British Journal of Psychiatry (1989), **155**, 501–504

The Measurement of Transmembrane Cation Transport *in vivo* in Acute Manic Illness

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We have used a novel technique to assess the transport of cations across the erythrocyte membrane *in vivo* in unmedicated patients suffering an acute manic illness. The results show that erythrocyte cation transport via the sodium-pump enzyme Na^+,K^+-ATPase is increased in manic patients compared with healthy controls.

Episodes of mania are the diagnostic hallmark of bipolar affective illness. Several biochemical mechanisms which might be important in the pathogenesis of affective illness have been investigated in patients suffering from episodes of mania, and one area of interest has been alterations in the transport of cations across cell membranes. Specifically, several groups of investigators have demonstrated an alteration in the *in-vitro* activity of the enzyme sodium- and potassium-activated adenosine triphosphatase (Na^+,K^+-ATPase) in the membranes of the erythrocytes and platelets of patients suffering from episodes of mania. However, the results of these studies have been conflicting. Some workers have shown a decrease in Na^+,K^+-ATPase activity during episodes of acute mania (Hokin-Neaverson *et al*, 1974; Naylor *et al*, 1980) or an increase in enzyme activity on recovery from a manic illness (Naylor *et al*, 1976). In contrast, other groups have found an increase in Na^+,K^+-ATPase activity in patients suffering from a manic episode (Sengupta *et al*, 1980; Akagawa *et al*, 1980; Nurnburger *et al*, 1982). The reasons for the discrepancies between the results of these studies are not clear, but may relate to differing diagnostic criteria for patients included in the studies, the effects of medication given to acutely disturbed patients before testing, or the *in-vitro* assays used to assess Na^+,K^+-ATPase activity.

Boon *et al* (1984) reported a technique which allows the assessment of cation transport *in vivo*. This technique is based on the measurement of plasma and erythrocyte rubidium concentrations after the oral administration of a dose of rubidium chloride. They subsequently showed changes in *in-vivo* rubidium transport in patients suffering from essential hypertension, or chronic renal failure, and after the short-term administration to volunteers of the Na^+,K^+-ATPase inhibitor digoxin (Boon *et al*, 1984; Boon *et al*, 1986). They showed that erythrocyte rubidium uptake *in vivo* can be inhibited not only by the prior administration of digoxin but also by the *ex-vivo* incubation of pre-loaded whole blood in the presence of digoxin (Wood *et al*, 1988*a*), and these results suggest that the majority of the accumulation of rubidium by erthrocytes *in vivo* during the period of study is attributable to the cation-transporting activity of Na^+,K^+-ATPase.

We have, therefore, used this technique to assess cation transport *in vivo* in a group of patients suffering an episode of manic illness, and who received no psychotropic medication before or during the study.

Method

Patients

We studied six patients shortly after their admission to hospital. All had been referred to one of the psychiatric hospitals in Oxford and were assessed by two psychiatrists, who agreed on a diagnosis of a manic episode requiring in-patient management. All the patients also met the DSM-III criteria for an acute episode of mania (American Psychiatric Association, 1980).

Three patients were admitted for a first episode of mania. None of these three patients had had previous episodes of affective illness, and so none was taking any regular prophylactic psychotropic medication at the time of admission. The other three patients had suffered previous episodes of mania or depression (or both). Two of the three patients had also received prophylactic lithium. None of the three received psychotropic drugs for at least one year before admission. All six patients were managed without drug treatment, apart from a single night-time dose of a benzodiazepine, from the time of their admission to the time of rubidium administration. Rubidium administration began within 18 hours of admission in all patients.

Control subjects

We compared the patients with healthy volunteers who had no personal or close family history of affective illness, and who were taking no regular medication. These healthy

volunteers were individually matched with manic subjects for age, weight, and sex.

Study protocol

The protocol for the study was approved by the Psychiatric Sector Ethics Committee of the Oxfordshire Health Authority. Shortly after clinical assessment on the day of admission the patients were interviewed, and the nature and details of the test were explained. They were approached again on the following morning and, if they were still willing to take part in the study, testing began immediately.

The patients and control subjects received an oral dose of rubidium chloride dissolved in tap water (Boon *et al*, 1984). The total dose of rubidium chloride was 8 mg/kg body weight, and this was given in eight separate doses of 1 mg/kg at intervals of 15 minutes. Samples of venous blood were taken immediately before the start of the rubidium load test, and one hour and five hours after the last dose of rubidium. The plasma and erythrocytes were separated by centrifugation at 4°C, and plasma and erythrocyte rubidium concentrations were measured by atomic absorption spectrophotometry using a Perkin Elmer 2380 spectrophotometer incorporating an HG 400 graphite furnace (Perkin-Elmer, Beaconsfield, Bucks). Analytical-grade rubidium chloride was obtained from British Drug Houses (Athelstone, Warwickshire).

In-vivo cation transport was assessed as the change in plasma and erythrocyte rubidium concentrations after the oral administration of rubidium chloride. In addition, the rate of uptake of rubidium by erythrocytes was calculated as the rate of increase in erythrocyte rubidium concentrations between one hour (RBC1) and five hours (RBC5), and corrected for the plasma rubidium concentration by dividing it by the plasma rubidium concentration at one hour (plasma1). Thus, rate of Rb uptake $= (Rb_{RBC5} - Rb_{RBC1})/4 \div Rb_{Plasma1}$. This value has the units of a rate constant (h^{-1}) and we refer to it as a pseudo-rate constant.

Plasma and intra-erythrocytic potassium concentrations and serum creatinine and urea were also measured in all the subjects in samples taken immediately before the rubidium load test.

During the period of rubidium loading the symptoms of mania were rated by one of us (AJW) using the Young Rating Scale for Mania (Young *et al*, 1978).

Differences in the results of cation concentration measurements were assessed stastically using Student's *t*-test for normally distributed paired data, these data being expressed as means with standard deviations. Data that were not normally distributed have been expressed as medians and ranges and have been assessed by the signed-rank test for paired data.

Results

The demographic data and results of *in vivo* cation transport measurement are shown in Table I. There was no significant difference in either age or weight between the two groups.

TABLE I

Demographic data and results of in vivo *cation transport measurement in acutely manic and matched control subjects*

	Acute mania	*Control*
Age range: years	21–75	24–65
Weight: mean (range) (kg)	70 (65–80)	72 (64–95)
In vivo cation transport (means ± s.d.)		
increment in plasma Rb conc. (μmol/l) (mean ± s.d.)		
1 h	9.13 ± 1.00	8.72 ± 1.60
5 h	6.67 ± 0.85	5.79 ± 1.30
increment in erythrocyte Rb conc. (μmol/l) (mean ± s.d.)		
1 h	29.46 ± 6.02	17.27 ± 3.96*
5 h	49.75 ± 10.9	32.70 ± 5.22*
pseudo rate constant (h^{-1})	0.48	0.33
(median range)	(0.35–0.65)	(0.26–0.42)*
Potassium concentrations (mmol/l) (mean ± s.d.)		
plasma	4.87 ± 0.33	4.48 ± 0.51
erythrocyte	87.51 ± 4.15	84.02 ± 5.08

*$P < 0.05$.

The median Young score for the patients was 23 (range 22–30). This implies a significant disturbance of behaviour but does not identify any particular subgroup of manic patients.

There was a significant increase in the intraerythrocytic rubidium concentration in the manic patients at both 1 h and 5 h after the rubidium load. The pseudo rate constant for rubidium uptake into erythrocytes was also significantly higher in the manic patients than in the matched controls ($P < 0.05$).

There was no significant difference in the plasma concentrations of rubidium after rubidium loading.

Because potassium and rubidium compete *in vivo* for transport by the Na^+,K^+-ATPase it is important to measure plasma and erythrocyte potassium concentrations in studies of this kind. There were no significant differences in these parameters between the two groups (Table I). All subjects had normal creatinine and urea concentrations (data not shown).

Discussion

There was a significantly greater intra-erythrocytic concentration of rubidium in patients than in controls, and the pseudo-rate constant for erythrocyte rubidium uptake was also significantly increased in the manic patients. Previous studies using this *in vivo* protocol have shown that the majority of erythrocyte rubidium uptake *in vivo* is mediated by Na^+,K^+-ATPase (Boon *et al*, 1984; Wood *et al*, 1988*a*), and we conclude that the changes shown here are consistent with an increase in the *in vivo* activity of erythrocyte Na^+,K^+-ATPase in acute manic illness.

However, it is possible that the increased uptake of rubidium into erythrocytes might result not from increased Na^+mK^+-ATPase activity but either from decreased efflux of rubidium from the cell or from increased uptake via some other pathway, such as the loop diuretic-sensitive Na/K cotransporter. Both passive rubidium efflux from cells and Na/K cotransport activity have been studied *in vitro* in the erythrocytes of patients with manic–depressive illness by Dagher *et al* (1984). They found no change in either cotransport activity or passive permeability, and this suggests that the increased rubidium uptake we have seen *in vivo* is due principally to an increase in Na^+,K^+-ATPase activity.

This finding is in agreement with the results of some *in-vitro* studies in manic patients (e.g. Sengupta *et al*, 1980), but conflicts with other, usually either, studies which demonstrated either a reduction in Na^+,K^+-ATPase activity during mania or an increase in activity following clinical recovery from a manic episode (e.g. Naylor *et al*, 1976). The reason for this difference is not clear, but it may reflect the fact that many of the patients included in the earlier studies were receiving medication, or it may be due to a difference in the severity of illness suffered by the patients included in the various studies previously undertaken.

The discrepancies between earlier *in-vitro* studies may also result from minor differences in the techniques used. All the *in-vitro* techniques involve repeated centrifugation, cooling, rewarming, and washing of the cells before being assayed. These manipulations might themselves have effects upon cellular cation concentrations or upon the activity of Na^+,K^+-ATPase, and they would inevitably remove plasma-borne factors which might be important in the physiological regulation of the sodium pump enzyme (deWardener & Clarkson, 1985). These technical limitations of the *in-vitro* assays re-emphasise the value of the *in-vivo* technique used in the present study.

The patients included in this study had Young mania rating scores in the range 22–30. This suggests a significant manic episode (not hypomania), but does not specify any particular pattern of symptoms. In fact, most of the patients were elated and had significant loss of insight. However, they were in the subgroup of manic individuals who are industrious and co-operative. They were neither particularly irritable nor extremely overactive. In fact, all were able to stay in a single room with the investigator throughout the two hours of rubidium administration. This may be important, in view of the changes in intra-erythrocytic cation concentrations which have been described following brief strenuous exercise (Bodemann *et al*, 1982), and which have been interpreted as being due to a change in Na^+, Ka^+-ATPase activity during exercise.

The mechanism whereby erythrocyte rubidium uptake is increased in manic illness is not known, nor is it possible to deduce the mechanism from the results of the present study. The potential role of the vanadate ion (the +5 oxidation state of vanadium) in the regulation of Na^+,K^+-ATPase activity in affective illness has been emphasised by Naylor & Smith (1981), and has recently been reviewed by Wood (1987). However, the present study would not support the suggestion (Dick *et al*, 1982; Naylor *et al*, 1984) that there is *decreased* activity of Na^+, K^+-ATPase due to increased vanadium concentrations in manic patients. Nevertheless, the changes in vanadium concentrations and in intracellular vanadium handling in affective illness are still poorly understood (see Wood, 1987), and the more recent work of Naylor *et al* (1987*a*,*b*) has stressed a possible role for vanadium in the *depressive* pole of affective illness, in which most *in-vitro* studies suggest a decrease in Na^+,K^+-ATPase activity.

It is possible that the concentration of some plasma-borne regulator of Na^+,K^+-ATPase is changed in manic illness. The possible effect of exercise and circulating catcholamines on Na^+,K^+-ATPase activity have been discussed in two reports (Bodemann *et al*, 1982, 1985), and may be relevant to the changes in physical activity and arousal during periods of affective illness as mentioned above. However, we have investigated the effect of the β_2-adrenoreceptor agonist salbutamol on rubidium uptake *in vivo* using the oral rubidium load, and have shown no stimulation of erythrocyte rubidium uptake (Wood *et al*, 1988*a*). Previous studies have shown that adrenoreceptor-mediated catcholamine stimulation may be important in the regulation of Na^+, K^+-ATPase activity in the brain (Phillis & Wu, 1981).

Many other circulating substances, including angiotensin, corticosteroids, thyroxine, and α-adrenoreceptor agonists, have been shown to modulate Na^+,K^+-ATPase activity in a number of tissues, and clearly an alteration in the circulating concentrations of any combination of these might underlie our present results.

It is also possible that the change in Na^+, K^+-ATPase activity is one manifestation of a more generalised change in membrane structure or function in manic illness. Some evidence for this comes from the work of Pettegrew *et al* (1982), who have inferred an alteration in membrane structure of both the erythrocytes and the lymphocytes of patients with manic–depressive illness.

The profile of plasma rubidium concentrations after oral rubidium administration is not altered in manic patients. This suggests that the increased rate of rubidium uptake into erythrocytes occurs only into a small intracellular volume, since a more generalised increase in tissue rubidium uptake ought to lead to an observable increase in the rate of clearance of rubidium from the plasma. Hence, the changes we have found in erythrocyte Na^+, K^+-ATPase activity cannot be assumed to reflect changes occurring in any other specific organ, including the brain. The direct study of cation transport *in vivo* in neurons is obviously desirable but must await the development of suitable and sensitive non-invasive techniques, such as nuclear magnetic resonance spectroscopy.

Conclusions

We have found an increase in the rate of uptake of rubidium into erythrocytes *in vivo* in unmedicated patients suffering from an acute manic illness. These patients fulfilled strict diagnostic criteria for acute mania, were suffering a significant symptomatic illness at the time of testing, and had received no medication before being studied. The changes we have found suggest that there is an increase in the *in-vivo* activity of Na^+,K^+-ATPase in the erythrocyte membrane in acute manic illness. This *in-vivo* model offers an extension of the *in-vitro* measurements of Na^+,K^+-ATPase activity used in previous studies. However, our data suggest that changes demonstrated in the erythrocyte membrane *in vivo* cannot necessarily be extrapolated to infer any similar changes in cation transport in the brain.

For acknowledgements, references and authors' details, see the following paper, on pp. 504–510

British Journal of Psychiatry (1989), **155**, 504–510

The Effect of Lithium on Cation Transport Measured *in vivo* in Patients Suffering from Bipolar Affective Illness

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We have investigated cation transport *in vivo* in patients being treated with lithium for bipolar affective illness by studying the disposition of rubidium after an oral load of rubidium chloride. The rate of erythrocyte cation transport was increased in the patients when compared with matched healthy volunteers. However, the rate of *in-vivo* erythrocyte rubidium accumulation in the euthymic treated patients was significantly lower than in a matched group of unmedicated manic patients. The regulation of specific pathways for cation transport may be altered in individuals predisposed to affective illness.

The specific therapeutic activity of lithium salts was first described in a group of manic men by Cade (1949). His discovery was made as a result of chance observations, and, despite almost 40 years of investigation of the effects of lithium on many aspects of neuronal biochemistry and neurotransmitter function, the pharmacological actions crucial to its well established clinical effects are not yet known (Wood & Goodwin, 1987).

One area of interest has been the changes in the transport of cations across cell membranes which occur in patients suffering from affective illness. Alterations in the activity of the sodium-pump enzyme Na^+,K^+-ATPase have been described *in vitro*