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Inhibition of glutaminase activity of rat brain by lithium

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The efficacy of lithium salts in the treatment of manic depressive psychosis is well documented [1–3] although the mechanism of its action is not well understood. Information available about its *in vivo* effects on glutamate metabolism [4–6] is however very scanty and *in vitro* experiments [7–9] indicate that lithium salts may influence the binding, uptake and metabolism of γ-aminobutyric acid and glutamate. No investigation seems to have been carried out regarding the possible effects of lithium salts on the synthesis and breakdown of glutamine. The present communication describes the effects of lithium chloride on glutamine hydrolysis in rat liver and brain.

Male albino rats (150–175 g) were killed by decapitation and the brain tissues were removed and homogenized in chilled 0.25 M sucrose to give a 10% suspension (w/v) and the crude mitochondrial fraction was prepared according to Brody and Bain [10] and served as the source of phosphate activated glutaminase (Glutaminase I). The liver extract was obtained by centrifuging a 20% (w/v) homogenate in ice cold glass distilled water [11] (pH 7.5) at 1000 *g* for 5 min and the resulting supernatant was then centrifuged at 105,000 *g* for 60 min. The supernatant obtained was used as the source of α-keto acid mediated glutaminase (Glutaminase II). Glutaminase I (E.C. 3.5.1.2) was assayed according to Horowitz and Knox [12], and α-keto acid activated glutaminase (E.C. 2.6.1.15) activity (Glutaminase II) was assayed according to the method of Errera [11]. Both glutaminase I and glutaminase II activities were determined by estimating the ammonia formed employing the diffusion technique of Conway and Byrne [13].

The enzyme was preincubated with lithium chloride for 15 min at 37° prior to addition of the substrate. Preliminary experiments indicated that under the experimental conditions employed the different enzyme activities assayed were linear as a function of time and concentration of enzyme.

Protein was estimated by the method of Lowry *et al.* [14] using bovine serum albumin as the standard. L-Glutamine, α-oxoglutaric acid, pyruvic acid, tri-hydroxy-methyl-aminomethane and maleic acid were the commercial products of Sigma Chemical Co., St. Louis, U.S.A. Other reagents used were of analytical grade.

Table 1 depicts *in vitro* effect of lithium chloride on glutaminase I activity of the crude mitochondrial fraction of rat brain. The assays were carried out at two different pH values, the optimum pH of 8.5 and the near physio-

Table 1. Effects of lithium on glutaminase I activity of rat brain mitochondria

Phosphate concentrations (M)	pH of assay system	Glutaminase I activity (μmoles of ammonia formed/hr/mg protein (±S.D.))		Per cent inhibition of glutaminase I activity
		Without lithium chloride	With lithium chloride (0.05 M)	
0.005	7.4	2.5 ± 0.02	0.1 ± 0.05	96
0.01	7.4	4.2 ± 0.02	1.6 ± 0.02	96
0.02	7.4	6.2 ± 0.05	1.7 ± 0.04	72
0.04	7.4	9.9 ± 0.20	0.9 ± 0.05	64
0.005	8.5	3.2 ± 0.16	0.6 ± 0.05	81
0.01	8.5	3.8 ± 0.07	1.2 ± 0.03	67
0.02	8.5	6.8 ± 0.16	3.9 ± 0.09	41
0.04	8.5	7.8 ± 0.05	6.7 ± 0.03	14

The reaction mixture contained 0.06 M Tris–maleate buffer of pH 7.4 or 8.5, 0.004 M L-glutamine and crude mitochondria of brain equivalent to 4 mg protein in a final volume of 3 ml. Varied concentrations of phosphate of respective pH values were added as indicated in the table. The enzyme was preincubated with lithium chloride adjusted to pH 7.4 or 8.5 for 15 min at 37° prior to the addition of the substrate and the results are average of six determinations. Other details are given in the text.

logical pH of 7.4 and at a series of different phosphate concentrations at and below the optimum level. Lithium chloride (0.05 M) was found to exert very strong inhibitory influence at both pH values, particularly at lower phosphate concentrations. On increasing the phosphate concentrations, this inhibition could be counteracted considerably, especially at the higher pH values. Thus at the optimum and usual assay condition of pH 8.5 and 0.04 M phosphate concentration, the degree of inhibition caused by lithium chloride is only 14%. At pH 7.4, higher phosphate concentrations were not so effective in counteracting the inhibitory effect of lithium chloride. On the other hand when the concentration of lithium chloride was decreased to 0.01 M, appreciable inhibition of glutaminase I occurred at lower phosphate concentrations (0.005–0.01 M) at pH 7.4 but not at pH 8.5. Further decrease of lithium concentration could not affect the enzyme activity at pH 7.4 and lower phosphate concentrations.

The nature of the interaction between phosphate and lithium chloride was analysed by the method of Dixon [15].

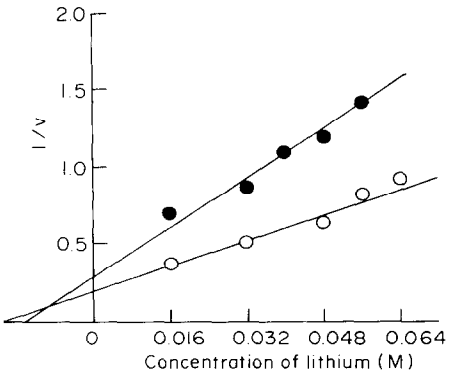

Fig. 1. Dixon plot [15] of competitive inhibition of glutaminase I by lithium with respect to phosphate. The reaction mixture contained 0.06 M Tris–maleate buffer pH 8.5, 0.004 M L-glutamine and an aliquot of mitochondrial suspension equivalent to 0.75 mg protein in a final volume of 3 ml. Varied concentrations of lithium were used as indicated in the figure and the respective phosphate concentrations were 0.01 M (—●—); and 0.02 M (—○—). Other details are given in the text.

In Fig. 1 the reciprocals of reaction velocities when plotted against inhibitor concentrations at two different phosphate concentrations, resulted in straight line intercepting on the left hand side of the ordinate indicating a competitive mode of inhibition by lithium chloride with respect to phosphate. K_i value calculated from the intercept was found to be 8×10^{-3} M. The inhibition of glutaminase I activity by lithium chloride was also found to be reversible (Fig. 2) employing the method of Ackermann and Potter [16]. It was also observed that washing the mitochondria after preincubation with lithium chloride produced reversal of the initial inhibition. α-Keto acid activated glutaminase of rat brain and liver tissues was also found to be strongly inhibited by lithium chloride (Table 2). The inhibition was found to be dependant on the dose of lithium chloride used. However, it was found that the brain enzyme was more susceptible to inhibition by lithium chloride than the liver enzyme. It was also observed (not shown) that at a higher dose of lithium chloride (0.05 M) glutamine synthetase (E.C. 6.3.1.2) activity measured according to Woolfolk *et al.* [17] is inhibited (50%) *in vitro* while at lower dose the inhibition was negligible.

It was observed that i.p. injection of lithium chloride to

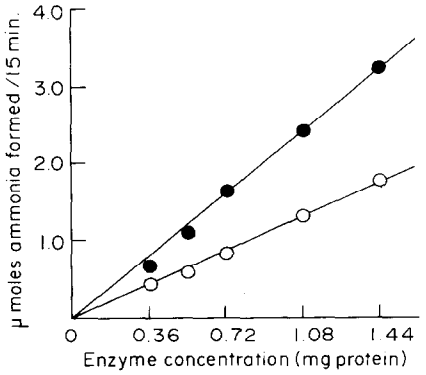

Fig. 2. Ackermann and Potter plot [16] of reversible inhibition of glutaminase I by lithium. The enzyme activity was measured with different concentrations of enzyme as indicated in the figure in absence (—●—) and in presence of 0.05 M lithium (—○—). Other details are given in the text.

Table 2. Effects of lithium on α-keto acid activated glutaminase of rat brain and liver tissue

Tissue preparation	α-Keto acid used	Lithium chloride concentrations (M)	μmoles of ammonia formed (± S.D.) without lithium chloride	μmoles of ammonia formed (± S.D.) with lithium chloride	Per cent inhibition
Brain mitochondria	2-oxoglutarate	0.025	2.8 ± 0.19	1.0 ± 0.15	64
		0.050	2.3 ± 0.68	0.3 ± 0.12	86
	pyruvate	0.025	2.1 ± 0.08	1.0 ± 0.16	51
		0.050	2.4 ± 0.19	0.6 ± 0.08	76
Liver supernatant	pyruvate	0.05	0.1 ± 0.01	0.07 ± 0.003	36
		0.10	0.1 ± 0.01	0.04 ± 0.002	63

The assay systems in the case of brain mitochondria contained 0.06 M Tris–buffer pH 7.4, 0.004 M L-glutamine, an aliquot of crude mitochondrial suspension containing 3.4 mg protein, 0.07 M pyruvate or 0.03 M 2-oxoglutarate in a final volume of 3 ml. The system used for liver tissue contained 0.01 M veronal–HCl buffer pH 7.7, 0.004 M L-glutamine, 0.02 M pyruvate and an aliquot of liver supernatant equivalent to 20 mg protein in a final volume of 4 ml. Lithium was added to the system as indicated in the table and preincubated for 15 min at 37° prior to the addition of L-glutamine. The reaction with brain and liver tissue was terminated after 60 and 50 min respectively. In case of brain the results are expressed as μmoles of ammonia formed/60 min/mg protein and in case of liver results are expressed as μmoles of ammonia formed/50 min/mg protein. Other details are given in the text. All values are average of six determinations.

rats (2.5 mmole/kg for 5 days or 5 mmole/kg for one day) failed to produce any effect on glutaminase activity. However glutamine synthesis was found to be inhibited when rats were given an acute dose of 5 mmole/kg and sacrificed within 90–150 min, but such an effect was not observed when the dose of lithium chloride was lowered. This indicates that at a therapeutical dose of lithium, as is generally administered, the possibility of inhibition of glutamine synthesis does not arise.

The above results indicate that lithium salts might interfere with the metabolic cycle of glutamic acid–glutamine as a result of which the homeostatic equilibrium of the amino acid transmitter of the glutamate–GABA system may be affected. The inhibition of phosphate activated glutaminase will produce a decreased formation of glutamate from glutamine which eventually is transformed to GABA by GAD [18–21]. However in view of high doses of lithium salts used in the above *in vitro* experiments than is used therapeutically, it appears that the effects observed are unlikely related to the mode of action of lithium *in vivo*.

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Effect of naturally occurring coumarins on the activity of drug metabolizing enzymes*

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Hepatic enzymes that metabolize foreign compounds have been shown to be affected by the treatment of the animals with a variety of environmental factors such as drugs, polycyclichydrocarbons, pesticides, industrial products and dietary factors [1–5]. It was demonstrated that these foreign compounds caused biphasic responses on the liver microsomal enzymes [6], eliciting marked changes in the pharmacological and toxicological action of drugs.

In continuing research on medicinal plants affecting drug-metabolizing function in the liver, it was found that *Angelica* spp exhibited potent biphasic effects on barbiturate action [7, 8]. Systematic fractionation of the roots of *Angelica koreana* monitoring by bioassay led to isolation of active principles which were identified as coumarins [9]. This finding and previous reports [10–12] that several synthetic anticoagulant coumarins induced drug-metabolizing enzymes (DME) prompted us to investigate the effect of some structurally related natural coumarins on DME in order to elucidate the structure–activity relationship.

Materials and methods

Animals. For *in vivo* studies, male albino mice weighing 20 ± 3 g were used. The animals were fed lab chows and tap water *ad lib.* Constant-temperature environments were maintained throughout the experiments. For enzyme preparations, male Sprague–Dawley (CD strain) rats weighing 200–250 g were used.

Materials. Coumarins shown in Fig. 1 were isolated from the Umbelliferae. SKF-525A was a gift from Smith, Kline & French (Philadelphia, PA).

Measurement of hexobarbital- (HB) induced sleeping time. The effect on the HB-induced sleeping time was investigated in two phases. During the first phase mice were administered i.p. with each compound 30 min prior to the injection of HB-Na (50 mg/kg i.p.) and the duration of the loss of the righting reflex was estimated. In the

* Part 5 in the series: Studies on crude drugs acting on drug-metabolizing enzymes. For Part 4 see ref. [9].