

# Erythrocyte phosphoglucomutase activity of bipolar I patients currently using lithium or carbamazepine

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## Abstract

Lithium has been used for the last five decades to treat bipolar disorder, but the molecular basis of its therapeutic effect is unknown. Phosphoglucomutase is a key enzyme in the metabolism of glycogen. In yeast, rabbit and human HEK293 cells, it is inhibited by lithium in the therapeutic concentration range. We measured the phosphoglucomutase activity in erythrocytes and the inhibitor constant for lithium in a population of healthy subjects and compared them to those of bipolar patients treated with lithium or carbamazepine. The specific activity of phosphoglucomutase measured *in vitro* in erythrocytes from control subjects presented a normal distribution, with the difference between the lowest and the highest activity being approximately 2-fold ( $0.53\text{--}1.10\text{ nmol mg Hb}^{-1}\text{ min}^{-1}$ ). Comparison of phosphoglucomutase activity in untreated bipolar patients and control subjects showed no significant difference, whereas comparison between bipolar patients treated with carbamazepine or lithium revealed significantly lower mean values in patients treated with carbamazepine ( $747.3 \pm 27.6$  vs  $879.5 \pm 35.9\text{ pmol mg Hb}^{-1}\text{ min}^{-1}$ , respectively). When we studied the concentration of lithium needed to inhibit phosphoglucomutase activity by 50%, a bimodal distribution among the population tested was obtained. The concentration of LiCl needed to inhibit phosphoglucomutase activity by 50% was 0.35 to 1.8 mM in one group of subjects and in the other it was 3 to 4 mM. These results suggest that phosphoglucomutase activity may be significant in patients with bipolar disorder treated with lithium and carbamazepine.

## Key words

- Phosphoglucomutase
- Lithium
- Carbamazepine
- Bipolar disorder

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## Introduction

An important strategy for investigating the molecular basis of bipolar disorder is to study the effects of the different drugs used for treatment on enzyme activity. Lithium has been used extensively for the treatment of bipolar disorder over the last 45 years (1).

Its therapeutic mechanism is unknown, although its effect on physiological processes and on a wide spectrum of enzymes has been described. Several enzymes that use  $\text{Mg}^{2+}$  as a co-factor are inhibited by lithium which is a monovalent alkaline metal ion with a hydrated radius similar to that of  $\text{Mg}^{2+}$ . Glycogen synthase kinase-3 $\beta$  is one of its targets,

and inhibition leads to severe defects in development in *Xenopus laevis* embryos and in *Dictyostelium discoideum* (2-6). Some of the enzymes inhibited by lithium share the structural motif Asp-Pro-(Ile/Leu)-Asp-Gly/Ser)-Thr/Ser that is responsible for lithium binding, such as inositol monophosphatase, inositol polyphosphate 1-phosphatase, fructose 1,6-biphosphate phosphatase, and biphosphate nucleotidase (7).

It has been recently shown that phosphoglucomutase is also inhibited by lithium in different species such as yeast (8) rats (9) and human HEK293 cells (8). We believe that the relevance of phosphoglucomutase activity in the manifestation of bipolar disorder and in the action of lithium deserves study. Phosphoglucomutase is an enzyme that is central to carbohydrate metabolism and protein glycosylation. It is responsible for the reversible interconversion of glucose 1-phosphate (Glu 1-P) to glucose 6-phosphate (Glu 6-P), both of which are key intermediates in the synthesis and breakdown of glycogen and in galactose metabolism. It is also important for the formation of UDP-glucose which is an essential intermediary metabolite in protein glycosylation. Inhibition of phosphoglucomutase by lithium in yeast cells has drastic effects on carbohydrate metabolism. During glucose metabolism lithium reduces the steady-state levels of UDP-glucose, resulting in a defect of glycogen and trehalose biosynthesis, while galactose metabolism is inhibited, leading to galactosemia, accumulation of galactose-1P and Glu 1-P and inhibition of fermentation (8,10). A recent report has shown that lithium treatment up-regulates phosphoglucomutase activity in various tissues of the rat and in bipolar patients, even though lithium inhibits phosphoglucomutase activity *in vitro* (11).

In the present study, we measured phosphoglucomutase activity in human erythrocytes and determined whether this activity is altered in patients with bipolar disorder treated or not with lithium or carbamazepine.

## Patients, Material and Methods

### Subject selection

Participants were men and women aged 18 to 60 years. Patients with bipolar disorder I (BPI) were classified according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, also known as the DSM-IV-tr (12). BPI is characterized by one or more manic or mixed episodes, usually accompanied by major depressive episodes (12,13). Patients who met DSM-IV criteria for other mood disorders were excluded. Patients were randomly recruited from a sample of 211 BPI patients currently receiving assistance in the inpatient unit of the Institute of Psychiatry, Federal University of Rio de Janeiro. We selected the BPI patients who had been taking lithium or carbamazepine for more than one year but had recovered from a recent manic episode and were ready to leave the inpatient clinic. Blood was collected into Vacuette® EDTA tubes which contain 8% liquid EDTA solution, when the patient left the inpatient ward. Women of childbearing potential were included if they were utilizing an effective method of birth control. The study was approved by the Ethics Review Board of the Institute of Psychiatry and all patients gave written informed consent to participate.

Patients who had been receiving lithium (N = 21; mean age:  $41.2 \pm 13.0$  years; 8 males and 13 females) or carbamazepine (N = 17; mean age:  $44.5 \pm 10.3$  years; 7 males and 10 females) for at least one year were selected. Two of the lithium patients were on lithium monotherapy, while the others were also receiving diazepam (N = 6); diazepam and olanzapine (N = 1); diazepam, haloperidol and promethazine (N = 5); diazepam and imipramine (N = 2); haloperidol (N = 1); haloperidol and olanzapine (N = 2), or valproic acid, haloperidol and chlorpromazine (N = 2). Serum lithium levels ranged from 0.40 to 1.10 mEq/L, with a mean  $\pm$  SD

of  $0.725 \pm 0.159$  mEq/L. Carbamazepine-treated patients were on monotherapy. Control subjects were volunteers (N = 22; 6 males and 16 females; mean age:  $31.0 \pm 11.2$  years) who declared that they had never sought psychiatric help. They were also interviewed by two psychiatrists using the Structured Clinical Interview (14) for DSM-IV. The age difference between patients and controls was not significant and the phosphoglucomutase-specific activity did not correlate with the age of the subjects under study (data not shown).

### Cell preparation

Blood samples were collected into EDTA-containing tubes and immediately centrifuged at 3,000 rpm for 10 min. The plasma and leukocyte layers were discarded. Three volumes of 160 mM KCl were added to erythrocytes and the preparation was centrifuged three times for 10 min each at 2,000 rpm. After the final centrifugation the red cell layer was resuspended in 0.5 mL 160 mM KCl and kept at  $-70^{\circ}\text{C}$  until use.

### Biochemical methods

Phosphoglucomutase activity was measured using a coupled system (14). Briefly, lysed erythrocytes (0.050-0.090 mg hemoglobin) were incubated at  $37^{\circ}\text{C}$  in 250  $\mu\text{L}$  buffer containing 50 mM Tris-HCl, pH 7.5, 0.5 mM  $\text{NAD}^+$ , 0.025 mM glucose 1,6-diphosphate, 2 units/mL recombinant *Leuconostoc mesenteroides* glucose-6-phosphate dehydrogenase, 1.5 mM  $\text{MgCl}_2$ , and 1.0 mM EDTA, unless otherwise stated. The reaction was started by the addition of 4 mM Glu 1-P, and NADH formation was monitored at 340 nm. Phosphoglucomutase activity is reported as pmol Glu 6-P  $\text{mg Hb}^{-1} \text{min}^{-1}$ . Hemoglobin concentration was determined by the cyanmethemoglobin method (15). The concentration of lithium that resulted in 50% inhibition of maximal activity (termed the

$\text{IC}_{50}$ ) was determined by measuring phosphoglucomutase activity as described above in the presence of increasing concentrations of lithium (0, 0.3, 0.6, 0.9, 1.2, and 3.0 mM). The inhibitor constant or  $\text{K}_i$  was determined by measuring phosphoglucomutase activity as described above in the presence of 0.1, 1.0 and 10 mM  $\text{MgCl}_2$  and increasing concentrations of LiCl. The  $\text{K}_i$  was determined by plotting the results in a Dixon's plot. The  $\text{K}_i$  of  $\text{Li}^+$  for yeast and human HEK293 cell phosphoglucomutase was determined earlier by Masuda et al. (8).

To test whether erythrocyte phosphoglucomutase is inhibited by carbamazepine, phosphoglucomutase activity was assayed as described above in the presence of varying concentrations of carbamazepine (10 to 500  $\mu\text{M}$ ). The therapeutic range for the steady-state plasma concentration of carbamazepine generally lies between 4 and 10  $\mu\text{g/mL}$  (10-40  $\mu\text{M}$ ).

## Results

### Erythrocyte phosphoglucomutase activity

When erythrocyte phosphoglucomutase was incubated with increasing concentrations of LiCl in a medium containing 0.1, 1.0, or 10 mM  $\text{Mg}^{2+}$  a typical competitive inhibition result was obtained, with a calculated apparent  $\text{K}_i$  for lithium of approximately 800  $\mu\text{M}$  (data not shown), confirming the report of Masuda et al. (8). This indicates that phosphoglucomutase is inhibited by about 50% within the therapeutic concentration range of lithium (0.4 to 1.0 mEq/L). The erythrocyte phosphoglucomutase-specific activity of control subjects (N = 22) and lithium-treated patients (N = 21) is reported in Figure 1A and B, respectively. The specific activity ranged from 530 to 1100 pmol  $\text{mg Hb}^{-1} \text{min}^{-1}$  in control subjects and from 600 to 1300 pmol  $\text{mg Hb}^{-1} \text{min}^{-1}$  in lithium-treated subjects, showing an approximate 2-fold range of minimum- and maxi-

Figure 1. Frequency distribution of phosphoglucomutase-specific activity in control subjects and bipolar I patients. Phosphoglucomutase (PGM) activity was measured in lysed erythrocytes from control healthy volunteers (A; N = 22) and from lithium-treated patients (B; N = 21). Data are the average of three replicates for each individual. Values are normally distributed according to the Shapiro-Wilk test performed at  $P = 0.05$  (values obtained were:  $W = 0.95732$ ,  $P = 0.56969$  for control group, and  $W = 0.89938$ ,  $P = 0.10989$  for lithium-treated groups).

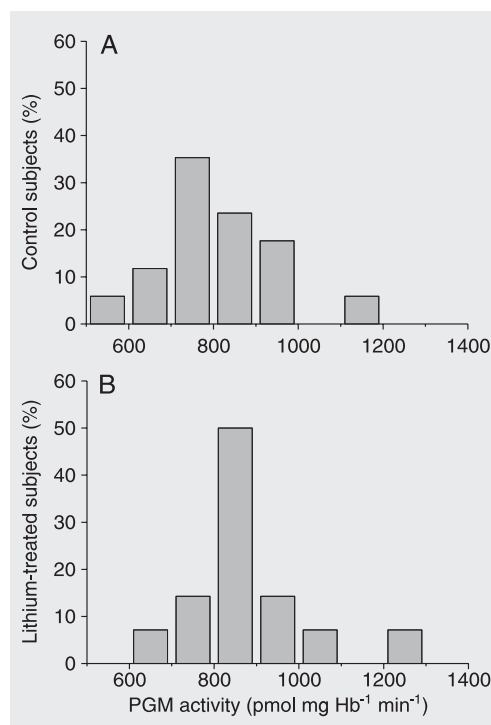
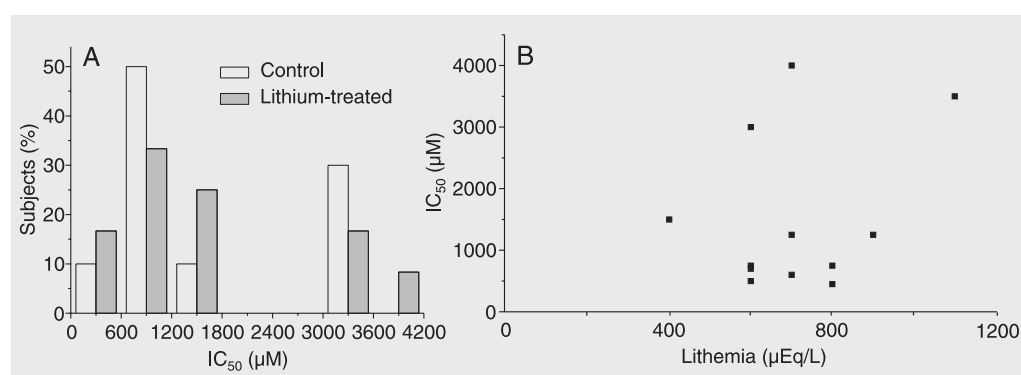


Table 1. Phosphoglucomutase-specific activity ( $\text{pmol mg Hb}^{-1} \text{min}^{-1}$ ) in erythrocytes and inhibition by lithium ( $\text{IC}_{50}$ , mM).

|   | Phosphoglucomutase activity<br>( $\text{pmol mg Hb}^{-1} \text{min}^{-1}$ ) | Lithium<br>$\text{IC}_{50}$ (mM) |
|---|---|----------------------------------|
| Control (N = 22)                        | $800.6 \pm 33.3$  | $1.44 \pm 0.34$                  |
| Lithium-treated subjects (N = 21)       | $879.5 \pm 35.9$  | $1.52 \pm 0.36$                  |
| Carbamazepine-treated subjects (N = 17) | $747.3 \pm 27.6$  | ND                               |

Data are reported as means  $\pm$  SEM. Although the mean phosphoglucomutase-specific activities were significantly different between the three groups ( $P = 0.02$ , one-way ANOVA), the *post hoc* Tukey test indicated that only the difference between lithium- and carbamazepine-treated subjects was significant. ND = not determined.

Figure 2. Inhibition of phosphoglucomutase activity by lithium. A, The concentration of lithium needed for 50% inhibition of phosphoglucomutase activity ( $\text{IC}_{50}$ ) was determined in three replicates from control (N = 22, open bars) and lithium-treated subjects (N = 21, filled bars). A frequency distribution histogram is shown. B,  $\text{IC}_{50}$  determined in lithium-treated subjects was plotted as a function of lithemia at the time the blood samples were collected.



mal-specific activity in each group (Figure 1). Shapiro-Wilk test performed at  $P = 0.05$  showed evidence that phosphoglucomutase activity values measured for both control and lithium-treated groups are normally distributed. The mean  $\pm$  SEM activity for control subjects was not significantly different from that of patients receiving lithium ( $800.6 \pm 33.3$  and  $879.5 \pm 35.9 \text{ pmol mg Hb}^{-1} \text{min}^{-1}$ , respectively; Table 1).

### Phosphoglucomutase activity in lithium- and carbamazepine-treated patients

A recent report showed that, although activity is inhibited *in vitro* by lithium, leukocyte phosphoglucomutase activity from a group of seven patients treated with lithium is increased in relation to healthy individuals (11). In our patients, a larger group, erythrocyte phosphoglucomutase activity was not significantly different from normal. In both cases it would have been better to analyze subjects before and after treatment. However, it was difficult for us to find a bipolar I patient that had been not medicated. Since we lacked this control we decided to test phosphoglucomutase activity in erythrocytes from patients treated with carbamazepine. We first assayed erythrocyte phosphoglucomutase activity *in vitro* in the presence of carbamazepine in order to test if carbamazepine modulates the activity of the enzyme. The results showed that carbamazepine does not alter phosphoglucomutase activity (data

not shown). Next we compared phosphoglucomutase activity between lithium-treated (N = 21) and carbamazepine-treated subjects (N = 17) (Table 1). Carbamazepine-treated patients had a lower erythrocyte phosphoglucomutase activity ( $747.3 \pm 27.6$  pmol mg Hb<sup>-1</sup> min<sup>-1</sup>) than subjects treated with lithium ( $879.5 \pm 35.9$  pmol mg Hb<sup>-1</sup> min<sup>-1</sup>). This difference was statistically significant (P = 0.02, one-way ANOVA). It is possible that there is a difference between erythrocyte and leukocyte phosphoglucomutase.

#### Distribution of lithium's (IC<sub>50</sub>) on phosphoglucomutase activity

An important characteristic of bipolar I patients is that lithium treatment is not always effective. Thus, we examined the distribution of the concentration of lithium needed to inhibit erythrocyte phosphoglucomutase activity by 50% (IC<sub>50</sub>), comparing control subjects (Figure 2A, open bars) with lithium-treated patients (Figure 2A, filled bars; Table 1). In this experiment, phosphoglucomutase activity was measured in the presence of 0.1 mM Mg<sup>2+</sup> since it has been shown that the intracellular free Mg<sup>2+</sup> level is approximately 0.16 mM (11). The IC<sub>50</sub> frequency distribution showed a best fit when the existence of two different populations was assumed. The distribution showed a bimodal curve, with some individuals clustered around a low IC<sub>50</sub> (0.35 to 1.8 mM) and others around a high IC<sub>50</sub> (3 to 4 mM). We also observed that the bimodal distribution was essentially the same for control subjects and for patients treated with lithium. Although there was no difference between controls and patients, the variation among individuals was significant.

Lithium treatment might lead to the expression of an isoform of phosphoglucomutase that is more resistant to lithium inhibition. However, Figure 2B shows that the IC<sub>50</sub> values of lithium-treated patients were

not correlated with lithemia (correlation coefficient = -0.27). We also analyzed if IC<sub>50</sub> values correlated with the rate of phosphoglucomutase activity and found no correlation (data not shown). Although the IC<sub>50</sub> values represent inhibition *in vitro*, it is likely that phosphoglucomutase is not affected by lithium *in vivo* in patients with a high IC<sub>50</sub> since lithemia was less than 1.1 mEq/L in all cases.

## Discussion

Phosphoglucomutase is a key enzyme in carbohydrate metabolism, glycogen synthesis and degradation, galactose metabolism and protein glycosylation (8). Although it has not been tested if bipolar disorder is linked to any metabolic disorder regulated by phosphoglucomutase, inhibition of this enzyme by lithium would probably lead to galactosemia and poor glycogen turnover, as has been shown in yeast (8). A recent report has shown that phosphoglucomutase activity is up-regulated in leukocytes from lithium-treated patients, even though lithium inhibits phosphoglucomutase activity *in vitro* (10). In the present study, we determined the distribution of phosphoglucomutase activity in erythrocytes from control, lithium-treated and carbamazepine-treated subjects, in order to determine if differences in phosphoglucomutase activity are linked to bipolar disorder. We found that patients treated with carbamazepine displayed a significant difference from lithium-treated patients activity ( $747.3 \pm 27.6$  vs  $879.5 \pm 35.9$  pmol mg Hb<sup>-1</sup> min<sup>-1</sup>, respectively; Table 1). Csutora et al. (11) reported that phosphoglucomutase activity is enhanced in leukocytes of lithium-treated patients compared to healthy subjects. This discrepancy might be due to the use of erythrocytes instead of leukocytes. The best way to study alterations in phosphoglucomutase activity after lithium treatment would be a time-controlled study in which blood samples could be taken from

individuals before and after prolonged lithium medication.

Phosphoglucomutase, fructose 1,6-bisphosphatase and glycogen synthase kinase 3 are inhibited by lithium and are all related to glycogen metabolism (Figure 3). However, the role of glycogen in brain metabolism and mood disorders is not well understood. Glial cells play an important role in glycogen metabolism in the brain. Recent findings indicate that glycogen in astrocytes can support axon function under both pathological and physiological conditions, with astrocytes

exporting lactate to neurons by degrading glycogen during an aglycemia insult (16). It has also been found that the number of glial cells is reduced in major depressive disorders, and bipolar patients not treated with lithium or valproate had a significant glial reduction (17). Inhibition of phosphoglucomutase and glycogen synthase kinase 3 enzymes could impair glycogen turnover. However, prolonged lithium treatment might result in increased glycogen metabolism in the brain.

An important aspect of our results is that phosphoglucomutase inhibition by lithium ( $IC_{50}$ ) in erythrocytes showed a bimodal behavior among the subjects studied: in part of the population the  $IC_{50}$  was less than 1 mM, close to the therapeutic range used, while in another part the  $IC_{50}$  was more than 3 mM, far from the range used therapeutically. Both control and lithium-treated subjects showed a bimodal distribution, which suggests that at least two different isoforms of phosphoglucomutase are normally expressed in erythrocytes. Expression of the resistant form is not correlated to prolonged lithium treatment since the distribution of  $IC_{50}$  in lithium-treated subjects was not related to lithemia (Figure 2B). If phosphoglucomutase is an important target for lithium therapy we might expect subjects with a high lithium  $IC_{50}$  not to be responsive. This issue must be studied further in patients undergoing lithium monotherapy.

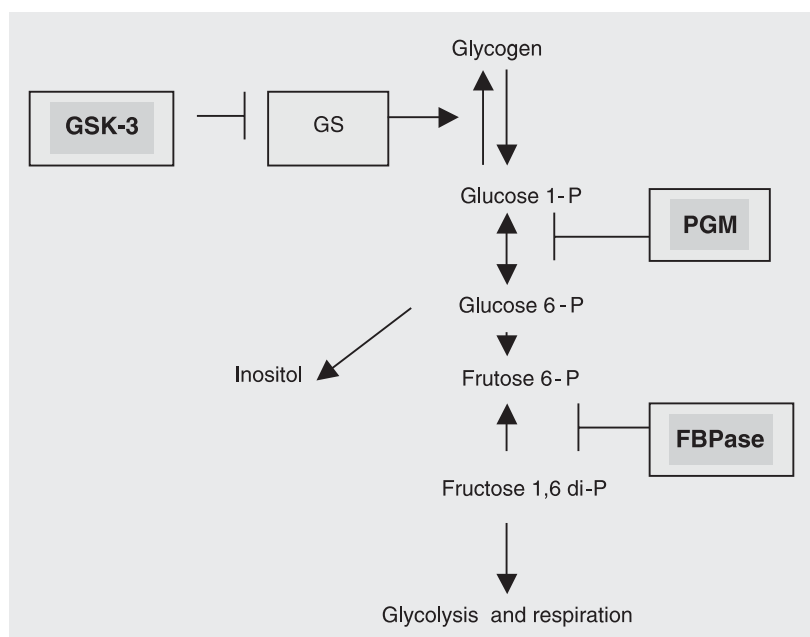


Figure 3. Lithium inhibits three enzymes involved in glycogen metabolism. Depending on the energy demands of the cell, glucose 6-phosphate (glucose 6-P) can be consumed by glycolysis and respiration or stocked in the form of glycogen. Two main enzymes involved in glycogen turnover are inhibited by lithium: phosphoglucomutase (PGM), which catalyzes the reversible formation of glucose 6-P to glucose 1-phosphate (glucose 1-P) and glycogen synthase kinase 3 (GSK-3), which in the active phosphorylated form inhibits glycogen synthase (GS). Lithium also inhibits fructose-1,6-bisphosphatase (FBPase) that converts fructose-1,6-bisphosphate to fructose-6-phosphate in gluconeogenesis. Inhibition of these enzymes suggests that the therapeutic effect of lithium may be related to regulation of glucose metabolism.

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