

Research report

# Chronic lithium and sodium valproate both decrease the concentration of *myo*-inositol and increase the concentration of inositol monophosphates in rat brain

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## Abstract

One of the mechanisms underlying lithium's efficacy as a mood stabilizer in bipolar disorder has been proposed to be via its effects on the phosphoinositol cycle (PI-cycle), where it is an inhibitor of the enzyme converting inositol monophosphates to *myo*-inositol. In contrast, sodium valproate, another commonly used mood stabilizer, appears to have no direct effects on this enzyme and was thus believed to have a different mechanism of action. In the present study, high resolution nuclear magnetic resonance (NMR) spectroscopy was used to study the chronic effects of both lithium and sodium valproate on the concentrations of *myo*-inositol and inositol monophosphates in rat brain. As predicted, lithium-treated rats exhibited a significant increase in the concentration of inositol monophosphates and a significant decrease in *myo*-inositol concentration compared to saline-treated controls. However, unexpectedly, sodium valproate administration produced exactly the same results as lithium administration. These novel findings suggest that both lithium and sodium valproate may share a common mechanism of action in the treatment of bipolar disorder via actions on the PI-cycle. © 2000 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

Bipolar disorder is a serious psychiatric illness affecting at least 1% of the population and is characterized by fluctuations between manic and depressed mood states [11]. While the physiological basis of this condition remains to be elucidated, the search for common mechanisms of action between mood stabilizers may provide insight into the causes of bipolar disorder.

Although lithium has remained the primary treatment of bipolar disorder for decades, other medications like sodium valproate are now commonly used as mood stabilizers [29]. One widely accepted hypothesis used to explain

lithium's mechanism of action is the inositol-depletion hypothesis [3] outlined in Fig. 1. This hypothesis proposes that uncompetitive inhibition of inositol monophosphatase (IMPase) by lithium [17,36] leads to an accumulation of inositol monophosphates and a corresponding depletion of *myo*-inositol, particularly in overactive cells. Regarding lithium's effects on the PI-cycle, animal studies have provided support for this hypothesis [1,19,28,41]. A number of in vitro cell studies have also found changes consistent with the inositol depletion hypothesis following stimulation of the PI cycle via receptor linked activation by a suitable agonist [15,18,42,46]. In contrast to lithium, valproate does not appear to inhibit IMPase [47] thus leading to the conclusion that this drug should not affect concentration levels of *myo*-inositol or inositol monophosphates.

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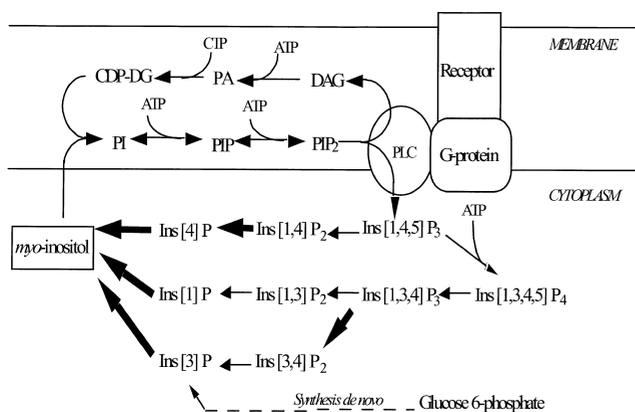


Fig. 1. Phosphoinositide cycle (PI-cycle). Lithium blocks the enzyme, inositol monophosphatase preventing the conversion of inositol-1-phosphate, (Ins[1]P), inositol-1-phosphate (Ins[3]P), and inositol-4-phosphate (Ins[4]P) to *myo*-inositol. It has been hypothesized that this leads to an accumulation of inositol monophosphates and a depletion of *myo*-inositol (steps blocked by lithium are shown by bold arrows). Reduced *myo*-inositol levels prevent the efficient formation of phosphoinositide (PI) from *myo*-inositol and cytidine diphosphodiacylglycerol (CDP-DG). Phosphatidylinositol-4,5-bisphosphate (PIP<sub>2</sub>), which is cleaved into two second messengers, inositol-1,4,5-trisphosphate (Ins[1,4,5]P<sub>3</sub>) and diacylglycerol (DAG) is subsequently decreased. An overall dampening of PI-cycle functioning is thus postulated to occur. Of note, *de novo* synthesis of *myo*-inositol is also prevented in the presence of lithium as the metabolism of glucose-6-phosphate to *myo*-inositol passes through Ins[3]P.

As observed in Fig. 1, the generation of two important intracellular second messengers, inositol 1,4,5-trisphosphate (Ins[1,4,5]P<sub>3</sub>) and *sn*-1,2-diacylglycerol (DAG), occurs within the PI-cycle. Their formation is thus dependent on the efficient breakdown of inositol phosphates and subsequent formation of inositol. A dampening of the PI-cycle by lithium has a number of implications for cell function based on the roles of Ins[1,4,5]P<sub>3</sub> and DAG in mediating Ca<sup>2+</sup> release from the endoplasmic reticulum and protein kinase C (PKC) activity, respectively.

*In vivo* magnetic resonance spectroscopy (MRS) is increasingly being used to examine the neurochemical basis of psychiatric illness, and the effects of psychiatric medications. MRS has gained popularity in the study of bipolar disorder because *myo*-inositol concentrations can be measured using <sup>1</sup>H MRS. Quantitative *in vivo* <sup>1</sup>H MRS studies of bipolar patients treated with or without lithium have revealed that *myo*-inositol decreases following lithium treatment [34,35] and that bipolar patients may have elevated pre-treatment levels of *myo*-inositol [51].

Human <sup>31</sup>P MRS has also been used extensively in studies of bipolar disorder, since the inositol monophosphates are contained in the phosphomonoester (PME) peak of <sup>31</sup>P spectra. In one study yielding results consistent with the inositol depletion hypothesis, an increase in the PME peak was found in lithium-treated healthy controls as compared to placebo-treated controls following amphetamine stimulation of the PI-cycle [44]. A number of other studies have found increased PME peak ratios in manic

and depressed bipolar patients [21,23,24] and decreased PME peak ratios during the euthymic phase of the illness compared to healthy controls [21,24]. However, these effects have been observed in both medicated and unmedicated bipolar patients [9,10,21] and thus it is difficult to isolate drug effects from the effects of the illness.

In the present study, we examined the effects of chronic lithium and sodium valproate administration on the concentrations of *myo*-inositol and inositol monophosphates in whole rat brain. Acute *D*-amphetamine was administered and tested as a possible *in vivo* stimulant of the PI-cycle. Also examined were the effects of these two drugs on glycine, the other brain neurochemical contributing to the human *in vivo* <sup>1</sup>H *myo*-inositol resonances, and glucose-6-phosphate (G6P) and phosphocholine (PC), two compounds which contribute to the human *in vivo* <sup>31</sup>P PME. High resolution *in vitro* NMR was used to resolve these peaks into their component parts, which cannot yet be accomplished in humans *in vivo*. Finally, we wished to examine the effects of lithium and valproate on the commonly used *in vivo* <sup>1</sup>H reference peaks of creatine+phosphocreatine (Cr+PCr) and *N*-acetyl aspartate (NAA) which are often used in the expression of ratio data where absolute quantification methods are not employed.

## 2. Materials and methods

This study was approved by the local ethics committee and all procedures were carried out in accordance with the guidelines of the Canadian Council on Animal Care. Adult male Sprague–Dawley rats (Ellerslie Biosciences), weighing 250–350 g were housed in Plexiglas cages. The rats were given free access to food and water and were maintained on an alternating 12-h light/12-h dark cycle. Injections of lithium, sodium valproate, and saline were started 4 days after the rats arrived giving them an opportunity to adjust to their new environment.

Each rat received a twice daily (b.i.d) intraperitoneal (IP) injection of either 2.0 mmol/kg lithium chloride (*n*=18) (Fisher Scientific, Fair Lawn, NJ, USA), 300 mg/kg sodium valproate (*n*=18) (Sigma Chemical Company, St Louis, MO, USA), or saline (*n*=18) at 07.30 h and 16.00 h for 14 days. Sodium valproate was initially administered in a dose of 400 mg/kg b.i.d but this was decreased after 2 days when the rats exhibited symptoms of excessive sedation. All injections were administered in volumes of 2 ml/kg and the doses used in this study have been previously shown to produce therapeutic serum levels of lithium [14] and valproate [6] in rats.

On day 15, the rats were administered their morning injection followed by an acute injection of 3.0 mg/kg of *D*-amphetamine or saline (*n*=9 for all six treatment groups) after 120 min. Thirty minutes later, they were decapitated. The brains were rapidly removed, immediately immersed

in ice-cold isopentane, and then maintained at  $-80^{\circ}\text{C}$  until extract preparation and NMR analysis.

Samples were prepared using a modified version of the total lipid extraction method as described by Bligh and Dyer [5]. Whole rat brains were homogenized in 4 volumes of methanol/chloroform (2:1,v/v; Fisher Scientific, Fair Lawn, NJ, USA). This was followed by the subsequent additions of one part of chloroform with homogenization, and one part of water with homogenization. Ten milliliters of homogenate was transferred to a test tube and centrifuged at 1000 *g* for 15 min in a bench top centrifuge (Sorvall GLC-2B, Dupont, Wilmington, DE, USA). Following centrifugation, 3.5 ml of the water/methanol layer was transferred to a 100×13 mm screw cap culture tube and maintained at  $-20^{\circ}\text{C}$  overnight. The next day, samples were dried using vacuum centrifugation (Speed Vac, Savant) and then reconstituted in 0.83 ml of deuterated water ( $\text{D}_2\text{O}$ ; Aldrich Chemical Company, Milwaukee, WI, USA) containing the  $^1\text{H}$  NMR standard, sodium 3-trimethylsilyl [2,2,3,3,  $^{-2}\text{H}$ ] propionate (TSP; Canadian Isotopes, Pointe-Claire, Que., CA), and the  $^{31}\text{P}$  NMR standard, methylenediphosphonic acid trisodium salt tetrahydrate (DSS; Sigma Chemical Company, St Louis, MO, USA), at concentrations of 1.56 mM and 0.98 mM, respectively. In addition to acting as internal chemical shift references, these two compounds made exact quantification of metabolite concentrations possible.

$^{31}\text{P}$  NMR spectra of the extracts were recorded at 11.75 T on a Varian Unity 500 NMR spectrometer using 45 degree (10  $\mu\text{s}$ ) pulses and an acquisition time of 1.6 s spanning 20 kHz. Typical  $^{31}\text{P}$  spectra were the sum of 3000–4000 scans each with  $^1\text{H}$  broad band decoupling using a standard Varian broad band 5 mm NMR probe.  $^1\text{H}$  NMR spectra of the extracts were obtained on the same spectrometer using 40 degree (4  $\mu\text{s}$ ) pulses, 32 acquisitions/spectrum, and an acquisition time of 3 s spanning 8 kHz.

Spectral analysis was carried out blind to the drug treatment received by the animals. After noise filtering and baseline correction, peak areas in extract spectra were calculated using a Gaussian total line shape analysis using the Peak Research (PERCH) spectrum analysis software package (distributed by PERCH project, Department of Chemistry, University of Kuopio, Kuopio, Finland). Concentrations were determined for *myo*-inositol, glycine, Cr+PCr, and NAA using  $^1\text{H}$  NMR while concentrations for inositol monophosphates+phosphoethanolamine ( $\text{IP}_1 + \text{PE}$ ), G6P, PC, glycerophosphoethanolamine (GPE), and glycerophosphocholine (GPC) were determined using  $^{31}\text{P}$  NMR. All metabolite concentrations were calculated by comparing peak areas to that of the added internal chemical shift standards of known concentration. To determine significant changes in concentration, two-way analysis of variance (ANOVA) with Tukey post-hoc analysis was employed (SPSS for Windows, Release 7.5.1) with chronic drug (saline, lithium, or sodium valproate) and acute drug

(saline, amphetamine) as the two factors. Results were deemed significant at  $P \leq 0.05$ .

### 3. Results

#### 3.1. $^1\text{H}$ NMR spectroscopy

*Myo*-inositol has been previously shown to give multiplet signals at 3.28, 3.54, 3.62, and 4.06 ppm in  $^1\text{H}$  NMR spectra of rat brain extracts [2]. Because the signals at 3.54 and 3.62 ppm are well resolved and relatively free of overlap from other metabolite signals, an average area of these two multiplets was used to quantify *myo*-inositol concentrations in the brain extracts. Fig. 2 shows a cropped 500 MHz  $^1\text{H}$  NMR spectrum of the brain extracts focused on the region where these two peaks are located. Concentrations for NAA, glycine, and creatine were calculated using the singlet peaks at approximately 2.02, 3.56, and 3.93 ppm, respectively [2].

Whole brain concentrations ( $\mu\text{mol/g}$  wet weight) of *myo*-inositol after chronic lithium, sodium valproate, or saline administration are illustrated graphically in Fig. 3 and numerically in Table 1. Significant drug effects of lithium and sodium valproate were observed ( $F=23.559$ , *d.f.*=2,  $P<0.001$ ). Tukey post-hoc results showed that both lithium- ( $P<0.001$ ) and valproate- ( $P<0.001$ ) treated rats exhibited significantly decreased whole brain *myo*-inositol concentrations compared to saline-treated rats. D-amphetamine had no effect on *myo*-inositol concentrations ( $F=1.274$ , *d.f.*=1,  $P<0.27$ ).

As shown in Figs. 4 and 5, significant drug effects of lithium and sodium valproate were also observed with Cr+PCr ( $F=16.284$ , *d.f.*=2,  $P<0.001$ ) and NAA ( $F=$

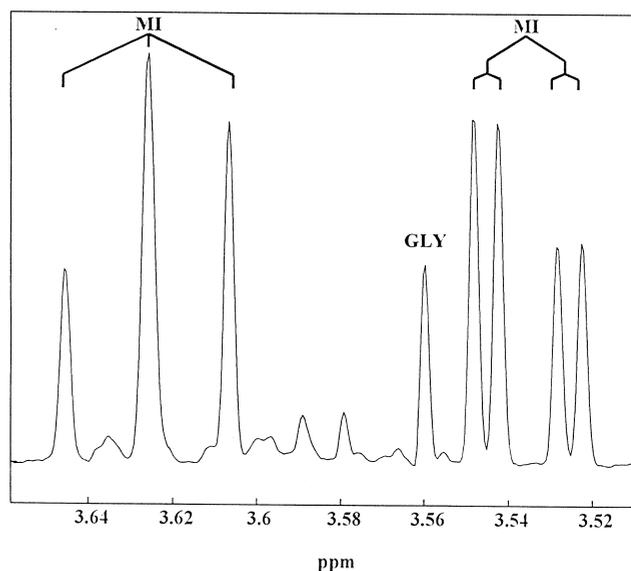


Fig. 2. 500 MHz  $^1\text{H}$  NMR spectrum from 3.51 to 3.66 ppm of rat brain extracts. GLY, glycine; MI, *myo*-inositol.

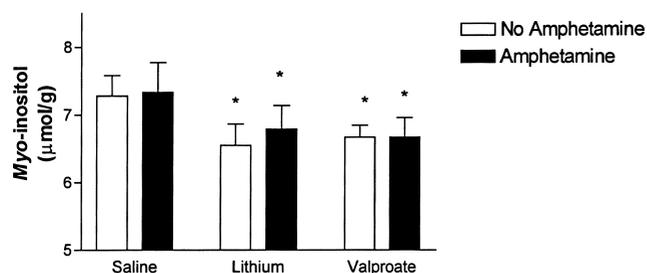


Fig. 3. Whole rat brain concentrations of *myo*-inositol following chronic saline, lithium or sodium valproate treatment and acute treatment of either saline or *D*-amphetamine. \*significantly different from rats treated chronically with saline,  $P < 0.001$ . *D*-amphetamine had no significant effects.

Table 1  
Concentration of metabolites in rat brain following chronic treatment<sup>a</sup>

Metabolite	Saline ( $\mu\text{mol/g}$ )	Lithium ( $\mu\text{mol/g}$ )	Valproate ( $\mu\text{mol/g}$ )
Cr+PCr	10.56 $\pm$ 0.40	9.65 $\pm$ 0.38*	9.84 $\pm$ 0.37*
<i>Myo</i> -inositol	7.28 $\pm$ 0.31	6.60 $\pm$ 0.33*	6.63 $\pm$ 0.17*
NAA	8.20 $\pm$ 0.40	7.47 $\pm$ 0.40*	7.26 $\pm$ 0.24*
Glycine	1.16 $\pm$ 0.14	1.12 $\pm$ 0.06	1.16 $\pm$ 0.08
IP <sub>1</sub> +PE	1.20 $\pm$ 0.07	1.39 $\pm$ 0.16*	1.39 $\pm$ 0.12*
G6P	0.226 $\pm$ 0.021	0.217 $\pm$ 0.013	0.222 $\pm$ 0.019
GPC	0.631 $\pm$ 0.241	0.525 $\pm$ 0.065	0.502 $\pm$ 0.049
GPE	0.261 $\pm$ 0.052	0.224 $\pm$ 0.027	0.237 $\pm$ 0.012
PC	2.55 $\pm$ 0.11	2.60 $\pm$ 0.18	2.65 $\pm$ 0.11
<i>Myo</i> -inositol/(Cr+PCr)	0.699 $\pm$ 0.021	0.688 $\pm$ 0.037	0.680 $\pm$ 0.027

<sup>a</sup> Values represent the mean $\pm$ S.D. Concentrations were calculated using the internal standards, TSP, in high resolution (500 MHz) <sup>1</sup>H NMR spectra and DSS, in high resolution (202.3 MHz) <sup>31</sup>P NMR spectra.

\* Significantly different ( $P < 0.05$ ) from saline-treated rats.

24.061, d.f.=2,  $P < 0.001$ ). Tukey post-hoc results showed that both lithium- ( $P < 0.001$ ) and valproate- ( $P < 0.001$ ) treated rats exhibited significantly decreased whole brain creatine and NAA concentrations compared to saline-treated rats. Whole brain concentrations of Cr+PCr and NAA following chronic saline or drug administration are illustrated in Table 1. *D*-amphetamine had no effect on Cr+PCr ( $F = 0.100$ , d.f.=1,  $P < 0.76$ ) or NAA ( $F = 0.206$ , d.f.=1,  $P < 0.66$ ) concentrations.

No significant drug effects were observed with whole brain concentrations of glycine ( $F = 2.607$ , d.f.=2,  $P <$

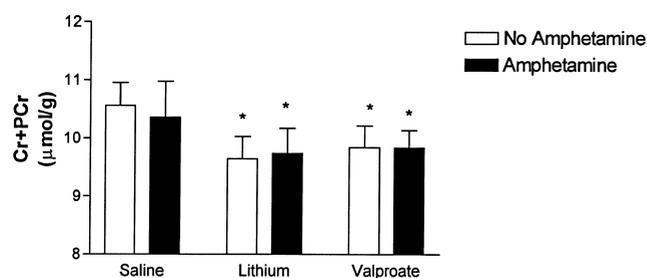


Fig. 4. Whole rat brain concentrations of Cr+PCr following chronic saline, lithium or sodium valproate treatment and acute treatment of either saline or *D*-amphetamine. \*significantly different from rats treated chronically with saline,  $P < 0.001$ . *D*-amphetamine had no significant effects.

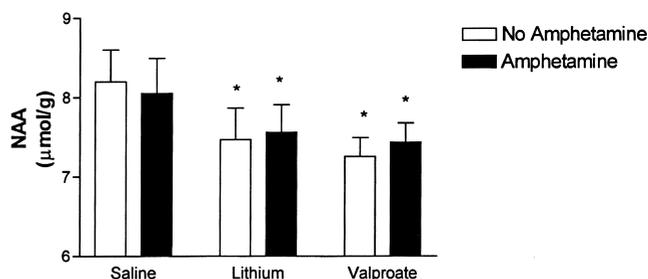


Fig. 5. Whole rat brain concentrations of NAA following chronic saline, lithium or sodium valproate treatment and acute treatment of either saline or *D*-amphetamine. \*significantly different from rats treated chronically with saline,  $P < 0.001$ . *D*-amphetamine had no significant effects.

0.09). Whole brain concentrations of glycine following chronic saline or drug administration are illustrated numerically in Table 1. *D*-amphetamine had no effect on whole brain glycine concentrations ( $F = 2.110$ , d.f.=1,  $P < 0.16$ ).

### 3.2. <sup>31</sup>P NMR spectroscopy

Fig. 6 shows a typical 202.3 MHz <sup>31</sup>P NMR spectrum of brain extract. It should be noted that the inositol monophosphates (IP<sub>1</sub>) are co-resonant with phosphoethanolamine (PE) in the region around 6.4 ppm [39]. Because it is not possible to resolve the IP<sub>1</sub> signal from the PE signal, a combined concentration for these two compounds was calculated from the observed peak area. Fig. 7 and Table 1 illustrate the brain concentrations of IP<sub>1</sub>+PE calculated from the observed peak following chronic lithium or sodium valproate administration. As with *myo*-inositol, there was a significant group effect with IP<sub>1</sub>+PE

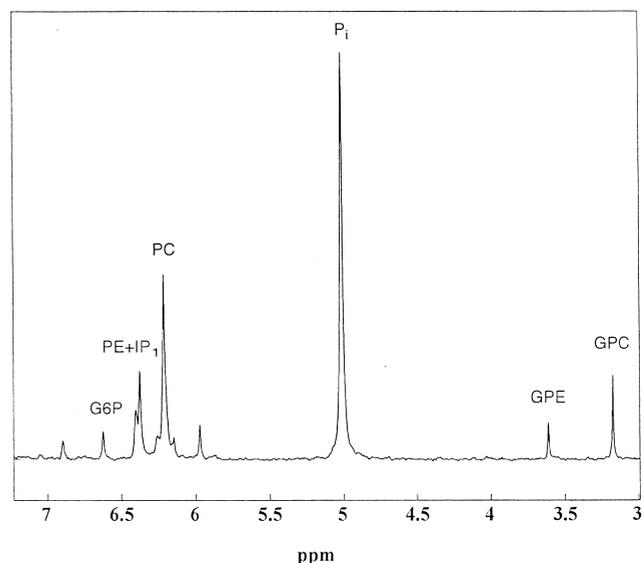


Fig. 6. 202.3 MHz <sup>31</sup>P NMR spectrum from 3.00 to 7.25 ppm of rat brain extracts. G6P, glucose-6-phosphate; GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; IP<sub>1</sub>, inositol monophosphates; PC, phosphocholine; PE, phosphoethanolamine; P<sub>i</sub>, inorganic phosphate.

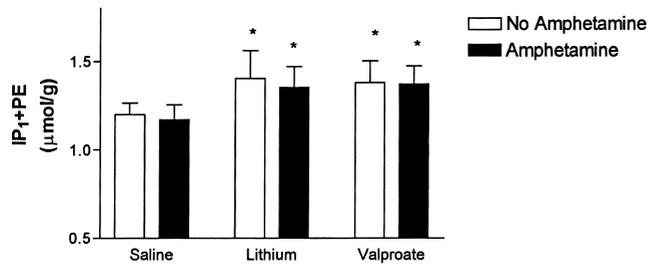


Fig. 7. Whole rat brain concentrations of IP<sub>1</sub>+PE following chronic saline, lithium or sodium valproate treatment and acute treatment of either saline or D-amphetamine. \*significantly different from rats treated chronically with saline,  $P < 0.001$ . D-amphetamine had no significant effects.

due to chronic drug treatment ( $F = 17.315$ ,  $d.f. = 2$ ,  $P < 0.001$ ). Tukey post-hoc results showed that both lithium- ( $P < 0.001$ ) and valproate- ( $P < 0.001$ ) treated rats exhibited significantly increased IP<sub>1</sub>+PE whole brain concentrations compared to saline-treated rats. D-amphetamine had no effect on IP<sub>1</sub>+PE concentrations ( $F = 0.880$ ,  $d.f. = 1$ ,  $P < 0.36$ ).

No significant changes were found with G6P ( $F = 0.642$ ,  $d.f. = 2$ ,  $P < 0.53$ ), PC ( $F = 2.322$ ,  $d.f. = 2$ ,  $P < 0.62$ ), GPE ( $F = 1.910$ ,  $d.f. = 2$ ,  $P < 0.16$ ), or GPC ( $F = 2.491$ ,  $d.f. = 2$ ,  $P < 0.10$ ). Whole brain concentrations of these compounds following chronic saline or drug administration are illustrated in Table 1. D-amphetamine had no effect on PC ( $F = 0.263$ ,  $d.f. = 1$ ,  $P < 0.11$ ), GPE ( $F = 0.014$ ,  $d.f. = 1$ ,  $P < 0.91$ ), or GPC ( $F = 0.089$ ,  $d.f. = 1$ ,  $P < 0.77$ ) concentrations but did have a significant effect on G6P ( $F = 5.035$ ,  $d.f. = 1$ ,  $P < 0.030$ ).

## 4. Discussion

### 4.1. Effects of lithium and sodium valproate on the PI-cycle

The inositol depletion hypothesis was originally proposed to explain lithium's clinical effectiveness following evidence of IMPase inhibition by lithium. For the first time we have demonstrated that therapeutic doses of either lithium or sodium valproate increase the concentration of inositol monophosphates and decrease the concentration of *myo*-inositol following chronic administration in rats. However, it is unlikely that both of these drugs act directly via IMPase, since only lithium has been shown to have an effect on this enzyme [17,41,47]. Thus, at present, the mechanism of valproate remains unknown even though lithium and valproate appear to be having similar effects on the PI-cycle. In contrast to our hypothesis regarding the stimulation of the PI-cycle, D-amphetamine did not potentiate these changes in the present study.

These findings add to other research suggesting that lithium and valproate may have common effects on PI-cycle functioning [45]. Incubation of mouse brain slices

with sodium valproate was shown to lead to an accumulation of Ins[1,4,5]P<sub>3</sub>, but not inositol monophosphates [12]. Another study found a significant attenuation of striatal agonist-stimulated inositol phosphate formation following chronic sodium valproate treatment suggesting effects of chronic sodium valproate on PI-cycle functioning [26].

Both lithium and sodium valproate have been shown to have effects on the protein kinase C (PKC) family of enzymes closely associated with PI-cycle functioning. PKC isozymes are activated when they are translocated from the membrane to the cytosol in the presence of DAG, one of the PI-cycle's two second messengers [7,30,32]. PKC isozymes  $\alpha$  and  $\epsilon$  have been found to be down-regulated in brain cells of rats following chronic administration of both lithium and sodium valproate [25,31].

Extending the effect of these two drugs on PKC activity, there have also been reported changes in PKC substrates following drug administration. Myristoylated alanine-rich-C-kinase substrate (MARCKS), associated with signal transduction and neurotransmitter release, is inactivated by PKC phosphorylation and has been found to be reduced in hippocampal cells following chronic lithium [49] or sodium valproate [48] administration. Interestingly, the down-regulation of PKC isozymes and MARCKS by lithium is prevented in the presence of excess inositol [7,48]. The effect of excess inositol in the presence of sodium valproate has not yet been studied. Finally, an increase in the binding activity of AP-1, another PKC substrate and a major transcription factor in the CNS known to regulate the activity of many genes, has been observed following the incubation of brain cells with both valproate [8] and lithium [38,50]. Thus it is possible that both lithium and sodium valproate have common effects on the PI-cycle second messenger system that are also reflected in actions on 'downstream' systems such as PKC isozyme activity.

### 4.2. Effects of lithium and sodium valproate on creatine and NAA

A number of in vivo <sup>1</sup>H MRS studies have used a ratio method of data expression where the Cr+PCr peak acts as a reference against which all compounds of interest are compared. Ratio data expression is usually employed where technology is not available to quantify metabolites using in vivo <sup>1</sup>H MRS. However, we have demonstrated that this method of data expression is inappropriate when lithium or sodium valproate are administered. Both drugs caused a significant decrease in Cr+PCr concentrations in whole rat brain following chronic administration. Therefore, the Cr+PCr peak is likely not an adequate internal standard for in vivo <sup>1</sup>H MRS research where lithium or sodium valproate is administered to patients or controls because significant changes in the ratio may reflect drug-dependent variations in Cr+PCr concentrations rather than those in the compound of interest. Also, non-significant changes may result when concentration changes in the

numerator are of the same magnitude as those in the denominator. This is illustrated in Table 1 where ratios of *myo*-inositol/(Cr+PCr) from the present study are shown to be non-significant.

In human in vivo  $^1\text{H}$  MRS research, NAA is another commonly used internal standard. Because it is found primarily in neurons, NAA is often used as a neuronal marker in MRS research [4]. Also there is evidence that a decrease in NAA correlates well with a decrease in neuronal density [13,37]. However, it is not known whether NAA concentrations may change in response to acute or chronic drug effects. In the present study, chronic lithium and sodium valproate both caused NAA concentrations to significantly decrease in rat brain. These results suggest that caution must be exercised when interpreting decreases in NAA as cell death when the possibility exists that neurons can have varying levels of NAA caused by an inherent dysfunction or the presence of drugs like lithium or sodium valproate. Further research is required to identify the mechanism by which neuronal NAA is decreasing in the presence of lithium or valproate.

In terms of MRS research and its use as an internal standard, like creatine, NAA is likely not an adequate internal standard where patients or controls have been medicated with lithium or sodium valproate. These findings with creatine and NAA suggest that future in vivo  $^1\text{H}$  MRS research of lithium- and sodium valproate-treated patients must employ quantification methods versus ratio data expression if the results obtained are to be reliable.

#### 4.3. Implications for human in vivo MRS research of lithium- or valproate-medicated subjects

Since magnetic field strengths of 4 tesla (T) or less are normally used on in vivo human brain, the resolution of  $^1\text{H}$  NMR spectra tends to be poor and the *myo*-inositol peaks are co-resonant with signals from glycine and the inositol monophosphates. At field strengths of 3 T or greater, two *myo*-inositol peaks can be adequately resolved for quantification at 3.54 and 3.66 ppm. However, the peak at 3.54 ppm also contains contributions from glycine and the inositol monophosphates, whereas the *myo*-inositol peak at 3.66 ppm is only contaminated with signals from the inositol monophosphates, but is usually of poorer quality than that at 3.54 ppm.

Previous studies have suggested that both lithium and sodium valproate may alter glycine levels in rat brain [20,27,33]. However, in the present study we have found that neither lithium or sodium valproate cause changes in rat brain glycine concentrations. The inconsistency in these findings makes it difficult to draw conclusions regarding the effect of lithium and sodium valproate on brain concentrations of glycine. In terms of the present study, the finding that glycine does not change following lithium or valproate treatment is important in terms of the inositol depletion hypothesis, since glycine and *myo*-inositol co-

resonate at 3.54 ppm in in vivo spectra at field strengths currently employed. Since no concentration changes in glycine were detected using high resolution  $^1\text{H}$  NMR following chronic lithium or sodium valproate administration in rat brain, this suggests that any observed significant changes in the 3.54 ppm peak observed in in vivo studies of the effects of lithium are most likely due to changes in *myo*-inositol.

The PME peak observed in in vivo  $^{31}\text{P}$  spectra has been reported to be abnormal in both medicated and unmedicated bipolar patients [9,11,14,21,22,24]. This peak is made up of contributions from the inositol monophosphates, PC, PE, and sugar phosphates like G6P [16] with the signal from the inositol monophosphates forming approximately 10% of the peak [43]. Therefore, it is difficult to know which of these contributing compounds is responsible for the changes in the PME peak observed in bipolar patients. Also, it is not known what the effects of lithium and sodium valproate are on the other contributors to the PME peak (i.e. PC, PE, sugar phosphates). However, one animal study used in vitro NMR of brain extracts to show that changes in the in vivo  $^{31}\text{P}$  PME peak following lithium treatment were correlated with increases in inositol-1-phosphate measured quantitatively [40].

In the present study, high resolution 202.3 MHz  $^{31}\text{P}$  NMR allowed us to isolate PC and G6P from the inositol monophosphates of the PME peak found in in vivo  $^{31}\text{P}$  MRS spectra. No significant changes in PC or G6P concentrations were found in the high resolution NMR extracts of whole brain following chronic drug treatment with lithium or valproate. Therefore it is unlikely that changes in the PME peak of in vivo  $^{31}\text{P}$  MRS spectra of medicated subjects is due to changes in PC or G6P concentrations. Also, because the concentrations of these two compounds did not change, it is very likely that changes in the PME peak of in vivo  $^{31}\text{P}$  MRS spectra represent changes in inositol monophosphate concentrations.

The current study has the limitations of being carried out with rat brain, and therefore may not be directly comparable to human studies. However, since it is not yet possible to do in vivo studies of human brain at the resolution necessary to examine each compound of interest individually, we must rely on animal data. Furthermore, the current study was conducted using post-mortem brain tissue, which again may not be directly comparable to human tissue in vivo. However, there were consistent changes in the lithium- and valproate-treated rats, which were not observed in the saline-treated rats, which suggests that there were no systematic post-mortem changes which could account for the data.

In conclusion, the primary finding of this study was that chronic treatment of rats with lithium or sodium valproate results in identical effects on the PI-cycle, namely a decrease in whole brain *myo*-inositol concentrations and an increase in whole brain inositol monophosphate concen-

trations. While the precise mechanism underlying these changes is not known at the present time, this finding suggests that lithium and sodium valproate may act through a common cellular mechanism involving the PI-cycle. We have also shown that both lithium sodium valproate decreased the concentrations of both Cr+PCr and NAA, two common internal references used in human in vivo <sup>1</sup>H MRS studies. This finding highlights the need for caution in the interpretation of in vivo MRS studies using ratio data.

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