The effect of lithium on choline transport in human erythrocytes

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SUMMARY Erythrocytes from lithium treated patients were separated according to the time they had been circulating in the plasma. Choline transport and choline content were measured in the erythrocyte fractions separated according to age, in order to investigate the relationship between decreased choline transport and increased choline content seen in lithium treated patients. The most recently formed erythrocytes of normal subjects had the greatest choline content and the most active choline carrier. The erythrocytes of lithium treated patients had reduced choline carrier activity and increased choline content in all age bands. However the greatest accumulation of choline and least inhibited choline carrier activity was seen in the most recently formed cells. The alteration in phospholipid concentrations measured could not of themselves account for elevated erythrocyte choline levels seen in lithium treated patients. It is concluded that the increase in choline content levels in lithiated erythrocytes does not have a simple inverse relationship with the deficiency in choline transport. The inhibition of the choline carrier is caused by modification due to circulation in lithiated plasma rather than a lack of its synthesis in reticulo-cytes.

Lithium is widely used as prophylaxis for the manic phase of bipolar affective disorder and there have been many studies on its biochemical effects.¹ One of the more striking effects is the irreversible inhibition (80-95%) of choline transport in erythrocytes² and the even more dramatic increase (10-20 fold) in their choline content.³ The questions we have addressed in this study are (a) is there a simple inverse relationship between the deficiency in choline transport and choline content as has been suggested?^{4,5} (b) what is the origin of the increased choline in erythrocytes? (c) what is the nature of the deficiency induced by lithium in the choline carrier?

As erythrocytes age their density increases.⁶ By using a simple density gradient technique to separate erythrocytes according to their age, the evolution of lithium effects in patients with affective disorder has been investigated. The age-separated erythrocyte fractions were examined for their ability to transport choline, choline content and membrane

phospholipid concentrations. The relationship of changes in choline uptake and content to the therapeutic effect of lithium is not understood. A major impediment is the interspecies variability of the lithium effect,² making the development of a suitable model difficult. Without an animal model a study of lithium effects on the CNS is impractical. However by defining the relationship in "lithiated" human erythrocytes (which are readily available) one may hope for clues that will allow a more focused approach to the effects of lithium on the brain and hence the explanation of its prophylactic effect.

Methods

Blood collection

Blood was collected by venepuncture from healthy volunteers and patients attending the lithium clinic at the Maudsley Hospital. All samples were taken into sodium heparin and washed 3 × with 133 mM NaCl, 4.5 mM KCl, and 10 mM HEPES pH 7.4. Unless stated in the text patients on lithium had been treated for at least six months.

Separation of erythrocytes according to age⁶

Solutions of different density of Percoll were formed containing 58%, 60%, 63% and 66% Percoll (density 1.072-1.100) in 5.263% Bovine serum albumin, 133 mM NaCl,

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4.5 mM KCl, and 10 mM HEPES (pH 7.4). Step gradients were prepared by layering 2.5 ml of each Percoll concentration. 1.5 ml of washed erythrocyte suspension (45–50% haematocrit) containing $1.5\text{--}2.0 \times 10^{10}$ cells was layered on top of each gradient and spun at 2000 g for 10 minutes at 20°C. The erythrocyte age fractions were collected by aspiration and washed three times in HEPES—buffered saline. Choline influx measurements were made immediately and a sample of the age separated fraction was stored at -80°C for measurement of choline and phospholipids.

Choline influx measurements

Choline influx was measured by a modification of a method first described by Martin.⁷ 60 μl of washed cells were added to an Eppendorf tube containing 190 μl of incubation buffer (137 mM NaCl, 4 mM KCl, 1 mM MgCl_2 , 2.2 mM CaCl_2 , 0.1% glucose, 0.01% chloramphenicol and 10 mM Tris HCl-Tris base pH 7.4). The cells were preincubated for 10 minutes, at 37°C and 2 μM -[^3H] choline (0.125 μCi) was then added. Uptake was terminated after 10 minutes, by centrifugation at 15,000 g for 4 seconds in a Janetzki centrifuge and the cells washed $3 \times$ in ice cold incubation buffer (pH 7.4). Choline was extracted by precipitating the proteins with 1 ml of 5% (w/v) trichoroacetic acid. Denatured protein was sedimented by spinning for 1 minute at 15000 g and 0.5 ml of the supernatant was counted. The scintillant was toluene containing 5 g PPO (2, 5-diphenyloxazole) and 0.2 g POPOP 1,4-bis-(phenyl oxazole-2-yl) benzene (POPOP) per litre/synperonic NXP/2M NH_4OH (100:50:1) vol).

Choline content assay

A radioenzymatic method⁸ was used to measure erythrocyte choline. This method is based on the phosphorylation of choline by choline kinase and [^{32}P]-ATP and the separation of ^{32}P -choline by an ion exchange resin.

Phospholipid measurements

Lipids were extracted from erythrocyte ghosts¹⁰ by the method of Bligh and Dyer.¹¹ Phospholipids were then separated by thin layer chromatography and lipid phosphorus determined.¹²

Results

The data presented in table 1 show a decrease in

choline influx and intra-erythrocyte choline levels throughout the age fraction of lithiated cells. This is consistent with the results previously reported^{2,3} for total unfractionated cells. However choline content is highest within the youngest cell fraction and decreases with increasing cell age; this tendency is more evident in lithiated erythrocytes. Choline influx also decreases with age at about the same rate in lithiated and normal cells but the decrease is less pronounced than that seen with choline content.

The inverse relationship between choline influx and content seen in total populations of erythrocytes is not observed when the erythrocytes are separated according to their age. This can be clearly seen by plotting erythrocyte choline influx vs choline content (fig. 1). The values plotted lie on a straight line with

Fig 1 The relationship between choline influx and choline content in erythrocytes separated according to age.

● Controls, ○ lithium treated patients, □ F54 1 week on lithium, ■ F54 2 weeks on lithium.

Straight lines were fitted by least squares to a linear equation. Figures placed next to the points indicate the age band (as in Table 2) of the erythrocytes.

Table 1 Distribution of choline transport and content in age-separated erythrocytes

Fraction no	Density	*RBC age (days)	Controls		Lithium treated patients	
			Choline influx (n = 5)	Choline content (n = 5)	Choline influx (n = 5)	Choline content (n = 5)
1	1.072	0–10	11.37 ± 0.9	1.097 ± 0.05	3.29 ± 0.6	8.76 ± 1.2
2	1.080	10–30	9.3 ± 1.4	0.935 ± 0.1	1.87 ± 0.4	8.72 ± 1.5
3	1.085	30–60	5.97 ± 1	0.88 ± 0.1	1.27 ± 0.2	5.72 ± 1
4	1.092	60–90	6.14 ± 0.8	0.535 ± 0.08	1.36 ± 0.1	3.5 ± 0.5
5	1.100	90–120	5.3 ± 0.8	0.421 ± 0.04	1.56 ± 0.5	2.68 ± 0.4

Values are means $\pm$ SEM of choline influx (nmols/min/cell $\times 10^{12}$) and choline content (nmol/cell $\times 10^9$) in the 5 erythrocyte age fractions. *The exact relationship between erythrocyte age and density is uncertain. However, from studies on the distribution of ^{59}Fe tagged human erythrocytes¹³ and similar labelling studies on rabbit erythrocytes,¹⁴ the ages of the various age bands have been approximated. Values obtained with total unfractionated blood are: lithium treated patients, choline influx 1.1×10^{-12} nmol/min/cell (n = 4), choline content 6×10^{-8} nmol/cell (n = 9); controls, choline influx 5×10^{-12} nmols/min/cell (n = 4) choline content 0.45×10^{-8} nmol/cell (n = 18).

positive relationships (that is choline content increases with decreasing cell age as choline influx also increases with decreasing cell age). The magnitude of this slope is increased by lithium treatment. Erythrocytes from patients treated with lithium for less than 3 weeks show an intermediate enhancement of the positive relationship of choline content to choline transport. This effect cannot be due to leakage of choline when the cells travel through the gradient because young and old cells were applied to a second Percoll gradient and choline concentration measured before and after centrifugation. No difference in choline concentration was found following a second density gradient centrifugation. Nor can the decrease in choline levels be due to increased passive diffusion of choline with increasing cell age because hemicholinium (200 μ m) insensitive flux (that is diffusion flux) was not found to increase with increasing cell age.

The most obvious source of the increased choline found as a result of lithium treatment would be uptake from the plasma. However plasma choline values are normal¹⁵ and at each age range the choline flux is less in lithiated cells than in control cells. An alternative explanation proposed^{4,5} is that choline produced from membrane phospholipid breakdown accumulates within the erythrocyte due to inhibition of choline transport. If erythrocyte membrane phospholipids are the non renewing source of choline the decrease in choline containing phospholipid concentration should be measurable (0.32–0.64 mg/ml of phospholipid would need to be catabolised to provide the elevated choline levels seen in lithium treated patients). However the decrease in the choline-containing phospholipid concentrations (phosphatidylcholine and sphingomyelin) in young and old erythrocytes, from lithium-treated patients and controls is insufficient to account for the increased choline concentration seen in the erythrocytes of patients treated with lithium. Unless there is a mechanism for renewing choline-containing membrane phospholipids these are not the source of the raised choline.

The deficiency in choline transport may be a consequence of the circulation of erythrocytes in lithiated plasma or it may be that lithium prevents the synthesis or incorporation of the choline carrier into the developing erythrocyte. In order to investigate this the erythrocytes from patients who had only just commenced a course of lithium treatment were investigated. Figure 2 shows age-separated erythrocyte choline influx, in two patients treated with lithium for 1–2 weeks, with for comparison patients treated with lithium for greater than 6 months and controls. Erythrocytes of patients commencing

Fig 2 Choline influx in erythrocytes at the commencement of lithium treatment. ● Controls, ○ lithium treated patients. □ F54 1 week on lithium, ■ 2 weeks on lithium. △ F24 1 week on lithium, ▲ 2 weeks on lithium.

lithium therapy show intermediate levels of inhibition at all erythrocyte age groups. If lithium was affecting the synthesis of choline carrier in the reticulocyte it would be expected that young cells would show an enhanced deficit. This is not the case so we conclude that lithium exerts a progressive inhibitory effect on the choline carrier of circulating erythrocytes.

Discussion

The alteration in choline metabolism and transport seen in the erythrocytes of patients treated with lithium, has previously been explained by an inhibition of the choline carrier system preventing the efflux of choline released from membrane phospholipids.^{4,5} However two types of data obtained in this study are inconsistent with this view. The decrease in erythrocyte phospholipid levels is not sufficient to account for the elevation of free choline. Assuming the average phospholipid concentration of erythrocytes is 3.75 μ moles/ml, a 30–50% decrease in choline containing phospholipid concentrations would be needed to account for the elevated choline levels. Secondly erythrocyte choline levels are lower in the older fractions of lithiated cells, which also have the lowest activity of the choline carrier.

Exchange between erythrocyte and plasma pools of sphingomyelin and phosphatidylcholine has been well documented^{16,17} and has been estimated to be 9

Table 2 The distribution of phospholipids within the erythrocytes of lithium-treated patients and controls

	Sphingomyelin	Phosphatidylcholine	Phosphatidylserine	Phosphatidylethanolamine
Controls (n = 7)	1.17 ± 0.05	1.39 ± 0.04	0.66 ± 0.04	1.34 ± 0.05
Control young cells (n = 4)	1.2 ± 0.04	1.46 ± 0.07		
Control old cells (n = 4)	1.1 ± 0.12	1.34 ± 0.08		
Lithium treated patients (n = 9)	1.12 ± 0.05	1.23 ± 0.08	0.68 ± 0.05	1.35 ± 0.09
Lithium young cells (n = 4)	1.15 ± 0.12	1.43 ± 0.13		
Lithium old cells (n = 4)	1.13 ± 0.11	1.24 ± 0.14		

Fractions 1-2 and 4-5 (see table 1) were aggregated to produce "young" and "old" cells respectively. Values are expressed as $\mu\text{moles phospholipid/cell} \times 10^{10}$ ($\pm$ SEM)

mmoles/ml per hour. It is not clear whether the exchange protein can catalyse net incorporation but if so it would be sufficiently active to replace any phosphatidylcholine broken down so that a net loss would not necessarily be evident. Therefore phospholipid breakdown with rapid replacement by processes that normally only exchange phospholipids could be the source of choline in lithiated erythrocytes. However, this would not explain the decrease in choline concentrations seen with increasing erythrocyte age. A scheme such as this would also require an active phospholipase in erythrocytes, which remains to be demonstrated. Unless the erythrocyte choline carrier had a greater affinity for external choline than internal choline during the early stages of lithium treatment, it could not be the cause of the increased choline. In addition a differential effect of lithium on efflux as compared to influx would require some fundamental mechanistic re-appraisal of how the carrier is thought to operate.¹⁸ (Such a possibility is currently under investigation).

All the erythrocytes from patients who had been treated with lithium for at least six months showed deficits in choline transport. The most recently formed erythrocytes however, in patients treated with lithium for 1-2 weeks show a comparatively unaffected choline carrier system. We therefore conclude that it is the circulation of erythrocytes in lithiated plasma, rather than any effect on the synthesis of carrier or its insertion into the membrane. Our study suggests some slow acting interaction between lithium and biological membranes such that the choline carrier is inactivated. Generally lithium prophylaxis develops 2 weeks after commencement of treatment. The time scale over which the majority of change in choline metabolism occurs would seem to parallel the development of the therapeutic effect. It would clearly be of a great interest to know whether or not the alteration in choline metabolism produced by lithium in erythrocytes is paralleled in the brain. Experiments have shown a decrease in choline influx into rat brain after lithium administration.¹⁹ But given the interspecies variability of this

effect² an extrapolation of this kind without some knowledge of its molecular basis is not without risk. The changes in patients and animals during lithium treatment will need to be characterised more fully but when both sets of information are taken together it may be possible to formulate testable hypotheses concerning affective disorder, lithium therapy and choline metabolism and transport.

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References

- Schou M. Possible mechanisms of action of lithium salts: Approaches and perspectives. *Biochem Soc Trans* 1973;1:81-5.
- Lingsch C, Martin K. An irreversible effect of lithium administration to patients. *Br J Pharmacol* 1976;57:323-7.
- Jope RS, Jenden DJ, Ehrlich BE, Diamond JM. Choline accumulates in erythrocytes during lithium therapy. *New Eng J Med* 1978;299:833.
- Jenden DJ, Jope RS, Fraser SL. A mechanism for the accumulation of choline in erythrocytes during treatment with lithium. *Comm Psychopharmacol* 1980;4:339-44.
- Hanin I, Mallinger AG, Kopp U, Himmelhoch JM, Neil JF. Mechanisms of lithium induced elevation in RBC choline content. An *in vitro* analysis. *Comm Psychopharmacol* 1980;4:345-55.
- Rennie CM, Thompson S, Parker AC, Maddy A. A human erythrocyte fractionation in Percoll density gradients. *Clin Chim Acta* 1979;98:119-25.
- Martin K. Extracellular cations and the movement of choline across the erythrocyte membrane. *J Physiol (London)* 1972;224:207-30.
- Goldberg AM, McCaman RE. Determination of picomole amounts of acetylcholine in mammalian brain. *J Neurochem* 1973;20:1-8.
- Wang FL, Haubrich DR. A simple, sensitive and specific assay for free choline in plasma. *Analyt Biochem* 1975;63:195-201.

- ¹⁰ Bjerrum, PJ. Haemoglobin depleted human erythrocyte ghosts. *J Membr Biol* 1979;**48**:43–67.
- ¹¹ Bligh EG, Dyer WJ. A rapid method for total lipid extraction and purification. *Can J Biochem Physiol* 1959;**37**:911–7.
- ¹² Bartlett GR. Phosphorus assay in column chromatography. *J Biol Chem* 1959;**234**:466–8.
- ¹³ Borun ER, Figueroa WG, Pessy SM. The distribution of Fe²⁹ tagged human erythrocytes in centrifuged specimens as a function of cell age. *J Clin Invest* 1957;**36**:676–9.
- ¹⁴ Piomelli S, Luninsky G, Wasserman LR. The mechanisms of red cell aging. *J Lab Clin Med* 1967;**69**:659–74.
- ¹⁵ Hanin I, Cohen BM, Kopp U, Lipinski JF. Erythrocyte and plasma choline in bipolar psychiatric patients: A follow up study. *Psychopharmacol Bull* 1982;**18**:186–90.
- ¹⁶ Reed CF, Murphy M, Roberts G. Phospholipid exchange between plasma and erythrocytes in man and the dog. *J Clin Invest* 1968;**47**:749–60.
- ¹⁷ Lovelock JE, James AT, Rowe CE. Lipids of whole blood. 2. The exchange of lipids between the cellular constituents and the lipoproteins of human blood. *Biochem J* 1960;**74**:137–40.
- ¹⁸ Marchbanks RM, Wonnacott S, Rubio MA. The effect of acetylcholine release on choline fluxes in isolated synaptic terminals. *J Neurochem* 1981;**36**(2):379–93.
- ¹⁹ McCall AL, Millington WR, Botticelli LJ, Wurtman RJ. Lithium treatment effects on choline transport at the blood brain barrier. *Proc Soc Neurosci* 1978;**4**:1360.