rubidium. Plasma and erythrocytes were separated by centrifugation (1500 g for 10 min at 4°C). Plasma and intra-erythrocytic rubidium concentrations were measured after dilution in distilled water. The results have been expressed as the increase in rubidium concentration over baseline concentration in μmol/l. A pseudo rate constant for rubidium uptake was calculated as the increment in erythrocyte rubidium concentration between 1 h (RBC1) and 5 h (RBC5) divided by the plasma concentration at t = 1 (plasmal)

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Pseudo \text{ rate constant } (h^{-1}) = \frac{Rb_{RBC5} - Rb_{RBC1}}{Rb_{plasmal}}
\]

Rubidium measurement. Rubidium concentrations were measured using an atomic absorption spectrophotometer incorporating a graphite furnace (Perkin-Elmer, Beaconsfield, Bucks., U.K.). An atomization temperature of 1900°C was used throughout. Concentrations were measured by the method of additions and all samples were measured in triplicate [21].

Statistical assessment of the data

All the results were assessed by the paired signed-rank test (Wilcoxon) or by Student's paired t-test, as appropriate.

Ethical approval

The study design was approved by the Oxford Psychiatric Sector Ethics Committee and subjects consented to participate after a written and full verbal explanation by one of the investigators (A.J.W.).

RESULTS

As early as the fourth day, the mean serum lithium concentration was 0.73 (range 0.58–0.89) mmol/l and subsequently remained stable within the range 0.6–0.8 mmol/l in all seven subjects.

Cation transport in vivo

The results are shown (mean ± sd) for plasma and erythrocytes in Figs. 1(a) and 1(b), respectively. The pseudo rate constants (median, range) are shown in Fig. 1(c). There was no significant change, at either 1 h or 5 h, in the measured plasma rubidium concentrations after 21 days of treatment with lithium chloride. The increment in plasma rubidium concentration was 8.47 ± 2.77 at t = 1, falling to 5.48 ± 1.09 μmol/l at t = 5 after lithium, and 7.87 ± 2.11 (t = 1) falling to 4.41 ± 2.08 μmol/l (t = 5) under control conditions (Fig. 1a).

The increase in erythrocyte rubidium concentrations was significantly lower at t = 1 after lithium administration (15.59 ± 4.70 μmol/l) than under control conditions (26.13 ± 6.15 μmol/l) (P < 0.05). However, there was no significant difference in erythrocyte concentrations at t = 5 (lithium 35.25 ± 6.35 μmol/l; control 36.50 ± 7.30 μmol/l; Fig. 1b).

The pseudo rate constant for rubidium uptake was significantly increased (P < 0.05) during the period of lithium treatment [0.54 (range 0.23–0.37) h⁻¹] when compared with control values [0.27 (range 0.10–0.30) h⁻¹].
**Lithium and cation transport**

Fig. 1. Increases in rubidium concentrations (mean ± so) after the oral administration of rubidium chloride (8 mg/kg) in (a) plasma and (b) erythrocytes of seven healthy volunteers after 21 days of lithium administration (●) and under control conditions (○). (c) Pseudo rate constant for rubidium uptake into erythrocytes (median, interquartile range and full range) for the same subjects. Statistical significance: *P< 0.05.

In a further group of experiments, to be presented elsewhere, we have shown that the uptake of rubidium into erythrocytes in vivo is linear in the presence of therapeutic serum concentrations of lithium over the entire period of the rubidium uptake test in vivo (A. J. Wood, M. Elphick, J. K. Aronson & D. G. Grahame-Smith, unpublished work).

**DISCUSSION**

These data demonstrate that lithium alters cation transport in vivo in healthy volunteers and suggest that its effects are mediated by more than one mechanism.

The decreased intra-erythrocytic rubidium concentration observed 1 h after the oral rubidium load suggests that lithium causes changes in both the absorption of rubidium and the early phase of its distribution. It is not known to what extent active cation transport systems modulate the absorption of rubidium across the gut wall. However, changes in cation-transporting systems in the gut wall have been reported in some animal models of hypertension [22], and some of our own data derived from similar cation transport measurement in vivo in patients with untreated essential hypertension were attributable to a change in the rate of intestinal absorption of rubidium [18].

If lithium causes a change in the absorption of rubidium, then one might have expected to have seen an alteration in the plasma profile of rubidium concentrations, and we have not demonstrated any such change here. This may have been because of our protocol for blood sampling, no samples having been taken either during the period of rubidium loading or at any time during the first hour after the last dose of rubidium. We would also have expected to have seen a change in the area under the concentration versus time curve for rubidium in the plasma after alterations in absorption, and whilst a decrease in the systemic availability of rubidium would account for our observations, this is, at present, only a suitable hypothesis, and there is clearly a need for the further study of intestinal cation handling.

There are other possible explanations for the decreased intra-erythrocytic rubidium concentration at 1 h. For example, it is possible that during lithium administration, the intracellular compartmentation of rubidium is altered such that the total intracellular rubidium concentration is reduced even though the concentration in some compartments of the cell is normal. Such compartmentation has been demonstrated for potassium [23]. Furthermore, some complex interaction of rubidium and lithium in the regulation of one or more of the active processes of cation transport may lead to a time-dependent change in net cation flux during the period of the test. Both mechanisms are plausible, but there is no direct experimental support for either one at present.

During lithium administration we have demonstrated a significant increase in the rate of rubidium uptake into erythrocytes between 1 and 5 h after a rubidium load, as reflected in the pseudo rate constant, and this suggests a stimulation of cation transport by lithium. Approximately 80% of total rubidium uptake into erythrocytes in vitro is sensitive to inhibition by cardiac glycosides [24], and we have previously shown that a significant fall in erythrocyte rubidium uptake occurs in vivo after the administration to healthy volunteers and patients of a clinically effective dose of digoxin [17, 19]. Both these observations suggest that the majority of rubidium uptake in vivo is via the sodium pump enzyme, Na+,K+-ATPase, and the results of this study are consistent with a direct stimulation in vivo of Na+,K+-ATPase after 21 days of lithium administration. The data for erythrocyte rubidium concentrations shown in Fig. 1(b) do not exclude some form of catch-up
phenomenon, leading to an increase in rubidium uptake only until control conditions are achieved. This could happen if, for example, rubidium regulated its own uptake even at low concentrations. However, we do not consider this explanation likely, since the rate of rubidium uptake by erythrocytes under control conditions is linear for at least 24 h after an oral load given to control subjects [25], and for at least 5 h in the presence of therapeutic serum lithium concentrations in patients (A. J. Wood, unpublished work).

It should be noted that the pseudo rate constant we have calculated here accurately reflects the true rate constant for rubidium influx only because it has been measured at an extracellular rubidium concentration which is well below the \( K_m \) for rubidium influx (about 1 mmol/l). Under these circumstances, the true rate constant is related to the \( V_{\text{max}} \) and \( K_m \) of transport as follows:

\[
\text{Rate constant} \propto \frac{V_{\text{max}}}{K_m}
\]

Thus, an increase in rate constant, as reflected here by an increase in the pseudo rate constant, could occur as a result of changes in \( V_{\text{max}} \), \( K_m \), or both. We have no way of distinguishing these possibilities in vitro and there are presently no data in vitro from which to draw conclusions.

The mechanism by which lithium stimulates \( \text{Na}^+, \text{K}^- \)-ATPase is not known. It is known that vanadate ion, the +5 oxidation state of the metallic element vanadium, is a potent inhibitor of the sodium pump [26], and there is evidence in vitro that lithium protects erythrocyte \( \text{Na}^+, \text{K}^- \)-ATPase from the inhibitory actions of vanadate [27]. A second possibility derives from the evidence in animals and man that lithium alters catecholaminergic function [28, 29] (and see [30]). It is also clear that, in some cell types at least, sodium pump activity is stimulated by catecholamines via a \( \alpha \)-adrenoceptor-mediated effect [31, 32]. However, there is only scant evidence for a population of \( \beta \)-adrenoceptors on the erythrocyte membrane [33].

It has also recently been proposed that the sodium/sodium countertransport pathway, the major route by which lithium efflux occurs from erythrocytes, might be identical with the sodium/proton antipporter in the renal tubular epithelium [34, 35]. This suggestion has yet to be fully explored, although the demonstration that the erythrocyte sodium/lithium exchanger is not sensitive to inhibition by amiloride [36] does not support the hypothesis. However, if intracellular lithium did compete with protons at a shared sodium/lithium (proton) countertransporter in erythrocytes, then a change in intracellular cytoplasmic pH might be predicted, which might in turn lead to an alteration in \( \text{Na}^+, \text{K}^- \)-ATPase activity.

If the uptake of rubidium into all cell species was increased, as it is into the erythrocyte, then we would have expected the profile of plasma rubidium concentrations between 1 h and 5 h after the oral load to be altered during the period of lithium treatment. We saw no such change, and must therefore conclude that the stimulation of rubidium uptake is confined to a relatively small cellular mass. The important specific corollary of this conclusion is the difficulty in extrapolating such studies of cation transport in peripheral cells to infer any similar alteration in sodium pump activity in the brain. There are very few data to allow direct comparison of our results with measures of cation transport in brain; indeed, all the studies of brain \( \text{Na}^+, \text{K}^- \)-ATPase to date have used animal tissues and assays of \( \text{Na}^+, \text{K}^- \)-ATPase activity in vitro. They have shown either no effect or else an inhibition of \( \text{Na}^+, \text{K}^- \)-ATPase activity after various regimens of lithium administration [37, 38].

The one previous study of the effect of lithium on cation transport in healthy volunteers showed no change in \( \text{Na}^+, \text{K}^- \)-ATPase activity in vitro measured as phosphate release, but a small, significant fall in the rate of uptake into erythrocytes of radioisotopic potassium in vitro [15]. These results in vitro are not in agreement with the results of our study in vivo. The discrepancies between the results of studies in vitro and in vivo may have a basis in technical problems relating to techniques in vitro. In all the studies cited in the Introduction [5–16], experimenters have assessed the rate of release of inorganic phosphate from adenosine 5'-triphosphate substrate in vitro, which occurs as the direct consequence of enzyme activity, and such assays are sensitive to small changes in pH and redox potential. Furthermore, the methods used for cell isolation and the preparation of cell-membrane fragments for assay are unphysiological. A few workers [15, 24] have also measured cation transport in vitro as the rate of uptake or release of radioisotopic substrates of defined transport systems. Such methods have the advantage of using whole cells, usually erythrocytes, but once again the preparative techniques, involving separation from plasma and repeated washing and centrifugation, may critically alter membrane function, and will inevitably remove plasma-borne factors which may have important modulatory effects upon cation transport (see [39]).

In contrast to the single study of healthy volunteers in vitro previous studies of patients treated with lithium salts have consistently shown stimulation of erythrocyte \( \text{Na}^+, \text{K}^- \)-ATPase in vitro [13, 14, 16]. This difference between patient and healthy volunteer studies may simply be due to the different periods of treatment with lithium in the two groups (14 days for volunteers, > 6 months for patients). It may also be explicable in terms of a different susceptibility of the enzyme in patients to the effects of lithium. Furthermore, it is possible that the primary effect of lithium in both patients and volunteers is either upon some other aspect of neuronal function, one consequence of this primary effect being altered cation transport, or else upon some other cellular mechanism important in regulating cation transport via \( \text{Na}^+, \text{K}^- \)-ATPase. The results of our study in vivo do not allow us to differentiate between these possibilities. However, they do suggest that the effect of lithium on cation transport via \( \text{Na}^+, \text{K}^- \)-ATPase is by a mechanism which is not confined to those individuals with a susceptibility to affective illness, nor in a system which is only susceptible to the effects of lithium when, as a consequence of some other functional disturbance, it is itself involved in the pathology of affective illness.
We conclude therefore that after 21 days of administration of lithium to healthy volunteers there are changes in cation transport in vivo, but that the changes seen in erythrocytes cannot be extrapolated to infer similar changes in all cell types. Our results also suggest that there are potentially important differences between activity and regulation of the active processes for transmembrane cation transport in vivo and in vitro.

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REFERENCES


